JOINT SPRING SYMPOSIUM 2018

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

Parasites shape the host

Dansk Selskab for Parasitologi

DSTMIS

Dansk Selskab for Tropemedicin og Internationel Sundhed

Friday 6th of April, 2018, 8:30-15:00

University of Copenhagen

Lecture room 1-01 (Festauditoriet),

Bülowsvej 17,

1870 Frederiksberg C

ORAL PRESENTATIONS – KEY NOTES

Pathogens as a driving force in mammalian genome evolution

Manuela Sironi

Bioinformatic Unit of Scientific Institute IRCCS Eugenio Medea, Bosisio Parini, Italy

The ever increasing availability of resequenced genomes from diverse human populations, mammals, and parasites is providing unprecedented opportunities to compare genetic diversity within and across species. The hypothesis whereby infectious diseases have been acting as a powerful selective pressure for human populations was formulated long ago, but it was not until the availability of large-scale genetic data and the development of novel methods to study molecular evolution that we could assess how pervasively infectious agents have shaped human genetic diversity. We now know that among the environmental factors that acted as selective pressures during the evolution of our species, pathogen load (and helminth diversity in particular) had the strongest influence. Thus, beside the textbook example of the major histocompatibility complex, selection signatures left by pathogen-exerted pressure can be identified at several human loci, including genes not directly involved in immune response. Comparisons among species have also provided important insights. This is because host-pathogen interactions often result in so-called arms races, whereby hosts are under pressure to evolve resistance against pathogens, while pathogens strive to develop countermeasures to evade host surveillance and achieve a successful infection. Thus, when resistance and counter-resistance are at least partially genetically determined, a constant genotype turnover may result in fast evolutionary rates over relatively long evolutionary times. For instance, the interaction between *Plasmodium falciparum* reticulocyte-binding protein homologue 5 and human basigin was shaped by positive selection acting on both partners. Similarly, *Palsmodium* parasites most likely represented the selective force underlying the evolution of several erythrocyte cytoskeleton proteins in great apes. Finally, the interaction between trypanosomes and their mammalian hosts resulted in mutual selective pressure at the interaction surface between mammalian hemoglobin and the parasite hemoglobin-haptoglobin receptor. Thus, evolutionary studies take advantage of experiments that natural selection has been performing over millenia. Hopefully, evolutionary biology and traditional biomedical research will be ever more integrated in the future. This will help to identify the determinants of infection susceptibility/resistance, to anticipate the zoonotic potential of non-human pathogens, and to predict the potential short- and long-term effects of new treatment strategies.

Sniffing out malaria: towards noninvasive diagnosis

Audrey Odum John

Washington University School of Medicine in St. Louis, USA

Test-and-treat strategies for malaria control rely on accurate, rapid, simple diagnostic strategies. The current generation of rapid diagnostic tests (RDTs) for malaria largely depend on detection of the *Plasmodium falciparum* protein, HRP2. Because HRP2 persists long after infection, false positives are common. In addition, increasing use of RDTs has begun to select for parasite strains that do not produce HRP2 and are undetected by standard tests. Previous evidence had suggested that malaria infection might result in characteristic changes in breath odors, but this possibility had not been examined in a field setting. We therefore performed a field study in Malawi, to analyze breath volatiles from children with and without uncomplicated falciparum malaria by thermal desorption-gas chromatography/mass spectrometry. Using an unbiased, correlation-based analysis, we find that children with malaria have a distinct shift in overall breath composition. Highly accurate classification of infection status was achieved with a suite of six compounds. In addition, we find infection correlates with significantly higher breath levels of two mosquito-attractant terpenes, α-pinene and 3-carene. These findings attest to the viability of breath analysis for malaria diagnosis, identifies candidate biomarkers, and identifies plausible chemical mediators for increased mosquito attraction to malaria-infected patients.

CONTRIBUTED ORAL PRESENTATIONS

Gut microbiome - helminth interactions between the tapeworm *Hymenolepis diminuta* and its intermediate beetle host

Inga Fossdal í Kálvalíð (1), C. Rune Stensvold (2), Henrik V. Nielsen (2), Christian M.O. Kapel (1), Brian L. Fredensborg (1)

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

There is increasing evidence that host-parasite interactions are influenced by either host and/or parasite associated microbes. This has potential impact on our understanding of disease ecology, and its applications in public health and commercially (e.g. drug development). Rigorous hypothesis testing at the organismal level may be difficult in parasite definitive hosts due to ethical and logistical restrictions. In this study, we tested the use of an insect model to explore reciprocal interactions between a parasite and the resident gut microbiota of its intermediate host. Based on 16S/18S rRNA microbial profiling we measured key parameters of the composition, abundance, and diversity of the host gut bacteriome and mycobiome in grain beetles, *Tenebrio molitor* in relation to infection status of the tapeworm *Hymenolepis diminuta*, which form chronic infection in the beetle hemocoel. In addition, we tested the effect of an experimentally induced dysbiosis of the gut microbiota on the establishment success of *H. diminuta*. We found significant changes to the community composition of the gut bacteriome and mycobiome in relation to infection status. In addition, the mycobiome experienced a significant decrease in abundance, but an increase in Shannon diversity in the infected beetles. Parasite establishment was negatively influenced by gut dysbiosis. We expect that experimental work using invertebrate intermediate hosts may provide a platform for detailed studies of host-parasite-microbe interactions of use to other hosts.

Dietary inulin influences gut health and immune response in helminth-infected pigs

Laura J. Myhill (1), Sophie Stolzenbach (1), Peter Nejsum (2), Tina V. Alstrup Hansen (1), Kerstin Skovgaard (3), Helena Mejer (1), Stig M. Thamsborg (1), and Andrew R. Williams (1)

(1) University of Copenhagen; (2) Aarhus University; (3) Technical University of Denmark

Dietary fibres, such as inulin, have been shown to influence host immunity and gut health, and may positively impact host responses during gastrointestinal helminth infection. Helminths such as *Trichuris suis* induce a polarised Th2 immune response, which modifies the gut environment by increasing mucin secretion and epithelial cell proliferation, eventually resulting in expulsion of the parasite. Our group have utilised a *T. suis*-infected pig model to study the interactions between an inulin-based diet, gut health and host immune responses during a helminth infection. Our study was designed as 2-factorial (diet and infection), with 8-9 pigs in each treatment group. Helminth infected pigs received a single dose of 10,000 embryonated *T. suis* eggs, and the prebiotic treated pigs received standard feed supplemented with 10% purified long-chain inulin daily. After 28 days post-infection, intestinal tissue samples were collected to measure local immune-related parameters, utilising techniques such as qPCR, histology and ELISA. Our results indicate that inulin has a positive effect on the gut health of helminth-infected pigs by increasing expression of intestinal epithelial barrier-related genes such as trefoil factor 3 (TFF3) and down-regulating proinflammatory immune genes. Also, 16S rRNA sequencing of proximal colon digesta samples revealed that inulin supplementation increased the ratio of Bacteroidetes:Firmicutes, with pigs treated with both inulin and *T. suis* showing the highest abundance of beneficial Bacteroidetes. Together, these data suggest that inulin may enhance Th2 responses and down-regulate inflammatory Th1 immune function in response to *T. suis* infection. This is potentially mediated by changes in host microbiota composition, or by direct stimulation of mucosal immune cells. To examine this, LPS-stimulated macrophages were cultured in vitro with inulin and/or *T. suis* soluble antigen. A clear anti-inflammatory effect of *T. suis* antigen was observed but not for inulin, suggesting two distinct modes-of-action and a likely involvement of the gut microbiota in the effects observed in vivo with dietary inulin. Our results indicate a profound effect of diet on immune function in helminth-infected pigs that may be exploited to improve gut health. Further work is still required to understand the mechanism(s) behind the diet-mediated immunomodulation observed *in vivo*.

Sub-lethal chemical stress increases parasite establishment in an insect-helminth model

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

University of Copenhagen

Environmental toxicants are abundant in nature and hosts and their parasites might frequently be exposed to chemical stress. 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. Here, we studied the effect of sub-lethal and multiple exposures of the pyrethroid insecticide alpha-cypermethrin to the establishment of the tapeworm Hymenolepis diminuta in the intermediate insect host *Tenebrio molitor*. We exposed *T. molitor* beetles with alpha-cypermethrin (LD20) before and after *H. diminuta* experimental infection, and measured the establishment success of cysticercoids. In addition, we conducted in vitro studies on the direct effect of the insecticide on parasite viability. Our results showed that there was no direct lethal effect of alpha-cypermethrin to the *H. diminuta* cysticercoids at the concentration ranges from LD10 to LD90. However, *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. We hypothesize that exposure to the insecticide alpha-cypermethrin increases parasite establishment success via physiological stress and immune suppression of the host. Thus, our results indicate that environmental toxicants can impact host-parasite interactions in terrestrial systems where parasites may experience more favorable conditions for establishment when their hosts are exposed to sub-lethal chemical stress.

Relationship between anti-*Fasciola hepatica* antibody levels in bulk tank milk and within-herd prevalence in Danish dairy farms

Nao Takeuchi-Storm (1), Matthew Denwood (1), Heidi L. Enemark (2), Stig M. Thamsborg (1)

(1) University of Copenhagen; (2) Norwegian Veterinary Institute

The common liver fluke, *Fasciola hepatica*, is a parasitic trematode of grazing livestock with a worldwide distribution. It causes substantial economic impact and prevalence is steadily increasing in Danish cattle farms. An appropriate diagnostic method is necessary to build a control strategy on a farm. Bulk tank milk (BTM) is taken at each milk collection and thus is easily obtainable. This study aimed to assess the usefulness of measuring antibody levels in bulk tank milk (BTM) by ELISA for approximation of within-herd prevalence. A total of 72 organic dairy farms with more than 99 animals, with negative, low, moderate, and high antibody levels were identified in spring 2016 by BTM pre-screening. In spring 2017, 4 to 7 individual milk samples and one BTM from these farms were collected and analysed by a commercial ELISA kit (IDEXX Fasciolosis Verification test). The relationship between BTM ELISA results and apparent within-herd prevalence according to dichotomized individual milk sample results were assessed by mixed effects logistic regression. The final dataset consisted of 72 BTM samples and 473 individual milk samples. Good correlation between BTM ELISA results and apparent within herd prevalence was seen, and the estimated apparent within-herd prevalence was ≤7.0% (95%CI: 3.7-11.4) if BTM ELISA negative, ≤30.5% (95%CI: 21.5-41.6) if low, <69.8% (95%CI: 61.2-77.5) if moderate, and ≥69.8% (95%CI: 61.2-77.5) if high. This showed that BTM ELISA is likely to be positive if more than 7.0% of lactating cows are positive. The model also predicted that with moderate BTM ELISA value, there is some risk that zero test positive animals would be found from a sample of only 5 animals, but almost no risk if 10 animals are sampled. In conclusion, BTM ELISA is a useful method for a herd level diagnosis and monitoring for *F. hepatica* on a dairy farm.

Liver worm (*Contracaecum osculatum*) infection status in cod (*Gadus morhua*) along a transect from the Skagerrak to the eastern Baltic

Maria Sokolova (1), Kurt Buchmann (2), Per W. Kania (2), Bastian Huwer (1), Uwe Krumme (3), Anders Galatius (4), Jane W. Behrens (1)

(1) Technical University of Denmark; (2) University of Copenhagen; (3) Thünen Institute of Baltic Sea Fisheries; (4) University of Aarhus

Since the mid-1990s the condition of Eastern Baltic cod (*Gadus morhua*) has decreased dramatically, and larger individuals have become rare in catches. Apart from expanding hypoxic areas and declining availability of main prey such as clupeids, infection with the parasitic nematode *Contracaecum osculatum* (liver worm) has increased drastically in the Eastern Baltic cod according to series of recent reports. Corresponding detailed information on the infection status of cod in the other adjacent areas is not available. In the present study, prevalence and abundance of the liver worm were determined and compared in cod from the nine study areas covering a transect from Skagerrak to the eastern Baltic. The investigation was based on analysis of a total of 321 cod livers, derived from fish between 35 and 50 cm, and sampled in Q4 2016 and 2017. The results showed that the prevalence and abundance of infection in the Eastern Baltic waters was significantly higher than in the remaining study areas. Moreover, a negative correlation between the infection abundance and Fulton's condition factor was found. Possible reasons for the differences between the areas such as grey seals (*Halichoerus grypus*) abundance and differences in salinity are discussed and mitigation measures are proposed.

A case of thelaziosis (eyeworm disease) in a Danish dog

Tommy Hardon (1), Helena Mejer (2), Stig M. Thamsborg (2)

(1) Haslev Veterinary Clinic; (2) University of Copenhagen

The oriental eyeworm (*Thelazia callipaeda*)(Spirurida) of dogs, cats, and other mammals, including humans, has been introduced to South-Europe from the Far East within the last 20 years. Autochthonous transmission is now reported in Portugal, Spain, France, Switzerland, Italy, Hungary, Greece and several Balkan states. The worms inhabit the lacrimal glands and conjunctival sac, particularly behind the nictitating membrane. It is a vector-borne infection transmitted by fruit flies which take up first stage larvae (L1) directly from the conjunctival fluid. Within the flies development to L3 takes 2-4 weeks before the infection is transmitted to a new host. A 3 year-old female Eurasian dog, bred in Denmark, was admitted to the clinic in September with persistent corneal ulcer, refractory to antibiotic treatment (late August), after a one month stay (July) in northern Italy. Examination showed bilateral conjunctivitis and ulcerative keratitis (right eye), and six 1-2 cm long milky-whitish worms were removed from the surface of conjunctiva under full anesthesia. It was evident that the female worms contained live larvae which measured around 200 µm. Worms were preserved and examined by microscope. The general morphology included fine transverse cuticular striations along the whole body, a vulva opening just anterior to the oesophagal-intestinal junction, two highly dissimilar spicules (approx. 140 versus 1800 long µm), a small cone stump buccal capsule (approx. 23 µm deep), and the worms were identified as *T. callipaeda*. Two worms were submitted to PCR-analysis (sequencing the *cox1* gene; courtesy of Dr. D. Otranto) and the diagnosis was confirmed. The dog was treated with oral milbemycin (Milbemax® VET) twice (1 week apart) together with supportive treatment. The infection was cleared and healing of cornea was observed after 2 weeks. Danish veterinarians should be aware of this emerging zoonotic infection in dogs and cats with a travel history of South-Europe.

Direct whole-genome sequencing of *Plasmodium falciparum* specimens from dried erythrocyte spots

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(1) University of Copenhagen; (2) Copenhagen University Hospital (Rigshospitalet); (3) Kolding Hospital, Denmark; (4) Bandim Health Project, Bissau, Guinea-Bissau; (5) Karolinska Institutet, Stockholm, Sweden; (6) Danderyds Hospital, Danderyd, Sweden; (7) Technical University of Denmark

Plasmodium falciparum malaria remains a major health burden and genomic research represents one of the necessary approaches for continued progress towards malaria control and elimination. Sample acquisition for this purpose is troublesome, with the majority of malaria-infected individuals living in rural areas, away from main infrastructure and the electrical grid. The aim of this study was to describe a low-tech procedure to sample *P. falciparum* specimens for direct whole genome sequencing (WGS), without use of electricity and cold-chain. Venous blood samples were collected from malaria patients in Bandim, Guinea-Bissau and leukocyte-depleted using Plasmodipur filters, the enriched parasite sample was spotted on Whatman paper and dried. The samples were stored at ambient temperatures and subsequently used for DNA-extraction. Ratios of parasite:human content of the extracted DNA was assessed by qPCR, and five samples with varying parasitaemia, were sequenced. Sequencing data were used to analyse the sample content, as well as sample coverage and depth as compared to the 3d7 reference genome. qPCR revealed that 73% of the 199 samples were applicable for WGS, as defined by a minimum ratio of parasite:human DNA of 2:1. WGS revealed an even distribution of sequence data across the 3d7 reference genome, regardless of parasitaemia. The acquired read depths varied from 16 to 99×, and coverage varied from 87.5 to 98.9% of the 3d7 reference genome. SNP-analysis of six genes, for which amplicon sequencing has been performed previously, confirmed the reliability of the WGS-data. This study describes a simple filter paper based protocol for sampling *P. falciparum* from malaria patients for subsequent direct WGS, enabling acquisition of samples in remote settings with no access to electricity.

A larger proportion of Ghanaian children generate an antibody response to group B than group A PfEMP1 DBLβ domains

Rebecca W. Olsen (1), Michael Ofori (2), Lars Hviid (1), and Anja T.R. Jensen (1)

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

Plasmodium falciparum is the most pathogenic human malaria parasite and cause the death of 450.000 individuals each year. The parasite live inside red blood cells and express proteins known as Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) on the surface of the infected cell. PfEMP1 proteins can adhere to various tissue by binding to receptors on the host endothelium. In areas with high malaria transmission individuals as they grow older develop immunity against *P. falciparum*. Immunity to falciparum malaria is mediated by a broad repertoire of IgG antibodies against PfEMP1. PfEMP1 proteins can be divided into three groups (A, B and C) and each PfEMP1 is composed of a number of Duffy-binding-like (DBL) domains and cysteine-rich interdomain regions (CIDR). Previous studies using recombinant DBL or CIDR domains (of any sub-type) or infected red cells have shown that antibodies against group A PfEMP1 are acquired earlier in life than antibodies to Group B and C PfEMP1. In this study, we looked at acquisition of antibodies against a subtype of DBL domains known as DBLÎ². Antibody reactivity to DBLÎ² was measured in plasma samples obtained from hospitalized Ghanaian children and in samples collected 2 and 6 weeks following hospitalization. By contrast, to previous studies children were found to have higher levels of IgG against group B DBLÎ² compared to Group A domains.

How do African women get placental malaria?

Michael F Ofori (1), Helena Lamptey (1), Emmanuel K Dickson (1), Eric Kyei-Baafour (1) and Lars Hviid (2)

(1) University of Ghana; (2) University of Copenhagen and Rigshospitalet

Plasmodium falciparum parasites causing placental malaria (PM) express the VAR2CSA type of the clonally variant antigen family PfEMP1. This enables evasion of pre-existing immunity and results in placental accumulation of infected erythrocytes (IEs). We present data on seasonal variation in levels of VAR2CSA-specific IgG and IgG specific for a PM-unrelated PfEMP1 protein among Ghanaian women at first antenatal visit. Our results indicate that PM does not require recent exposure to infected mosquitoes, in contrast to malaria in general. This has implications for the impact of insecticide-treated bed nets on PM incidence, and for antenatal care in woman with pre-existing immunity.

POSTER PRESENTATIONS

Trichuris suis and inulin change the gut microbiota towards a healthier profile

Sophie Stolzenbach (1), Laura J. Myhill (1), Peter Nejsum (2), Rune Stensvold (3), Lee O'Brien Andersen (3), Dennis S. Nielsen (1), Tina V. Hansen (1), Helena Mejer (1), Andrew R. Williams (1) and Stig M. Thamsborg (1)

(1) University of Copenhagen; (2) Aarhus University; (3) Statens Serum Institut

Dietary inulin beneficially affects the gut microbiota as well as the immune system, but little is known regarding how the microbiota is affected by a gastrointestinal helminth infection. We therefore set out to explore the interaction between prebiotic, microbiota, and gastrointestinal helminth infection to improve the gut health of pigs and to identify novel means of control. To do so, we performed a 2-factorial study with parasite-naïve two-months old pigs: Nine pigs were fed the fermentable fructo-oligosaccharid inulin (10% w/w feed) and infected with 10,000 *Trichuris suis* eggs, eight pigs received inulin only, nine pigs were infected only and eight pigs served as controls. Faeces were sampled fortnightly, and ileum, caecum, proximal and distal colon samples were obtained at necropsy 4 weeks post-infection. All samples were sequenced at the V3-V4 region of the 16S rRNA gene in a single Illumina MiSeq run. Subsequent assembly was done using the bespoke bioinformatics pipeline BIONmeta, and analyzed using QIIME. Across treatments, digesta samples showed a distinctly different composition of bacteria in the ileum, whereas the difference between the caecum and colon were more subtle and showed a similar bacterial profile. A clear difference was found between inulin- and control-fed groups, with an increase of Bifidobacterium and the Bacteriodetes:Firmicutes ratio. Infection with *T. suis* affected low-abundance species, and was mainly characterized by an increase in Lactobacillus and a slight decrease in Proteobacteria. *T. suis* infection and inulin supplementation together enhanced the changes from each treatment, and resulted in a greater increase in the probiotic-related Lactobacilli and Bifidobacteria, and an elimination of the disease-associated Campylobacter. Overall, the results indicate that *T. suis* can modulate the microbiota in an advantageous way, and when used in combination with inulin, will create a shift towards a more beneficial gut microbiota, ultimately increasing the gut health of pigs.

Schistosoma mansoni microbiome and its origins - how to find a microbiome in a tiny worm and its egg

Agate Auzane, Anna M O Kildemoes, Dennis S Nielsen

University of Copenhagen

Schistosoma mansoni (commonly known as a blood fluke) is the causative agent of acute and chronic intestinal schistosomiasis. From socio-economic and public health perspective, schistosomiasis is considered the second most important parasitic disease after malaria, with more than 290 million people being affected. Multiple studies indicate that many parasitic worms from both Nematoda and Platyhelminthes phyla have persistent associations with microorganisms. For some species these symbioses are crucial for survival and development, but in the majority the role of these associations is unknown. Helminthic microbiomes could influence parasite's virulence, disease manifestation, reproduction rate, lifespan and many other aspects of the parasite's physiology and interactions with the host. A parasite can also function as a vector for bacteria that can be pathogenic to the host. Within a human host schistosomes inhabit an almost sterile and nutrient rich environment - blood vessels, but during its lifecycle schistosomes get exposed to microorganism rich environments like host feces, digestive glands of a snail, and freshwater. There are a few studies that indicate a presence of microorganisms in adult schistosomes, including a human pathogen - Salmonella spp. The aim of this study was to determine whether adult male and female S. mansoni have a distinctive microbiome, whether it is vertically transmitted to the next generation and how it is affected by administration of antibiotics to the host. Here we will present method development data for isolation of microbiomes from adult worms and eggs.

Extracellular vesicles secreted from the parasitic helminth, *Ascaris suum*, show unique proteomic profile

Sidsel D Andersen (1,2), Anne Borup (1,2), Eline P Hansen (3), Allan Stensballe (4), Yan Yan (5) and Peter Nejsum (1,2)

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Helminth infections are among the most neglected of the tropical diseases, even though they have a great impact on human health. In addition, helminths are a major constraint on livestock production and animal welfare worldwide. Parasitic worms often establish long-term chronic infection by manipulating the host immune responses, but very little is known about the specific mechanisms by which they do so. Excretory-secretory antigens from parasites are important in this context but few studies have indicated that parasitic extracellular vesicles (EVs) are crucial for host-parasite interaction. The aims of this study were to characterize the size profile of EVs secreted from *Ascaris suum* and their protein content. A. suum were collected from naturally infected pigs slaughtered at a Danish abattoir and incubated under sterile conditions for 3 days. EVs were isolated using ultracentrifugation and/or size exclusion chromatography (qEV). Their size profile was established using nanoparticle tracking analysis (NTA) by Nanosight and showed that EVs were around 163 nm in size. The proteomic content of EVs were determined using UPLC-nanoESI MS/MS followed by bioinformatics analysis using the software package Perseus. Proteomic analysis showed that EVs had a unique proteomic profile with a number of enriched proteins (n=154) which was not present in the the non-EV supernatant. Further characterization of the EV proteome identified several proteins previously reported in parasite EVs.

IMMUNOSUPPRESSIVE EFFECTS OF HELMINTHS ON INFLAMMATORY RESPONSES *IN VITRO* AND *IN VIVO*

Amin Zakeri, Martin R Jakobsen and Peter Nejsum

University of Aarhus

Epidemiological studies suggest an inverse relationship between helminth infections and inflammatory diseases. Innate immunity, especially antigen presenting cells (APC), play a key role in the initiation of allergic responses. Toll-like receptor (TLR) 4 is an important pattern recognition receptor, which play a central role in asthma. Interestingly, several studies have also shown that helminth parasites are masterful modulators of TLR signaling. In this study, we evaluate TLR4 expression in a murine model of asthma, treated with helminth antigens. The immunomodulatory effects of *Trichuris suis* somatic antigens on murine bone marrow macrophages and human monocytes are undertaken to assess whether these antigens can inhibit LPS-induced inflammatory cytokines. Allergic airway inflammation was induced in BALB/c mice by sensitization with ovalbumin/Alum. The effect of helminth-derived antigens on the suppression of inflammation was evaluated by histology and cellular infiltration in the lungs and expression of TLR4 transcripts was assessed by quantitative real-time PCR assay. To further dissect the immunomodulatory effects of helminths on LPS-induced inflammatory responses, we use the human derived monocytic THP-1 cell line and murine bone marrow macrophages exposed to *T. suis* antigens to validate pro- and anti-inflammatory cytokines profiles. These analyses are ongoing and will be presented at the meeting. Helminth antigens were found to attenuate ovalbumin induced hyperplasia and mucus production in the lungs as well as cellular infiltration in the bronchoalveolar lavage fluid. In addition, helminth antigens suppressed the expression of TLR4 mRNA in the splenocytes of ovalbumin sensitized mice. Our preliminary in vitro studies show that IL-6 is significantly suppressed by *T. suis* antigens. This study has so far demonstrated that TLR4 signaling is involved in helminth-mediated immune modulation and use of helminth products can lead to suppressed inflammatory responses *in vivo* and *in vitro*.

Parasites and plants - looking for bioactive compounds in plants with anti-parasitic effect

A. H. Valente (1), A. R. Williams (1), H. T. Simonsen (2), S. M. Thamsborg (1)

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

Increasing resistance to the limited number of existing drugs against gastrointestinal parasites (GIP) has led to an urgent need to explore new control options. Cattle grazing on chicory (*Cichorium intybus*) have lower levels of infection with the GIP *Ostertagia ostertagi*, indicating that chicory could be a promising anti-parasitic agent in cattle. The putative active compounds in chicory are sesquiterpene lactones (SL). In this project, we aim to isolate and characterize the active compounds from chicory and assess their anti-parasitic activity against Ostertagia and several other GIP, such as *Heligmosomoides polygyrus*, *Ascaris suum*, Cryptosporidium spp. and *Giardia duodenalis*. Extracts from initially five different cultivars of chicory have been prepared and their SL profiles assessed by HPLC-MS. The extracts have then been tested for anti-parasitic effects against the swine nematode *A. suum* using *in vitro* assays. The composition of bioactive compounds will be correlated with the anti-parasitic effect, and this may indicate which of individual or combinations of bioactive compounds are responsible for the effect, guiding further fractionation and compound isolation. Furthermore, anthelmintic effects of purified compounds will be determined by *in vivo* experiments with *H. polygyrus*-infected mice. Finally, mode of action will be investigated using transcriptomic data from GIP exposed to sublethal doses of the active compound *ex vivo*.

Host immunopathology in response to *Schistosoma mansoni* infection in mice with altered gut microbial composition

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(1) University of Copenhagen; (2) Research Center Borstel; (3) Bispebjerg University Hospital

Chronic infection with the parasitic blood fluke, *Schistosoma mansoni*, present the most severe pathology related to egg-induced host immune responses and fibrosis development. Commensal gut microbial composition influences host immune homeostasis and hence potentially affects systemic immunopathology induced by pathogens such as *S. mansoni*. Both humans and experimental models show metabonomic changes related to gut microbial and liver metabolism associated with *S. mansoni* infection. This points towards a role for gut microbiota in *S. mansoni* pathology regulation via the host immune response. To investigate whether different gut microbial compositions would result in differences in pathology induced by *S. mansoni* eggs, a controlled mouse model combining antibiotics treatment and infection was established. The commensal gut microbial composition was altered by oral administration of ampicillin/vancomycin and combined with subsequent *S. mansoni* infection in a C57BL/6-NTAC mouse model. Here egg-induced hepatomegaly and relative degree of inflammation were quantified in liver and ileum tissues by stereological principles. A significantly lesser degree of inflammation in liver tissue in the combined antibiotics treated and *S. mansoni* infected group compared to the infected only group was seen. This difference in degree of inflammation was not observed in ileum tissue and could not be explained by infection burden. Furthermore, differences in liver panel parameters, number of collagen depositions and cytokines (IFN-ɣ, IL-33) underscore an altered response to *S. mansoni* eggs in the liver when the gut microbial composition is strongly altered before infection. Our results implicate a role for intestinal immune milieu influenced by gut microbial composition in liver pathology development and hence morbidity. Further research is necessary to determine whether more subtle alterations of gut microbial compositions by factors such as diet, other infections, food-associated antibiotics or drugs facilitate a strong enough change in immune milieu to affect systemic pathology.

Serological evidence of exposure to *Toxoplasma gondii* in extensively farmed wild boars (*Sus scrofa*) in Denmark

Celine Kaae Laforet (1), Gunita Deksne (2, 3), Heidi Huus Petersen (4), Pikka Jokelainen (5, 6, 7), Maria Vang Johansen (1) and Brian Lassen (1)

(1) University of Copenhagen; (2) Institute of Food safety, Animal Health and Environment "BIOR", Latvia; (3) University of Latvia; (4) Technical University of Denmark; (5) Statens Serum Institut; (6) University of Helsinki; (7) Estonian University of Life Sciences

We conducted a cross-sectional seroepidemiological study to estimate the seroprevalence of the zoonotic parasite *Toxoplasma gondii* in extensively farmed wild boars in Denmark. A total of 101 samples were tested for anti-*T. gondii* antibodies with a commercial indirect enzyme-linked immunosorbent assay. We found serological evidence of substantial exposure to *T. gondii* among the wild boars included in our study. Eating undercooked meat of Danish wild boars should be considered a potential source of *T. gondii* infection for humans and other hosts.

Risk assessment of *Toxoplasma gondii* in Danish indoor sows

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Toxoplasma gondii is found worldwide in up to one-third of the human population. Pork consumption is considered a primary route of infection in humans. Denmark has a substantial export of pork and a large national consumption. A recent study revealed a seroprevalence of 33.7 % in Danish indoor sows, and cats' access to pig feed has previously been identified as a risk factor for transmission of *T. gondii*. The present cross-sectional study aimed to risk rank Danish indoor sow herds and identify risk factors for transmission of *T. gondii*, related to management practices. Data were collected from September 2017 to March 2018 and combined questionnaire interviews, observational surveys and sampling from cats and mice at sow herds randomly selected from all Danish herds. Cat faecal samples were examined for *T. gondii* oocysts. Mice brains were isolated and kept frozen until examined by PCR. In total, 39 sow herds were visited, 45 cat faecal samples were collected from 17 farms and 79 mice were caught by snap traps from 21 farms. All faecal samples were microscopically negative for *T. gondii* but will be further analysed by PCR. Both cats and mice were abundant in the sow herds, and hygiene, feed storage and water practices all posed a potential risk of transmission of *T. gondii*. Final analyses of the data are expected to elucidate the transmission of *T. gondii* in Danish indoor sow herds.

Is *Ancylostoma celaynicum* a zoonotic parasite in Mwanza, Tanzania?

Molecular identification of hookworm species in human and dog feces from Mwanza, Tanzania

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The hookworm *Ancylostoma ceylanicum* is the common hookworm of domesticated cats and dogs in Asia and the Pacific. However, recent studies have uncovered the parasite's zoonotic potential. Studies done in several places in Asia showed that 6 to 51% of people infected with hookworms harbored *A. ceylanicum*. This was done using molecular techniques for species identification as hookworms are extremely difficult to distinguish based on morphology. Using this same technique on samples taken from dogs in Morogoro, Tanzania, a recent study documented the presence of *A. ceylanicum* in Africa for the first time. This project will focus on the zoonotic potential of *A. ceylanicum* in Mwanza, Tanzania. The parasite has not been documented in humans in Africa which might be due to routine diagnostics being done by microscopy, where hookworm egg-based species identification is not possible. Therefore, the main purpose of this project will be to identify hookworm species in human and dog feces using molecular techniques. *A. ceylanicum is* of special interest, as this hookworm species is zoonotic and potentially present an unaddressed public health problem. Here we will present the project aims and methodology for differentiating hookworm species and documenting the first *A. ceylanicum* infection in humans in Tanzania.

A new report of the carnivore-transmitted *Taenia ovis* cysts infesting the heart muscles of sheep in Denmark

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To our knowledge, this is the first report of *Taenia ovis* infections in Danish sheep. The cysts infested the hearts, diaphragm and skeletal muscles of at least 130 lambs from one specific farm localized in South Jutland in spring 2016. Morphological diagnosis is impossible due to high resemblance to other Taeniid infections, hence molecular typing of the mitochondrial cytochrome c oxidase I (cox1) gene was implemented. We could not identify the definitive hosts, but we suspect that infection might either be due to the importing of infected dogs to the farm or due to the recent localization of wild carnivores in the area; namely wolves, raccoon dogs and jackals. The widespread infection of *T. ovis* in this farm is of concern to veterinary authority and to meat producers; the meat is condemned due to aesthetic reasons which causing economic losses to the farmers and to stop the possible spread of the infection to susceptible wildlife definitive hosts in the area.

Antibody response to recombinant and native PfEMP1 proteins in Colombian individuals

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The intensity of *Plasmodium falciparum* transmission in Colombia is low. Although at least one million women of reproductive age live in malaria-endemic areas of the country, malaria in pregnancy, including placental malaria, is not common. Protection from malaria is mainly antibody-mediated, and member of the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family expressed on the infected erythrocyte (IE) surface are important target antigens. The VAR2CSA subtype of PfEMP1 mediates IE binding in the placenta and IgG specific for this protein is normally acquired only after exposure to placental parasites. Nevertheless, it was recently reported that a large proportion of men and children from Colombia have high levels VAR2CSA-specific IgG. This potentially undermines the current understanding of malaria immunity in pregnant women, and we therefore set out to shed additional light on the prevalence and specificity of VAR2CSA specific IgG in Colombian individuals. We found that plasma IgG against two full-length recombinant PfEMP1 proteins, produced in baculovirus-transfected insect cells (one VAR2CSA-type and one not), was frequent among Colombian men, children, and pregnant women with previous malaria exposure. In contrast, IgG reactivity with a homologous full-length VAR2CSA-type protein, expressed in CHO cells, was low and infrequent among the Colombian plasma samples, as were reactivity to both the corresponding native PfEMP1 proteins. Likewise, specific antibodies to P. vivax Duffy Binding Protein (PvDBP) from human and rabbit did not react with VAR2CSA-type recombinant or native proteins, although a mouse monoclonal antibody was weakly reactive with recombinant proteins expressed in the baculovirus system. Our data indicate that the previously reported Colombian IgG reactivity to recombinant VAR2CSA is not malaria-specific, and that acquisition of VAR2CSA-specific IgG is restricted to pregnancy.

Prevalence of Onchocerca in Danish wild deer

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Hunters and game processing units in Denmark have the last few years recorded an increasing prevalence of subcutaneous nodules on red deer (*Cervus elaphus*) carcasses at meat inspection. The nodules host the filarial worm Onchocerca, a genus comprising of >30 species with a worldwide distribution mainly associated with wild and domestic ungulates. In Europe, four species of Onchocerca are represented in red deer; *O. flexuosa, O. jakutensis, O. skrjabini and O. garmsi*. Adult worms are located within the subcutaneous connective tissue, while the microfilariae are present in the skin. Both adults and microfilariae have species specific locations on their host. E.g. in red deer, nodules of *O. flexuosa* are situated on the dorsal areas of back and flank, while the microfilariae are located in the skin on the ventral abdomen. The geographical distribution of Onchocerca in the Danish deer population has not previously been studied. In the present study Onchocerca microfilaria in skin samples from the abdomen was analysed from 121 red deer (*Cervus elaphus*), 51 roe deer (*Capreolus capreolus*) and 119 fallow deer (*Dama dama*) sampled from 18 locations during October-January 2017/2018. Solely, red deer were found positive for Onchocerca microfilaria with a prevalence of 21.5%. Prevalence were associated with age where mature animals have a higher infection rate (38.3%) compared to yearlings (10.8%). Onchocerca were observed from 54.5% (6/11) of the sampled red deer locations indicating that Denmark has favourable conditions for the vectors (simuliids and ceratopogonids) and the abundance of the deer provide optimal environment for the maintenance of the parasite. To our knowledge, this is the first systematic study of Onchocerca in Denmark.

Unsafe water - a source of Taeniid and other helminth infections for pigs and people in Mbeya region, Tanzania

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Elimination of *Taenia solium* taeniosis/cysticercosis in low-income countries has, so far, been unsuccessful. This could be linked to the incomplete explanation of the transmission of *T. solium*. This study used a case-control design and mixed methods to evaluate water sources and water management practices at household level. Six villages (three case and three control) were selected based on the prevalence of porcine cysticercosis as established by local veterinary surveillance programs. From each village, four pig producing households and three water sources were examined between September and December 2017. Taeniid eggs were found in all water sources in case villages and 4/9 water sources in control villages. Eggs from other helminths (primarily *Schistosoma* spp., *Hymenolepsis* spp., and *Ascaris* spp.) were also found in both case and control villages. Water from these sources was used for drinking by humans and pigs primarily during the dry season (April - November) with rain replacing ground water sources as the primary source for pigs in 22/24 households during the rainy season. The lack of hand-washing facilities, continuous dipping of ladles and hands, prolonged storage of HH water in wide mouth containers and on the floor, as well as the failure to regularly treat water in the HH all allow for the contamination of water stored in the HH and subsequently provided to animals and humans for consumption. At water sources, the use of buckets to retrieve water, relative placement of latrines, poor maintenance, poor drainage, access to animals and the potential of surface run-off water to enter water sources were all present and contributed to an elevated risk of contamination of the water source. Together with the extended survival of up to one year of Taenia eggs in the environment, the findings demonstrate the potential importance of water as an environmental component in the transmission of *T. solium* and further illustrate the importance of providing access to safe drinking water to rural communities in Tanzania.

Immune-modulating effects of parasites and dietary compounds

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Helminths are known to strongly modulate host immune and inflammatory responses in order to establish chronic infections. These immune-modulating effects can also be considerably influenced by the host diet and microbiome. In my bachelor's thesis in biology, I am exploring the cellular mechanisms of how antigens from the parasite *Trichuris suis*, together with certain dietary carbohydrates synergistically modulate inflammatory immune responses. The laboratory work has contained functional experiments with stimulation of macrophages with compounds of *T. suis*, lipopolysaccharides (LPS), and dietary components. In these experiments, I am looking into to the secretion of different cytokines using ELISA. And the results have shown an increased production of IL-19 in the presence of *T. suis* products together with LPS. The next step in my project is to analyse media from indirect co-cultures of epithelial cells and macrophages. Using flow cytometry to analyse the effect of products produced by stimulated epithelial cells on macrophages.

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