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Host-Parasite Communication

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Friday 31st of March, 2017, 9:00-16:10
Faculty of Science, Lecture room 1-01 (Festauditoriet), Bülowsvej 17,

1870 Frederiksberg C

ORAL PRESENTATIONS – KEY NOTES

Extracellular vesicles from parasitic helminths and biomedical applications

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A brief introduction of milestones on the EVs studies, EVs main characteristics and their role in cellular communications, will be followed by the description of their finding in parasites (protozoans and helminths), and their possible roles in parasite-host communications. Our group has been pioneering in describing the presence of EVs in parasitic helminths. We have been working on the characterization of trematode EVs, identifying their components, mainly proteins and small RNAs, as well as investigating their possible applications in controlling parasitic. In this sense, we have further studied the potential of trematode EVs as new diagnostic tools (biomarkers), as well as their usefulness in vaccination assays. Preliminary results indicate that parasite EVs can be detected in host samples like urine and plasma form infected animals, revealing high specificity and sensitivity. Immunization of mice with trematode EVs has shown that, although they do not prevent from being experimentally infected, they alter the immune response in the host, balancing it to Th2 responses, and resulting in amelioration of clinical symptoms and increasing the animal survival.

Along with parasitic diseases, we have also addressed the usefulness of trematode EVs in preventing autoimmune diseases like inflammatory bowel disease (IBD). Several studies have supported the "hygiene hypothesis", where the presence of helminths is inversely correlated with the incidence of autoimmune diseases. A very recent report has shown that infections with gastrointestinal helminths can protect against IBD in murine models, as well as in humans, by mean of altering the balance of commensal and pathogenic bacteria in the intestine. In this context, we and others previously suggested the immunomodulatory effects of extracellular vesicles (EVs) from helminth parasites.

To evaluate the protective and therapeutic effects of EVs from *Fasciola hepatica* adults on the inflammatory response in IBD, we have used a dextran sodium sulphate (DSS) induced murine model of acute ulcerative colitis. In this model we have detected that *F. hepatica* EVs modulate the immune response when injected before inducing the disease. Pretreatment with EVs from *F. hepatica* provoked an amelioration of clinical symptoms in immunized mice, as evidenced by macroscopic and histological observations. EVs reduced the disease activity index (DAI), and protected from colon shortening induced by DSS. EV treatment also changed the profile of inflammatory mediators in the mice intestines, including cytokines (i.e. TNFα, IL-6 and IL-17), and signaling molecules (i.e. serine kinases and transcription factors). Finally, preliminary studies regarding the identification of immune cells and *in vivo* routes involved in this response will be discussed.

Malaria Parasite Networking

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Cells use extracellular vesicles to communicate, coordinate social activities and, in the case of pathogens, export various effectors to target host cells. The lethal malaria parasite *Plasmodium falciparum* (Pf) was recently shown to transfer episomal plasmids via vesicles produced at the ring stage of their asexual life cycle. However, a comprehensive characterization of the cargo delivered by these vesicles and its function is still lacking.

Here, we identify these nanovesicles as exosomes and determine their molecular composition: nucleic acids, proteins and lipids. We established advanced nano-resolution techniques for analysing exosome content, with which we discovered that malaria parasite-derived exosomes deliver not only a large group of non-coding RNAs but also parasite nuclear, apicoplast and mitochondrial genes, in a time dependent manner. We reveal that these vesicles are taken up by human monocytes and that the exosomal DNA is then directly recognised by the STING DNA-sensing pathway. This results in the translocation of host transcription factor IRF3 into the nucleus, where it activates a modified type I interferon response, which, in turn, may promote infection. This work also identifies previously unknown molecular players in the Pf signalling pathway and provides new insight into how malaria parasites manipulate their host environment.

CONTRIBUTED ORAL PRESENTATIONS

Extracellular vesicles of parasitic nematodes in pigs: An examination of contents and uptake in host cells

Eline P Hansen (1), Kasper L Andersen (1), Antonio Marcilla (2), Aaron Jex (3), Robin B Gasser (3), Merete Fredholm (1), Stig M Thamsborg and Peter Nejsum (4)

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Endoparasites from all major parasitic groups have been shown to release extracellular vesicles (EVs) containing protein and RNA which appear to be important in the host-parasite interplay. The aim of this study was to examine EVs from three gastrointestinal nematodes of pigs, *Ascaris suum*, *Trichuris suis* and *Oesophagostomum dentatum*. These parasites have different patterns of migration and location in the host, as well as the immunological response they evoke. They could therefore represent suitable candidates to explore unique as well as common modes of host immune modulation. Adult worms were incubated in RPMI under sterile conditions for 72 hours where after EVs were purified from the RPMI by differential centrifugations, with two ultracentrifugations at 110,000 x g, and identified by transmission electron microscopy. RNA was purified and sequenced and reads were aligned to the genomes of *A. suum*, *T. suis* and *O. dentatum*, respectively. miRNAs unique and common for the three helminth species were identified using the miRDeep2 algorithm. Predicting miRNA targets and potential functions is part of an on-going analysis, but preliminary results indicate immune-related properties of highly expressed miRNAs. In order to visualize the uptake of EVs and the subsequent transfer of EV RNA into host cells, EVs were treated with RNase, labelled with EV membrane stain as well as RNA stain, transferred to intestinal epithelial cells (Caco-2) and examined by confocal microscopy at 37°C. We found that EVs of all three species were taken up by intestinal epithelial cells followed by a release of RNA, which had a tendency to accumulate in the cell nuclei. Next step will be to explore how parasite EVs influence the cytokine production of various types of immune cells in vitro. The findings of this study contribute to unraveling the complex interplay between parasites and their hosts, which may provide novel targets for diagnostic tests and parasite control in the future.

Zebrafish as a model organism to study host/parasite interactions

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The zebrafish has become an important model to study a wide spectrum of vertebrate biological processes. It is used within all major biological sciences and has been especially valuable within developmental biology and genetics. Why the zebrafish is such a good model? First of all, it is a vertebrate, secondly it has external embryological development and is fully transparent for the first 24 h post hatch. Thirdly development is fast; the vertebrate organs can be identified within 48 hours (ears, eyes, brain, internal organs). On top of that 70 % of annotated human genes have true orthologues in the zebrafish genome with significant homology also at the protein level. The embryos are simple to genetically manipulate, the genome has been mapped, thousands of transgenic lines exist and a pair of breeders are able to produce up to 300 eggs per week making it a high-throughput but low-cost model organism. Unique tools for non-invasive in vivo imaging has been developed and high quality intravital microscopy can be conducted without comparison. The parasite *Ichthyophthirius multifiliis* is the causative agent of white spot disease and a major problem for the aquaculture and ornamental fish industry. It infects skin and gills of fish and cause high mortality during outbreaks. I have used the zebrafish as a model to study immunological responses including the behaviour of neutrophils during an acute infection with the parasite. Using adult fish of a transgenic line with GFP-tagged neutrophils, I got an unprecedented view into the interactions between the parasites and the neutrophils. During the first 24 h of the infection the number of neutrophils in the tail fin increased 4 fold but during the following 48 h the neutrophil number decreased even though the size of the parasites increased. Video-recordings of the interface between the parasites and the neutrophils showed parasites ingesting neutrophils as a possible way of evading and fighting the immune system of the host.

A novel SURFIN4.2 protein complex at the merozoite apex and surface implicated in *Plasmodium falciparum* invasion

Maria del Pilar Quintana (1), Sherwin Chan (1), Jun-Hong Ch'ng (1,2), Arash Zandian (3), Maryam Imam (1), Kjell Hultenby (4), Peter Nilsson (3), Ulrika Qundos (3), Kirsten Moll (1) and Mats Wahlgren (1)

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Plasmodium falciparum invasion into the red blood cells (RBCs) is a complex process, engaging proteins on the merozoite surface or contained and then sequentially released from the apical organelles of the merozoite. SURFIN4.2 was initially identified at the surface of the parasitized RBCs (pRBCs) but also apically associated with the merozoite. Using antibodies against the N-terminus of the protein, we show here that the protein is present at the surface of the merozoite but more strikingly is also present in the neck of the rhoptries. The protein is shed into culture supernatant upon schizont rupture and is associated with GLURP (Glutamate Rich Protein) and RON-4 (Rhoptry Neck Protein 4) to form a complex we have named SURGE (SURFIN4.2-RON-4-GLURP complEx). Importantly, SURFIN4.2 is detected at the apex of the merozoite during initial attachment and active invasion into the RBCs. The exact functional role of SURGE remains to be determined, but the presence of RON-4, a protein confined to the moving junction (MJ), strongly suggests a role in strengthening the stable contact between the merozoite apex and the RBC, possibly as and additional RBC adhesion molecule. Data indicating that the CRD (Cystein Rich Domain) of the protein, both as recombinant protein or expressed on the surface of CHO cells binds RBCs and the fact that antibodies against SURFIN4.2 partially inhibited invasion reaffirm the proposed role of SURFIN4.2 during the invasion process.

Cross-reactive antibodies targeting a PfEMP1 motif associated with cerebral malaria inhibit ICAM-1 binding

Rebecca W. Olsen (1), Gertrude Ecklu-Mensah (1,2), Anja Bengtsson (1), Lars Hviid (1) Yvonne Adams (1) and Anja T.R. Jensen (1)

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Cerebral malaria, a potential deadly outcome of *Plasmodium falciparum* infections, is precipitated by infected erythrocytes adhering to brain endothelial cells. Increased risk of developing cerebral malaria is associated with infected erythrocytes (IE) expressing PfEMP1 with a conserved DBLβ motif; such PfEMP1 show dual receptor specificity and synergistically bind both ICAM-1 and EPCR. We used ELISA to measure IgG reactivity and cross-reactivity to DBLβ domains of ICAM-1 binding and non-binding PfEMP1 in plasma from Ghanaian children with severe or uncomplicated malaria and in plasma from rats immunized with different DBLβ domains. The ICAM-1 adhesion-inhibitory capacity of motif-specific antibodies was assessed by ELISA using recombinant DBLβ and under physiological flow using IE. Children with severe malaria demonstrated significantly lower levels of DBLβ motif-specific IgG compared to children diagnosed with uncomplicated malaria. Antibodies, either human or rat, targeting the ICAM-1 binding motif prevented homologous and heterologous recombinant DBLβ domains and IE from binding to ICAM-1 under static- and physiological flow conditions. Higher levels of motif-specific IgG in children with uncomplicated compared to severe malaria suggest a protective role in severe disease. This encourages future efforts to raise broadly reactive antibody responses against these PfEMP1.

The MHC class II associated invariant chain, engineered for proteolytic cleavage increases MHC class I and II restricted antigen presentation and antibody responses towards PfEMP1 domains

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Severe malaria has been shown to be associated with the binding of PfEMP1, presented by infected erythrocytes, to the host endothelial protein C receptor (EPCR), after *P. falciparum* infection. PfEMP1 have a very high level of diversity as they are encoded by over 60 different "var" genes. Children can acquire immunity from severe disease after repeated infections; nevertheless the induced antibodies are not sufficient for protection against the next parasite infection. A vaccine providing protective immunity towards the EPCR binding var genes could prevent the morbidity and mortality that occurs until immunity is acquired. We engineered a new adenovirus based vaccine targeting two "var" gene domains (IT4var19 and PFCLINvar30 from the CIDR1.1 family) containing the MHCII chaperone invariant chain as an adjuvant that can enhance the T cell response to tethered antigen. We then modified this adjuvant by insertion of a furin protease recognition site to obtain a secreted version of the adjuvant-antigen complex leading to an antigen that theoretically should be partly targeted for MHC class II presentation and partly secreted in an oligomerized form. Our data show that the T cell effect from the invariant chain is largely retained while the modified adjuvant strongly enhance and broadens the specific antibody response against our targeted antigen to include responses to different var genes although largely within the same CIDR family. We also show that the combination of an endosomal targeting sequence, secretion and a trimerization domain in the invariant chain C-terminal domain is needed for the full adjuvant effect on antibody responses.

Identification of single-nucleotide polymorphisms in mitochondrial DNA and the Kelch 13 gene of *Plasmodium falciparum* in different geographical populations

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The emergence of resistance to artemisinin-based antimalarial drugs in *Plasmodium falciparum* in Southeast Asia poses a threat against malaria control and elimination. It is therefore crucial to understand the interconnectedness of parasite populations and be able to monitor resistance spread. Recently, a barcoding system using geographically restricted single nucleotide polymorphisms (SNPs) in mitochondria and apicoplast genes of *P. falciparum* was developed. Combined with SNP detection in the artemisinin resistance marker, K13, use of this system could offer important insights into parasite population structure and diversity and possibly detail the route of drug resistance spread. This study aims to explore the diversity and validity of geographically restricted SNPs in mitochondrial DNA as well as in the K13 gene of *P. falciparum* samples obtained from malaria patients in Southeast India (Jharkhand state) and Northern Senegal (Districts of Richard Toll and Podor). The extracted DNA was amplified using PCR and subsequently Sanger sequenced. SNPs were identified by comparing to reference genomes. Of 44 sequenced Indian samples, the following mitochondrial SNPs were found: T2175C (n=25), G1367A (n=6), A827G (n=3) and G1511A (n=3). According to available data, A827G has not been detected before, while T2175C and G1367A have been detected in Central Asia. Together, these findings suggest a possible SNP barcode for Central Asia. Of 48 sequenced Senegalese samples, the following mitochondrial SNPs were found: G1692A (n=17), G1108T (n=1) A848G (n=1), A939T (n=1) and G1076A (n=1). Analysis of K13 sequencing data is ongoing and will be presented. The mitochondrial SNPs identified in this study can likely be incorporated into the *P. falciparum* mitochondrial barcoding system, thereby expanding its usability. Potential SNP findings in the K13 gene of the Indian and Senegalese samples would be valuable to the worldwide molecular surveillance activities of artemisinin resistance.

Risk factors affecting haemoglobin levels and physical fitness in schoolchildren living in *Schistosoma mansoni* and malaria endemic areas bordering Lake Victoria in Kenya and Tanzania

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Infection with schistosomes can result in numerous complications, including malnutrition, impaired physical fitness and anaemia. Since 2011, cohorts of schoolchildren have been investigated for *Schistosoma mansoni* infection, malaria and related morbidity in regions bordering Lake Victoria in Kenya and Tanzania. Despite being neighbouring countries with similar lifestyles and environments, there were considerable differences in both *S. mansoni* prevalence and intensity and several morbidity parameters between the two countries. In order to determine the basis for the differences, the parasitological and morbidity data, which was collected in 2013-14, was compared with information on socioeconomic statuses (SES) collected for the present study (2015) using questionnaires. A total of 490 schoolchildren (163 Kenyans and 327 Tanzanians) aged 9-11 years provided data. The SES data showed that a higher proportion of Tanzanian pupils knew where to wash hands after toilet visits compared to Kenyan pupils (69.4% vs. 48.5%, $P<0.0005$). Similar proportions of children in the two countries took breakfast, lunch and dinner, but the content of the meals differed. Thus, at all three meals, a higher proportion of Tanzanian pupils consumed meat (mostly fish) compared to their Kenyan peers (35.0% vs. 0%, $P<0.0005$; 69.0% vs. 43.6%, $P<0.0005$ and 67.2% vs. 53.4%, $P=0.003$, respectively). In contrast to this, more Kenyan than Tanzanian pupils consumed vegetables at dinner (44.2% vs. 30.4%, $P=0.003$). Multivariable analyses investigating risk factors for important morbidity markers for schistosome infections at child level, revealed that eating fish and knowing where to wash hands after toilet visits were significant predictors for both haemoglobin levels and physical fitness (measured as VO2 max). These results suggest that the community should put more emphasis on providing hand washing facilities at school, on education on good personal hygiene and in a healthy and nutritional diet.

Molecular identification of hookworm species in dog faeces from Morogoro, Tanzania

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Four species of ancylostomatids are considered as canine hookworms, *Ancylostoma caninum*, *A. braziliense*, *A. ceylanicum* and *Uncinaria stenocephala*. Despite their veterinary relevance, they have zoonotic potential and are able to cause different clinical manifestations and patent infections in humans. Little is known about the presence and distribution of different canine hookworm species in Africa. Consequently, the main objective of this study was to identify, by molecular techniques, the hookworm species present in canine faecal samples from Morogoro, Tanzania. Faecal samples from 160 local dogs were collected and hookworm positive samples processed to recover larvae for further molecular characterisation. We extracted DNA from 66 samples that subsequently was analysed by two different molecular approaches, PCR-RFLP and species-specific PCR coupled with sanger sequencing. Using PCR-RFLP we only detected the presence of the ubiquitous *A. caninum*. However, by species-specific PCR, we identified ten samples with *A. braziliense*, two with *U. stenocephala* and five with *A. ceylanicum*. Thus, in the present study, we have identified all four canine hookworm species in Morogoro, Tanzania, and it is to our knowledge, the first report documenting the presence of *U. stenocephala* and *A. ceylanicum* in Africa using molecular techniques. *A. ceylanicum* causes patent infections in human populations and its presence among Tanzanian dogs may have public health importance.

Disease behaviours of sows naturally infected with *Taenia solium* in Tanzania

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"Neurocysticercosis (NCC) is a disease caused by the zoonotic parasite *Taenia solium* lodging in the central nervous system. Both humans and pigs can get NCC. The impact of the disease in pigs has so far been little explored. The aim of this study was to describe the effect of NCC on social and feeding behaviours as well as the pattern of activity as indicators of reduced welfare in naturally infected sows. In total 13 *T. solium* naturally infected and 15 non-infected control sows were videotaped for 2 consecutive weeks using close circuit television cameras at research facilities at Sokoine University of Agriculture, Morogoro, Tanzania. Videos were analysed at the beginning, in the middle and at the end of the 2 week recording period. For each time point, videos were analysed during feeding, while the enrichment was provided, and by recording every half an hour the sows' behaviours performed over the course of a whole day. Sows with NCC spent significantly less time at the feeding trough, especially during the second half of the feeding period. Infected sows were also more passive e.g. lying and standing still significantly more during a whole day period and showed social isolation compared to non-infected control sows by performing behaviours more distant to their nearest neighbour. Results of this study indicated that NCC changed the behaviour of infected sows. The behavioural changes are indicative of decreased welfare. Efforts to reinforce the animal welfare aspect are needed as this has so far been neglected.

Farm level risk factors for liver fluke infection in Danish dairy cattle as evaluated by two diagnostic methods

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Infection with liver fluke (*Fasciola hepatica*) in cattle is increasing in Denmark but appropriate guidelines for control is yet lacking. Therefore farm-level risk factors for fasciolosis in Danish dairy farms (>50 animals slaughtered) were investigated by a case-control study. Infection status of farms was determined using two different diagnostic methods: recordings of liver condemnation at slaughter, and herd-level *F. hepatica* antibody levels in bulk tank milk (BTM). A case farm was defined by a minimum of 3 incidents of liver condemnation due to liver fluke at slaughter (in any age group) during 2013, and control farms were located within 10 km of at least one case farm and had no history of liver condemnation due to liver flukes during 2011-2013. Information about grazing and control practices was collected through telephone interviews and BTM was analysed by ELISA in 2014. Two logistic regressions were performed according to different diagnostic methods using a final dataset consisting of 132 case and 63 control farms. A case farm was associated with heifers grazing on wet pastures with access to surface water, dry cows grazing on wet pastures, herd size and concurrent beef cattle production. A BTM ELISA positive farm was associated with heifers grazing on wet pastures with access to surface water, dry cows grazing on wet pastures, and purchase of calves/grazing of calves with animals from other farms. BTM ELISA showed positive in 75% of case and 12.7% of control farms. The reasons behind disagreement between the two diagnostic methods include detection limit of the farm-level prevalence by the BTM ELISA test, time span between slaughter data and BTM, and the relatively low sensitivity of liver inspection at slaughter. In conclusion, bovine fasciolosis is associated with heifers and dry cows grazed on wet areas and therefore should be targeted when considering for control.

Host size-dependent anisakid infection in Baltic cod *Gadus morhua* associated with differential food preferences

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A significant increase in the infection level of Baltic cod *Gadus morhua* with the anisakid nematode larvae *Contracaecum osculatum* and *Pseudoterranova decipiens* has been recorded during recent years due to the expanding local population of grey seals *Halichoerus grypus*, which act as final hosts for these parasites. Here, we report from an investigation of 368 cod that the infection level of juvenile cod (TL6−30 cm) with larvae of *C. osculatum* and *P. decipiens* is absent or very low, whereas it increases drastically in larger cod (TL 31−48 cm). A third nematode *Hysterothylacium aduncum* was rarely found. The study indicates that the prey animals for large cod act as transport hosts for the parasite larvae. Analyses of stomach contents of cod caught in the same area (2007−2014) showed that small benthic organisms are preferred food items by small cod, the isopod *Saduria entomon* is taken by all size classes, and sprat *Sprattus sprattus* are common prey items for cod larger than 30 cm. Parasitological investigations of *H. sarsi* and *S. entomon* did not reveal infection in these invertebrates, but 11.6% of sprat was shown to be infected with 1−8 C. osculatum third stage larvae per fish. Analyses of sprat stomach contents confirmed that copepods and cladocerans are the main food items of sprat. These observations suggest that the *C. osculatum* life cycle in the Baltic Sea includes grey seals as final hosts, sprat as the first transport host and cod as second transport host. It may be speculated that sprat obtain infection by feeding on copepods and/or cladocerans, which could serve as the first intermediate hosts. One cannot exclude the possibility that the size-dependent *C. osculatum* infection of cod may contribute (indirectly or directly) to the differential mortality of larger cod (>38 cm) compared to smaller cod (<30 cm) recently recorded in the Baltic cod population.

POSTER PRESENTATIONS

Field studies on parasite-induced biting behaviour of red wood ants in relation to temperature and time of day

Simone Norstrand Gasque (1) and Brian L. Fredensborg (1)

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The trematode, *Dicrocoelium dendriticum* provides one of the best-known examples of parasite-induced host behaviour manipulation. In the ant intermediate host, one cercaria lodge itself against the sub-esophageal ganglion and cause the infected ant to crawl up in the vegetation and lock its mandibles unto a leaf. Previous laboratory studies showed that ant biting behaviour (tetany) is primarily observed at low temperature, but the diurnal rhythm of the behaviour is poorly understood, and systematic field studies of the phenomenon are lacking. This study was conducted to examine the effects of temperature, relative humidity, and time of day on the number of ants displaying the manipulated phenotype (biting behaviour) under field conditions. Field studies on *Formica polyctena* ants infected with *Dicrocoelium dendriticum* were conducted on nine occasions from August-September 2016, at the Bidstrup forests in Hvalsø, Denmark. On each occasion ants locked to the vegetation were observed around four ant nests from sunrise to sunset. Temperature and humidity loggers were placed as close to the microclimate of the infected ants as possible and infected ants were individually marked. Each nest was observed every second hour to describe the relationship between time of day, abiotic conditions and ant biting behaviour. The results showed that infected ants were most commonly observed in the morning and again late in the day at times when ruminant hosts are most active. However, temperature was the most important determinant for ant biting behaviour. Thus, on cool days infected ants remained attached to the vegetation throughout the day. Our results suggest that temperature-sensitivity of the infected ants serves the dual purpose of exposing infected ants to the next host at an opportune time, and at the same time protects infected ants from exposure to high temperatures which could increase host mortality.

Diseases of Greenland muskox (Ovibos moschatus): Wildlife health and disease transmission to livestock and humans

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"Muskox (*Ovibos moschatus*) and caribou (*Rangifer tarandus groenlandicus*) are the only wild ungulates in Greenland. Overlapping habitats of these species and sharing of grassland with domestic livestock (e.g. sheep (*Ovis aries*)) may lead to unexpected transmission of parasites and other pathogens. Adaptation of caribou parasites to muskoxen has been suggested as indication of such dynamics occurring in Greenland. Recently, zoonotic transmission of muskox/caribou parasites to man has been observed in Greenland. Other parasites, which in other Arctic regions are widespread in sheep, reindeer and muskoxen, may be relevant for Greenland, such as *Toxoplasma gondii*, which can lead to abortion in sheep, muskox and humans.

The present knowledge of the pathogen diversity in muskoxen in Greenland is limited to preliminary studies and a few case studies. Therefore, baseline studies on the prevalence and geographical distribution of pathogens are highly relevant to outline and understand risks of emergence of disease in animal populations and zoonotic transmissions in Greenland.

The present PhD project aims at providing baseline knowledge on pathogen diversity in Greenland muskoxen, and will investigate the differences in occurrence of pathogens in populations living under distinctly different habitats: 1) without other ruminants, 2) overlapping with caribou, and 3) grassland shared with sheep. In addition, it is the aim to study pathogen transmission in various climatic regions of Greenland.

Parasites and plants – exploring the anti-parasitic activity of a bioactive livestock forage

Angela Hørdum Valente (1), Andrew Richard Williams (1), Henrik Toft Simonsen (2) and Stig Milan Thamsborg (1)

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Increasing resistance to the small 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 several GIP such as *Ascaris, Ostertagia* and *Giardia*. Extracts from initially five different cultivars of chicory will be prepared and their SL profiles will be assessed by HPLC-MS. The extracts will then be tested for anti-parasitic effects against the swine nematode *Ascaris suum* using in vitro assays. The distribution of SL's will be correlated with the anti-parasitic effect, and this may indicate which of the individual or combination of SL's are responsible for the effect, guiding further fractionation and compound isolation. Moreover, previous grazing experiments in cattle have shown only an effect in vivo towards abomasal nematodes and not intestinal nematodes, suggesting host physiology or digestive processes may influence activity or availability of compounds. To investigate this issue, the content of the abomasum, small intestine and the large intestine from cattle fed chicory will be analyzed for the content of SL and reveal possible digestive and/or chemical modifications of the SL's. Finally, in vivo experiments in *Heligmosomoides polygyrus*-infected mice will be the first investigation of anti-parasitic activity of chicory in monogastric animals. The knowledge obtained from this work can provide information to breed for cultivars with anti-parasitic effects and thereby increasing the sustainable production of meat and milk from livestock.

Coccidia infections in Danish farmed mink

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Although Danish farmed mink are frequently infected with Coccidia, knowledge of factors affecting the infection is scarce. Thus, we studied the age, geographical and season-related factors affecting coccidia prevalence. Oocysts excretion was examined every 7-14th day from Apr-Oct 2016 from bitches and cups on 30 farms (n=4142 fecal samples, n=335 mink) located in South- and North Jutland or Zealand. Unsporulated oocysts were quantified microscopically and characterized by size and thickness of the wall. In total, 60.9% (n=204) mink were *Eimeria* positive, 56.7% (n=190) were *Isospora* positive and 20.9% (n=70) excreted both parasites at least once. Mink at all 30 farms excreted oocyst at least once. *Eimeria* prevalence was highest on the Zealand farms (25.4±2.2%, P<0.0001) compared to the farms located in Jutland (5.4±2.9%; 7.5±4.1%). Contrary, *Isospora* prevalence was unrelated to geography (12.2±2.9%, 11.8±3.5%, 9.2±7.1%). The *Eimeria* prevalence peaked in June to July (12.6%-24.9%), while most animals were *Isospora* positive in July to August (12.1%-27.6%). For cups (15.7%) and bitches (10.5%) the *Eimeria* prevalence was similar, while the *Isospora* prevalence was age related with more positive cups (19.5%) than adults (4.6%). The *Eimeria* prevalence peaked in cups aged 7-11 weeks old and again when 18-24 weeks old. *Isospora* prevalence peaked in cups aged 13-15 weeks old. Three *Eimeria* types were characterized (not confirmed by PCR); A, B and C, with types B and C (40.9%, 39.8%) being more prevalent than A (19.3%). Bitches were primarily infected with type B (B: 50.4%, A: 20.4%, C: 29.9%), while type C (48.0%) predominated in cups (A: 19.0%, B: 33.0%). Mink from Zealand were mostly infected with type B (56.5±13.7%), while mink from Jutland were primarily infected with type C (55.6±28.6% and 81.9±19.4%). This study showed high coccidia prevalence in farmed mink with age related *Isospora* prevalence and geographical related prevalence and distribution of *Eimeria* type.

Synergistic adhesion of *Plasmodium falciparum* and association with cerebral malaria

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Malaria remains one of the major causes of morbidity and mortality in sub-Saharan Africa, with children under 5 years old most at risk. The causative agent, *Plasmodium falciparum* is capable of infecting erythrocytes (IE). Survival of the malarial parasite, *P. falciparum*, within the human host is facilitated by the phenomenon of sequestration, whereby infected erythrocytes avoid splenic clearance by adhering to receptors expressed on the lining of blood vessels. Infected erythrocytes bind to a variety of receptors such as CD36, ICAM-1, and EPCR via the high molecular weight malaria protein, PfEMP-1. This protein is expressed on the surface of IE and has been demonstrated to mediate the adhesion of to numerous cells and tissues causing endothelial dysfunction and vessel occlusion. Here we present data showing that specific *P. falciparum* subsets expressing a particular subset of PfEMP1 proteins strongly associated with cerebral malaria are capable of binding both ICAM-1 and EPCR simultaneously. This synergism enhances the adhesive interaction in response to increasing shear stress on both recombinant proteins and primary human brain endothelial cells (HBMEC) These data highlight an important adhesive phenotype associated with severe disease and a potential vaccine target for the prevention of cerebral malaria.

Survival and development of chicken ascarid eggs in temperate pastures

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Eggs of chicken ascarids (*Ascaridia galli* and *Heterakis* spp.) are thick-shelled and are believed to be hardy and survive for long periods outdoors when protected by soil. However, this has not been evaluated quantitatively and our study therefore aimed to determine development and recovery of ascarid eggs after burying in pasture soil. Unembryonated chicken ascarid eggs of faecal origin were mixed with soil (approximately 1300 eggs g-1 soil), placed in sealed nylon bags (mesh size: 25 µm) and buried at 7 cm depth in pasture plots April 2014 (spring, n=72) and December 2014 (winter, n=72). Further, eight randomly selected bags per season were used to estimate the pre-burial egg recovery (i.e. 0 weeks post burial (wpb)). Eight random bags were removed at 5, 12, 23, 38, 52, 71 wpb per season and additionally at 104 wpb from the spring burial. The contents of each bag was analysed for numbers and developmental stages of eggs. Results showed that eggs buried in the spring were fully developed (larvated) within 12 wpb. In contrast, eggs buried in the winter were developing between 23-38 wpb so that all viable eggs seemed to be fully developed by 38 wpb. The majority of eggs (~90%) disappeared from the soil within 23 wpb (spring) and 38 wpb (winter). Small proportions (2-3%) of seemingly viable and infective eggs were still recovered up to 2 years after deposition. In conclusion, it is obvious that the current recommended scheme (60-120 days) for spelling (resting) pastures in Denmark does not sufficiently reduce soil contamination of chicken ascarids eggs. We therefore recommend that pastures and yards contaminated in early spring are not to be used for hens before the following spring whereas pastures contaminated in late autumn/winter should not to be used until the following winter/spring.

Naturally acquired antibodies target the glutamate-rich protein on intact merozoites and predict protection against febrile malaria

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Plasmodium species antigens accessible at the time of merozoite release are likely targets of biologically functional antibodies. Immunoglobulin G (IgG) antibodies against intact merozoites were quantified in the plasma of Ghanaian children from a longitudinal cohort using a novel flow cytometry–based immunofluorescence assay. Functionality of these antibodies, as well as glutamate-rich protein (GLURP)–specific affinity-purified IgG from malaria hyperimmune Liberian adults, was assessed by the opsonic phagocytosis (OP) assay. Opsonic phagocytosis activity was strongly associated (hazard ratio [HR] = 0.46; 95% confidence interval [CI] = .30–.73; P = .0008) with protection against febrile malaria. Of the antimerozoite-specific antibodies, only IgG3 was significantly associated with both OP and protection (HR = 0.53; 95% CI = .34–.84; Pcorrected = .03) against febrile malaria. Similarly, GLURP-specific antibodies previously shown to be protective against febrile malaria in this same cohort were significantly associated with OP activity in this study. GLURP-specific antibodies recognized merozoites and also mediated OP activity. These findings support previous studies that found OP of merozoites to be associated with protection against malaria and further shows IgG3 and GLURP antibodies are key in the OP mechanism, thus giving further impetus for the development of malaria vaccines targeting GLURP.

Culture and characterization of Blood Outgrowth Endothelial cells from children with malaria

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The endothelium represents a critical player in severe malaria pathology. Specifically, endothelial cells express cell surface receptors such as ICAM-1 and EPCR which the pathogenic parasite protein PfEMP-1 interacts with in a process called cytoadhesion. Adhesion leads to sequestration of IEs in endothelial beds of various organs; results in endothelial activation and a compromise in the vascular barrier integrity. There is an ever increasing interest to elucidate the molecular details of cytoadhesion which may offer therapeutic interventions. We report the successful isolation and characterisation of blood outgrowth endothelial cells (BOEC) from small volumes of peripheral blood obtained from children with malaria. BOECs are differentiated matured endothelial cells and are found in peripheral blood mononuclear cells. They are culture adaptable cells with a very high proliferative potential and are also known to express parasite adhesion proteins such as ICAM-1, EPCR and PECAM. BOECs thus represent a promising model for studying both autologous and heterologous malaria parasite adhesion.

Modulation of human macrophage activity by *Ascaris* antigens is dependent on macrophage polarization state

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Helminths are strong modulators of host immune responses and inflammation to ensure their own survival within their hosts. Identification of the mechanisms responsible for this immunomodulation 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. The aim of this study was to investigate if adult body fluid (ABF) from the helminth *Ascaris suum* has immunomodulatory effects on different subtypes of human monocyte-derived macrophages (Mϕ) in vitro. Mϕs were exposed to A. suum ABF at different stages of their differentiation and/or polarization. MØ were first differentiated from monocytes into either uncommitted (M0), classically activated (M1) and alternatively activated (M2) phenotypes. ABF strongly supressed lipopolysaccharide-induced TNF-α, IL-6 and IL-10 secretion in M1 MØ, however in M2 MØ only TNF-α was supressed, with these cells secreting high levels of IL-10 which was not affected by ABF treatment. To determine if ABF modulated the differentiation of previously uncommitted MØ to either M1 or M2 MØ, monocytes were differentiated into M0 and then polarized by IFN-γ or IL-4 treatment in the presence of ABF. Under these conditions, ABF did not modulate cytokine secretion but did reduce CD80 expression in M1 but not M2 MØ. Finally, we demonstrate that when monocytes are differentiated into M1 MØ in the presence of ABF, subsequent inflammatory responses are markedly suppressed. Our data suggest that ABF inhibits cytokine secretion and co-stimulatory molecule expression in classically activated MØ but not in alternatively activated MØ, indicating selective action of ABF depending on MØ subtype. Moreover, ABF appears to exert stronger activity when acting upon MØ that have already been polarized to the M1 phenotype, rather than influencing the polarization process per se.

Immunomodulatory effects of *Ascaris suum* antigens on porcine lymphocytes and epithelial cells

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In vivo experiments have shown that the porcine roundworm, *Ascaris suum*, is known to modulate host immune responses in order to dampen inflammatory responses. The aim of this project is to assess the ability of *A. suum* to modulate the in vitro inflammatory responses of both porcine small intestine enterocytes using the cell line IPEC-J2 and peripheral lymphocytes as an infection model. The immunological activity of the cells in response to bacterial lipopolysaccharide in the presence or absence of *A. suum* antigens will be assessed. Finally, directly co- or cross-culturing the IPEC-J2 line with porcine peripheral blood mononuclear cells, after stimulation with Ascaris antigens, will further evaluate the cross-talk between small intestine enterocytes and lymphoid cells. The ability of Ascaris antigens to modulate this response, could prove useful in understanding the mechanisms of parasite immuno-modulation.

Quantification of the sub-lethal cost of infection in relation to the developmental stage of larval tapeworms

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By definition, parasites are harmful to their host and incur a cost to host fitness. However, the cost of infection can be difficult to predict, as it depends on the energy requirements of the parasite, and infected hosts may alter key life-history traits to compensate for losses caused by infection. In addition, most studies have been based entirely on the number of offspring produced, without considering the offspring quality. In this study, we measured both the body mass (quality) and number (quantity) of larvae produced by infected hosts using the tapeworm *Hymenolepis diminuta* – flour beetle *Tenebrio molitor* model. Our results showed that beetles infected with *H. diminuta* cysticercoids reduce offspring production. This decrease in larvae production is only evident within the first 21 days after infection, corresponding to the development of larval tapeworms into mature cysticercoids. We did not see any evidence for host compensation in the form of increased offspring quality (i.e. increased body mass) during the first 21 days of infection. Thus, our results indicate the host is not able to compensate for the energy demands required by parasite development.

An epidemiological survey on gastrointestinal parasites of cats in Denmark and evaluation of concentration McMaster technique

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A total of 99 euthanized cats; feral cats (n=92) and household cats with outdoor access (n=7), were collected March to May 2014 from the Zealand region, Denmark. The sedimentation and counting technique (SCT) was used to isolate helminths and coproscopy was done by a concentration McMaster technique (c-McMaster). Overall, 90.1% of the cats were infected with a total of 10 species determined by SCT: five nematodes: *Toxocara cati* (84.8%), *Ollulanus tricuspis* (13.1%), *Aonchotheca putorii* (7.1%), *Personema* spp. (3.0%), *Strongyloides* spp. (1.0%); three cestodes: *Hydatigera taeniaeformis* (36.4%), *Mesocestoides* sp. (3.0%), *Dipylidium caninum* (1.0%); and two trematodes: *Cryptocotyle* spp. (5.1%) and *Pseudamphistomum truncatum* (1.0%). *Ollulanus tricuspis* was the second most common gastrointestinal nematode with highest intensity. Prevalence and worm burden of *T. cati* were significantly higher in feral than household cats. No juvenile cats were infected with *H. taeniaeformis*, thus age had a significant effect on prevalence and worm burdens of this species. Rural cats had a higher prevalence and worm burden of A. putorii than urban cats. By c-McMaster, ascarid, capillarid, strongylid or taeniid type eggs were found in 77.9% of the cats while *Cystoisospora felis* was found in 2.1%. The sensitivity of the c-McMaster was high (82.5%) for *T. cati* but low (26.5%) for taeniid eggs, compared to SCT. A positive correlation between faecal egg count and worm burden was seen for T. cati. Inconsistent findings of capillarid eggs in faeces compared to necropsy were likely to be due to the presence of extraintestinal Capillariidae species including *Eucoleus aerophilus* and *E. boehmi*, although further necropsy studies are needed to confirm these findings.

Bioactive diets influence gut health and immune response in helminth-infected pigs

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Dietary fibres, such as prebiotic chicory-derived inulin, have been shown to influence host immunity and gut health, and may have anti-parasitic activity against gastrointestinal helminths. Infections with helminths such as *Trichuris suis* leads to a polarised Th2 immune response, resulting in release of Th2 cytokines and increased antibody secretion in the host. This host immune response also 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 a prebiotic-based diet, gut health and host immune responses during helminth infection. Intestinal tissue samples were collected to measure local immune related parameters, utilising techniques such as qPCR, histology and ELISA. Preliminary findings indicate that diets containing prebiotic inulin can have positive effects on the gut health of helminth-infected pigs by increasing expression of intestinal epithelial barrier-related genes such as trefoil factor 3 (TFF3) and sodium/glucose co-transporters, and down-regulating proinflammatory immune genes. In *T. suis*-infected pigs fed inulin, co-operative suppression of proinflammatory Th1-type genes and enhancement of genes encoding TFF3 was observed, suggesting that the host response to prebiotic inulin may enhance the host-protective Th2 immune response usually observed during helminth infections. Thus, our results indicate a profound effect of diet on immune function in helminth-infected pigs that may be exploited to improve gut health.

Minyoo Matata - The Vicious Worm – A Taenia solium taeniosis/cysticercosis health education tool – in Swahili

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Taenia solium is a zoonotic parasite that contributes to substantial public health and economic consequences across the globe. In 2014 "The Vicious Worm" a computer based health education tool was developed with the hope to have evidence-based health education included as a specific control tool in any control strategy. In East Africa Swahili is the most commonly spoken language. In order to use and increase the impact of the health education tool "The Vicious Worm" also in communities where English is not always spoken the need of a translated version of the tool was recognised. The aim of this project was to develop a new version of "The Vicious Worm" tool, in Swahili. "The Vicious Worm" is an open access computer based education tool for *T. solium* taeniosis/cysticercosis. The program provides information on transmission, diagnosis, treatment, risk factors, prevention and control of the parasite and targets different stakeholders across different sectors and disciplines. The educational materials included in "The Vicious Worm" are illustrated short stories, videos, scientific texts and policy and information sheets. The information is displayed using an interactive map showing a village, a town, and a city addressing three levels of stakeholders. For this project the education material of the English version was systematically translated, formatted and implemented in the new version. The beta version of the "The Vicious Worm" in Swahili is now available and can be downloaded for free through the homepage theviciousworm.org. The beta version will be distributed in the coming months. We welcome everyone to test it and participate in its evaluation. As lack of knowledge is among the major risks for the spread of the parasite, evidence-based health education should be included as a specific control measure in any control programme and we hope "The Vicious Worm" will serve as example.

Comparison of prevalence and intensity of infection of the parasitic nematode *Contracaecum osculatum* (liver worm) in Baltic cod from different areas.

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The Baltic Sea cod is divided into a western and an eastern stock. For the eastern Baltic stock (EB) weight at age has declined drastically since the mid-2000s resulting in poor condition of individuals while in the meantime number of fish has increased. One of the possible reasons for these changes is the increase of the abundance of grey seal (*Halichoerus grypus*). It has historically been low, but the population size has increased drastically and was estimated to be ca. 40 000 individuals in the entire Baltic. Grey seal is the final host of the parasitic nematode *Contracaecum osculatum* (liver worm) and cod is one (of several) transport hosts to this parasite. Recent field investigations have documented a marked increase in prevalence and intensity of infection for this parasite in EB cod in the areas near Bornholm and Christiansø where a colony of seals lives, compared with the 1980s when seal abundance was lower. Infection intensity correlates positively with length, i.e. older/larger specimens have more parasites and single cod livers in the area east of Bornholm now host up to 320 worms. The current study aims to investigate prevalence and intensity of infection with *C. osculatum* (number of parasitic nematodes per infected fish). Received results show that the prevalence of the parasitic nematode *Contracaecum osculatum* in cod caught near Bornholm (39 G5 and 40 G6 ICES squares) is 100% and the intensity of infection is 28 and 44 worms per fish (mean values). Contrary to these high infection rates, prevalence was 62 and 55% in cod from the Sound and the Southern Kattegat respectively, with mean intensity of infection being 1 and 4 worms per fish. The study demonstrates a positive correlation of increasing *C. osculatum* infection with the rise of grey seal abundance.

Diagnosis of soil-transmitted helminthes in the Caribbean: does the diagnostic method impact prevalence?

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Soil-transmitted helminths (STHs) infect over 1.5 billion people world-wide and are one of the most neglected tropical diseases. Mass drug administration and improvements in hygiene, sanitation and education have decreased STH infection intensity and prevalence. However, the decrease in prevalence might be overestimated. The standard diagnostic methods used are most appropriate for moderate to heavy infections; therefore, false negative results can occur with the lower intensity infections. In veterinary medicine, more sensitive diagnostic methods are used compared to human medicine with the goal of identifying and treating low level infections to decrease environmental contamination. The adaptability of these methods to human health has not been well studied. The purpose of this project was to determine if using standard veterinary diagnostic methods had an impact on prevalence results. Stool samples from 269 individuals (aged 4 to 70) were obtained from medical laboratories in Saint Lucia and St Kitts after being examined by the respective laboratory using Formalin-Ether (FE) or Kato-Katz (KK) for *Necator americanus*, *Ascaris lumbricoides*, *Trichuris trichiura* and *Giardia* sp. The veterinary methods used were: double centrifugation with Sheather's sugar flotation solution, double centrifugation with Zinc sulfate flotation solution and a modified Baermann technique. The diagnostic sensitivity of each technique was calculated using the combination of all four techniques as the gold standard and results were recorded by eggs per gram per feces (EPG). None of the samples were positive based on the medical laboratory results while 14 (5.2%) samples tested positive for at least one of the targeted parasites using the veterinary methods. In conclusion, alternative methods impacted the prevalence data for STH and suggest that there is a need to change diagnostic methods in human parasitology when infection intensity is low.

Comparison of malaria diagnosis by rapid diagnostic test (RDT), microscopy and PCR in a cross-sectional study in asymptomatic patients from Ranchi, India

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Monitoring of prevalence of *Plasmodium* infections by cross-sectional studies is necessary for measuring the impact of malaria control strategies. Microscopy and malaria rapid diagnostic tests (RDTs) are the most commonly used diagnostic techniques since they are inexpensive and fast with a relatively high specificity and sensitivity. Molecular tools, such as PCR is not used for routine diagnostics but is considered superior to these tools and of high relevance to identify the true prevalence of *Plasmodium* infections. This study aimed to compare the efficacy of microscopy and RDTs in identifying *P. falciparum* infections with a nested species PCR (gold standard) by using in vivo diagnostics data from cross-sectional studies (CSS) carried out in India in 6 different hamlets in the region of Ranchi,. Blood samples (n≈400) were taken from subjects attending the health centers regardless of their clinical symptoms during Oct-Nov 2014 (CSS1, high season), June-July 2015 (CSS2, lower season) and Nov-Dec 2015 (CSS3, high season). Independently of the 3 methodologies assessed, CSS1 displayed the highest parasite prevalence (at 42.3% by PCR) followed by CSS3 (at 32.3%) and CSS2 (at 16.6%). The sensitivity of RDT compared to PCR was 67.1% in CSS1, 46.9% in CSS2 and 52.9% in CSS3. Moreover, the sensitivity of microscopy compared to PCR was 39.4% in CSS1, 16.6% in CSS2 and 32.3% in CSS3. Thus, both methodologies showed lower efficacy compared to PCR. However, RDTs were more sensitive than microscopy, probably because of the ability of RDTs to detect parasites below the threshold of microscopy. Moreover, some samples (n=91, 33.8%) were RDT positive while PCR negative. Possibly, these are false positives due to persistent antigenicity caused by previous infections identified by the RDTs only. Even though expensive, PCR provides more reliable epidemiologic information and should be considered as an adjunct diagnostic methodology to assess true prevalence of malaria.

Prevalence of helminths in dogs from training clubs in the area of Copenhagen and Roskilde

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Helminth infections in dogs constitute a health risk for both the dog and potentially the public because of the zoonotic aspects. The prevalence of helminths in Danish dogs has not been described since 2009. The aim of this cross-sectional study was to determine the prevalence of a range of gastrointestinal and pulmonal helminths in dogs by coproscopy. It is expected that the sample size will reach a minimum of 200 dogs of more than 6 months of age. The study was initiated in February 2017 and is expected to be completed in May 2017. Dog owners, who are members of training clubs in the area of Copenhagen and Roskilde, were contacted through trainers and volunteered to hand in fresh faecal samples from three consecutive days, and complete a questionnaire. The questionnaire provided basic data on the dog (age, sex, breed, etc.), focusing on risk factors associated with helminth infections, e.g. the waking routines, potential coprophagy and anthelminthic treatments. The three stool samples were examined by a Baermann method for pulmonal nematodes and the most recent sample was examined by a McMaster method for helminth eggs and oocysts. To differentiate between the hookworms *Uncinaria stenocephala* and *Ancylostoma caninum*, larval cultures were prepared from faeces positive for typical strongyle eggs and afterwards analyzed by PCR. When *Toxocara* spp. eggs were detected, these were, documented by photo and speciated according to their size. Faeces was stored for later PCR to confirm identify as *T.canis* or *T. cati*. Currently 36 dogs have been examined. In total, helminth eggs or larvae were detected in 6 dogs (16.7%). The specific prevalence is 3/36 for Strongyle eggs, 1/36 for unidentified larvae very similar to *Crenosoma* spp. and 2/36 for *Angiostrongylus vasorum*. In addition, 2 samples contained what appeared to be poultry ascarid eggs and *Eimeria* spp., which was probably due to coprophagy. The preliminary prevalences are in accordance with previous studies in Denmark. However, the prevalence of *A. vasorum* is higher than expected.

Assessment of modified Baermann methods for diagnosis of heart- and lungworm first-stage larvae

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Lungworms and French heart worms are diagnosed as first-stage larvae (L1) using many different modifications of the Baermann method which rely on migration of L1 from faeces submerged in water. The current study compared four different Baermann methods (sedimentation glass (GB), wide plastic bag for freezing food (FB), narrow pastry piping bag (PB) and a commercial plastic funnel (L-ID)), incubation time (8 and 24h) and storage time (5^{o}C for 10, 17 and 24 days) in relation to recovery of *Angiostrongylus vasorum*, *Dictyocaulus arnfieldi* and *Dictyocaulus viviparus*. Ten to 15 replicates were set up for each treatment. Within 8 hours incubation the majority (compared to 24h) of *D. arnfieldi* (71-89%) and *A. vasorum* (84-86%) L1 had sedimented in GB, FB and PB, whereas *D. viviparus* sedimented with a slower velocity and only 14-42% was recovered after 8 hours. Many samples were false negatives and recovery was low using the L-ID. The two plastic bag methods were equally effective for recovery of *A. vasorum* but GB yielded significantly more L1 (approx. 30% higher). For *D. arnfieldi* GB tended to give slightly better yields than FB which in turn was a little better than PB though there were no significant differences. GB and FB were equally good for *D. viviparus* after 24h while recovery using PB appeared to be lower, but there was no significant effect. In general, *A. vasorum* experienced the most severe mortality during storage and no L1 could be detected by day 24. Recovery of *D.* viviparus was reduced by half day 10 and day 24 only 12% of the larvae could be detected. In general, *D. arnfieldi* survived best and by day 24, 32% of the L1 could still be recovered. It is recommended to set up samples as quickly as possible, combining 24h incubation with the GB as it gave the best overall results. The PB was probably too narrow to allow for efficient recovery of the L1, but FB can be a low cost disposable option compared to GB. The commercial L-ID is not recommended.

Survival of *Taenia saginata* eggs in the environment

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Taenia saginata eggs ingested by cattle cause bovine cysticercosis, a zoonotic disease, which can have considerable economic consequences for the meat sector. The environmental contamination *of T. saginata* eggs plays an important role in the transmission of bovine cysticercosis.This study aimed to investigate the potential survival of *T. saginata* eggs in freshwater streams and to assess the importance of temperature for egg survival. A total of 84 freshwater and silt samples were spiked with *T. saginata* eggs, of which half were maintained at 5 °C and the other half at 20 °C. The duration of the experiment is 6 months, with 3 sampling time points at 2, 4 and 6 months, respectively. Additionally, two smaller experiments were carried out; 8 samples were kept in a freshwater stream for a period of 4 months; 6 water samples were kept for one week in the freezer at -18 °C, and 6 other samples were kept outdoor at freezing temperatures ranging from -1 °C to – 4 °C. A recovery technique of eggs from soil was adapted to isolate *T. saginata* eggs from the silt samples. For each sample, viability of the eggs was tested in vitro, by hatching and activating. After 2 months at 5 °C, water and silt samples showed a survival proportion of 54% and 48%, respectively, while at 20 °C the survival proportions were 28% and 22%, respectively. After 2 months in the fresh water stream, 39% of the eggs were viable. At -18 °C, 44% of the eggs survived one week, while at outdoor freezing temperatures 50% survived. These preliminary results show considerable differences between the survival of *T. saginata* eggs at low and high temperatures, and an important survival at low temperatures was confirmed. This study indicates that *T. saginata* eggs can survive a Northern European winter, thus contributing to transmission, and contact with contaminated water sources can be considered an important risk factor of bovine cysticercosis in this region.