JOINT SPRING SYMPOSIUM 2019

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

Microbiota in host-parasite interaction

Dansk Selskab for Parasitologi

DSTMIS

Dansk Selskab for Tropemedicin og International Sundhed

Friday 5th of April, 2018, 8:30-16:15

University of Copenhagen

Lecture room 1-01 (Festauditoriet),

Bülowsvej 17,

1870 Frederiksberg C

ORAL PRESENTATIONS – KEY NOTES

Worms and Germs – the wild gut as an interacting biome

Sarah E Perkins (1), Emily Pascoe(1,2), Julian Marchesi (2,3), Heidi C Hauffe (2)

(1) School of Biosciences, Cardiff University, UK (2) Department of Biodiversity and Molecular Ecology, Centre for Research and Innovation, Italy (3) Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, UK.

Bacterial communities (microbiota) and eukaryotic parasites (macrobiota) have shared space and evolutionary history in the gut for millions of years, such that biotic interactions between them have likely shaped the phenotypic characteristics of bacteria, parasites and host, as well as the conditions for their coexistence. All host species are typically coinfected by multiple parasite species, but despite this, the current paradigm of intestinal homeostasis - the equilibrium balance between healthy and harmful microbes - overlooks the potential effects of a diverse and dynamic macrobiota (typically helminths).

Despite a growing number of microbiome research projects, few if any, currently have the capacity to fully tease apart the relationship between the host, its microbial gut community and macrobiota. Previous studies have been observational, leaving doubts about cause and effect in this dynamic system, while experimental studies are usually limited to laboratory animals; highly selected species with depauperate micro- and macrobiota communities. Instead, we propose this tripartite relationship (host-microbes-parasites) is best elucidated using a 'wild biome'.

Our group has been examining the **gut as an interactive 'biome', and the interplay between microbiota and macrobiota**. Here, we describe the value of using wild mice as a model system, and the association between microbiota and natural infection of multiple helminth species in wild mice (*Apodemus flavicollis*), using 16S rRNA gene catalogues (metataxonomics). We find parasitism is associated with high microbiota diversity, and helminths themselves appear to harbour a microbiota that is unique and different to their host, leading us to question whether helminths directly or indirectly alter the gut microbiota. Regardless, helminths clearly have the potential to alter gut homeostasis. We should therefore view the gut as a 'biome' – a large and species-rich ecosystem, and increase our acceptance of wildlife species as a utilitarian model for future gut microbiota studies.

Natural protective mechanism driven by gut microbiota against plasmodium infection

Bahtiar Yilmaz

Gastroenterology & Mucosal Immunology, Department for BioMedical Research, University of Bern

Malaria is an infectious disease of humans and other animals including birds, reptiles and most mammals and transmitted *via* the inoculation of *Plasmodium* sporozoites into the skin through the bite of an infected female *Anopheles* mosquito. Although every year, around 500.000 lives are perished, mainly children under the age of 3-5 years old, to *Plasmodium* infection this deadly parasite has a relatively low efficiency of transmission from mosquitoes into humans. This argues for a natural protective mechanism, which presumably acts during the early stages of *Plasmodium* infection in the human host. Using a rodent model of *Plasmodium* infection, we demonstrated that such a mechanism does exist and is mediated through the action of natural antibodies recognizing specifically the α-gal glycan. Moreover, these antibodies can be produced upon antigenic exposure by α-gal expressing specific components of the intestinal microbiota and target *Plasmodium* sporozoites in the skin. Additionally, we found out that the food components have also a role on production of these natural antibodies and additional chemically induced colitis, to our surprise, it triggers antibody production against α-gal glycan even higher. Additional to mice studies, we demonstrated that anti-α-gal antibodies are associated with protection against *P. falciparum* infection in humans. Mechanistically, complement and polymorphonuclear cell-dependent mechanisms contribute to this protective mechanism in which these antibodies prevent the migration of sporozoite from the skin into the liver and inhibit hepatocyte invasion, suppressing the establishment of liver stage of infection. Our findings also reveal that this natural host defense mechanism reduces the rate of disease transmission and provides sterile protection against malaria. Importantly, vaccination against α-gal protects mice against malaria transmission, suggesting that a similar vaccination approach may reduce malaria transmission in humans.

CONTRIBUTED ORAL PRESENTATIONS

Interaction of parasite and sub-lethal chemical stress results increase establishment of parasite and synergy on insect host fitness.

Suraj Dhakal (1), Elizabeth J. Cassidy (1), Kathrine E. Pedersen (1), Nicolai V. Meyling (1), Nina Cedergreen (1), Brian L. Fredensborg (1)

(1) University of Copenhagen, Denmark

Environmental toxicants are abundant in nature and hosts and their parasites might frequently be exposed to chemical stress. This could have significant negative effects on host and parasite biology depending on the nature of those interactions. Pathogens interacting with chemical stress may act independently of each other (no interaction), enhance (synergy) or reduce (antagony) the effect of each other to affect the fitness of host organisms and disease biology. The association between heavy metals, aquatic hosts and parasites has been extensively studied, but there is a lack of studies on the interaction between terrestrial hosts and their parasites exposed to agricultural toxicants. We used the insect model *Tenebrio molitor* to quantify individual and interactive effects of *Hymenolepis diminuta* and multiple exposures to a sub-lethal concentration of the pyrethroid insecticide alpha-cypermethrin on host fitness and parasite establishment success. Our results demonstrated significantly higher host mortality in the combined treatment than predicted from the individual effects of the two stressors providing evidence for a synergistic effect of parasite and insecticide. Also, *T. molitor* exposed to alpha-cypermethrin (LD20) after experimental infection with *H. diminuta* significantly increased parasite establishment in the beetles compared to the beetles infected with *H. diminuta* only. Thus, our results indicate that environmental toxicant can impact host-parasite interactions in terrestrial systems where parasites may experience more favorable conditions for establishment and cause significant fitness effects when their hosts are exposed to sub-lethal chemical stress. This might be accomplished by trade-off between immune response to infection and activation of detoxification enzymes to insecticides.

Predicting outbreaks of mosquito borne *Setaria tundra* parasites in Finnish reindeer: a mechanistic transmission model approach

René Bødker (1), Sauli Laaksonen (2,3), Lene J. Kjær L (1), Antti Oksanen (4), Najmul Haider (5)

(1) University of Copenhagen, Denmark; (2) University of Helsinki, Finland; (3) WAZAMA Media; (4) Finnish Food Safety Authority Evira; (5) Technical University of Denmark

In northern Finland, reindeer are reared as semi-domesticated animals. The region has a short summer season of 2-3 months, yet reindeer are being infected with the mosquito-borne filarioid parasite *Setaria tundra*. The infection causes peritonitis and perihepatitis, leading to significant economic losses due to reduced body weight and condemnation of organs at slaughter. The objective of this study was to describe the spatial and temporal pattern of outbreaks in Finnish Lapland, and to construct a temperature-driven mechanistic transmission model able to predict the observed pattern. We developed a temperature-driven transmission model to quantify the number of S. tundra worms potentially transmitted from an infectious reindeer. We then applied the model to the years 2004-2015, and compared the model predictions to the proportion of reindeer whose livers were condemned due to *S. tundra* infection at the time of slaughter. The mean proportion of liver condemnation increased in reindeer slaughtered in late autumn/winter compared to earlier dates. Outbreaks were geographically clustered each year but there were no fixed foci where outbreaks occurred. Larger outbreaks were recorded in the southern regions of reindeer-herding areas compared to the central or northern parts of Lapland. Our model showed that temperatures never allowed for transmission of more than a single generation of *S. tundra* each season. In southern and central Lapland, our model predicted an increasing trend from 1979 to 2015 for both the duration of the effective transmission period of *S. tundra* ($P<0.001$) and for the potential number of L3 *S. tundra* larvae being transmitted from an infectious reindeer ($P<0.001$). The effective transmission period for *S. tundra* in reindeer is very short in Lapland, but it increased over the period studied. Increasing temperatures may facilitate a range expansion and increasing duration of effective transmission period for *S. tundra*.

Susceptibility of *Plasmodium falciparum* field isolates to Artemisinin – based combination therapies in a Ghanaian population

Samuel Yao Ahorhorlu (1), Michael Alifrangis (2), George Obeng Adjei (1), Neils Ben Quashie (1)

(1) University of Ghana; (2) University of Copenhagen, Denmark

Malaria morbidity and mortality has declined in recent years globally due to the many interventions aimed at prevention, control and possible elimination of the disease. However, with reports of emerging artemisinin resistance in the Thai-Cambodia border, the need for continuous monitoring of local parasite populations for potential early parasite resistance identification in endemic areas such as Ghana is essential. The aim of this study was to determine the response of *P. falciparum* field isolates to artemether-lumefantrine (AL) in vivo and to dihydroartemisinin (DHA, the main metabolite of artemether) ex vivo in a Ghanaian population. 143 children aged 6 months to 14 years with uncomplicated malaria were between June-November 2018 recruited from Danfa Health Centre, Shai Osudoku District Hospital, and Princess Marie Louise children hospital in Accra after obtaining informed consent. AL was given and participants were followed up at 6 hours and 12 hours intervals where possible, and then on days 3, 7, and 28, to evaluate parasite clearance in vivo by microscopy. Fresh blood samples (1% parasitemia and 2% hematocrit) were exposed to 700nM DHA for 6 hours and their survival assessed 72 hours later in the ex vivo ring-stage survival assay (ex vivo RSA). There was significant difference in mean day 0 parasitemia and mean parasitemia after 12 hours of AL intake ($p < 0.01$), and only 3 patients (3.5%) ($n = 85$) had day 3 parasitemia. No parasites were seen on days 7 (0/70) and 28 (0/35). However, 8/97 field isolates recorded >10% survival of the parasite ring stage after DHA exposure, by microscopy and this was confirmed by flow cytometry. Strong correlation between ring stage survival rates and in vivo parasite clearance rates were reported in Cambodia, hence this warrants further investigations.

Vaccine induced mice monoclonal antibodies reactive across the highly diverse CIDRα1 antigens

Rasmus W. Jensen (1), Charlotte Harmsen, (1) Louise Turner (1), Thor G. Theander (1), Thomas Lavstsen (1)

(1) University of Copenhagen, Denmark

Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1)-mediated sequestration of parasites in host organs is a major cause of *P. falciparum* malaria pathogenesis. PfEMP1 are key targets of natural acquired immunity to malaria, and an effective vaccine targeting PfEMP1 could significantly reduce malaria burden. In semi-immune children or adults, severe malaria is precipitated by parasites binding endothelial protein C receptor (EPCR) via their CIDRα1 PfEMP1 domains. However, the antigenic diversity of CIDRα1 imposes a challenge for vaccine development. Here, we have characterized two mouse monoclonal antibodies elicited by vaccination using two different CIDRα1 variants. These antibodies exhibited a remarkable ability to react and inhibit EPCR-biding across diverse CIDRα1 variants. Moreover, the antibodies were found to bind the same site on the CIDRα1 molecules. These findings encourage efforts to pursue the development of a broadly reactive poly-epitope PfEMP1 based vaccine against severe malaria.

Comprehensive analysis of Fc-mediated IgM binding to the *Plasmodium falciparum* erythrocyte membrane protein 1 family in three parasite clones

Maria del Pilar Quintana (1), Gertrude Ecklu-Mensah (1, 2), Sergey O. Tcherniuk (3), Sisse Bolm Ditlev (1), Andrew V. Oleinikov (3), Lars Hviid (1, 4), and Mary Lopez-Perez (1)

(1) University of Copenhagen, Denmark; (2) University of Ghana; (3) Florida Atlantic University, USA; (4) Rigshospitalet, Copenhagen, Denmark

PfEMP1 is a family of adhesive proteins expressed on the surface of *Plasmodium falciparum*-infected erythrocytes (IEs), where they mediate adhesion of IEs to a range of host receptors. Efficient PfEMP1-dependent IE sequestration often depends on soluble serum proteins, including IgM. Here, we report a comprehensive investigation of which of the about 60 var gene-encoded PfEMP1 variants per parasite genome can bind IgM via the Fc part of the antibody molecule, and which of the constituent domains of those PfEMP1 are involved. We erased the epigenetic memory of var gene expression in three distinct *P. falciparum* clones, 3D7, HB3, and IT4/FCR3 by promoter titration, and then isolated individual IEs binding IgM from malaria-unexposed individuals by fluorescence-activated single-cell sorting. The var gene transcription profiles of sub-clones measured by real-time qPCR were used to identify potential IgM-binding PfEMP1 variants. Recombinant DBL and CIDR domains corresponding to those variants were tested by ELISA and protein arrays to confirm their IgM-binding capacity. Selected DBL domains were used to raise specific anti-sera to select IEs with uniform expression of candidate PfEMP1 proteins. Our data document that IgM-binding PfEMP1 proteins are common in each of the three clones studied, and that the binding epitopes are mainly found in DBLε and DBLζ domains near the C-terminus, although IgM-binding DBLβ and DBLγ domains, and DBLα-CIDRα and DBLδ-CIDRβ domain cassettes, were also identified.

Identification of mimotopes (epitope mimics) for anti-RH5 monoclonal antibodies by random peptide phage display library pannings

Lena Höbel (1), Maria Bassi (1), Daniel Alanine (2), Michael Foley (3), Simon Draper (2), Lea Barfod (1)

(1) University of Copenhagen, Denmark; (2) University of Oxford, UK; (3) La Trobe University, Australia

Malaria is a considerable problem in many countries across the world, causing morbidity and mortality as well as economic problems. Developing a vaccine has been the goal for some years now, with still many challenges left. One of the main problems is finding a suitable vaccine target, since the parasites are skilled at evading immune responses through antigenic variation. A potential target protein that has received attention recently is Reticulocyte-binding protein homolog 5 (RH5). It is anchored on the surface of the parasite's merozoite stage and is essential for invasion into red blood cells. Antibodies that target RH5 can inhibit parasite invasion in vitro. However, the specific epitopes these antibodies bind to on the RH5 protein are not yet known. To generate a more potent vaccine it is practical to vaccinate with the pure epitope in order to avoid unspecific responses and reach higher titers of the protective antibodies in the blood. We want to find mimotopes, small peptides that mimic the epitopes that protective antibodies bind to, in order to achieve this. The advantage of mimotopes is that due to their small size they will not generate unspecific responses while also avoiding the problem that discontinuous epitopes pose if parts of the real protein were to be used. We tried to generate mimotopes for six RH5 specific protective antibodies obtained from RH5 vaccinated volunteers. We used phage display technology, where we screened a random peptide library with these antibodies. So far, we have found three mimotopes for two of the antibodies. These are being characterized further with the goal of using them in mouse experiments to test if protective antibodies against RH5 can be induced by vaccinating with the mimotope. This way a second-generation vaccine can be developed to help combat malaria and the strain it poses on societies.

Differential immune gene response in gills, skin, and spleen of rainbow trout, *Oncorhynchus mykiss* infected by *Ichthyophthirius multifiliis*

Khairul Syahputra (1), Per W. Kania (1), Azmi Al-Jubury (1), Huria Marnis (1), Agung Cahyo Setyawan (1), Kurt Buchmann (1)

(1) University of Copenhagen, Denmark

Infection of rainbow trout with the parasitic ciliate *Ichthyopthirius multifiliis* induces differential responses in gills, skin and spleen. A controlled experimental infection was performed and expression of immune-relevant genes in skin, gills, and spleen were recorded by qPCR at day 1 and 8 after parasite exposure. Infection induced a marked immune gene reaction involving innate and adaptive immune defenses in rainbow trout at day 8 post-infection. The expression level of a total of 22 out of 24 investigated genes was significantly higher in gills compared to skin reflecting the more sensitive and delicate structure of gills. Especially pro-inflammatory cytokines IL-6, IL-17 C1, regulatory cytokines IL-4/13A, IL-10, TGFβ, complement factor C5, chemokines CK10, CK12, acute phase proteins (precerebellin, hepcidin) and immunoglobulins (IgM, IgT) displayed differential expression levels. The spleen, a central immune organ with no trace of the parasite, showed elevated expression of IgM, IgT, complement factor C5 and chemokine CK10 (compared to skin and gills directly exposed to the parasite), indicating an interaction between the infected surface sites and central immune organs (mediated at least by chemokines CK10 and CK12 and cytokine IL-4/13) leading to a systemic response in rainbow trout against the parasite.

The Nordic seaweeds *Saccharina latissima* and *Laminaria digitata* have potent in vitro anthelmintic effects against the pig nematode *Ascaris suum*

Charlotte Smith Bonde (1), Louis Bornancin (2), Andrew Richard Williams (1), Helena Mejer (1), Henrik Toft Simonsen (2), Stig Milan Thamsborg(1)

(1) University of Copenhagen, Denmark; (2)Technical University of Denmark

Seaweed contains an abundance of bioactive compounds, and some seaweed species have been used as natural deworming agents for centuries in traditional Chinese medicine. In this study, we investigated the in vitro anthelmintic (AH) activity of extracts of seaweed, from Nordic waters. We prepared three different extracts: hexane, dichlormethan:methanol (DCM), and water:methanol (WM), from four seaweed species: *Saccharina latissima*, *Laminaria digitata*, *Ascophyllum nodosum*, and *Palmaria palmata*. The AH activity was assessed using an *Ascaris suum* third stage larvae (L3) mortality assay (1 mg DM/mL dissolved in DMSO). Moving or coiled-up larvae were counted as alive, and immobile or straight larvae as dead. Extracts with more apolar compounds (hexane, DCM) showed higher AH activity than extracts with polar compounds (WM), and the most potent extracts originated from *S. latissima* and *L. digitata*, with an average larval mortality of >95% after 48 hours. Extracts from *A. nodosum* had significantly lower AH effect after 48 hours. Fractionation of the DCM extracts of *L. digitata* and *S. latissima* showed that activity was spread over several fractions, indicating that the observed AH activity may arise from the combined effect of several compounds. We conclude that the Nordic seaweeds *Saccharina latissima* and *Laminaria digitata* have strong in vitro AH effects against the most common nematode in pigs, and the AH activity is mainly linked to apolar compounds. Further bio-guided fractionation will be used to identify active compounds, and feeding trials of infected livestock will reveal whether seaweeds as a bioactive forage can play a role in future nematode control.

Seroprevalence of *Toxoplasma gondii* in domestic pigs, sheep, cattle, wild boars, and moose in the Nordic-Baltic region: Methodological considerations

Abbey Olsen (1), Rebecca Berg (2), Maarja Tagel (3), Kärt Must (3), Gunita Deksne (4), Heidi L Enemark (5), Hans Houe (2), Henrik V Nielsen (6), Marianne Sandberg (1), Anna Lundén (7), Christen R Stensvold (6), Sara M Pires (8), Pikka Jokelainen (6), Lis

(1) Danish Agriculture & Food Council; (2) University of Copenhagen, Denmark; (3) Estonian University of Life Sciences; (4) Institute of Food Safety, Animal, Health & Environment "BIOR", Latvia; (5) Norwegian Veterinary Institute; (6) Statens Serum Institut, Denmark

One of the ways humans may become infected with the zoonotic parasite *Toxoplasma gondii* is if they consume undercooked meat of infected animals. In the Nordic-Baltic region, *T. gondii* seroprevalence in humans varies markedly between the countries. This may be due to differences in food consumption habits and geographic variation in *T. gondii* prevalence in animals consumed by humans. However, data on *T. gondii* in different animal species are scattered. We conducted a systematic review and meta-analysis of *T. gondii* seroprevalence in domestic pigs, sheep, cattle, wild boars and moose in the Nordic-Baltic region. We included studies from January 1990 to June 2018. Thirty-two studies qualified for the meta-analysis: 13 on domestic pigs, 6 on sheep, 3 on cattle, 6 on wild boars, and 4 on moose. For each host species, we estimated the pooled apparent seroprevalence using a random effects model. To identify variables influencing seroprevalence, subgroup analyses were performed using mixed-effects models. The estimated pooled *T. gondii* seroprevalence was 6% in domestic pigs (CI95%: 3–10%), 23% in sheep (CI95%: 12–36%), 7% in cattle (CI95%: 1–21%), 33% in wild boars (CI95%: 26–41%), and 16% in moose (CI95%: 10–23%). In all host species except wild boars, the pooled seroprevalence estimate was significantly higher in >1-year-old animals than in younger animals. The results indicate that a substantial proportion of animals used for human consumption are exposed to *T. gondii* in the region, but the low number of studies did not allow identification of geographical patterns at country-level. We found large variations in seroprevalence estimates between the studies, and information on sensitivity and specificity of the serological test was not reported in several of the studies. Future seroepidemiological studies should report the age of the animals tested and sensitivity and specificity of the serological test, whenever possible.

Taenia solium cysticercosis - still a major problem in smallholder pigs in the Southern Highlands of Tanzania

Mwemezi L Kabululu (1), Helena A Ngowi (2), James E D Mlangwa (2), Ernatus M Mkupasi (2) and Maria V. Johansen (3)

(1) Tanzania Livestock Research Institute; (2) Sokoine University of Agriculture, Tanzania; (3) University of Copenhagen, Denmark

Taenia solium taeniosis/cysticercosis is a disease of substantial economic and public health importance particularly in the developing world. In Mbeya and Songwe regions in the Southern Highlands of Tanzania, sero-prevalence of up to 32% was reported in 2008. From 2012, through a national campaign against schistosomiasis, mass drug administration (MDA) with praziquantel has been provided to school aged children in the regions. Being efficacious against *T .solium* as well, praziquantel could potentially also control the disease in the area. After years of the MDA with praziquantel, this study aimed at estimating current prevalence and intensity of *T. solium* in pigs; and understand farmers' knowledge, perceptions and practices that may perpetuate the disease in the area. A total of 890 households were surveyed by a questionnaire and 282 pigs examined by postmortem tissue dissections. Only 6% of the respondents perceived PC to be an important disease. About 19% were aware of *T. solium* taeniosis, 32% of whom did not know how the infection is acquired. Also, 61% of the respondents who had seen cysts in pork were not aware that consumption of infected pork could cause taeniosis/cysticercosis. More than 90% of latrines lacked doors; and 45% of them were accessible to pigs. Twenty seven pigs (9.6%) had *T. solium* cysts. About half (52%) of the infected pigs had light infections (1-100 cysts), 15% had moderate infections (101-1000 cysts) and 33% had heavy infections (>1000 cysts). This study provides evidence that despite the MDA with praziquantel the disease is still endemic in the area. Also, poor farmers' knowledge, perceptions and risky practices have been revealed, which are probably responsible for disease perpetuation. One Health approach is needed to control the parasite, incorporating improvement in farmer's knowledge regarding the disease.

The zoonotic potential of the dog hookworm *Ancylostoma ceylanicum* in Mwanza and Simiyu regions, Tanzania

Anne M Jensen (1), Mita E Sengupta (1), Safari Kinunghi (2), Birgitte J Vennervald (1), Anna M O Kildemoes (1, 3)

(1) University of Copenhagen, Denmark; (2) National Institute for Medical Research, Tanzania; (3) Leiden University Medical Center, The Netherlands

Necator americanus and *Ancylostoma duodenale* are traditionally considered the only hookworms capable of establishing patent infections in humans. However, using molecular tools to investigate *Ancylostoma* at species level recent studies have shown the zoonotic potential of *Ancylostoma ceylanicum*. This hookworm species was considered a parasite of dogs and cats until several studies in the Asia-Pacific region found it capable of establishing patent infections in humans. In fact, it has been shown to be the second most prevalent hookworm species in humans in Asia and the Pacific, where it is known to be endemic in dogs and cats. These accepted geographical constraints of *A. ceylanicum* are now being put to the test as it was recently identified in dogs in Morogoro, Tanzania, using molecular tools. The current study therefore set out to investigate the zoonotic potential of *A. ceylanicum* in Tanzania. Samples from 1387 people from 7 different villages in the Mwanza and Simiyu regions along with 264 samples from dogs were screened for hookworm eggs using the Kato-Katz method. Larvae were reared and recovered from hookworm positive samples for further molecular work. Positive samples from humans and dogs were analysed using polymerase chain reaction coupled with restriction fragment length polymorphism and Sanger sequencing to identify the species of hookworms. Using these methods on human derived samples, the current study did not only observe the presence of the two common anthropogenic species *N. americanus* and *A. duodenale*, but also identified the zoonotic species *A. ceylanicum*, *A. caninum* and *A. braziliense*. This is, to the best of our knowledge, the first study to have documented the presence of A. *ceylanicum* in humans in Africa.

POSTER PRESENTATIONS

Helminth-mediated modulation of inflammatory responses in Systemic Lupus Erythematosus

Laura B Dall (1, 2), Bent W Deleuran (1, 2), Maibritt Mardahl (1, 2) Paul W. Denton (1, 2), Lars Østergaard (1, 2), Peter Nejsum (1, 2)

(1) Aarhus University, Denmark; (2) Aarhus University Hospital, Denmark

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease caused by complex immunopathological mechanisms. Existing treatment options are suboptimal and new therapeutic strategies are needed. Therapeutic effect of helminths has already been suggested for several autoimmune diseases, and studies in murine models of SLE have shown promising results. The aim of this study is to evaluate possible immune-modulating effects of helminth products from *Ascaris suum*, *Anisakis simplex* and *Trichuris suis* on peripheral blood mononuclear cells (PBMCs) and serum from SLE patients ex vivo. We stimulate PBMCs from SLE patients and healthy controls ex vivo with helminth products and, to enhance the pro-inflammatory activity in a SLE-like manner, we further stimulate with TLR-4 and TLR-9 agonists and activators of the STING pathway. Furthermore, we stimulate PBMCs from healthy controls with helminth products and SLE serum or isolated microparticles from SLE serum. Next, we characterize the inflammatory responses by analyzing the cytokine profile (e.g. CXCL-10, Gal-9, TNF-α, IL-6, IL-10), key lymphocyte subsets (e.g. T-cells memory subsets, T regulatory cell subsets, B regulatory cell subsets), early activation marker status (e.g. CD69), gene expression and specific proteins. The experiments will be conducted from autumn 2018 to spring 2019. We expect that the helminth products will suppress the pro-inflammatory immune responses in PBMCs ex vivo and induce a regulatory environment. If this study shows that helminths can reduce inflammation in SLE, this may pave the way for developing novel efficient drugs based on helminth products.

Immunosuppressive effects of helminth antigens on TLR-mediated induction of inflammatory responses in macrophage

Amin Zakeri (1), Martin R Jakobsen (2), Andrew R Williams (3), Peter Nejsum (1)

(1) Aarhus University hospital, Denmark; (2) Aarhus University, Denmark; (3) University of Copenhagen, Denmark

Toll-like receptors (TLRs) are an important compartment of the innate immunity and highly expressed by macrophages. TLRs are able to initiate inflammatory signals and polarize macrophages toward overactivation that leads to onset of an autoimmune condition. Helminth-derived antigens are masterful modulators of host inflammatory responses. This intervention can be mediated via manipulating TLRs signalling and modifying extracellular vesicle (EV)-associated immune cells communication. In this study, we explore the effects of *Trichuris suis* antigens on mouse bone-marrow derived macrophages (BMDM) in the presence of different TLRs agonists. Also, our investigation on the effect of parasite antigens on macrophages exosome is undertaken to assess whether the parasite antigens are able to modify macrophages to release regulatory EVs. Bone marrow (BM) cells were isolated from 8- to 12-week-old female C57BL/6 for generation BMDM. BMDMs were pre-treated with *T. suis* antigens for 0.5h and then stimulated with different TLRs agonists. After 24h, cytokine production was assessed by ELISA. In order to produce helminth-induced EVs, BMDMs were stimulated with *T. suis* antigens for 48h, then EVs were isolated by size exclusion chromatography. *T.suis* antigens were found to significantly attenuate most TLR-induced IL-6 and TNFa production in BMDM while IL-10 was increased. Our preliminary data show that isolated EVs also are able to alter macrophage responses. This study has so far demonstrated that *T. suis* antigens can interrupt with TLR signalling in BMDMs. In addition, we showed EVs derived from *T. suis*-treated BMDM might potentially alter inflammatory responses.

Immunomodulatory properties of *Anisakis simplex*

Veronika Potocka (1), Anders T Boysen (1), Maibritt Mardahl (1), Peter Nejsum (1)

(1) Aarhus University, Denmark

Helminths are known for their ability to modulate the immune system of a host to promote survival. *Anisakis simplex*, is a common marine mammal parasite that has fish and crustaceans as intermediate hosts. Larvae develop into adult worms and complete their life cycle when reaching their final marine mammal host. Human can become an accidental host. When larvae are ingested by eating raw or under processed seafood, they will penetrate the intestines and cause Anisakidosis. The purpose of this work is to investigate, if *A. simplex* products have immunomodulatory properties when co-cultured with the human monocyte cell line THP-1. These are stimulated with different concentrations of *A. simplex* products, in order to see possible effect on immune system. ELISA is afterwards used to measure cytokine production, to see whether parasite product can reduce proinflammatory cytokines released by LPS-stimulated cells. We hypothesize, that this parasitic worm-derived products may have a therapeutic potential in immune-related diseases.

Dietary polyphenols and their immuno-modulating effects: Implications for parasite infection

Audrey I Andersen-Civil (1), Milla Leppä (2), Juha-Pekka Salminen (2), Stig M Thamsborg (1), Andrew R Williams (1)

(1) University of Copenhagen, Denmark; (2) University of Turku, Finland

Polyphenols are a group of intensively studied compounds and their diverse effects on the immune system have encouraged a multitude of interdisciplinary research to investigate them. We are investigating proanthocyanidins, which are among the most common dietary polyphenols, to assess their impact on gut inflammation during helminth infection. Proanthocyanidins are characterized by their high molecular weight consisting of flavan-3-ol oligomers and polymers (Manach et al., 2004; Williams et al., 2015). In this study, the compounds were purified from cocoa, grape seeds and *Ribes alpinum* by series of extractions, sephadex separation (19 samples), and Semi-preparative Liquid Chromatography (152 samples). The samples were analyzed by Ultra high Performance Liquid Chromatography Mass Spectrometry (UPLC-MS/MS), and their mean degree of polymerization (mDP) and procyanidin/prodelphinidin ratios were assessed.

The scope of the project is to identify the most active compounds, and each sample will initially be tested in-vitro on RAW 264.7 macrophages, to assess their impact on cytokine secretion before selected samples are used to assess the effects on mucosal immune responses to parasitic infection.

Detection of *Giardia* in Algerian Individuals Using Real-Time PCR

Salem Belkessa (1, 2, 3), Farida Ghalmi (4), Elhosseyn Ait-Salem (1), Karim Houali (1), Nassima Chikhaoui (5), Imen Boussaada (6), Christen Rune Stensvold (3)

(1) Mouloud Mammeri University of Tizi Ouzou, Algeria; (2) Mohamed Khider University of Biskra, Algeria; (3) Statens Serum Institut, Denmark; (4) Higher National Veterinary School of Algiers, Algeria; (5) CHU Nafissa Hamoud-EX Hopital Parnet, Hussein Dey, Algeria

Giardia is an important zoonotic flagellate parasitizing the gastrointestinal tract of humans and animals. In humans, symptoms include diarrhea, malabsorption, and impaired growth in children with chronic infections. In Algeria, no surveys have been published on this parasite. A community-based cross-sectional descriptive study was carried out from 2012 to 2018 across two hospitals in Algiers and two hospitals in Biskra. A total of 108 faecal samples were collected from 108 individuals (44 females, 59 males, five of undetermined sex; age range, 1–74 years; mean age, 17 years) suspected of parasitosis and/or suffering from diarrhoea, vomiting and abdominal cramps and referred to the clinical laboratories of these hospitals. The samples had all been scored as parasite-positive by microscopy for ova and parasites and were screened by real-time PCR for *Giardia* with a view to evaluating the diagnostic sensitivity of microscopy and identifying the *Giardia* infection rate across age groups (children [≤ 15 years] vs. adults [> 15 years]). Of the 108 positive samples, 74 (69%) were real-time PCR-positive for *Giardia*, of which 66 were positive by both microscopy and real-time PCR (mean Ct value, 27.51 [SD ± 5.99]); eight were negative by microscopy but positive by real-time PCR (mean Ct value, 34.24 [SD ± 4.25])(P = 0.0030). The remaining 34 samples (31%) were real-time PCR-negative for *Giardia*. Among these, seven were positive for *Giardia* by microscopy, and the remaining 27 were negative by both microscopy and real-time PCR. Children were more commonly infected than adults (85% vs. 29%; P < 0.00001 [Fisher's Exact Test]). In conclusion, we found that most of the parasite-positive children had *Giardia* and children were more often colonized by *Giardia* than adults. We moreover confirmed that real-time PCR is more sensitive than microscopy for detection of *Giardia* in stool.

Immunomodulation of human cytokine responses by the porcine whip worm, *Trichuris suis*, and the porcine nodular worm, *Oesophagostomum dentatum*

Maibritt Mardahl (1), Sidsel Dahl Andersen (1), and Peter Nejsum (1)

(1) University of Aarhus, Denmark

Trichuris suis and *Oesophagostomum dentatum* are parasitic gastrointestinal nematodes that occupy the cecum and colon of their porcine host. While *T. suis* promotes a rapid Th2 response resulting in expulsion from the host about 9 weeks after infection, *O. dentatum* is able to establish a chronic infection. *O. dentatum* seems to induce a Th1 response and a delayed Th2 response. Co-infections with *T. suis* and O. *dentatum* do occur, and studies have shown that *T. suis* can suppress *O. dentatum* and *O. dentatum* can prolong infection by *T. suis*. We are interested in dissecting the responsible molecules of *T. suis* and *O. dentatum* focusing on their excretory-secretory products (ESP) and their worms extracts (soluble products=SP). We size-fractionated the molecules in the ESP of both parasites. In addition, as recent studies have shown that parasite extracellular vesicles (EVs) may have potent immune stimulatory properties we enriched our samples for EVs and investigated their immunomodulatory effects in vitro. Interestingly, we find that the *O. dentatum* ESP and EVs stimulate TNF production in the human monocytic leukemia cell line THP-1, whereas *T. suis* does not. In the presence of the TLR4 agonist, LPS, *T. suis* ESP, but not EVs, is able to suppress TNF production in a dose-dependent manner, whereas *O. dentatum* ESP or EVs cannot. *T. suis* ESP, but not EVs, seem to induce production of IL-10, whereas neither ES nor EVs of *O. dentatum* are able to produce IL-10. In contrast, *O. dentatum* SP is able to induce IL-10 in a dose-dependent manner, whereas *T. suis* SP cannot. These findings indicate that both SP, ES, and EVs contribute to host modulation. If we can unlock the underlying mechanisms of how *T. suis* and *O. dentatum* interact with their host we may identify new targets for intervention and control.

Characterization of *Plasmodium falciparum* binding to blood group A and B determinants

William van der Puije (1), Christian wang (2), Casper Hempel (3), Rebecca Olsen (2), Nanna Dalgaard (2), Michael Ofori (1), Jørgen Kurtzhals (2,4) and Trine Staalsø (2,4)

(1) University of Ghana; (2) University of Copenhagen, Denmark; (3) Technical University of Denmark; (4) Copenhagen University Hospital, Denmark

Plasmodium falciparum-infected erythrocytes can express variant surface antigens (VSA) that bind A and B glycan antigens of the human AB0 blood group system. This leads to rosette formation with uninfected non-0 erythrocytes. Rosetting is associated with malaria severity. We investigated the binding properties, serological changes and the transcription of the most prominent VSA gene family, the var genes in *P. falciparum* strains selected for adhesion to blood group A or B. Adhesion assays were done using primary human dermal and aortic endothelial cells and BeWo cells. Rosetting assays were done by fluorescence microscopy using erythrocytes of different AB0 types. VSA-surface expression was measured by flow cytometry using sera from 93 Ghanaian children with malaria. The var-transcript levels of selected and unselected 3D7 and FMG/It parasites were assessed by quantitative real-time PCR. Selection for blood group A or B oligosaccharide increased binding to endothelial cells, and a strong preference for rosette formation with non-0 erythrocytes was induced. Serum antibody reactivity to A/B binding parasites was slightly increased in children older than 5 years relative to the isogenic non-binding parasites, with no major changes in the overall recognition pattern. In 3D7 parasites transcript levels of one var-gene, (3D7Pf13_0003), previously associated with blood group A rosetting was transcribed at the same level after selection whereas most other var genes decreased their level of transcription. In FMG/It parasites a var gene previously associated with EPCR binding, (IT4var32b) was transcribed at a higher level in A/B binding parasites. An overall decrease in the total number of var transcript detected in blood group A or B selected parasites was however seen in both parasite genotypes. Thus, we conclude that no consistent changes in var-gene expression other than a decrease in overall transcription were associated with binding to blood group A and B.

Functional expression of *Trichuris suis* acr-16 in Xenopus laevis oocytes – a pharmacological screening tool for the anthelmintic effect of synthetic and natural compounds on nicotinic acetylcholine receptor.

Tina V. A. Hansen (1), Dan A. Klaerke (1), Kirstine Callø (1), Susanna Cirera (1), Richard J. Martin (2)

(1) University of Copenhagen, Denmark; (2) Iowa State University, Ames, IA, USA

Anthelmintic resistance and/or decreased susceptibility to anthelmintics is a major global problem in parasitic nematodes of livestock. Therefore, there is an urgent need to understand the molecular mechanisms of existing drugs. Trichuris is an interesting nematode genus due to its broad host spectrum and its low susceptibility to several anthelmintic drug classes. However, the cholinergic agonists oxantel has a high efficacy against *Trichuris* spp. Despite this knowledge, the exact molecular target of oxantel within Trichuris is still unknown. In *Ascaris suum*, oxantel has a low effect on the nicotinic Ach receptor (nAChR) acr-16. We therefore hypothesized that the sensitivity of *T. suis* acr-16 to oxantel is different from *A. suum* acr-16. The sensitivity of *T. suis* acr-16 was evaluated using PCR, molecular cloning, functional expression of *T. suis* acr-16 cRNA in Xenopus laevis oocytes and two-electrode voltage clamp electrophysiology. In electrophysiological experiments, oxantel produced a high and robust current response of *T. suis* acr-16, whereas pyrantel activated acr-16 moderately. The cholinergic anthelmintic compounds morantel and levamisole did not activate the receptor significantly. Other nAChR agonists, including epibathidine, nicotine, cytisine, dimethylpiperazine and 3-bromocytisine resulted in minor responses. *T. suis* acr-16 is more sensitive to oxantel than *A. suum* acr-16. *T. suis* acr-16 may not be the only target of oxantel, but the functional expression of this nAChR can prove a valuable screening tool to evaluate the effect of new synthetic or natural compounds against this genus.

Toxoplasma gondii in cats in Denmark: seroprevalence and risk factors

Anna Kassow Grønlund (1), Andreas Persson (1), Charlotte Reinhard Bjørnvad (1), Maria Vang Johansen (1) and Pikka Jokelainen (2)

(1) University of Copenhagen, Denmark; (2) Statens Serum Institut, Denmark

Despite domestic cats are important hosts for the zoonotic parasite *Toxoplasma gondii*, information about feline *T. gondii* infections has been lacking in Denmark. In this study, we estimated *T. gondii* seroprevalence among cats visiting five veterinary clinics in Copenhagen, Denmark. Samples from 139 pet cats, collected from September 2018 to January 2019, were tested for antibodies against *T. gondii* using a commercial indirect ELISA. The apparent seroprevalence was 21% (95% confidence interval: 15-28); exposure to *T. gondii* was common in the investigated pet cat population. Seropositivity was positively associated with some lifestyle-factors, in particular with having outdoor access and with diet including raw meat. Seropositive cats have presumably shed oocysts of the parasite after acquiring the infection; after sporulation, these oocysts can serve as infection source to other hosts. It was noteworthy that 40% of the cats had outdoor access and 18% of the cat owners reported flushing their cats' faeces down the toilet – both activities allow oocysts, if present in the faeces, to end up in the environment. Based on the results of this study, preventing feline *T. gondii* infections and preventing environmental contamination with oocysts should merit more attention in Denmark.

Investigation of transmission patterns of *Fasciola hepatica* in ruminants in a Danish Nature Area

Leah Lourenço (1), Mita E Stengupta (1), Nao Takeuchi-Storm (1), Stig M. Thamsborg (1) and Anna-Sofie Stensgaard (1)

(1) University of Copenhagen, Denmark

Fasciola hepatica is a snail-borne, parasitic trematode that causes fasciolosis in livestock. In Denmark, there has been an increasing prevalence in cattle and the dominant transmission pattern of *F. hepatica* was found to be "summer infection" 40 years ago. This study aimed to characterise the current transmission patterns of *F. hepatica* in a fasciolosis hotspot (>90% infection in ewes) in Vestamager by 1) investigating the time of exposure in the definitive sheep host (*Ovis aries*); and 2) describing the infection and population dynamics of the intermediate snail host (*Galba truncatula*). Blood and faecal samples from 22 lambs were collected four times during June-December 2018. The liver fluke infection status was analysed through egg counting by sedimentation and detection of antibodies by ELISA. Livers were also dissected at the time of slaughter in November/December. Snails were collected monthly at 7 different sites in the nature area (April-December 2018). Snail shell morphology was used to identify G. truncatula (n=321) and potential *Galba* morphs (n=32). The presence of *F. hepatica* DNA in the snails was determined through PCR, by targeting the ITS-2 region on pooled snail DNA samples and the COX1 partial gene on individual snails of positive pools. In addition, snail species identification was verified by sequencing the Lymnaid ITS-2 region in morph snails and from two snails from each site and time period. Overall results showed that only one lamb (4.5%) became infected, possibly in relation to the unusual dry, hot summer. ELISA results indicated seroconversion of the lamb as a probable case of "winter infection." Preliminary data from snail surveys suggests that the prevalence of *F. hepatica* in *G. truncatula* is ~2%. Overall, the study will further our knowledge on the seasonal transmission patterns of *F. hepatica* in Denmark, and optimize control of liver flukes in ruminants in conservation areas.

Modulation of inflammation by helminth-derived EVs

Anne Borup (1), Bent W. Deleuran (2), Peter Nejsum (1)

(1) Aarhus University, Denmark; (2) Aarhus University Hospital, Denmark

Developed countries have for several decades experienced a dramatic increase in the incidence of autoimmune diseases, while the incidence of infectious diseases has markedly decreased. The inverse relationship between immune dysregulation and exposure to microorganisms suggests that our immune system needs to be exposed to a variety of pathogens, including parasitic worms (helminths) in order to maturate in a proper way. Recent discoveries show that helminths produce anti-inflammatory and/or immunomodulatory compounds that can balance a dysregulated immune system. A promising component with great therapeutic potential is Extracellular Vesicles (EVs) due to their heterogeneous cargo containing bioactive proteins and nucleotides participating in cell-communication. Despite the great potentials of EVs, a major challenge relates to EV-isolation. Optimized protocols for EV isolation, which gives both high yield and enrichment of the most potent immunomodulatory EVs still needs to be established. Improved characterization and classification of EVs from helminths are therefore needed in order to achieve sufficient EV isolation. In this study, I will establish a 'golden standard' for isolation of EVs from the helminth *Ascaris suum* by comparing the often used isolation methods ultracentrifugation and size exclusion chromatography and compare their ability to recover the highest number of EVs with lowest protein contamination. Furthermore, I will test the immunomodulatory ability of the helminth-derived EVs in vitro by stimulating immune cells with different subpopulations of EVs in order to identify the most potent EV fraction.

Temporal correlates of host and parasite fitness and its effects on the gut microbial composition

Christian Simonsen(1), Cæcilie Steinhausen(1), Christian M.O. Kapel(1), Brian L. Fredensborg(1)

(1) University of Copenhagen, Denmark

The gut microbiome is known to have a significant influence upon health. Manipulating the composition of the gut microbiome may have detrimental or beneficial adjustments to gut homeostasis and host health. During an infection, helminths are known to adjust the gut microbiome to up-regulate anti-inflammatory bacteria. This helminth property could be utilized to possibly ameliorate known intestinal disorders, but the underlying mechanisms to host gut microbiome modification are still mostly unknown. In this study, a pin worm - cockroach model is developed to investigate if an intestinal helminth infection cause consistent modifications in the composition and function of the gut microbiome. These modifications will be correlated with the age and development of the host and parasite to determine any difference during different stages of infection. Currently, an infection protocol for the model is being developed through the infection of *P. americana* cockroaches with the eggs of the pin worm *L. appendiculata*.

Immune modulatory effects on innate immune responses by adult body fluid from the parasitic worm, *Ascaris suum*

Sidsel D. Andersen(1), Martin R. Jacobsen(1) and Peter Nejsum(1)

(1) Aarhus University, Denmark

Parasitic worms are known as strong modulators of their host immune responses. They suppress multiple signaling pathways of the immune system and induce an anti-inflammatory environment, which increases their survival in the host. Interestingly, this immune modulation has shown to cause positive bystander effects in the host, as evident from murine models of autoimmune disorders like inflammatory bowel disease, psoriasis and allergies. However, the specific mechanisms behind this immune modulation remain elusive. Here, we try to isolate the responsible parasitic molecules from the soil-transmitted helminth, *Ascaris suum*, that interfere with the innate immune pathways regulated by toll-like receptors (TLRs). To elucidate this, we challenge human macrophages with adult body fluid from *A. suum*, prior to stimulation with various stimulus. Interestingly, we find that helminth product suppresses the production of cytokines from macrophages. If we can identify the intracellular pathways targeted by helminth-derived molecules, this may pave the way for novel agents that can modulate as dysregulated immune system. In addition, our work will contribute to the limited knowledge on the field of immunomodulatory effects of parasitic worms.

Purification and characterization of *Plasmodium falciparum* K+ channels, PfKch1 and PfKch2

Karen Molbaek (1,3), Kirstine Calloe (1), Maria de los Angeles Tejada (1), Peter Ellekvist (2), Peter Scharff-Poulsen (1), Nirbhay Kumar (4), Per Amstrup Pedersen (1) and Dan A. Klaerke (1)

(1) University of Copenhagen, Denmark, (2) Herlev Hospital, Denmark

Plasmodium falciparum, the species responsible for the majority of malaria- associated fatalities, encodes two putative K channels, PfKch1 and PfKch2, which have been cloned in our laboratories. The *Plasmodium* K+ channels may be potential drug targets, and we have shown in the model parasite P. berghei, that Kch1-null parasites exhibit a total inhibition of oocyst development in the mosquito midgut (transmission block). The Plasmodium channels are difficult to express in conventional expression systems; however we have shown that PfKch1, PfKch2 and a C-terminally truncated version of PfKch1 (PfKch1-1094) are indeed functional K+ channels in vivo, since a K+ uptake deficient Saccharomyces cerevisiae strain is complemented by the *P. falciparum* cDNAs. It is the aim of the present study to express PfKch1 and PfKch2 in yeast and to characterize the purified channel proteins by electrophysiological methods. PfKch11-1094-GFP and GFP-PfKch2 fusion proteins were overexpressed in yeast and affinity purified to homogeneity. Purified proteins were reconstituted into giant unilamellar vesicles (GUVs). Lipid bilayers were formed on NPC-1 borosilicate glass chips with a resistance of 2-5 MW using symmetrical K+ concentrations the bilayer (130 mM KCl, 10 mM HEPES, 10 µM CaCl2, pH = 7), and single channel activity was monitored on the Port-a-Patch, Nanion Technologies, Germany. For both purified channels, PfKch1 and PfKch2, single channel events could be measured after reconstitution into planar lipid bilayers. Consistent with the prediction of regulator K+ conductance (RCK) domains in the C-terminals of both channels, the channel activity was dependent on the presence of Ca2+. Single channel conductances were estimated to 16 pS for PfKch1 and 28 pS for GFP-PfKch2. The *Plasmodium* K+ channels, PfKch1 and PfKch2, may be expressed in yeast and purified in a functional form. This may allow for future structural characterization.

Are wild deer a potential reservoir of the common liver fluke *F. hepatica* in Denmark?

Anna-Sofie Stensgaard AS (1), Martin Niemann (1), Søren Salomonsen (1), Nao Takeuchi-Storm (1), Heidi Huus Petersen (2), Mita Eva Sengupta (1), Stig Milan Thamsborg S (1)

(1) University of Copenhagen, Denmark; (2) Technical University of Denmark

The common liver fluke, *Fasciola hepatica*, is a snail-borne trematode that infects a wide range of mammalian hosts. In Denmark the prevalence of fasciolosis in cattle is increasing, but the reasons behind this are unclear. The Danish populations of wild deer (red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and fallow deer (*Dama dama*)) have also been increasing and could present a risk for increased transmission of the parasite between wildlife and cattle. Here, we investigated the potential role of deer as a wildlife reservoir *F. hepatica* in Denmark. A cross-sectional study was conducted during the hunting season of 2017/2018 and 2018/2019 to examine the prevalence and geographical distribution of liver flukes in Danish wild deer. Furthermore the parasite isolated from the livers of sheep, cattle, hares and deer were analyzed for molecular characterization based on the nad1 gene. A total of 698 livers from wild deer (roe, fallow, sika and red deer) were examined for presence of *F. hepatica* by dissection. The overall prevalence of *F. hepatica* in deer was found to be very low (1.9%), and typically only few parasites (1-3) per liver. A total of 14 closely related haplotypes were found and two of these were found in both deer, cattle, hares and sheep. Our results indicate that transmission of *F. hepatica* between wildlife and domestic animals in Denmark is possible. However, given the low prevalence and few numbers of parasites found in individual deer livers, the potential size of this reservoir must be regarded as minimal.

Cercarial dermatitis in Danish freshwater lakes during summer 2018

Azmi Al-Jubury(1), Eva Susanna tracz(2), Louise von Gersdorff Jørgensen (1), Anette Bygum (2), Per Walter Kania (1) and Kurt Buchmann (1)

(1) University of Copenhagen, Denmark; (2) Odense University Hospital, Denmark

Summer temperatures in Denmark 2018 were unusual high and freshwater lakes were used to a higher extent for bathing and swimming. Several clinical cases of cercarial dermatitis were reported at different locations in Denmark. The disease occurs when people have direct contact with freshwater lakes containing free swimming cercaria of bird schistosomes. Birds are the definitive host of the parasite while the snails are the intermediate hosts. We samples 1,095 snails (581 *L. stagnalis*, 449 *R. Balthica* and 65 *P. corneus*) from 19 Danish lakes. Avian schestosome cercariae were identified by light microscop. Four out of 19 lakes were positive for *Trichobilharzia* spp. Findings from the field trail is discussed in the light of the clinical cases.

Transcriptomic analysis of Baltic cod (*Gadus morhua*) liver infected with *Contracaecum osculatum* third stage larvae indicates parasitic effects on growth and immune response

Huria Marnis (1), Per W. Kania (1), Khairul Syahputra (1), Shaozhi Zuo (1), Ron P. Dirks (2), Kurt Buchmann (1)

(1) University of Copenhagen, Denmark; (2) Future Genomics Technologies B.V., Leiden, The Netherlands

Baltic cod (*Gadus morhua*) is an important fish species on the world market but during the latest decades a marked increase of infections with third-stage larvae of the anisakid nematode *Contracaecum osculatum* in cod from eastern part of this brackish sea has been observed. The worm larvae mainly infect the liver of the fish and prevalence of infection has reached 100 % with a mean intensity up to 80 parasites per host in certain areas. Marked increases of the grey seal (*Halichoerus grypus*) population in this marine area explains the rise in infection level as this marine mammal is the final host of the parasite species. Concomitant with the rise in parasite abundance condition and growth parameters have decreased suggesting a parasite-induced effect. To investigate any association between parasite infection and physiological status of the host we performed a comparative transcriptomic analysis of liver obtained from *C. osculatum* infected and non-infected cod. A total of 47,025 predicted gene models showed expression in cod liver and sequences corresponding to 2,085 (4.43%) unigenes were differentially expressed in infected liver when compared to non-infected liver. Of the differentially expressed unigenes (DEGs) 1,240 unigenes were up-regulated while 796 were down-regulated. The Gene Ontology (GO) enrichment analysis showed that 845 DEGs were highly represented in cellular process and single-organism process, cell and cell part, binding and catalytic activity. As determined by the Kyoto Encyclopedia of Gene and Genomes (KEGG) Pathways analysis, 241 DEGs were involved in 753 pathways. Eighty DEGs were related to metabolic pathways including carbohydrate, lipid, and amino acid metabolism. Twenty-four regulated genes were playing a role in immune response such as complement and coagulation cascades, B-cell receptor signalling, chemokine signalling and twenty-five genes (most of which were down-regulated) were associated with growth of Baltic cod which indicated that the nematode infection had significant effects on molecular mechanism involving metabolism, immune function and growth.

Mapping the epitope(s) binding α2-macroglobulin and non-immune IgM in the malaria parasite protein PfEMP1

Nexhibe Beciri (1), Lars Hviid (1), Maria del Pilar Quintana (1)

(1) University of Copenhagen, Denmark

Plasmodium falciparum malaria is the most serious form of malaria in humans. The ability of *P. falciparum*-infected erythrocytes (IEs) to adhere to host receptors, on the surface of endothelial cells (cytoadhesion) and/or on the surface of the erythrocytes (rosetting), is a hallmark event in the pathogenesis of the disease. The parasite antigen PfEMP1 is the most prominent parasite ligand responsible for cytoadhesion and rosetting. We have previously shown that the human serum factors IgM and α2-macroglobulin (α2M) can bind to IEs via interactions with PfEMP1, and that this binding often is required for rosetting to occur. This suggests that parasite appropriation of host factors to the IE surface contributes to the disease pathogenesis. The precise PfEMP1 amino acid sequence required for binding of IgM and α2M is not known. We therefore studied an IgM- and α2M-binding domain (DBLζ2) from a rosette-mediating PfEMP1 variant (HB3VAR06) in an attempt to identify the minimal region(s) mediating binding to these two host factors. We measured the ability of full-length DBLζ2 and smaller sub-regions to bind IgM or α2M by ELISA. Overall, optimal binding of IgM and α2M required the entire DBLζ2 domain, probably indicating the involvement of conformational epitopes only present in the full-length domain.

Characterising protective monoclonal antibodies to *Plasmodium falciparum* merozoite antigens PfRAMA and PfCyRPA

Anne Schlitterlau Knudsen (1), Maria Bassi (1), Melanie R. Walker (1) and Lea Klingenberg Barfod (1)

(1) University of Copenhagen, Denmark

Malaria caused by *Plasmodium falciparum* remains a significant public health and economic burden in Sub-Saharan Africa. A highly effective vaccine would be a valuable weapon toward malaria control and elimination; however, has the development of such faced many challenges. These challenges are primarily due to a vast number of potential targets as well as high levels of polymorphism seen in malaria antigens. Where the first generation malaria vaccines focused on single pre-erythrocytes targets, an effective second-generation vaccine will need to target multiple antigens simultaneously. Antigens expressed by merozoites are likely to be important targets of human immunity and are promising vaccine candidates, as some of these antigens give rise to growth inhibitory and synergistic antibodies. Characterising monoclonal antibodies (mAbs) against antigens that operate at distinct steps in the erythrocyte invasion, will allow a better understanding of protective epitopes, which could form the bases of a second-generation malaria vaccine. Merozoite antigens PfRAMA and PfCyRPA are promising vaccine candidates as they have been shown to elicit antibodies with growth inhibitory effects in vitro. In this study, anti-PfRAMA and anti-PfCyRPA mAbs are produced from immunised mice using Hybridoma Technology. MAbs are characterised for their growth inhibitory activities in in vitro assays both to determine individual inhibition as well as synergistic inhibition utilising mAbs against different antigens. Western blots on recombinant antigens can give insights to whether the mAb bind a linear or conformational epitope. Furthermore, surface plasmon resonance will be used to perform epitope-binning experiments to classify mAbs into different competitive groups. To date, 13 anti-PfRAMA and 11 anti-PfCyRPA mouse mAbs have been produced. Individual mAbs have been tested for growth inhibitory effects and it has been confirmed whether they bind a linear or conformational epitope. Understanding the characteristics of protective mAbs and their epitopes may give rise to novel malaria vaccine designs.

Study on survival of nonstrangulating intestinal infarctions associated with *Strongylus vulgaris*: Treatment outcomes of 39 horses (2008–2019)

M. L. Honoré (1), M. K. Nielsen (2), S. N. Olsen (1), P. S. Leifsson (1), S. Jacobsen (1) and T. H. Pihl (1)

(1) University of Copenhagen, Denmark; (2) University of Kentucky, USA

Strongylus vulgaris is the most pathogenic gastrointestinal parasite in the horse and it is reemerging, in amongst other places, Denmark. If nonstrangulating intestinal infarctions (NSII) caused by *S. vulgaris* are not diagnosed in time, the horse may die. This could most likely be prevented with an increased awareness and recognition of the disease and the initiation of the correct treatment. The objective was to investigate the survival rate in horses with NSII undergoing exploratory laparotomy. Sample collection and recording of data was carried out at 2 different time points; from 2008 to 2016 and 2017 to 2019. During the latter there has been an increased awareness of NSII and a more rapid initiation of exploratory laparotomy. NSII was diagnosed in horses with a localized intestinal infarction and concurrent signs of *S. vulgaris* migration and no signs of intestinal strangulation or enterocolitis. Data were obtained from medical records. 30 cases was diagnosed from 2008 to 2016 and 9 cases from 2017 to 2019. Exploratory laparotomy was undertaken in 21 out of 30 cases in 2008 to 2016. 11 was euthanized intraoperatively due to a poor prognosis. Of the remaining 10 horses 3 (30 %) survived to discharge and 7 (70 %) was euthanized postoperatively. None of the 9 horses that were treated medically survived (100 %). In the 9 cases from 2017 to 2019 exploratory laparotomy was undertaken in 5 cases. Of these 4 (80 %) survived to discharge and 1 was euthanized intraoperatively due to a poor prognosis. None of the 4 horses that were treated medically survived (100 %). In conclusion the findings indicate that an exploratory laparotomy seems to be justified in cases with NSII in order to improve the survival rate. Additionally the survival rate increases with increased awareness and a more rapid initiation of surgery.

The role of Vδ1+ γδ T cells in malaria and endemic Burkitt's lymphoma,

Cecilia Smith-Togobo (1,2) and Lars Hviid (1,3)

(1) University of Copenhagen, Denmark; (2) University of Ghana, (3) Rigshospitalet, Copenhagen, Denmark

Naturally acquired protective immunity to *Plasmodium falciparum* malaria is mainly antibody-mediated. However, other cells of the innate and adaptive immune system also play important roles. These include so-called unconventional T cells, which express a γδ T cell receptor (TCR) rather than the TCR αβ expressed by the majority of T cells. The γδ T cell compartment can be divided into distinct subsets. One expresses a TCR involving Vγ9 and Vδ2, while another major subset uses instead a TCR composed of Vδ1 paired with one of several types of γ chains. We have proposed that Vδ1+ cells play an immuno-regulatory role in malaria. Our recent evidence indicates that these cells play a similar role in the aggressive B-cell tumour endemic Burkitt's lymphoma, which is a common childhood cancer in areas with stable transmission of malaria parasites.

Adverse effects of high (above optimal) temperature on *Schistosoma mansoni* intermediate host snails.

Møller ND (1), Steensgaard AS (1), Vennervald BJ (1).

(1) University of Copenhagen, Denmark

Schistosoma mansoni, is a snail-borne parasite which causes big health problems in Africa, and through long term exposure can be fatal. Through its life cycle it uses fresh water snails of the genus *Biomphalaria*. The snails can only exist in areas where certain climatic conditions allow it to proliferate. Climate change will cause many of these areas in Africa to periodically become hotter than the snails current preferred temperatures. This is expected to have adverse effects on for example snail behavior and immune function and may ultimately alter disease dynamics by reducing hosts' ability to resist infections. The parasite can typically tolerate higher temperatures better than the vector snails, which can lead to complications for the vector snail's immune system if it is exposed to temperatures above their optimal. In this study we would like to investigate the effect of high (above optimal) temperatures (30-35°C) on the vector snail, *Biomphalaria glabrata* immune function on non-infected snails. We will do so by conducted a controlled experiment, where we expose *Biomphalaria glabrata* to temperatures at 23^0C (control) and at 30-35^0C (above optimal temp.), through intervals of 24 hours and 1 week of exposure, in replicas of 3 small aquariums per setup. The high temperature is expected to cause stress-responses in the vector snail. These responses will be measured in hemolymph through determination of PO-like activity by an ELISA assay. Through results from articles studying similar topics and using same measuring methods as us, we expect the PO-like activity in the vector snails to be lower at temperatures above optimal and through longer exposure. We expect this to lead to an increase in the parasites infection success rate of the vector snail, because the snail isn't living under optimal conditions which affect the immune system.

Toxoplasma gondii seroprevalence in feral and farmed Danish mink

Heidi H Petersen (1), Mita E Sengupta (2), Sussie Pagh (3), Mariann Chriel (1)

(1) Technical University of Denmark; (2) University of Copenhagen, Denmark; (3) University of Aalborg, Denmark

In Denmark, American mink (*Neovison vison*) have been bred for their fur since the mid-1920s, and Denmark is currently one of the largest producers of mink skins with 17 million skins annually. Farmed mink is originally native to North America. However, feral populations are now common in Europe and South America. In Denmark, feral mink is regarded as an invasive species originating from farm escapes. The zoonotic parasite *Toxoplasma gondii* has a wide host range, including mink. A Danish study from 1994 observed 3% sero-positive farmed mink by latex agglutination test. However, the literature on *T. gondii* in mink is generally scarce, and to our knowledge, no studies on *T. gondii* in feral Danish mink have been carried out. *Toxoplasma gondii* infections in mink might be of public health importance, when dead mink are handled. This study examined the antibody titer against *T. gondii* in feral- and farmed mink. In total, 112 farmed mink submitted to DTU-VET for diagnostic examination, and 228 feral mink sampled by hunters from Jutland, Funen, Zealand and Bornholm were included in the study. At necropsy, the heart was removed and stored at -20°C until analysis. Meat juice was extracted from the hearts when thawed and analysed for anti-*T. gondii* antibodies using a commercial indirect ELISA. None of the farmed mink were sero-positive, while 50.5% of the feral mink were sero-positive. Of the positive feral mink, 13.4% (15/112) had fur colors other than brown, indicating a recent escape from farms. Significantly ($p=0.0002$) more feral male (63.5%) than female (36.2%) were sero-positive. In view of the results, precautions should be taken when handling feral mink. In contrast, handling farmed mink, pose a neglectable risk of acquiring *T. gondii* infections.

Temporal pattern of mutations in the knockdown resistance (kdr) gene of *Aedes aegypti* mosquitoes sampled from Southern Taiwan

Sandrine Biduda (1), Chia-Hsien Lin (1), Fatma H. Saleh (2), Flemming Konradsen (1), Helle Hansson (1), Karin L. Schiøler (1), Michael Alifrangis (1)

(1) University of Copenhagen, Denmark; (2) The State University of Zanzibar, Tanzania

Aedes aegypti mosquitoes are the principal vector for dengue transmission in Taiwan and vector control, including the use of chemical insecticides is one of the main strategies for breaking dengue transmission. However, over time the vector population has developed resistance to most insecticides, such as pyrethroids which have been used extensively in the attempt to control disease vectors, including *Aedes* mosquitoes. In this study, immature *Ae. aegypti* were sampled from various settings in Kaohsiung City and Pingtung County in Southern Taiwan during dry season 2013-2014, wet season 2014 and wet season 2015 and analysed for occurrence and temporal trends of single nucleotide polymorphisms (SNPs) in the voltage-gated sodium channel (VGSC)-gene resulting in knockdown resistance (kdr) to pyrethroids. Fragments of the VGSC-gene were PCR amplified followed by restriction fragment length polymorphisms (RFLP) to identify known SNPs in the VGSC-gene. Our study identified three kdr mutations in *Ae. aegypti*, namely V1023G, D1794Y and F1534C, while only wildtypes were observed at position 989 and 1016. In the first dry season, the mutant alleles 1023G, 1794Y and 1534C were observed at frequencies of 0.36, 0.55 and 0.33, respectively. Exploring for temporal changes, the most important observations were that the 1794Y mutant allele decreased significantly in frequency, to 0.42 in wet season of 2015 ($P=0.02$), while the 1534C mutant allele increased significantly in the following season to 0.60 ($P<0.05$). When combining the three SNPs, an insignificant decrease in double mutants were observed with time, while the triple mutants increased between dry season (at 0.14) to wet season 2014 (to 0.22) and continued to be high in wet season 2015 (at 0.21) ($P=0.3$). Both double and triple mutations were observed in our mosquito population which suggest that continued insecticide pressure is driving the observed mutational changes, although the selection is not unambiguous in the mosquito population.

Fermentable dietary inulin modulates mucosal immune responses and prevents *Trichuris muris* expulsion

Laura J. Myhill (1), Sophie Stolzenbach (1), Helena Mejer (1), Tina V.A. Hansen (1), Simon Jakobsen (1), Peter Nejsum (2), Stig M. Thamsborg (1), Andrew R. Williams (1)

(1) University of Copenhagen, Denmark; (2) Aarhus University, Denmark

Fermentable dietary fibres, such as inulin, have been shown to influence mucosal immunity and gut health. In pigs, we have shown that dietary inulin modulated the characteristic Th2-immune response induced by the porcine whipworm, *Trichuris suis*, by synergistically up-regulating the expression of Th2 and mucosal barrier genes (e.g. IL13, TFF3), and down-regulating inflammatory genes (e.g. IFNG). We have subsequently used the murine whipworm infection model to further explore this novel diet-parasite interaction, where inoculation with either a low (20 eggs) or high (300 eggs) *T. muris* egg dose results in worm persistence or expulsion, respectively, in C57BL/6 mice. Interestingly, dietary inulin enhanced worm numbers and size in both low- and high-dosed mice, and prevented worm expulsion in high-dosed mice typically resistant to infection. In these high-dosed mice, immune responses were markedly skewed towards a Th1-dominant state, as evidenced by increased numbers of T-bet expressing T-cells and IFNG and NOS2 caecal gene expression, and reduced mast cell numbers. Moreover, the inulin-induced persistence of *T. muris* altered gut microbiota profiles, with expansion of Proteobacteria and depletion of 'healthy' microbial phyla, such as Bacteroidetes and Actinobacteria observed. Conversely, in uninfected mice, inulin promoted the growth of Bacteroidetes, Actinobacteria and Verrucomicrobia (mainly *Akkermansia muciniphila*). Our results indicate a profound effect of diet on *T. muris* infection and immune regulation in C57BL/6 mice. Elucidation of the inulin-mediated mechanisms responsible for *T. muris* persistence is still required, nevertheless these findings have clear implications for the use of diet in regulating helminth infection and host gut health.

Characterising protective monoclonal antibodies to *Plasmodium falciparum* antigens PfMSRP5 and PfSERA9

Melanie R Walker (1), Asger Frank (1), Maria Bassi (1), Anne Schlitterlau Knudsen (1) and Lea Klingenberg Barfod (1)

(1) University of Copenhagen, Denmark.

Due to the large health and economic burden of malaria, designing a preventative vaccine is a major global priority. The current approach to vaccination is to induce antibodies against the merozoite form of the parasite. However, despite the fact that naturally acquired immunity is directed against the blood stage antigens, developing an efficient vaccine against *Plasmodium falciparum* has been challenging. This is primarily due to the high levels of polymorphism seen in malaria antigens as well as a complex parasite lifecycle which presents a large repertoire of potential vaccine targets. An effective second-generation vaccine will therefore need to include multiple antigens that elicit synergistic antibodies. Characterising monoclonal antibodies (mAbs) against antigens that operate at distinct steps in the same pathway will allow a better understanding of protective epitopes which could form the bases of a second-generation malaria vaccine. Antibodies against *P. falciparum* merozoite antigens PfSERA9 and PfMSRP5, have been shown to inhibit *P. falciparum* in vitro. Therefore, both antigens are promising candidates for vaccination. Nevertheless, there are limited studies investigating the humoral immune response against either antigen. In this study, anti-PfSERA9 and anti-PfMSRP5 mAbs are produced from naturally immune Ghanian donors using EBV immortalisation, and from immunised mice using hybridoma technology. MAbs are characterised using Attana QCM technology to assess affinity and map targeted epitopes. Additionally, growth inhibition activity assays are performed to determine individual inhibition as well as synergistic inhibition utilising mAbs that target a range of merozoite antigens. Finally, human and mouse mAb characteristics are compared. To date, seven anti-PfMSRP5 and four anti-PfSERA9 mouse mAbs have been produced. Additionally, one anti-PfMSRP5 human mAb has been isolated and characterisation of all mAbs is currently underway. Understanding the characteristics of protective mAbs and the epitopes they bind to may inform preventative vaccine strategies.

LIST OF PARTICIPANTS

	First name	Last name	Institution
1	Abbey	Olsen	PARZOO (Maria Johansen's group), PAP, KU
2	Agung	Cahyo Setyawan	AQUA (Kurt Buchmann's group), PAP, KU
3	Alice	Olsen	Idéværkstedet De Frie Fugle
4	Amin	Zakeri	Aahus University (Peter Nejsum's group)
5	Anders	Toftegaard Boysen	Aahus University (Peter Nejsum's group)
6	Angela	Pinot de Moira	Public Health, KU
7	Anna	Grønlund	PARZOO (Maria Johansen's group), PAP, KU
8	Anna-Sofie	Stensgaard	PARENVI (Birgitte Vennervald's group), PAP, KU
9	Anne	Schlitterlau Knudsen	CMP, KU
10	Anne	Majgaard Jensen	PARENVI (Birgitte Vennervald's group), PAP, KU
11	Anne	Borup	Aahus University (Peter Nejsum's group)
12	Appiah-Korang	Labi	CMP, KU
13	Asger	Meldgaard Frank	CMP, KU
14	Asma	M.Karami	AQUA (Kurt Buchmann's group), PAP, KU
15	Audrey	Andersen-Civil	PIGH (Andrew William's group), PAP, KU
16	Azmi	Al-Jubury	AQUA (Kurt Buchmann's group), PAP, KU
17	Bahtiar	Yilmaz	University of Bern
18	Birgitte Jyding	Vennervald	PARENVI (Birgitte Vennervald's group), PAP, KU
19	Bogdan	Cristinoi	CMP, KU
20	Bradley	Whitehead	Aahus University (Peter Nejsum's group)
21	Brian	Fredensborg	Organismal Biology, PLEN, KU
22	Cæcilie	Steinhausen	Organismal Biology, PLEN, KU
23	Charlotte	Bonde	VetPar (Stig Thamsborg's group), PAP, KU
24	Christian	Kapel	Organismal Biology, PLEN, KU
25	Christian	Simonsen	Organismal Biology, PLEN, KU
26	Dan	Klaerke	Veterinary and Animal Sciences, KU
27	Duncan	Ng	Staten Serum Institute
28	Foojan	Mehrdana	AQUA (Kurt Buchmann's group), PAP, KU
29	Grethe	Gomme	Honorary members
30	Heidi	Mathiessen	AQUA (Kurt Buchmann's group), PAP, KU
31	Heidi Huus	Petersen	DTU-Vet
32	Helena	Mejer	VetPar (Stig Thamsborg's group), PAP, KU
33	Helene	Kringel	Paratech
34	Helle	Hansson	CMP, KU
35	Helle	Gotfred-Rasmussen	Staten Serum Institute
36	Henrik Vedel	Nielsen	Staten Serum Institute
37	Huria	Marnis	AQUA (Kurt Buchmann's group), PAP, KU

38	Jørgen	Kurtzhals	CMP, KU
39	Jørn	Andreassen	Honorary members
40	Julie	Torvund-Jensen	Aahus University (Peter Nejsum's group)
41	Khairul	Syahputra	AQUA (Kurt Buchmann's group), PAP, KU
42	Lars	Hviid	CMP, KU
43	Laura	Myhill	VetPar (Stig Thamsborg's group), PAP, KU
44	Laura Boysen	Dall	Aahus University (Peter Nejsum's group)
45	Lea	Barfod	CMP, KU
46	Leah	Lourenco	PARENVI (Birgitte Vennervald's group), PAP, KU
47	Lena	Höbel	CMP, KU
48	Lena	Baukart	CMP, KU
49	Lise-Lotte	Christiansen	VetPar (Stig Thamsborg's group), PAP, KU
50	Louise	Von Gersdorff Jørgensen	AQUA (Kurt Buchmann's group), PAP, KU
51	Magnus	Hilarius Ohlin Jepsen	Organismal Biology, PLEN, KU
52	Maibritt	Mardahl	Aahus University (Peter Nejsum's group)
53	Maiken	Visti	CMP, KU
54	Maria Vang	Johansen	PARZOO (Maria Johansen's group), PAP, KU
55	Marie Louise	Honoré Jørgensen	Veterinary Clinical Sciences, KU
56	Mary	Lopez-Perez	CMP, KU
57	Melanie	Walker	CMP, KU
58	Mette	Schjelde	PIGH (Andrew William's group), PAP, KU
59	Milla	Leppä	PIGH (Andrew William's group), PAP, KU
60	Mita Eva	Sengupta	PARENVI (Birgitte Vennervald's group), PAP, KU
61	Morten	Pontoppidan	CMP, KU
62	Mwemezi	Kabululu	PARZOO (Maria Johansen's group), PAP, KU
63	Nao	Takeuchi-Storm	VetPar (Stig Thamsborg's group), PAP, KU
64	Nexhibe	Beciri	CMP, KU
65	Nikoline Dupont	Møller	PARENVI (Birgitte Vennervald's group), PAP, KU
66	Pascal	Magnussen	CMP, KU
67	Peter	Nejsum	Aahus University (Peter Nejsum's group)
68	Pikka	Jokelainen	Staten Serum Institute
69	Rasmus	Jensen	CMP, KU
70	René	Bødker	Veterinary and Animal Sciences, KU
71	Rune	Stensvold	Staten Serum Institute
72	Salem	Belkessa	Staten Serum Institute
73	Samuel Yao	Ahorhorlu	CMP, KU
74	Sandrine	Biduda	CMP, KU
75	Sara	Perkins	Cardiff University
76	Shaozhi	Zuo	AQUA (Kurt Buchmann's group), PAP, KU
77	Sidsel	Andersen	Aahus University (Peter Nejsum's group)
78	Stig	Thamsborg	VetPar (Stig Thamsborg's group), PAP, KU
79	Suraj	Dhakal	Organismal Biology, PLEN, KU
80	Trine	Staalsø	CMP, KU
81	Uffe Christian	Braae	Staten Serum Institute
82	Valdemar	Vejlstrup	CMP, KU
83	Veronika	Potocka	Aahus University (Peter Nejsum's group)