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Friday 1<sup>th</sup> of April, 2015, 8:30-16:10  
Frederiksberg Campus, Main Lecture Hall 1-01 (Festauditoriet),  
Bülowsvej 17, 1870 Frederiksberg C

## ORAL PRESENTATIONS – KEY NOTES

WAR OF THE WORMS: New methods for investigating anthelmintic modes of action and resistance

Richard Martin

Department of Biomedical Sciences, Iowa State University, United States of America

Anthelmintic drugs play an important role in the war against nematode parasites of animals and humans. The continuous use of a limited number of classes of anthelmintic drugs has given rise to the development of resistance in domestic animals and there are concerns about the development of resistance in humans. New classes of weapons are required which include 'resistance-busting' anthelmintic drugs and new methods for investigating anthelmintic modes of action and resistance. In this paper I will describe: 1) new microfluidic techniques for testing anthelmintic drugs that can quantitate drug effects in more detail; 2) *Xenopus* oocyte expression studies on nematode parasite anthelmintic receptors and; 3) Electrophysiological techniques for testing anthelmintic combinations using *Ascaris suum*. We are optimistic about the future for the development of new techniques and anthelmintics but caution that the worms may also develop new responses.

### Different evolutionary paths to neurotransmitter recognition in flatworms and roundworms

Timothy P. Lynagh

Department of Drug Design and Pharmacology, University of Copenhagen, Denmark.

Receptor recognition of the neurotransmitter glutamate underlies functions from thought in the human brain down to locomotion in parasitic roundworms. The importance of the latter is exemplified by the frontline anthelmintic ivermectin, which kills roundworms by over-activating glutamate receptors called glutamate-gated chloride channels (GluCl<sub>s</sub>). It was recently revealed that GluCl<sub>s</sub> also occur in flatworms, leading us to question the differences in the GluCl<sub>s</sub> of different phyla. Experiments showed that chemically, glutamate recognition is remarkably similar in both phyla. However, phylogenetic analysis indicated that the amino acids required for the chemical recognition of glutamate evolved from different parts of the receptors in the different phyla. This shows that flatworm and roundworm receptors have converged on a common means of glutamate recognition. We suggest that despite their differences, both hold potential as targets for new anthelmintics.

### Using an evolutionary refined malaria antigen to target cancer

Ali Salanti

Centre for Medical Parasitology, Department of Immunology and Microbiology, University of Copenhagen, Denmark

*Plasmodium falciparum* malaria and cancer are two of the most deadly diseases affecting humanity. The pathogenic complications and underlying mechanisms in these diseases are radically different; though sharing one important feature. In placental malaria (PM) chondroitin sulphate A (CS) is the receptor for parasite adhesion to the placenta, while in cancer CS, as a molecule itself, has a function in almost all the defined hallmarks of cancer development. The link between these two very different diseases is interesting due to the high specificity of the PM parasites for the placenta, despite CS being present in almost every tissue of the human body. This suggests that placental CS is distinct and that the PM parasites through evolution have been selected to adhere to this type of CS only. Given the similarities between placental and cancer cells in terms of cellular growth, migration, and invasion, and CS's well documented involvement in this, we hypothesized that these cells share a specific type of oncofetal CS that enables these functions. The specific structure, which by current methods cannot be distinguished from CS in other tissues, can be targeted with a recombinant malaria protein (VAR2CSA), mimicking parasite adhesion to CS in the placenta. This talk will describe the role of oncofetal-CS in cancer and malaria and discuss the possibilities in utilizing this as a target for the development of anti-cancer treatments.

## CONTRIBUTING ORAL PRESENTATIONS

### Modulation of the immune response to a helminth parasite through bioactive dietary supplementation

Andrew R. Williams (1), Lukasz Krych (2), Dennis S. Nielsen (2), Stig M. Thamsborg (1)

(1) Department of Veterinary Disease Biology and (2) Department of Food Science, University of Copenhagen, Denmark.

Polyphenol-rich dietary components (e.g. grapes, berries) have been proposed as health-promoting dietary components due to their immune-modulating and anti-inflammatory effects. Here, we investigated the interactions between a polyphenol-rich diet and intestinal parasite (*Ascaris suum*) infection on host immunity and gut microbiota composition. Pigs were fed either a basal diet (n=12) or a diet supplemented with polyphenol-rich grape pomace (GP; n=12). Half of the pigs in each group were then inoculated with infective *A. suum* eggs and killed at 14 days post-infection. *A. suum* infection resulted in an increase in parasite-specific plasma antibodies, tissue eosinophils along the small intestine and intra-epithelial T-cells in the jejunum. This response was modulated by GP supplementation; significantly more eosinophils and mast cells, but less basal lamina T-cells, were observed in the jejunum in pigs fed the GP-containing diet, indicating a synergistic enhancement of Th2-type immunity induced by *A. suum*. In vitro experiments with primary human cell cultures demonstrated that purified polyphenols and helminth products synergistically reduced Th1-type cytokine secretion in dendritic cells and induced a Th2-type phenotype in naïve T-cells, suggesting a cellular basis for the observed in vivo effects. Furthermore, *A. suum* infection significantly impacted on the composition of the commensal gut microbiota in the proximal colon, however GP supplementation restored the gut microbiome to that resembling uninfected control animals. Our results thus demonstrate that parasite-induced changes in mucosal immune responses and microbiota composition are significantly modulated by a polyphenol-rich diet, and suggest a profound interaction between dietary components and gastrointestinal pathogens on host immunity and gut function.

### Induction of potent humoral immune responses with virus encoded VAR2CSA virus-like particles

Anne-Marie Andersson (1), Morten A Nielsen (1), Mafalda Resende (1), Ali Salanti (1), Peter J. Holst (1).

(1) University of Copenhagen

The ID1-ID2a region of VAR2CSA has been identified as a promising vaccine target against placental malaria. *Plasmodium falciparum* malaria parasites cause pregnancy associated malaria by its particular binding to chondroitin sulphate A (CSA). Infected erythrocytes present the *P. falciparum* membrane protein 1 (PfEMP1) protein VAR2CSA on their surface that upon binding to CSA expressed on vascular endothelium leads to parasite sequestration. We previously analysed the potential of adenovirus encoded HIV and SIV derived virus like particles (VLPs). Here we designed adenovirus encoded ID1-ID2a VLPs by co-encoding the ID1-ID2a region together with SIVmac239 gag. Two ID1-ID2a VLP anchored constructs were produced by fusing ID1-ID2a to an N-terminal signal peptide and either the transmembrane (TM) and cytoplasmic tail (CT) domains of the mouse mammary tumour virus (MMTV) or the hemagglutinin of influenza A virus at the C-terminus. For a non-VLP incorporation control a third design was made where SIVmac239 gag was expressed together with ID1-ID2a lacking a TMCT region. In the primary immunogenicity study in Balb/c mice the ID1-ID2a fused to HA TMCT was statistically superior in total humoral immune response but also in their functionality when tested in their potential to inhibit parasite binding to CSA. A sequential study was performed to include a comparison to the soluble VAR2CSA protein vaccine which has entered a phase I clinical trial. The results from immunized Balb/c mice once again revealed superiority of fusing ID1-ID2a to HA TMCT, with VLP encoded ID1-ID2a HA TMCT boosted with VAR2CSA protein outperforming VLP encoded ID1-ID2a MMTV TMCT and also a prime boost regimen with VAR2CSA protein. Consequently, modification of VLP anchors are an important point of optimization in virus-encoded retroviral VLP-based vaccines, and adenovirus VLPs boosted by recombinant proteins offer hope of increasing the levels of protective VAR2CSA specific antibodies.

### Hair cortisol and dehydroepiandrosterone (DHEA) concentrations in naturally *Taenia solium* infected pigs in Tanzania

Chiara Trevisan (1), Marta Montillo (2), Alberto Prandi (2), Ernatus M Mkupasi (3), Helena A Ngowi (3) and Maria V Johansen (1)

(1) University of Copenhagen; (2) University of Udine, (3) Sokoine University of Agriculture

Hair analysis has increasingly been used as a non-invasive method to obtain information on stress responses during medium and long-term periods and to assess adaptation to potentially stressful environmental changes. The aim of this study was to assess the activity of the hypothalamic–pituitary–adrenal axis (HPA-axis) by measuring hair cortisol and dehydroepiandrosterone (DHEA) concentrations in naturally *Taenia solium* infected and non-infected control pigs and assess the effect of an environmental change on the aforementioned parameters. Hair samples were collected at 3 time points (prior to, at and 30 days after arrival at the research facility) from 13 *T. solium* infected and 15 control pigs. Hair cortisol and DHEA were extracted using methanol and analysed by radio immune assay. Mean hair cortisol concentrations were significantly lower in *T. solium* infected (mean  $4.7 \pm$  SD  $3.0$  pg/mg) compared to control pigs ( $9.0 \pm 3.7$  pg/mg) prior to arrival at the research facility, however no significant difference was observed between the two groups at arrival and after 30 days. Similar patterns were also observed for DHEA concentrations, where concentrations were significantly different in *T. solium* infected pigs ( $253.9 \pm 82.3$  pg/mg) compared to control pigs ( $387.7 \pm 116.4$  pg/mg) only prior to arrival at the research facility. Cortisol and DHEA concentrations however did drop significantly ( $p < 0.001$ ) in all the pigs over time. Results of this study have shown that an environmental change has an effect on the hormonal levels of the pigs suggesting an undergoing important adaptation process. After the pigs were kept under the same conditions, fed and watered ad libitum, no significant differences were observed between the groups, but a drop of both hormones was observed in all the pigs. Finally, we have shown that *T. solium* can alternate hormones in pigs but more studies are warranted in order to investigate this further.

### Induction of high-titrated malaria transmission blocking antibodies using novel virus like particle display technology.

Susan Thrane (1), Christoph M. Janitzek (1), Sungwa Matondo (2), Mafalda Resende (1), Tobias Gustavsson (1), Wian de Jongh (3), Stine Clemmensen (1) (3), Will Roeffen (4), Marga van de Vegte-Bolmer (4), Geert Jan van Gemert (4), Robert Sauerwein (4), John T. Schiller (5), Morten A. Nielsen (1), Thor G. Theander (1), Ali Salanti (1), Adam F. Sander (1)

(1) Centre for Medical Parasitology at the Department of Immunology and Microbiology, University of Copenhagen, and Department of Infectious Diseases, Copenhagen University Hospital, Denmark; (2) Kilimanjaro Clinical Research Institute, KCMC, Moshi, Tanzania; (3) ExpreS2ion Biotechnologies, SCION-DTU Science Park, Hørsholm, Denmark; (4) Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands; (5) Laboratory of Cellular Oncology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA.

CONFIDENTIAL

### Levels and kinetics of merozoite-specific IgG in Ghanaian children with *Plasmodium falciparum* malaria

Frederica D. Partey(1,2), Gordon Awandare (1), Simon Draper(3), Michael Ofori(1), Lars Hviid(2) and Lea Barfod(3)

(1) Department of Biochemistry, Cell and Molecular biology, University of Ghana, Legon, Ghana, (2) Centre for Medical Parasitology, University of Copenhagen, (3) Jenner Institute, University of Oxford

Successful invasion of erythrocytes by *P. falciparum* merozoites requires the parasite antigen reticulocyte binding-like homologous protein 5 (PfRh5) which is specific for the host erythrocyte receptor basigin. PfRh5 forms a complex with another parasite antigen, Cysteine-rich protective antigen (CyRPA). It is still unclear to what extent PfRh5 antibodies are acquired naturally following natural parasite exposure and contribute to clinical protection from malaria. Some studies have suggested PfRh5 is a poor target of naturally acquired immunity whereas others have reported that PfRh5 is both immunogenic and associated with protection from clinical disease among children. We have used two strategies to answer the question better 1) Analysis of plasma levels of RH5-specific antibodies in longitudinal samples from naturally infected children and 2) Generation of human monoclonal antibodies from immune Ghanaian adults. Merozoite antigen-specific IgG levels were measured by ELISA as described by Ampomah et al, 2014. An appreciable percentage of the patients had detectable levels of PfRh5 which persisted 6 weeks after infection but the levels were relatively low compared to the control antigens. We are currently generating human monoclonal anti- PfRh5 and anti- PfCyRPA IgG from seropositive adults to investigate their ability to inhibit merozoite invasion of erythrocytes. The mode of action of the antibodies will be elucidated through the binding/disruption of basigin- RH5. The findings from this study are useful in the consideration of PfRh5 as a vaccine candidate.

Experimental *Echinococcus multilocularis* inoculation in lab-bred wild rodent species: A model system for assessment of ecologically relevant intermediate hosts.

Ian David Woolsey (1), Per Moestrup Jensen (1), Peter Deplazes (2), and Christian Moliin Outzen Kapel (1).

(1) Department of Plant and Environmental Sciences, University of Copenhagen; (2) Institute of Parasitology, University of Zurich.

In Europe, *Echinococcus multilocularis* transmission predominantly occurs between the red fox (*Vulpes vulpes*) and various species of vole intermediate hosts. The geographical distributions of these intermediate host species is thought to play a key role for the transmission of the parasite, but there is a dearth of information pertaining to comparative infection dynamics including establishment, growth and fertility of the metacestode. This can only be obtained through experimental infections. We infected 5 species of rodent, *Microtus arvalis*, *Microtus agrestis*, *Myodes glareolus*, *Mesocricetus auratus* and outbred *Mus musculus* with 100 viable oncospheres of *E. multilocularis* and performed post mortem examination 6, 8 and 10 weeks post inoculation (wpi). Conventional C57BL/6j mice were used as positive controls as they have been shown to exhibit macroscopic liver lesions 4 wpi. Significant differences were observed in establishment, growth and fertility in the rodent species. Although the initial lesion numbers were comparable in *M. arvalis* and the closely related *M. agrestis*, the lesion growth was significantly greater in *M. arvalis* potentially underlining the importance of this species to parasite transmission. Clear differences in establishment were observed between inbred and outbred mice with the former demonstrating increased establishment. Lesions were only detected in <5% bringing into question their role as transmitters of the parasite. Likewise, no lesions were found in *M. auratus*. The present study clearly demonstrates that comparative experimental inoculation in ecologically relevant rodent species may provide information on establishment and growth of *E. multilocularis* that may be key for epidemiological modelling.

### Molecular Paleoparasitology – towards whole-genome sequencing of ancient parasites

Martin Jensen Søe (1,2), Peter Nejsum (3), Frederik Seersholm (2), Mikkel W Petersen (2), Brian Lund Fredensborg (1), Eske Willerslev (2), Kurt Kjær (2), Christian Moliin Outzen Kapel (1)

(1) Department of Plant and Environmental Sciences, University of Copenhagen, (2) Centre for Geogenetics, Natural History Museum, University of Copenhagen, (3) Department of Veterinary Disease Biology, University of Copenhagen

Paleoparasitology is the study of parasitic infections in pre-historic animal and human populations. Traditionally researchers have relied on their ability to identify morphological characteristics of recovered parasite eggs. One major drawback of morphological characterization is its general inability to distinguish eggs from different species in a genus and thereby infer the exact species and in many cases the host of a given egg. To overcome this limitation, we have implemented a broadly applicable next generation shotgun sequencing and analysis method that allows us to identify any organism in a sample providing that it's mitochondrial or nuclear reference genome is available. Applying this method on DNA extracts of filtered archeological contexts containing parasite eggs have allowed us to identify: a broad range of human and animal helminths to species level, DNA of their potential hosts as well as a range of bacteria and plants in the same samples. During the course of the presentation, two case studies will be presented. The first describes how analysis of midden deposits in Greenland have provided novel insight into the resource economy of pre-historic Greenlandic cultures dating back to the earliest settlers (4500 BC). This is achieved by combining the identification of animal DNA with identification of specific parasite life-cycles. The second describes how latrine samples from across Europe and the Middle East up to 2000 years old, have provided novel insights into pre-historic human parasitic worm infections in present day non-endemic areas. In doing so, it was possible to reconstruct complete mitochondrial and in some cases even nuclear genomes of ancient human specific parasites. This gives promises of addressing phylogeographic questions such as how parasites were dispersed across the globe possibly mediated by human migration or in connection to dispersal of domesticated animals.

### Assessment of the dominant PfEMP1 gene families expression in *Plasmodium falciparum* parasites isolated from hospitalized Tanzanian children with severe malaria

Sixbert Mkumbaye (1), Christian W. Wang (2), Jakob S. Jespersen (2), Jens E.V. Petersen (2), Reginald Kavishe (1), John Lusingu (2,3), Thor G.Theander (2), Thomas Lavstsen (2)

(1) Kilimanjaro Christian Medical University College and Kilimanjaro Clinical Research Institute, Moshi, Tanzania; (2) Centre for Medical Parasitology, Department of International Health, Immunology & Microbiology, University of Copenhagen and Department of Infectious Diseases, Rigshospitalet, Copenhagen, Denmark; (3) National Institute of Medical Research, Tanga Centre, Tanga, Tanzania

**Introduction:** Severe malaria syndromes are precipitated by *Plasmodium falciparum* parasites binding to endothelial receptors on the vascular lining. This binding is mediated by highly variant *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family. Previously, it was identified that a subset of PfEMP1 are associated with severe malaria and found that the receptor for these PfEMP1 variants is Endothelial Protein C Receptor (EPCR). The binding is mediated through the amino-terminal of cysteine-rich interdomain region (CIDR $\alpha$ 1) more specifically CIDRa1.1 and CIDRa1.4 to 1.8. In this study we wanted to measure the transcript of genes encoding EPCR-binding CIDRa1 in hospitalized children with severe and uncomplicated malaria. **Method:** In this study parasitized blood samples were collected from hospitalized Tanzanian children presented with severe malaria syndromes. PfEMP1 var genes expression was tested by quantitative PCR using primers targeting CIDRa1 subtypes, DBL $\alpha$ ,  $\beta$ ,  $\epsilon$  and  $\zeta$ . **Results:** From designed primers to amplify all known CIDR $\alpha$ 1-subtypes and DBL subtypes: CIDR $\alpha$ 1 subtypes 1.1 and 1.4 up to 1.8 show high transcript levels among children with severe anemia, hyperlactate, and cerebral malaria: Though there were very high CIDR $\alpha$ 1 transcript levels in some isolated parasites from children with uncomplicated malaria, indicating that at some point they developed/were developing to severe malaria. **Conclusion:** High transcript level of CIDRa1 subtypes is associated with severe malaria among the hospitalized children.

### The absorptive function of the bacillary band of *Trichuris muris*

Tina V. A. Hansen (1), Michael Hansen (1), Peter Nejsum (1), Helena Mejer (1), Stig M. Thamsborg (1)

(1) Department of Veterinary Disease Biology, University of Copenhagen

**Background:** A common characteristic of *Trichuris* spp. infections in humans and animals is a low to varied efficacy of single-dose benzimidazoles, used in mass drug administration programmes against human trichuriasis. The bacillary band, a specialised morphological structure of *Trichuris* spp., as well as the unique partly intra- and extracellular habitat of adult *Trichuris* spp., may be involved in restricted drug absorption, resulting in a low drug accumulation. However, the function of the bacillary band is still unknown. **Methodology:** We studied whether adult worms of *T. muris* were dependent on glucose and/or amino acids for survival in vitro and if the bacillary band has an absorptive function. The viability of the worms was evaluated microscopically using a motility scale from 0 - 3, and by measuring emission in response to metabolic activity utilizing the colorimetric assay Alamar Blue. The absorptive function of the bacillary band was explored using a fluorescent glucose analogue (6-NBDG) detected in the living worms by confocal microscopy. To exclude oral ingestion of 6-NBDG, the oral cavity of *T. muris* worms was sealed. **Principal Findings:** Adult worms of *T. muris* incubated in media devoid of glucose were immotile after 17 hours incubation and generated an emission signal similar to controls (RPMI media without worms). The fluorescent glucose analogue was detected in the pores of the bacillary band and accumulated inside the worms, primarily within the stichocytes in living worms with and without a sealed oral cavity. **Conclusions/Significance:** The whipworm *T. muris* is dependent on glucose for survival in vitro, and the bacillary band has an absorptive function in relation to the glucose analogue 6-NBDG which accumulates within the stichocytes of the worms. This new insight into the absorptive function of the bacillary band may encourage for further explorations of the bacillary band in relation to anthelmintics.

### Integrated control of *Taenia solium*: effect on taeniosis and porcine cysticercosis in rural communities of Tanzania

Uffe Christian Braae (1), Pascal Magnussen (1), Wendy Harrison (2), Benedict Ndawi (3), Faustin Lekule (4), Maria Vang Johansen (1)

(1) University of Copenhagen; (2) Imperial College London; (3) Bora Professional Consultancy Services; (4) Sokoine University of Agriculture

This study aimed to assess over a four year period the effect of school-based mass drug administration of praziquantel on the prevalence of taeniosis and porcine cysticercosis. *Taenia solium*, the cause of neurocysticercosis, is found throughout sub-Saharan Africa and co-endemic with schistosomiasis in many regions. Praziquantel, effective against a range of trematodes and cestodes, creates the potential for integrated control. Five cross-sectional surveys, four including humans, were carried out in two endemic districts, Mbozi and Mbeya, in Tanzania from 2012 to 2015. Stool samples were collected from humans and prevalence of taeniosis estimated by copro-Ag-ELISA. Blood samples from pigs were collected to estimate cysticercosis prevalence by Ag-ELISA. School-based mass drug administration of praziquantel was delivered thrice in Mbozi and twice in Mbeya. More than 12000 human stool samples and 4500 porcine serum samples were collected. Children ( $\leq 15$ ) from Mbozi had significant decreased risk of being infected throughout the study compared to children from Mbeya that only showed a significant decrease after the first treatment. Adults in Mbozi had significant decreased risk of infection during the last survey ( $p=0.031$ , OR 0.40, CI: 0.17-0.89), where also the prevalence of porcine cysticercosis had dropped significantly ( $p=0.002$ , OR 0.49, CI: 0.32-0.76). The study showed that a prolonged single approach intervention of repeated mass drug administration targeting a proportion of the definitive hosts not only had an effect on the target population, but spilled over into the intermediate host population and the remaining definitive host population.

## POSTER PRESENTATIONS

Zoonotic hookworm infections. Molecular identification of the species harboured by dogs in Morogoro, Tanzania.

Ana Merino Tejedor (1), Ernatus M. Mkupasi (2), Helena A. Ngowi (2), Maria Vang Johansen (1), Peter Nejsum (1), Annette Olsen (1)

(1)University of Copenhagen, Department of Veterinary Disease Biology, Denmark; (2) Department of Veterinary Medicine and Public health, Sokoine University of Agriculture, Tanzania.

The most common zoonotic canine hookworms are *Ancylostoma caninum*, *A. braziliense* and *A. ceylanicum*, which besides their veterinary importance, are recognized agents for eosinophilic enteritis and the characteristic dermatological lesion known as Larva Migrans Cutanea (LMC) in humans. Human infections with canine hookworm species have been widely reported but considered to be rare or isolated cases of little epidemiological importance. Nevertheless, recent studies in Asia have demonstrated, by use of molecular techniques, that *A. ceylanicum* was present in 6 to 51% of the people infected with hookworms, pinpointing dogs and cats as important reservoirs for zoonotic ancylostomiasis. However, as the knowledge of the distribution of canine hookworm species in SSA is very limited, this study aimed to identify the hookworm species among dogs in an endemic area to evaluate the risk of zoonotic infection and their possible role in the epidemiology of human hookworm infection. The field work took place in Morogoro, Tanzania, at Sokoine University of Agriculture, where 159 dogs from 4 different non-randomized settings were sampled and screened for gastrointestinal helminths. Three different helminths were found, hookworm (57.9%), *Toxocara* spp (5.7%) and *Dipylidium caninum* (1.3%). Infective L3 from hookworm positive individuals were recovered and brought to the University of Copenhagen for species identification by molecular methods.

### Effects of environmental factors on behavioral modification of ants, *Formica polyctena* infected by the trematode *Dicrocoelium dendriticum*

Camilla F. Botnevik (1), Joanna Malagocka (1,2), Annette B. Jensen (1,2), and Brian L. Fredensborg (1)

(1) Department of Plant and Environmental Sciences, University of Copenhagen (2) Centre for Social Evolution, University of Copenhagen

Parasite manipulation of host behavior with the aim of increasing transmission of infective stages to a next host is a widespread phenomenon described from most parasite phyla. The lancet fluke, *Dicrocoelium dendriticum* is a poster child for parasite manipulation of host behavior, but we know oddly little about factors inducing and maintaining behavioral changes in its intermediate hosts. This study examined the importance of 3 environmental factors on the biting behavior of red wood ants, *Formica polyctena* infected with *D. dendriticum*. The state of tetany, a cramping of the mandibular muscles making the ant cling to vegetation, was observed in naturally infected *F. polyctena* under controlled temperature, light, and humidity conditions. We found that low temperature significantly stimulated and maintained a state of tetany in infected ants, while light, humidity, ant size, and infection intensity did not influence the probability of ants entering tetany. Temperature likely serves as a simple but reliable indicator of the encounter rate between infected ants and ruminant definitive hosts. In addition, temperature-sensitive manipulation of biting behavior may protect infected ants from exposure to temperatures in the upper thermal range of the host.

### On the hunt for parasites in shotgun metagenomic datasets

Casper S Poulsen (1), Sofie H Nielsen (1), Oksana Lukjancenko (1) and Sünje J Pamp (1)

(1) National Food Institute, Technical University of Denmark, Lyngby, Denmark

The number of metagenomic-based studies is increasing rapidly. Most studies are performed on Illumina platforms providing a large amount of short-read sequences that are often used to characterize microbial communities in terms of bacterial composition and function. Metagenomic studies that assess the presence of parasites are limited, in part because relatively few reference genomes are available. In the present study we created a database that includes all available genomes from flatworms, roundworms, and protists (NCBI, January 2016). This database was incorporated into MGmapper, an automated pipeline for mapping and stratification of metagenomics sequence data. Benchmarking of the setup was performed to assess the specificity and sensitivity of the pipeline. The benchmarking datasets consisted of simulated metagenomic datasets based on the genome sequences from 12 parasites. The simulated datasets were constructed as paired end reads with length of 150 bp, insert size of 200 bp, and three different base error rates (0.01, 0.1 & 0.2). Sensitivity testing included spiking pig feces and sewage samples with three concentrations of a mock community containing 6 different bacterial species representing 5 different bacterial phyla, a fungi (*Saccharomyces cerevisiae*), and an apicomplexan (*Cryptosporidium parvum*). The spiking with *C. parvum* was performed by adding oocysts into samples at three different cell numbers (10<sup>6</sup>, 10<sup>5</sup> and 10<sup>4</sup> oocysts/gram). The samples were sequenced on a HiSeq 3000 and a minimum of 28,000,000 reads/sample were obtained. By extending metagenomic pipelines to enable detection of eukaryotic organisms, a more holistic picture of the microbial ecology is obtained, revealing novel insights into interactions between organisms of all domains of life. The setup can also be applied to specific fields including surveillance and diagnostics where all types of pathogens can be detected in a single sample simultaneously.

### Plasma levels of Angiopoietin-2 predict malaria outcome in Ghanaian children

Gertrude Ecklu-Mensah (1,2), Michael F. Ofori (1), Filip Castberg (2), Lars Hviid (2), Anja TR Jensen (2)

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

*Plasmodium falciparum* malaria remains the number one cause of death in children from Sub-Saharan Africa. It is widely accepted that the combined effects of endothelial adhesion of infected erythrocytes and the host inflammatory response provoke the endothelium to become more permeable. This involves release of activation markers, including angiopoietin-2 (Ang-2), which may intensify the pathological processes of malaria. In this study, the relationship between plasma concentrations of Ang-2 and disease severity in paediatric malaria was investigated in Ghana. Ang-2 levels were quantified from *P. falciparum*-infected patients with uncomplicated malaria (UM) and severe malaria (SM) at days 0 and 14 using a commercial ELISA kit (R&D Systems). Overall, high Ang-2 levels were observed in both SM and UM samples at day 0 compared with febrile controls, with levels decreasing after treatment. However, Ang-2 levels were significantly higher in SM than UM patients at day 0. The results demonstrate the clinical utility of Ang-2 to discriminate between SM and UM. Other endothelial markers including soluble ICAM-1 and EPCR are in the process of being measured to provide a more robust assessment of risk of malaria associated mortality.

### Occurrence and control of *Trichuris vulpis* and *Uncinaria stenocephala* in dogs - a cohort study from a Danish breeding kennel

Ida J Kolthoff (1), Pernille I B Møller (1), Rebecca Langhorn (2) and Helena Mejer (1)

(1) Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen; (2) Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen

Gastrointestinal parasites are frequently diagnosed and treated in small animal veterinary practices. However, transmission dynamics are seldom considered in relation to pets such as dogs, treatments are often random and there is a need for further knowledge about the transmission dynamics for each parasite to support parasite control programmes. In this cohort study, the gastrointestinal parasite status (i.e. faecal egg counts) of 39 dogs in a Danish breeding kennel was monitored for 11 months (February-December 2015) during which the dogs were treated once (March 2015) with fenbendazole (3-5 consecutive days) and praziquantel. The soil from four areas of the kennel was examined for *Trichuris vulpis* and *Toxocara canis* eggs using a sieving and flotation technique. All outdoor areas were contaminated with infective *T. vulpis* (4.4 eggs/g dry soil) and *T. canis* eggs (0.4 eggs/g dry soil). Prior to treatment, 94.9% of the dogs were infected with one or more parasites: *Uncinaria stenocephala* (79.5%), *T. vulpis* (66.7%), *T. canis* (20.5%) and *Taenia* spp. (5.1%). Treatment was successful but starting at the end of the respective prepatency periods re-infections gradually appeared in an increasing number of dogs. After three and nine months, respectively, *U. stenocephala* and *T. vulpis* prevalence and faecal egg counts were similar to those prior to the treatment. Prevalence and egg counts of *T. canis* and *Taenia* spp. were low compared to those of *U. stenocephala* and *T. vulpis*. In conclusion, a comprehensive control programme involving four yearly treatments will be necessary to control particularly *T. vulpis* and *U. stenocephala* infections in the dogs. Until egg counts are reduced to an acceptable level, the kennel owner should also clear outdoor areas for faeces daily, mow the grass frequently, crop trees and bushes, dry out concrete areas with a weed burner and repair concrete runs to provide unfavourable conditions for the free-living parasite eggs and larvae.

### Comparing and evaluating schistosomiasis associated morbidity indicators in schoolchildren participating in SCORE cohort studies in regions near Lake Victoria of Kenya and Tanzania

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Schistosomiasis is a public health problem in Sub-Saharan Africa as it results in numerous complications. A cohort of pupils aged 7-8 years was investigated for *Schistosoma mansoni* infection and related morbidity in 2011 in regions bordering Lake Victoria in Kenya and Tanzania. Despite comparable *S. mansoni* prevalence and intensities in the two countries, anthropometric and other morbidity markers showed significantly poorer indicators among Kenyan compared to Tanzanian pupils. In order to assess the causes of this difference, information of socio-economic statuses (SES) in the two countries were investigated through questionnaires, and ten microsatellite loci and mitochondrial partial cox1 markers of *S. mansoni* obtained from infected pupils were determined to clarify possible differences in parasite strains. The overall SES data shows a great emphasis on hygiene/sanitary practices in Tanzania, as all had sanitary facilities in their compounds and usage of bore hole/well water was popular (48.7%). In the Kenyan cohort, many did not have sanitary facilities (28.6%) and 91.9% used open water sources, which was significantly higher than in the Tanzanian cohort (57.2%,  $p < 0.0005$ ). There was also a significant difference in the availability of quality food at all meals, especially with regard to fish consumption with 38.0% and 70.6% of the Kenyan and Tanzanian pupils consuming fish for lunch ( $p < 0.0005$ ). The overall diversity of *S. mansoni* found in the infected pupils from the microsatellite loci and mt partial cox1 highlights the fact that *S. mansoni* is not a uniform population, possibly exhibiting several phenotypic differences. There were no clear differences between the two countries; however, the Kenyan cohort had slightly more private alleles and heterozygosity for the microsatellite markers but further work with an expanded sample size is required to clarify whether these genetic findings are related with the morbidity of schistosomiasis.

### An ongoing longitudinal study of the infection dynamics of *Fasciola hepatica* in organic and conventional dairy cattle.

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The liver fluke, *Fasciola hepatica*, is a parasite of cattle which causes substantial economic losses due to liver condemnation, retarded growth and reduced milk yield. The cattle is infected by eating encysted metacercariae that hatch upon entering the gastrointestinal tract and the larva burrow into the liver where they mature and produce eggs that are passed with the feces approximately 6 weeks after ingestion. In order to create better management strategies, it is important to understand the infection dynamics of *F. hepatica* at the farm-level. For this purpose, a 2-year longitudinal study was initiated surveying 2 conventional and 2 organic dairy farms. Samples were so far taken from 44 cattle of 4 different age groups (calves, heifers, 1. time lactating cows and 2. time lactating cows) in April, July and November 2015. Serum and bulk tank milk (BMT) ELISA tests and a sedimentation technique (ST) were conducted to detect any changes in the number of infections. For the entire surveyed period, relatively high prevalence were shown in organic farms with 62% and 49% for 1. and 2. year lactating cows respectively, compared to the same age groups in conventional farms (11% and 26%). Heifers had low prevalences in both types of farms with 7% and 8% prevalence for organic and conventional farms respectively. Only one farm had incidences of infected calves and only in november with a prevalence of 64%. The ELISA tests showed an increase in prevalence in november indicating that *F. hepatica* infections occur more heavily towards the end of the season. However the 4 farms generally had very different results which can be the result of different grazing management by each farm. Thus it is important to examine how each farm manages its cows and pastures so it can be determined how and when the animals become infected.

### Identification of extracellular vesicles in three parasitic nematodes of veterinary importance

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Parasitism has been denoted the most successful way of life and their success relates to their ability to evade or modulate the host immune response. However, the underlying mechanisms behind are poorly understood. Parasites from all major parasitic groups have been shown to release extracellular vesicles (EVs) containing protein and RNA species and seem to be of importance in the host-parasite interplay. The aim of this study was to examine the secretion of EVs for three most important parasitic nematodes of pigs, *Ascaris suum*, *Trichuris suis* and *Oesophagostomum dentatum* for later to functionally test their role in host-parasite interactions. Adult worms of *A. suum*, *T. suis* and *O. dentatum* were incubated in RPMI 1640 containing Streptomycin, Penicillin, Amphotericin B and Gentamycin (Gibco®) for 24-48 hours. EVs were purified from parasite-depleted RPMI by an initial filtering step (200 nm) followed by differential centrifugations with two ultracentrifugations at 110.000 x g (Beckman Optima™ L-70). The final pellet was analyzed with a CM 100 BioTWIN transmission electron microscope (TEM) and RNA was purified using miRCURY™ RNA Isolation Kits (Exiqon A/S). The RNA concentration was measured using a Nanodrop 2000 spectrophotometer. The TEM analysis revealed that all three species produce EVs in the size of ~100 nm and the Nanodrop measurements show that the purification products contain RNA. These results suggest that adult worms of the three porcine parasite species, *A. suum*, *T. suis* and *O. dentatum* secrete RNA-containing EVs. Future studies should focus on an in-depth characterization of the EVs and their contents through RNA sequencing and proteomic analysis. With this, we may unravel the complex interplay between parasites and their hosts and potentially discover novel targets for diagnostics and disease control.

### Risk factors associated with the transmission of *Enterobius vermicularis* in Danish kindergarten children

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Infection with *Enterobius vermicularis* (pinworm) is known to cause discomfort and sleep disturbance in children due to pruritus ani and the parasite has also been associated with pain in the genital and urinary tract of female children. Pruritus ani causes the child to itch the perineal region where the female pinworm deposits eggs. The child can autoinfect himself and/or contaminate the environment with unclean hands. Eggs are light enough to flow on air currents which helps distribute the eggs in the environment where they are viable for up to three weeks under optimal conditions. The parasite benefits from the close contact in institutions where personal hygiene is sub-optimal and pinworm infections are often prevalent in kindergartens. Studies in Asia, South America and Norway have shown that a number of risk factors are associated with the transmission and infection with pinworms, including the knowledge of parents and kindergarten staff about pinworms, the family size, the child's age and personal hygiene. The aim of this study is to identify risk factors associated with the transmission of *Enterobius vermicularis* in Danish kindergarten children. The study consists of a literature review using PubMed database to investigate previously identified risk factors, a questionnaire survey involving kindergarten staff and parents of kindergarten children, an observational study to observe hygiene and placement of hands, and collection of samples of the environment and from children in a kindergarten prone to pinworm infections to determine presence of eggs in the environment and prevalence of asymptomatic infections. Preliminary results from the surveys show that 23.6% of 51 parents from 3 kindergartens answered, that their child has been infected once or several times with pinworms. Risk factors associated with pinworm infection identified in the preliminary results are number of siblings, the age of the child and not washing hands with water and soap before meals.

### Changing patterns of malaria in a time of migration: do we only see the tip of the iceberg?

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Denmark is experiencing an increase in the number of cases of imported malaria. The aim of this study was to investigate changes in identified malaria cases in the Region of Southern Denmark (RSD) with regard to both total number and subspecies. Odense University Hospital is a regional referral hospital for treatment of malaria. Patients suspected of suffering from malaria were diagnosed by microscopy of Giemsa-stained thick and thin films from capillary blood and by rapid antigen detection test (RDT) using EDTA-blood. Remaining blood was stored at -80 °C. Highly sensitive real-time PCR assays were used for screening for *Plasmodium* sp. and for specific detection of *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae* in the stored blood. We selected eleven patients with microscopy-confirmed *Plasmodium* infection, three patients suspected of infection undetected by microscopy and fifty-five patients with microscopy- and RDT negative samples admitted to the hospital from July 2015 to February 2016. Sixty-eight cases of malaria were identified in the period from 2010 to 2015. The annual number of malaria cases had increased from 6 in 2013 to 15 in 2014 and to 17 in 2016. The increase was caused by a large number of *P. vivax* infections. Species-specific PCR confirmed the results from microscopy. DNA from the parasites was detectable in blood up to eighteen days after microscopy showed negative in response to treatment. One mixed *P. falciparum*/*P. vivax* infection was detected by PCR. Furthermore, two infections by *P. falciparum* and *P. vivax* were detected from microscopy-negative samples. A dramatic increase in the number of cases of malaria was recorded in RSD since 2013, mainly due to *P. vivax*. PCR analyses revealed infections by *Plasmodium* species not detectable by microscopy. The observed changes thus indicate an underestimation of the occurrence of malaria in RSD and possibly in the country as a whole.

### Fluidic Chips for the selection of *Plasmodium falciparum* field isolates.

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Current methods for selecting *Plasmodium falciparum* genotypes of interest range from antibodies coupled to magnetic beads, panning on recombinant proteins, or panning on endothelial cells. Antibody selection requires the generation of antibodies targeting specific domains of interest, whilst receptor and endothelial panning require multiple round of selection to generate a population with the desired phenotype. These methods routinely take weeks, if not months before sufficient parasite numbers are generated. Here we present data on the use of fluidic chips for the selection of field isolates. The fluidic chip mimics the shear stress found in the microvasculature and can be coated with a variety of cell, recombinant proteins or antibodies to target IE from flow. Preliminary data from recent field isolates (Tanzania), using the recombinant receptors ICAM-1, EPCR and CD36, has shown parasites can be captured from flow and be ready for assessment within 4 -10 days. In addition, this method can also produce sufficient sample for transcription analysis 24 hours after selection. We propose this method may provide a means to analyze the adhesion and transcription profiles of both field and lab isolates more rapidly than existing methods.

### *In vitro* anthelmintic activity of plant extracts against different life stages of drug-resistant cattle nematodes

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Gastrointestinal nematodes are economically important parasites with a potential major impact on the health of cattle worldwide. In recent years their successful control has been threatened by development of drug resistance. Bioactive plants with natural anthelmintic activity are an alternative control option, which has been widely studied in sheep while scientific evidence for their effect in cattle remains scarce. *In vitro* anthelmintic activity is known to vary according to the plant sources, *in vitro* assays and parasites. Here, we evaluated the susceptibility of three cattle nematode isolates to six crude plant extracts containing condensed tannins (CT) with different chemical structures, and trans-cinnamaldehyde in one case. Two *Cooperia oncophora* (ivermectin-resistant and susceptible) and one drug susceptible isolate of *Ostertagia ostertagi* were subjected to egg hatch assay (EHA), larval feeding inhibition assay (LFIA) and adult motility assay (AMA). Subsequently, we calculated EC<sub>50</sub> values and 95% intervals for the three isolates in the LFIA and for the two isolates of *C. oncophora* in the AMA after 48 h. EC<sub>50</sub> values based on LFIA and AMA were not significantly different for the two *C. oncophora* isolates with any of the plant extracts, and no obvious difference was seen in the EHA. In contrast, the *O. ostertagi* isolate was significantly more susceptible than the drug-susceptible isolate of *C. oncophora* with four extracts in LFIA. The plant extracts showed distinctly different potencies and modes of action depending on the life stages. The trans-cinnamaldehyde containing extract was the most potent demonstrating a strong inhibiting effect on egg embryonation in EHA and showing lowest EC<sub>50</sub>s in AMA (172 µg/mL). Regarding the other extracts, CT with larger size greatly inhibited the larval eclosion in EHA and larval feeding in LFIA. The percentage of prodelphinidin subunits in CT-containing extracts likely explained the greater effect on the worm motility in AMA.

### The effect of mebendazole and flubendazole solely and in combination with praziquantel on *Schistosoma mansoni*: An in vitro study

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The study aimed to investigate the survival of different stages of *Schistosoma mansoni* when subjected to different doses of mebendazole (MBZ), praziquantel (PZQ) and flubendazole (FLBZ). Since PZQs introduction for treatment of schistosomiasis in the 1980s it remains the main drug and control tool against schistosomiasis. However, recent reports of reduced cure rates have resulted in the requirement of repeated treatments. This, and PZQs inability to prevent reinfection, its reduced effect against juvenile stages, no vaccine and the difficulty in targeting the intermediate host, suggests the need for alternative measures to prevent and control schistosomiasis. A potential measure is treatment plans that combine PZQ with other drugs. In this study, *Biomphalaria glabrata* infected with *S. mansoni* were subjected to illumination to induce shedding of cercariae followed by mechanical transformation to induce separation of the cercariae tail to obtain schistosomula. Juvenile stages were grown from schistosomula in supplemented growth media and provided human erythrocytes approximately 48 hours after being transformed. Schistosomula were hereafter exposed to varying concentrations of MBZ, PZQ and FLBZ in an in vitro assay based on microscopic evaluation on movement and structure. Initial results show that 98% of day 1 old schistosomula were damaged when exposed to MBZ at of concentration of 100  $\mu\text{M}$  for 2 hours. After 24 hours of exposure to 100  $\mu\text{M}$  MBZ all schistosomula had died. FLBZ at concentrations up to 1000  $\mu\text{M}$  had no effect on day 1 old schistosomula. Exposure to 100  $\mu\text{M}$  PZQ for 24 hours on day 1 old schistosomula showed damage to 54% of the worms. In vitro MBZ was more efficacious compared to PZQ, and both drugs were superior to FLBZ against day 1 old schistosomula. However, no conclusion on the best treatment regime can be made as further experiments on eggs, juvenile and adult stages, and infected mice subjected to different doses and drug combinations are required.

### Cultivation of chlamydospore from *Duddingtonia flagrans* - influence of pH and temperature

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The nematode- trapping fungus *Duddingtonia flagrans* is a promising candidate for biological control of helminths in grazing animals. In order to obtain large quantities of chlamydospores for treatment fungal cultivations needs to be optimized. We here examined the growth characteristics of *D. flagrans* in different culture media (corn kernels, small wheat, big wheat) under different temperature and pH. A specific time points the chlamydospores were isolated from the culture media, counted and were then inoculated on corn meal agar (CMA) at either pH 5.5, 6.0, 6.5, 7.0 under different incubation temperatures (15°C, 20°C, 25°C, 30°C, 35°C). The length of the hyphae was measured daily, until the CMA media surface was covered by hyphae. The medium matrix of barley was easiest to inoculate and yielded the highest number of chlamydospores. The optimum pH and temperature were pH6.5~7.0 and 35°C, respectively. The hyphae were transparently and radially in CMA media. It also produced chlamydospores at the late of the culturing.

### The effect of abiotic and biotic factors on movement patterns of *Schistosoma mansoni* and *Fasciola hepatica* – as a background for developing a tele-diagnostic tool

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*Schistosoma mansoni* and *Fasciola hepatica* causes severe problems in both humans and animals despite many control programs. Although successfully implemented for both diseases, infections numbers for schistosomiasis and human fascioliasis have increased. For both species the sensitivity of the diagnostic methods, i.e. PCR and Kato-Katz, will decrease significantly after interventions, be costly and require specialized technical staff particularly in developing countries. This problem with detecting and diagnosing is a great barrier when implementing control programs. Furthermore, abiotic and biotic factors may impact both transmission and survival of larval stages (miracidia) of both parasites as well as change the behavioural patterns of miracidia. A new way of detecting movement and identify the specific species of miracidia is tele-diagnostics. In this study, it will be tested whether tele-diagnostics can be used for determining the movement and/or behavioural patterns of miracidia from *S. mansoni* and *F. hepatica*, and to verify whether it is possible to distinguish and diagnose the species early in their lifecycle. Furthermore, it will investigate if there are detectable differences in these possible patterns, when the miracidia are affected by abiotic and biotic factors. This could lead to a more efficient diagnostic tool to differentiate between parasites and to more effective control programs by understanding ways to interfere with transmission and elimination earlier in their lifecycle. Miracidia was obtained by hatching the *S. mansoni* eggs from intestinal tissue and the *F. hepatica* eggs from faeces. The miracidia were placed in single compartments retaining water in lids of 96 well culture plates to create a suitable space for miracidial movement. Factors such as type of water, light and temperature that affect the miracidia are recorded at different time points to capture changes. The recordings are then analysed by Ethovision XT software.

### *Ascaris suum* products suppress lipopolysaccharide-induced cytokine secretion in human dendritic cells and macrophages

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Helminth parasites are known to strongly modulate host immune responses and inflammation to ensure their own survival within their hosts. This immune-modulation can lead to chronic infections causing major health problems for humans in the developing world and posing serious constraint on livestock production. Paradoxically, deliberate infection with helminths has been shown to alleviate symptoms of autoimmune disorders in animal models and human patients. Identification of the mechanisms responsible for this immune-modulation may be a major step towards design of effective vaccines for helminth infection, and for identification of helminth-derived molecules which may be developed as novel anti-inflammatory agents. *Ascaris suum* is a swine helminth which is closely related to the human *A. lumbricoides*. Here, we investigated if *A. suum* products have immunomodulatory effects on human monocyte-derived dendritic cells (DCs) and macrophages in vitro. We measured the secretion of the pro-inflammatory Th1-polarising cytokines interleukin (IL)-6, IL-12 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) induced by lipopolysaccharide (LPS) in DCs with or without exposure to *A. suum* adult body fluid (ABF) or excretory/secretory products (ESP) from larvae or adult worms. We also assessed whether *A. suum* ABF modulated TNF- $\alpha$ , IL-6 and the anti-inflammatory cytokine IL-10 secretion by LPS-primed human macrophages. We demonstrated that ABF, and to a lesser extent ESP, suppressed TNF- $\alpha$ , IL-6 and IL-12 secretion by LPS-primed human DCs. Moreover, ABF dose-dependently suppressed LPS-induced TNF- $\alpha$ , and IL-6 secretion in macrophages. Interestingly, IL-10 secretion in macrophages was also strongly suppressed by ABF. Our data suggest that *Ascaris* strongly modulates human dendritic cell and macrophage activity by suppressing both pro- and anti-inflammatory cytokine production. This indicates a profound influence of the parasite on the development of innate host immune responses.

### Dose-response study on the establishment success of *Hymenolepis diminuta* in intermediate host *Tenebrio molitor*

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The tapeworm *Hymenolepis diminuta* – flour beetle *Tenebrio molitor* model has been used extensively for ecological and evolutionary host-parasite studies. Transmission success of *H. diminuta* from the rodent definitive host to *T. molitor* depends upon the ingestion of viable eggs, penetration of the gut wall by the onchosphere and establishment of cysticercoids in the hemocoel of *T. molitor*. Unfortunately, in previous studies it has not been possible to standardize the infection load of cysticercoids in beetles, resulting in a large variation in infection load among beetles in experiments. The aim of this study was to implement a standardized infection protocol, and to investigate the establishment success of cysticercoids in relation to the exposure dose of *H. diminuta* eggs over a wide range of doses. Suspensions of five different concentrations (mean±SE; 27±1, 52.5±5.5, 100±3, 229.5±4.5, and 409±16) of *H. diminuta* eggs isolated from infected rat feces were fed individual beetles 7-10 days after eclosion. Establishment success was measured after two weeks of infection by counting the number of cysticercoids which developed inside the beetle host, which were 0.99±0.69, 1.9±0.9, 9.54±6.09, 26.33±0.67 and 58.79±2.66, respectively. Our results show that the number of established cysticercoids increased linearly with an exposure dose exceeding 100 eggs, and that the variation in the infection load was limited. The exposure dose of eggs therefore serves as a good proxy of establishment success of cysticercoids in the beetle host. The establishment success was substantially lower than expected at exposure doses lower than 100 eggs. Ongoing studies are investigating the potential underlying mechanisms for the lower transmission success at lower exposure doses.

### Profiling circulating miRNAs in serum from pigs infected with the porcine whipworm, *Trichuris suis*

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MicroRNAs (miRNAs) are recently discovered as key regulators of gene translation and are becoming increasingly recognized for their involvement in various diseases. This study investigates the miRNA profile in pig serum during the course of an infection with the gastrointestinal parasite, *Trichuris suis*. Of this panel, the expression of selected miRNAs in serum from *T. suis* infected and uninfected pigs were determined by quantitative real time PCR using Exiqon Human Panel assays at 0, 2, 4, 6, 8 and 10 weeks post first infection (wpi). One miRNA, ssc-let-7d-3p, was significantly up-regulated in infected pigs 8 wpi. The let-7 family miRNAs have been shown to post-transcriptionally regulate the translation of the helminth-controlling cytokine, IL-13, in a murine model for asthma and interestingly, the most highly transcribed miRNA in *T. suis*, tsu-let-7a, is a homolog of let-7d. The tsu-let-7a and the ssc-let-7d-3p are highly complementary and we hypothesize possible interactions between these host- and parasite-derived miRNAs and their immune modulating roles.

### *Giardia duodenalis* modulates human dendritic cell activity through cytokine secretion and apoptosis

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*Giardia duodenalis* is a protozoan parasite with a worldwide distribution that can infect range of hosts, including humans. *G. duodenalis* commonly causes chronic infections, persisting over extended periods of times and possibly causing repeated bouts of symptoms in untreated patients. Symptoms range from asymptomatic to severe including diarrhoea, dehydration, abdominal pain and, if infection persists, it is associated with malnutrition and retarding mental and physical development in children. The ability to cause chronic infection suggests that *G. duodenalis* employs modulatory mechanisms to evade the human immune system. In this study we have investigated the modulatory effects of *G. duodenalis* on human dendritic cell (DC) function in vitro. DCs were activated with toll-like-receptor (TLR) 4, 1+2 and 2+6 agonists either in the presence or absence of *G. duodenalis* trophozoite lysate. Secretion of the pro-inflammatory cytokines interleukin (IL)-6, IL-12 and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) along with the anti-inflammatory cytokine IL-10 were quantified by ELISA. Cell surface expression of major-histocompatibility complex class II (MHC-II) and CD86 were also assessed using flow cytometry. Preliminary results indicate that *G. duodenalis* modulates the human inflammatory response by down-regulating secretion of IL-6, IL-12, TNF-  $\alpha$  and up-regulating secretion of IL-10, most noticeably following TLR4 stimulation. Initial flow cytometry data suggests that *G. duodenalis* enhances CD86 expression of DCs, even without TLR activation, but also induces significant apoptosis in the presence of TLR stimulation. Our preliminary data indicates that *G. duodenalis* modulates human DC activity by inducing apoptosis and modifying cytokine secretion to dampen local inflammation. These mechanisms may protect the parasite against elimination from the human host.