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Emerging and reemerging parasitic diseases in Europe

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Friday 20th of March, 2015, 8:30-16:10
Frederiksberg Campus, Main Lecture Hall 1-01 (Festauditoriet),
Bülowsvej 17, 1870 Frederiksberg C

ORAL PRESENTATIONS – KEY NOTES

Expected and unexpected (re)emergent parasites and vectors in Europe: understanding the current situation and predicting future scenarios

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A pathogen, a vector or a disease can be defined emergent when occurring in a new host and/or a new geographic area, or re-emergent when their incidence/prevalence increases above the expected threshold in previously endemic areas. Often the term "emergent" is confused with "neglected" or just "previously ignored". A parasite/vector can be at the same time emergent and neglected, depending on the geographic area of concern: for example canine leishmaniosis is neglected in southern Europe and is emergent in northern Italy and Spain.

Many parasites are considered to be emergent or re-emergent in areas of Europe, for example the dog tapeworm *Echinococcus granulosus* and the fox tapeworm *E. multilocularis*, the dog filarial nematodes *Dirofilaria immitis* and *D.repens*, the eye worm *Thelazia callipaeda,* the dog lung worm *Angiostrongylus vasorum*, the fish trematode *Opistorchis felineus*, the protozoa *Besnoitia* spp. and *Babesia* spp.. Other parasites, such as the dog filarial nematodes *Cercopithifilaria spp.* and the cat lung worms *Troglostrongylus spp.,* were only recently increasingly reported, but their high prevalence throughout Europe suggest they were undiagnosed in the past rather than currently emergent. Also, the use of new diagnostic tools, from PCR to next generation sequencing, has facilitated the identification of novel or previously undetected pathogens, such as many rickettsiae in ticks or viruses in mosquitoes.

Several factors have been claimed to drive emergence of parasites and vectors, i.e. climate change, global trade of humans, animals and goods, domestic and wildlife animal variation of density, urbanization, land use and fragmentation, socio-economic factors including political instability and wars. There is however an increasing evidence that human interventions can affect the emergence of pathogens and diseases more rapidly and effectively than ecological conditions. A paradigmatic example is the dramatic increase of trichinellosis in domestic pigs and humans in Eastern Europe after the fall of the Berlin Wall, which was ultimately followed by the collapse of the veterinary and industrial production systems. On the other hand, the factors driving the emergence of some vector-borne diseases in central Europe (i.e., blue tongue serotype 8 and Schmallenberg viruses) are still debated or unknown.

Finally, the increasing invasion of alien animal species for Europe, such as the raccoon (*Procyon lotor*) and some Aedes mosquitoes, poses a new threat for animal and human health, i.e. the likely emergence of old pathogens and the introduction and spread of new ones.

In this current complex situation, are we able to predict future scenarios?

Re-emerging Leishmaniasis in Southern Europe

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Endemic leishmaniasis in Europe is mainly caused by *Leishmania infantum* and is associated to a zoonotic cycle with domestic dogs as the main reservoir and sandflies of the subgenera *Larroussius* and *Adlerius* as vector. Anthroponotic cutaneous leishmaniasis due to *L. tropica* is also endemic, but geographically restricted to Greece; and within the last years *L. donovani* has emerged as responsible of autochthonous cutaneous and visceral leishmaniasis in Cyprus and Turkey. Nevertheless, the highest burden of leishmaniasis is attributed to *L. infantum,* traditionally considered a disease of childhood but also affecting adults in varying proportion. The high ratio of asymptomatic/symptomatic infection in humans and the elevated infection rate in dogs point to a latent public health problem. This was demonstrated in the past with the advent of HIV infection, when *Leishmania*/HIV co-infection dramatically emerged in southern Europe (the highest proportion of cases was reported from Spain). While *Leishmania* infections in Europe are most frequent in the Mediterranean basin, several factors including human and animal travel, immunosuppression, and environmental changes may influence its spread to other previously non-endemic countries. There is a risk of (re-) emergence, and examples are the recent propagation of leishmaniasis to northern Italy and Spain, autochthonous canine and human cases in Hungary and Germany, as well as equine and bovine leishmaniasis in Switzerland and Germany. Reports of imported human and canine infections from Mediterranean countries to the north, and also of exotic species in different European countries are increasing. To this scenario adds the GIS framework models combining entomological and environmental variables, indicating that global warming could lead to the spread of sandfly populations to previous non-colonized areas.

And last but not least: an urban outbreak of visceral and cutaneous leishmanaisis is taking place in the southern region of Madrid, Spain. The average incidence in this region was 12-25 human cases per year. However, the outbreak starting in 2009 has caused several hundreds of human cases up to now. All age groups are affected, without significant association to immunosuppression. The origin of this outbreak, attributed to environmental changes, will be discussed.

CONTRIBUTING ORAL PRESENTATIONS

Lack of age related physiological susceptibility to *Echinococcus multilocularis* in the common vole (*Microtus arvalis*), a key intermediate host

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The small fox tapeworm, *Echinococcus multilocularis* is a zoonotic parasite distributed within arctic to temperate climates in the Northern Hemisphere. Rodents serve as intermediate hosts, becoming infected after ingesting eggs excreted from worms in foxes. Temporal fluctuations in prevalence of *E. multilocularis* in intermediate hosts is affected by the age structure of populations as once established, a metacestode remains in the rodent for the remainder of its life. In two key intermediate hosts, the water voles (*Arvicola* spp.) and common vole (*Microtus arvalis*), highest prevalence of animals with fertile metacestodes (containing protoscolices) has been observed in spring in animals that had procured their infections in winter, however variances in physiological susceptibility due to the age of the rodent host at infection has not yet been determined. Such differences could affect transmission dynamics of *E. multilocularis*. Via oral inoculation, 4 groups of *M. arvalis* at various ages (35 days, 56 days, 84 days, and c. 263 days) were infected with 100 viable *E. multilocularis* eggs, with the oldest animals split into two groups and terminated at 6 and 10 weeks post infection (p.i.). Eight C57BL/6j mice were used as positive controls. No effect of age at infection was observed on protoscolex production however data suggest that protoscolex development time takes a minimum of six weeks in *M. arvalis* hinting at interesting ramifications for parasite transmission. Fundamental differences between metacestode growth in *M. arvalis* and C57BL/6j mice were also revealed.

Emerging and re-emerging helminth parasites of wildlife in Denmark, an open field for “One-Health” approach

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In DTU-VET, we experienced emergence and re-emergence of parasites in several terrestrial and marine mammals, which may have impact on animal and human health and economy.

In terrestrial mammals, roe deer infections with *Taenia krabbei* were in coincidence with the recent invasion of grey wolves infected with the same parasite. Two mosquito-borne filarids were recently found in roe deer: Setaria tundra, that inhabits the peritoneum and *Onchocerca cervipidis*, which induce subcutaneous nodules. The invading raccoon dog had high prevalence and abundance of *Alaria alata, Isthimiophora melis* and *Dipylidium caninum*, which exceeded that of the native red fox; an indication of a spill-over or recent emerging of potentially zoonotic parasites. *Echinococcus multilocularis* was found in raccoon dogs inhabiting an endemic focus that was mainly a niche for foxes. In foxes, new species were recently recovered, including the zoonotic *Metorchis bilis, Pygidiopsis summa* and *Brachylaima tokudai*, and the tumour-inducing parasite of canids; *Spirocerca lupi*. Zoonotic and highly pathogenic parasites were reported for the first time in Danish otters, including *Molineus patens, Physaloptera sp., Eucoleus aerophilus, Schistocephalus solidius, Metorchis bilis* and *Plagiorchis sp*.

Several parasites were recovered from heart, lung, liver and stomach of marine mammals, for example: *Anisakis sp., Pseudoterranova decipiens, Contracaecum osculatum* and *Pseudamphistomum truncatum*. Emergence of these parasites has negatively affected the health of marine mammals and the fish intermediate hosts, which obviously harmed the fish industry in the Baltic sea.

This parasite emergence might be a result of the recent environmental changes. However, other factors could have affected the ever dynamic nature of wildlife and their parasites, which might bring more surprises in the future. Given the broad spectrum of affected hosts, collaborative “One-Health” approach is advised for combating such infections.

Post-treatment infection dynamics of *Schistosoma haematobium*

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Schistosomiasis is a debilitating disease affecting hundreds of millions of people, especially in sub-Saharan Africa. Among the responsible parasitic trematodes is *Schistosoma haematobium*, the causative agent of urinary schistosomiasis, which causes significant pathology in the genito-urinary system. Traditional parasitological methods for determining infection such as urinary egg counts and detection of haematuria has been supplemented with both immunological and molecular methods, and recently antigen detection assays have proven useful. In this study we have used eight different diagnostic tests (measuring eggs in urine, haematuria, urinary IL-6, IL-8, eosinophil cationic protein (ECP) and a circulating schistosome antigen (CAA), parasite DNA by Q-PCR, and urinary tract pathology by ultrasound) to determine the prevalence and intensity of infection in 55 school-aged children from Tanzania before treatment with Praziquantel and to follow the short term post-treatment infection dynamics of *S. haematobium*, with sampling 6, 24, 48 hours and 1, 3 and 12 weeks after treatment.

We found that the antigen detection test was very sensitive, also after treatment, where many other methods began to lose sensitivity. Even 12 weeks after treatment the CAA assay diagnosed more than 60 % of the study participants as positive where assays such as the urinary egg count showed 0 %. Furthermore, we saw that most markers of infection were affected rapidly by the treatment, as soon as 6-8 hours after ingestion of the tablets. Interesting to note was that the urinary egg count increased after treatment, even though evidence pointed towards the successful killing of at least some of the worms, which might indicate that treatment with praziquantel forces surviving worms to expulse eggs at an increased rate.

Evaluation and optimization of the circulating cathodic antigen (cca) cassette test for *schistosoma mansoni* in rural areas of Tanzania

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There is a need for diagnostic techniques which are sensitive, specific, rapid and easy to perform at the point-of-care. The aim of this study was to evaluate the diagnostic performance of the Circulating Cathodic Antigen (CCA) cassette test for *Schistosoma mansoni* in three rural districts of Mwanza region, Tanzania. Our study was conducted among 404 school-age children (aged 9-12 years) across four different schools, adjacent to Lake Victoria in Tanzania. Stool and urine samples were collected for three consecutive days. For S. mansoni diagnosis, stool samples were examined for eggs with duplicate Kato-Katz, whereas urine samples were tested for presence of antigen by CCA cassettes. The proportion of positive individuals for S. mansoni by three CCA cassettes was higher (75.0%; 303) than for six Kato-Katz (42.6 %; 172). The 'gold standard' was built considering the combined results obtained for six Kato-Katz smears and three CCA cassettes from three consecutive days. For CCA cassette performance, three CCA cassettes were more sensitive (94.7%) than six Kato-Katz smears (53.7%). In terms of optimization of the CCA cassette test, a Software tool (Image Studio Lite®) has been able to detect the result band in all positive CCA cassettes and pixel quantification has been performed, showing a positive correlation between pixels and CCA cassette intensities and between pixels and egg counts. In conclusion, CCA cassette test seems to be a more appropriate tool for *S. mansoni* diagnosis compared to Kato-Katz in endemic communities such as Mwanza region. Optimization of the tool in terms of cassette-reading could be assessed by computer software which quantifies the cassette colour band.

Mitochondrial genome analyses suggest multiple *Trichuris* species in humans, baboons, and pigs from different geographical regions

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The whipworms *Trichuris trichiura* and *T. suis* are two parasitic nematodes of humans and pigs, respectively. Although whipworms in human and non-human primates historically have been designated *T. trichiura*, recent reports suggest that several *Trichuris* spp. are found in primates. Parasites with wide global occurrence might comprise several cryptic species. Complete mitochondrial genomes of *Trichuris* recovered from a human in Uganda, two Olive baboons held in captivity (Texas, US), and two pigs from Denmark and Uganda were sequenced and annotated. Comparative analyses with other published mitochondrial genomes of *Trichuris* recovered from a human and porcine host in China and from François' leaf-monkey (China) were performed, including phylogenetic analyses and pairwise genetic and amino acid distances. Genetic and protein distances between human *Trichuris* in Uganda and China were high (~19% and 15%, respectively) suggesting that they represented different species. One baboon *Trichuris* was genetically related to human *Trichuris* in China while the other was nearly identical to human *Trichuris* from Uganda. Baboon-derived *Trichuris* were genetically distinct from *Trichuris* from François' leaf-monkey suggesting multiple whipworm species circulating among non-human primates. The genetic and protein distances between pig *Trichuris* from Denmark and other regions were roughly 9% and 6%, respectively, while Chinese and Ugandan whipworms were closely related. Our results suggested that *Trichuris* species infecting humans and pigs differed across geographical regions, which might have important implications on implementing suitable and effective control strategies in different regions. Moreover, we found further support that *Trichuris* infecting primates comprises a cryptic species complex and may transmit to humans.

Coping with anthelmintic resistance in ruminants: the potential use of chicory (*Cichorium intybus*) as an antiparasitic forage in cattle

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Studies were performed to test the anthelmintic activity of chicory against cattle nematodes and to investigate the role of sesquiterpene lactones (SL) as active compounds. In study 1, 2-4 months-old calves were allocated into a chicory (CHI, n=9) or control (CON, n=6) group. CHI and CON were stabled and fed with chicory silage (cv. Spadona) or hay, resp., ad libitum. After 2 weeks all calves were infected with 10,000 *Ostertagia ostertagi* and 65,000 *Cooperia oncophora* third-stage (L3) larvae. In study 2, 4-6 months-old calves were allocated into a chicory (cv. Spadona, CHI, n=10) or ryegrass/white clover (CON, n=10) pasture. After 1 week all calves were infected with 20,000 *O. ostertagi* L3 larvae. Fecal egg counts were calculated as number of eggs per g of dried feces (FECDM). At day 56 (study 1) and day 36 (study 2) post-infection calves were killed for worm recovery. FECDM and worm counts were analysed by ANOVA. In study 3, SL extracts were purified from leaves of chicory cv. Spadona and cv. Puna II. *O. ostertagi* adults were incubated at decreasing concentrations of SL extracts and worm motility was evaluated after 6, 24 and 48 h of incubation (37oC). SL profile in the extracts was analysed by liquid chromatography (LC). In study 1 mean FECDM were not significantly different between groups. *O. ostertagi* mean worm counts were 1599 and 3752 in CHI and CON groups, respectively ($P<0.05$). *C. oncophora* burdens were not statistically different between groups. In study 2 FECDM was decreased in CHI by 48-65% as compared to CON ($P<0.05$). Worm counting of study 2 is undergoing. In study 3 Spadona-SLs showed higher potency and exerted faster worm paralysis than Puna II-SLs. LC analyses revealed a different composition of SL between cultivars. In conclusion, chicory demonstrated a marked in vivo anthelmintic effect against *O. ostertagi*, but not on *C. oncophora*. Different anthelmintic potency of chicory SL can guide the identification and selection of antiparasitic cultivars.

Identification of the ICAM-1 binding site in DBLβ of PfEMP1 proteins associated with severe malaria

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The virulence of *Plasmodium falciparum* is linked to the ability of infected erythrocytes (IE) to adhere to endothelium. The adhesion is mediated by a family of surface expressed proteins known as P. falciparum erythrocyte membrane protein 1 (PfEMP1). A sub-group of group A PfEMP1 mediates binding to host ICAM-1 on inflamed endothelium and is associated with development of severe malaria, including fatal cerebral malaria. We predict a PfEMP1-based vaccine that induces antibodies interfering with ICAM-1 adhesion to protect against severe malaria and we are looking into the molecular details of the PfEMP1-ICAM-1 interaction. In this study, we identified a conserved 69-amino acid motif that predicts ICAM-1 binding of PfEMP1 DBLβ subdomains despite low overall sequence identity. The motif is found in 1-4 copies per parasite genome and is exclusively present in DBLβs from group A PfEMP1s. Recombinant DBLβs proteins with the motif bind ICAM-1 in enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance (SPR) assays. Introduction of the motif into a non-binding DBLβ domain results in gained ICAM-1 binding. The actual binding site was further narrow down to 23 amino acids using hydrogen deuterium exchange mass spectrometry (HDX-MS) and this finding was confirmed by small angle x-ray scattering (SAXS) analysis of the DBLβ::ICAM-1 complex. Furthermore, using site-directed mutagenesis we identified two amino acids critical for ICAM-1 binding. Importantly, experimental rat antibodies targeting the motif were able to interfere with ICAM-1 binding in adhesion assays. These results raise the possibility of designing a peptide-based vaccine protective against severe malaria.

A Novel ICAM-1 Binding Motif is a Target of Cross-reactive and Cross-inhibitory Human Antibodies

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Malaria is responsible for the death of nearly 600.000 people every year most of these are children under the age of five years. One of the most fatal forms of malaria is cerebral malaria, which is caused by accumulation of malaria parasites in the small vessels of the brain. Malaria parasites live inside the red blood cells and export proteins known as *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) onto the surface of the infected cell. The PfEMP1 proteins enable the parasite to bind various tissues and thereby avoid being killed by the host immune system. Binding to host tissue is mediated by receptors and binding to the human ICAM-1 receptor has been associated with the development of cerebral malaria. In this study we have identified the minimal ICAM-1 binding site motif of DBLβ domains from a group of PfEMP1 proteins (see presentation by Bengtsson et al.). We find that human antibodies targeting this motif inhibit the binding of homologous and importantly also heterologous DBLβ domains to ICAM-1 and that levels of such antibodies are significantly increased during severe malaria infections, but not during uncomplicated malaria infections In addition, real-time Q-PCR analysis show an increased transcription level of transcripts encoding the ICAM-1 binding DBLβ in patients with cerebral malaria compared to hospitalized patients with less complicated malaria. These findings indicate that our identified motif does play a role during cerebral malaria and that to obtain optimal protection against cerebral malaria a PfEMP1-based vaccine needs to include motif-containing proteins capable of inducing cross-reactive antibodies that inhibit ICAM-1 adhesion of infected erythrocytes.

Multiple *Plasmodium falciparum* erythrocyte protein 1 variants in a single parasite genome bind IgM via Fcμ

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The high-molecular weight *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) adhesive parasite antigens on the surface of infected erythrocytes (IEs) are of key importance in the pathogenesis of P. falciparum malaria. Several structurally and functionally defined PfEMP1 types have been associated with severe clinical manifestations such as cerebral malaria in children and placental malaria in pregnant women. The ability to bind non-specific IgM characterizes one such severity-related PfEMP1 type, although the functional significance of the IgM binding remains unclear. In this study we report the identification and functional analysis of five IgM-binding PfEMP1 proteins encoded by P. falciparum NF54. In addition to the VAR2CSA-type PFL0030c, already known to bind non-specific IgM and to mediate CSA-specific adhesion of IEs in the placenta, we found four PfEMP1 proteins not previously known to bind non-specific IgM. Although they all contained DBL domains similar to those in VAR2CSA-type PfEMP1, they did not mediate IE adhesion to CSA, and binding of IgM to them did not shield IEs from phagocytosis of IgG-opsonized IEs. Instead, they shared functional and structural similarities with the rosette-mediating and IgM-binding PfEMP1 protein HB3VAR06. However, they did not form rosettes. We could map the capacity to bind non-specific IgM to C terminal DBL domains in four of the five PfEMP1 proteins. Our study provides new evidence on the function of binding of non-specific IgM to PfEMP1, which appears to be an important marker of PfEMP1 proteins causing severe *P. falciparum* malaria.

Haplotype H3 of the Endothelial Protein C Receptor (EPCR) gene associated with elevated plasma levels of soluble EPCR is not associated with protection against severe malaria in Tanzania

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Endothelial protein C receptor (EPCR) was recently identified as a receptor for *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) mediating sequestration of *P. falciparum* infected erythrocytes in patients suffering from severe malaria. Soluble EPCR (sEPCR) inhibit binding of *P. falciparum* to EPCR in vitro and increased levels of sEPCR have been associated with the H3 haplotype of EPCR. Thus, it has been hypothesized that elevated sEPCR levels, possibly linked to the H3 genetic variant, may confer protection against severe malaria. This study determined the frequencies of the haplotypes and plasma levels of sEPCR in a Tanzanian study population to investigate a possible association with severe malaria.

Study participants were children under 5 years of age admitted to the Korogwe District Hospital (N = 143), and diagnosed as having an infection other than malaria (N = 67), uncomplicated malaria (N = 24) or severe malaria (N = 52). In addition, blood samples from 71 children from nearby villages were included. The single nucleotide polymorphism (SNPs) defining the haplotypes were determined by post-PCR ligation detection reaction-fluorescent microsphere assay. Individuals carrying at least one H3 allele had significantly higher levels of sEPCR compared to individuals with no H3 alleles ($P < 0.001$). No difference in the frequency of H3 was found between the non-malaria patients, malaria patients or village population ($P > 0.1$). Plasma levels of sEPCR were found to differ between these three groups, with higher levels of sEPCR in the village population compared to the hospitalized patients ($P < 0.001$) and higher levels in malaria patients compared to non-malaria patients ($P = 0.001$). Frequencies of the SNPs determining haplotypes were in concordance with other African studies. H3 was associated with higher levels of sEPCR, confirming earlier findings, however, in this study; neither haplotype nor level of sEPCR was associated with protection against malaria.

New but old parasite – *Alaria alata*: a fluke with unique abilities

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Alaria alata is a small parasitic trematode that has the ability to infect a range of hosts. Despite lack of reported human cases, *A. alata* is considered zoonotic, as other *Alaria* spp. have caused clinical diseases in humans including one fatal outcome in USA. Although *A. alata* has a long history of existence in Europe, the presence of its mesocercarial infective stage in meat has been re-recognised only in recent years due to increasing numbers of accidental findings in wild boar meat during *Trichinella* inspection. As high prevalences of *A. alata* in the definitive hosts (canids) have been reported in Denmark previously, this epidemiological survey was conducted to provide preliminary baseline prevalence data in potential paratenic hosts intended for human consumption and other paratenic/definitive host. Muscles of 406 domestic pigs, 153 wild boars, 62 horses, 9 badgers and 99 cats were collected by convenience sampling during October 2013 until September 2014 and analysed by a modified *A. alata* mesocercariae migration technique (AMT). The intestines of 99 cats were examined using the sedimentation and counting technique (SCT) to recover adult flukes. No mesocercariae were found in pigs, wild boars, or horses, whereas *A. alata* mesocercariae were isolated from 66.7% of badgers and 3.0% of cats, the later were all pregnant or lactating. No adult flukes were detected by SCT in 99 cats. Several *Alaria* spp. have the unique ability of transmission called “amphiparatenesis” i.e. that male and non-lactating female cats are definitive hosts, but lactating cats are primarily paratenic hosts. Our findings indicate this may also be the case for *A. alata* thereby increasing the chance of vertical transmission from mother to offspring.

Severe seizures in pigs infected with *Taenia solium*

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Neurocysticercosis caused by the intermediate stage of the pig tapeworm *Taenia solium* is a serious neurological disease in humans. In endemic areas it is estimated to be responsible for one third of all late onset epilepsy. Whilst in humans these neurological symptoms have been well documented there is limited information on the effect of the parasite in pigs.

The aim of the study was to assess the behaviour of naturally infected *T. solium* pigs in Tanzania.

An experimental behavioural study was carried out including 17 naturally *T. solium* infected and 14 non-infected pigs. These were continuously videotaped for 14 consecutive days using close circuit television cameras. After the video recording period, all animals were euthanized and the brain, tongue, masseters and heart were sliced and the number of cysts counted.

During recording period, two infected pigs were observed having seizures. This is the first recorded observation of seizures in pigs ostensibly caused by *T. solium*. Although only two pigs were observed, multiple linear regression analysis found this to be significantly related to age ($p=0.0008$) but not to total number of cysts in the brain ($p=0.7$). Although preliminary, these observations run contrary to the notion of asymptomatic neurological effects in the porcine host due to *T. solium*, potentially opening up a new experimental pathway to explore the aetiology of neurological symptoms in humans.

POSTER PRESENTATIONS

Detection of *Trichobilharzia* sp. infected snails using a genus specific and sensitive PCR assay

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Bird schistosomes are widespread parasites of freshwater snails and waterfowl and members of the genus *Trichobilharzia* are common in Europe. Cercariae can penetrate human skin and cause swimmer´s itch and, moreover, the larvae may migrate within the body of experimentally infected mammals, indicating a potentially more severe pathology in accidentally infected humans. Cases of swimmer's itch have previously occurred in Denmark and the disease is considered a re-emerging problem in Europe. However, distribution studies are limited and there is no surveillance of bird schistosomes in Danish lakes. Presence of the parasites is usually confirmed by cercarial shedding from collected snails but only patent infections are detected hereby, while a PCR method offers the opportunity to detect immature larval stages by amplification of minute amounts of DNA within the snail host. Therefore, the aim of this project was to validate and test a PCR assay, hypothesized to be more sensitive than shedding, for detection of *Trichobilharzia* sp. DNA within snails. Primers that target a *Trichobilharzia* sp. specific tandem repeated region (ToSau3A) were applied in a conventional PCR and initially validated on cercariae and experimentally infected and uninfected snails. Subsequently, 3 shedding and 54 non-shedding field sampled snails were investigated. While DNA of *Trichobilharzia* sp. originating from cercariae and infected snails (both experimentally infected and field sampled) was successfully amplified, none of the negative field sampled snails showed a clear positive signal and further testing of the method is needed to verify the ability of the assay to detect pre-patent stages. Detection of parasite DNA may be an important approach for identification of areas with transmission and new tools are indeed needed in the light of the public health risk represented by these parasites and with the continuing changes in distribution and transmission dynamics driven by e.g. climate warming.

Feeding behaviour of cage-cultured rainbow trout – consequences for parasite transmission and prevalence

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Salmonids cage-cultured in marine environments are generally considered to be free from infections with zoonotic nematode larvae. The nematode-free status is explained by the confined life style of caged fish and the provided diet consisting of heat-treated feed without viable parasite larvae. Hence, fish from modern cage-culture are theoretically excluded from participating in complex parasite life cycles such as that of *Anisakis simplex*. In order to investigate this notion in more detail ten rainbow trout, i.e. five runts (= individuals showing inferior growth) and five harvest quality fish were sampled from each net-cage mariculture facility in Denmark at the time of slaughter in 2012 and 2013. Trout (*n* = 190) were examined for endoparasites by visual inspection of the gastrointestinal tract and body cavity, and by pepsin digestion of the belly flap musculature. Stomach content analysis was performed to assess the feeding behavior of the caged trout. All trout were negative for nematode larvae in the musculature and no zoonotic parasites were found. However, *Hysterothylacium aduncum* (Nematoda), *Eubothrium* sp. (Cestoda) and *Neoechinorhynchus* sp. (Acantocephala) were found in the gastrointestinal tract of runts (*n* = 95; mean total body mass = 0.813 kg) with prevalences of 9.5%, 3.2%, and 1.1%, respectively. In contrast, 2.1% of the harvest quality trout (*n* = 95; mean total body mass = 2.390 kg) harbored *H. aduncum* as the only parasite detected. The higher number of parasite infections and the increased prevalence of *H. aduncum* among runts were associated with a markedly higher intake of small fish (three-spined sticklebacks) and crustaceans (amphipods) as well as sessile mussels and algae associated with the nets of the net-cages. These findings suggest that feeding behavior of rainbow trout runts is skewed towards predation on net-cage-associated fauna and that infection status of runts may serve as an indicator of possible endoparasite transmission to cage-cultured fish.

Occurrence of *Aelurostrongylus abstrusus* (Railliet, 1898) in Danish cats: A modified lung digestion method for isolating adult worms

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Aelurostrongylus abstrusus is a metastrongyloid nematode causing respiratory symptoms in domestic cats and other feline species. The parasite has an indirect life cycle, which requires snails and slugs as intermediate hosts as well as frogs, birds and small mammals as paratenic hosts. The adult worms are localised in the lung parenchyma and in the terminal branches of the respiratory tract where the eggs are deposited. After hatching, the first stage larvae (L1) are coughed up and swallowed and the L1 can subsequently be detected in the faeces. As *A. abstrusus* has not previously received any attention in Denmark, the study investigated the occurrence of *A. abstrusus* amongst outdoor cats from three regions (Zealand, Møn and Falster). Faeces and lungs were collected from a total of 147 feral (n = 125) and domesticated cats (n = 22) that were euthanized for reasons outside of this project. Using a modified Baermann technique, 13.6% of the cats were found to be positive for L1. A new lung digestion technique utilising direct infusion of the digestion fluid into the lungs was developed to isolate the parasite from the lungs and this revealed an overall prevalence of 15.6%. There was no difference between feral and domesticated cats just as sex and age of the cat did not appear to influence prevalence and worm burden. Lungs from 87% of the positive cats had the gross appearance compatible with damage caused by *A. abstrusus* and the severity of lung damage was proportional to faecal LPG and number of adult worms.

Feline aelurostrongylosis is more common than previously believed and should be considered as a differential diagnosis of outdoor Danish cats with clinical signs related to the respiratory system in all age groups of cats.

Giardiasis and cryptosporidiosis in children, Southern Denmark

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Little is known about the current occurrence of infections with *Giardia lamblia* and Cryptosporidium species in children less than 17 years of age in Denmark. To obtain basic information on this patient group we analysed cases of microscopically confirmed giardiasis and cryptosporidiosis.

Giardia lamblia or Cryptosporidium species was detected in fecal samples from a total of 627 patients identified from the records of the laboratory systems at the departments for clinical microbiology at Slagelse Hospital (1998 – jan 2015) and Odense University Hospital (2005 to 2014). Of these patients, 179 were children less than 17 years old. *Giardia lamblia* was detected in fecal samples from 166 children and Cryptosporidium spp. was seen in samples from 13 children. Of the children with giardiasis 83 were boys and 75 were girls. Gender was not indicated in eight cases. 37 of the cases of giardiasis were in children one to three years of age. Some seasonal variation was observed as most cases were detected from June to January.

The present study showed that childhood giardiasis is not often diagnosed in southern parts of Denmark. Affected children are mainly very young; from one to three years old. Cryptosporidiosis is very infrequently diagnosed in children. In contrast to *Giardia lambia*, Cryptosporidium spp. are only easily recognized by microscopy when using special staining techniques, requested only on specific suspicion of cryptosporidiosis. This may contribute to underestimation of the number of cases of cryptosporidiosis.

Prospective studies employing sensitive assays for both parasites are needed to reveal the true frequencies of these infections in children.

Blood Outgrowth Endothelial Cells: A novel tool for the study of adhesive interactions between *Plasmodium falciparum* infected erythrocytes and the endothelium

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Survival of the malarial parasite, *Plasmodium falciparum*, within the human host is facilitated by the phenomenon of sequestration. Infected erythrocytes (IE) bind to a variety of receptors such as CD36, ICAM-1, chondroitin-4-sulphate and most recently, EPCR. The high molecular weight malaria protein, PfEMP-1, has been demonstrated to mediate the adhesion of infected erythrocyte to numerous cells and tissues causing endothelial dysfunction and vessel occlusion. Investigation of this phenomenon requires tissues expressing the desired receptors, however current standard methods for investigating adhesive interactions rely on static adhesion assays and commercially available cell lines of non-human origin, i.e. CHO or mammalian cell lines such as HBEC-5i, C32 or BeWo. These cells express native receptors to which IEs may bind, but some (i.e. C32 and BeWo) also express receptors not found on healthy cells. We propose the use of a sub-population of human endothelial cells found within the peripheral blood stream which can be isolated from the blood in a minimally invasive manner. These cells are highly proliferative and retain their endothelial characteristics whilst in culture. We are currently investigating their ability to support adhesion of IE and their potential as a cost effective source of human endothelial cells for the study of the adhesive interactions between the malaria parasite and its host.

Structure of the DBL4γ domain of a malaria parasite Erythrocyte Membrane Protein 1 and Identification of the binding site for a DBL4γ-specific monoclonal antibody

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Malaria remains a serious health problem affecting 200.000.000 people worldwide each year. In 2012 alone, WHO reported 627.000 deaths caused by malaria. The main source of fatal complications is the ability of infected erythrocytes (IE) to adhere to host endothelium cells in brain or placenta capillaries where they obstruct blood circulation and cause inflammation. Adhesion of IE is mediated by the parasite *Plasmodium falciparum* Erythrocyte Membrane Protein 1 (PfEMP1). PfEMP1 is a large group of multimodular adhesion receptors consisting of an N-terminal segment, a variable number of Duffy-binding-like (DBL) domains together with one or two Cysteine-rich Inter-domain Region (CIDR) domains. DBL domains are further divided into α, β, γ and δ subclasses based on sequence similarity.

We present the structure of PFD1235w DBLγ a 400 residues long PfEMP1 domain, which is known to bind a specific antibody (AB01) found in plasma of malaria-infected humans.

DBL4γ had proven to be a difficult target that presented several major problems starting from purification and crystallization to diffraction data analysis and structural determination. Despite the presence of 20 cysteines and 9 disulfide bonds, we managed to express DBL4γ in E. coli as folded protein. The initial structure was determined by SeMet MAD phasing at 3.5Å and followed by SAD phasing using Hg derivative at 2.8Å. Finally, the 2.0Å structure of PfEMP1 DBLγ domain was refined using native data collected at the ESRF microfocus beamline. In addition, we also identified the AB01 binding site in DBL4γ by hydrogen-deuterium exchange mass spectroscopy (HDX-MS) technique.

An automated method for determining the cytoadhesion of *Plasmodium falciparum*-infected erythrocytes to immobilised cells

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The parasite *Plasmodium falciparum* exports parasite antigens to the surface of the infected erythrocyte in order to adhere to the host vasculature. As it is believed to be central in severe malaria pathogenesis this process has been studied in detail. The study of cytoadhesion in vitro relies mainly on manual counting methods and the current study aimed at developing an automated method to allow high throughput testing.

Erythrocytes possess pseudoperoxidase activity due to the presence of haemoglobin and this was utilized to quantify binding of infected erythrocytes chromogenically. *Plasmodium falciparum*-infected erythrocytes selected on and binding to Chinese hamster ovary (CHO) cells was used to develop the method. Binding of infected erythrocytes to primary endothelial cells was also performed. Regression analyses and Bland-Altman plots were performed to compare the method with microscopical examination.

By using this set-up, binding to adherent cells could automatically be quantified, and importantly with a precision comparable to microscopical examination. The method is sensitive and has lower limit of detection compared with microscopical examination. Its relevance for quantification of binding studies was assessed in two well-controlled set-ups: i) binding of P. falciparum-infected erythrocytes to CHO cells over-expressing chondroitin sulphate A; and, ii) CHO cells transfected with human CD36. The assays both showed the expected dose-dependent reduction in binding to CHO cells when blocking with soluble chondroitin sulphate A and CD36 antibody, respectively. Furthermore, quantification of binding to endothelial cells was performed revealing clear distinction between selected vs. non-selected parasite lines.

The assay is simple and in a reproducible manner quantifies erythrocyte adhesion to several types of immobilized cells.

Determination of the prepatent period and sporocyst development of *Schistosoma mansoni* in *Biomphalaria glabrata* snails at constant high temperatures

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Global warming is ongoing with temperatures increasing during the last century and is also expected to continue in the future. The rising temperatures related to climate change are expected to affect the distribution of most species, including many infectious organisms, such as *Schistosoma mansoni*. This parasite is particularly sensitive to the changes as parasite development and survival are highly temperature dependent. It is important to acknowledge these changes as they can lead to infections being introduced into currently non-endemic areas. Monitoring the current distribution of S. mansoni, by snail collection to identify parasites and predicting the future distribution using models predicting how the parasites development will respond to changes in temperature, is therefore crucial. However traditional methods for detecting infected snails can underestimate the infection rates, molecular methods might be a better approach. Additionally, predictive models often use the Growing Degree-Day approach which is based on a 35 year old study that has not been validated since then. The aim of this study is to determine the prepatent period and sporocyst development of *S. mansoni* in *Biomphalaria glabrata* snails at constant high temperatures to generate better parameters for predictive models. The approach is to set up three aquaria containing infected *B. glabrata* snails with temperatures of 33, 34 and 35°C and observe the snails for cercarial shedding at regular intervals. Additionally snails will be sampled during the experiment and dissected to physically look for parasite stages using microscopy. In addition DNA will be extracted from the snail tissue for subsequent qRT-PCR analysis for the presence of *S. mansoni* DNA. Preliminary results based on snail microscopy indicate that parasite development is faster at 33°C. However, problems with high mortality in the negative controls, rotifer contamination and low infection rates have seriously impacted the results.

Control of *Taenia solium*: impact of repeated mass drug administration with praziquantel on taeniosis prevalence in rural communities of Tanzania

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Taenia solium infection is a zoonotic problem in many areas where poverty and poor sanitation exist. Control tools are available, but an evidence-based algorithm for the most effective combination remains to be developed and evaluated. This study aimed to describe the effect on the prevalence of taeniosis, of mass drug administration (MDA) with praziquantel administered to school-age children. Three cross-sectional surveys were performed in Mbeya Region, Tanzania in 2012 (R0), 2013 (R1), and 2014 (R2). In each survey approximately 3,000 people representing all ages participated from Mbeya (intervention 1) and Mbozi (intervention 2) districts. Participants were tested for taeniosis using a copro-Ag-ELISA. In total 9,064 stool samples were collected. There was significant difference in taeniosis prevalence between the two areas at R0 (X2-test, $p=0.007$) with an estimated prevalence of 1.5% and 3.0% in the intervention 1 and intervention 2 area, respectively. At R2 the difference had diminished (X2-test, $p=0.514$). In the intervention 1 area a decrease in infection was seen at both R1 ($p=0.024$) and R2 ($p<0.001$) compared to R0. The same analysis for the intervention 2 area showed a significant decrease in infection from R0 to R1 ($p=0.004$) and a borderline significant decrease from R0 to R2 ($p=0.051$). Logistic regression analysis based on stool samples from school-aged children (70% of samples) showed that in the intervention 2 area infection significantly decreased at R1 ($p=0.005$) and R2 ($p=0.001$), compared to R0. In the intervention 1 area infection significant decreased at R1 ($p=0.03$), but no difference was found at R2 ($p=0.29$), compared to R0. The study indicates that MDA might have effect on the targeted age-group, and a spill-over effect into the general population may occur when treatment is carried out over a prolonged period. The results supports control of *T. solium* as part of schistosomiasis control as a reasonable control approach to be explored further.

Capability of spatial distribution models in predicting schistosomiasis and fascioliasis

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Knowledge of the geographical distribution of schistosomiasis and fascioliasis is lacking in many parts of Africa and pose a challenge to health interventionists when planning intervention schemes. This knowledge gab can be filled with help from statistical prediction models by making use of sub-samples of parasites and vectors to produce proxy-maps of infection risk. But, as such risk maps are based on models and not true observations; there is a need to assess the quality before using them as decision support.

In this study we compared predictions of different stages in the infection complex: parasites (observations of eggs in faeces) and intermediate hosts (observations of snails), as a reality check for predictive performance. Furthermore, changes in spatial distribution over the past three decades of both parasite and snail were assessed, and subsequently used to discuss predictions of distributions in a future climate scenario.

We found an overlap of the schistosomiasis- and fascioliasis parasites with their respective vector snail species though the distributions of vector snails were somewhat larger than that of the parasites. Predictions of snail distribution can, conclusively, be used for conservative estimates of locations with risk of infection. Additionally, the magnitude of changes in historical time supports the projected distribution in the future climate.

This is a rare opportunity to assess the output performance of statistical species distribution models by the use of observational data, spaced well in time, and by assessing distribution of different stages in the lifecycles of the parasites.

The commercial diagnostic ELISA kit for antibodies to the filarial recombinant antigen Bm14: What is it measuring?

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A newly developed and marketed ELISA kit for measuring antibodies to the filarial recombinant antigen Bm14 (Bm14 CELISA, Cellabs Pty Ltd, Australia) has been proposed as a promising tool for monitoring transmission in lymphatic filariasis control programmes. Lymphatic filariasis is a major health problem in many parts of Africa, where it is caused by infection with the mosquito borne parasitic nematode *Wuchereria bancrofti*. Transmission occurs when infected mosquitoes land on the skin for blood feeding and thereby expose humans to infective larvae. Although the human antibody response to Bm14 is commonly claimed mainly to be a reflection of exposure to infective larvae, the direct association of exposure to antibody response has not been investigated.

The availability of sera collected from well characterized individuals with regard to age, sex, circulating filarial antigenemia, microfilaraemia, household vector biting and household exposure to infective filarial larvae provided a unique opportunity for analyzing the role and relative effect of these variables on the Bm14 antibody response. A total of 836 sera, collected from these individuals from two villages in Tanzania (Masaika and Kirare) and one village in Kenya (Kingwede), were analysed for IgG4 antibodies to Bm14, with the aim of determining the key drivers of this response. Associations between antibody response and the different variables were first analysed pairwise and thereafter in multivariable regression analyses, - first for individual villages and thereafter for the villages combined. The measured responses were highly related to circulating filarial antigenemia (a marker of infection with adult *W. bancrofti* worms) and age. However, an expected association with transmission intensity was not found. The Bm14 antibody response thus appeared more to reflect actual infection of individuals with adult filarial worms than ongoing exposure to transmission.

Distribution of species and subtypes of *Cryptosporidium* identified in ***Cryptosporidium***-positive human faecal samples in Denmark

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In Denmark, *Cryptosporidium* is screened for mainly in cases of chronic and travel-associated diarrhoea. Surveillance of human cryptosporidiosis in Denmark remains to be developed, and so little is known regarding the epidemiology of *Cryptosporidium* cases identified in the country, including the species involved in human cryptosporidiosis, transmission (endemic vs. imported cases), reservoir hosts, differences in disease severity, and 'hidden' outbreaks. Species and subtype identification by analysis of small subunit (SSU) rRNA and gp60 genes represent subtle and robust tools for molecular characterization and are widely applied in studies aiming to map the epidemiology of *Cryptosporidium*. The objective of this study was to identify the distribution of species and subtypes of *Cryptosporidium* identified in human faecal samples in Denmark by SSU rRNA and gp60 gene analyses. A total of 44 DNAs extracted directly from faeces using the NucliSENS® easyMAG protocol (bioMérieux, France) and positive by routine screening for *Cryptosporidium* by genus-specific real-time PCR were included in the study. Nested PCR targeting the SSU rRNA and gp60 loci, respectively, was applied to all 44 DNAs. PCR products were sequenced, and sequences were edited, aligned, and compared with reference sequences available in GenBank. Subtypes were identified according to the gp60 subtype consensus terminology and based on differences in trinulecotide repeats (TCA and TCG) and polymorphism in the post-repetitive region of gp60. Thirty-six samples were positive for *C. parvum*, seven samples for *C. hominis*, and one sample was positive for *C. meleagridis.* Regarding the distribution of subtypes, at least 18 different subtypes were detected; in five cases, the subtype could not be called by confidence mainly due to issues regarding sequencing across the trinucleotide repeat encoding the gp60 poly-serine tract. A predominance of the two common cattle subtypes IIaA15G2R1 (n = 10) and IIaA16G3R1 (n = 5) was observed. Zoonotic transmission of this subtype was suspected in some cases, since the clinical information accompanying the samples showed evidence of contact to calves. Renal transplantation had been performed in the patient positive for *C. meleagridis* (IIIgA27G3R1). Human cases of cryptosporidiosis in Denmark appear to be caused primarily by *C. parvum*, especially by the common calf subtypes IIaA15G2R1 and IIaA16G3R1, while other species, including *C. hominis* and *C. meleagridis* appear to account for about 15%. The fact that so many different subtypes were detected indicates that the samples represented mainly sporadic cases, although minor, 'hidden' outbreaks could not be ruled out.

Effect of vacuum packing and temperature on survival and hatching abilities of strongyle eggs

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Strongyle eggs usually hatch within a few hours or days if not refrigerated. This poses a problem during sampling in the field and as oxygen is needed for development, it is recommended to reduce air supply during transport. The present study therefore investigated the effect of vacuum packing and temperature on survival of strongyle eggs and their subsequent ability to hatch and develop into L3. Fresh fecal samples were collected from calves infected with *Cooperia oncophora*, pigs infected with *Oesophagostomum dentatum*, and horses infected with *Strongylus vulgaris* and cyathostomines. The samples were allocated to four treatments: vacuum packing and storage at 5°C or 20°C (5V and 20V), no vacuum packing and storage at 5°C or 20°C (5N and 20N). The number of eggs per gram feces (EPG) was estimated every fourth day until day 28 post set up (p.s.) by a concentration McMaster. Larval cultures were prepared day 0, 12 and 28 p.s. and the number of larvae per gram feces (LPG) determined. For *C. onchophora*, the EPG was significantly higher in vacuum packed samples day 28 p.s., regardless of temperature However, *O. dentatum* EPG was significantly higher in samples kept at 5°C, irrespective of packing mode. For the horse strongyles, vacuum packed samples at 5°C had a significantly higher EPG compared to the other treatments after 28 days. L3 recovery resembled the EPG trends for *C. onchophora* and *O. dentatum*, while recovery of horse L3 was very low for both strongyle species at day 28 p.s.

Removal rate of *Ascaris suum* eggs from solid surfaces

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Helminthiasis is an important public health problem worldwide, especially in low and middle-income countries. Helminth eggs adhere to different surfaces, e.g. soil particles, hands, particles in water, but also laboratory utensils. Despite these characteristics which have direct implications on egg transmission, little is known about the adhesion properties of helminth eggs. The aim of this study was to estimate the removal of adhered *Ascaris suum* eggs from PVC plastic and glass surfaces by flushing the surfaces with different solutions. The experiments were performed with perfusion chambers mounted on a surface with adhering *A. suum* eggs (1,000 eggs deposited with 10µl of water), and then flushed with different solutions (ddH2O, 1% Tween20, and 0.5M NaOH) and flow velocities ranging from 1.68 ml/min (low shear stress, 0.11 Pa) up to 10.06 ml/min (high shear stress, 0.67 Pa). Each experiment ran for 30 min, and the number of eggs removed was enumerated every 5 min. Different factors of importance to egg removal were tested, such as drying time of eggs to the surface (0, 1.5, 2.5, 12 and 24 hrs), coating of PVC and glass surfaces (1% Tween20, and RainX repellent), and flushing solutions (ddH2O, 1% Tween20, and 0.5M NaOH). The results showed that only freshly deposited eggs (drying time 0) were removed from both PVC and glass surfaces at both high and low shear stress (100% removal of the eggs after 30 min at high shear stress and 96.63% removal at low shear stress). No removal at all was seen for eggs dried on a surface for 1.5 hrs (where all the water containing the eggs had just evaporated) or longer periods, regardless of surface type, coating, flushing solution, or flow velocity. In conclusion, *A. suum* eggs showed a very strong adherence towards the surfaces tested. Further investigations are being carried out with other plastic types, detergents/surfactants, e.g. Benzethonium chloride 0.1% which is a quaternary ammonium compound that lowers the surface tension.

Use of microfungi for reducing the number of chicken ascarid eggs in soil

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Chicken ascarid (*Ascaridia galli/Heterakis* spp.) eggs can survive for years in the environment. Recent agar based models have shown a promising effect of some species of microfungi in their ability to degrade chicken ascarid eggs. The current study went one step further examining the application of three species of naturally occurring soil microfungi (*Pochonia chlamydosporia* Biotype 10, *Metarhizium brunneum* and *Trichoderma harzianum*) for limiting the number of chicken ascarid eggs in soil. Unembryonated chicken ascarid (mainly *A. galli*) eggs were added to sterilised soil in Petri dishes, with or without a fungus (*P. chlamydosporia* Biotype 10, *M. brunneum* or *T. harzianum*) at 22 °C. The eggs were isolated from the soil by a sieving and flotation technique, and counted using McMaster slides at day 0 and 30 post treatment (p.t.). With reference to day 0 p.t., the number of eggs in the *P. chlamydosporia* Biotype 10, *M. brunneum, T. herzianum* and the untreated Control plates at day 30 p.t. was reduced by 45.7%, 30.2%, 4.7% and 4.6%, respectively. The results showed that *P. chlamydosporia* Biotype 10 could be used as a potential biocontrol agent for reducing ascarid egg contamination on farm. However, further studies using unsterilized soil are important to examine the influence of biotic factors on the efficacy of the fungus.

Secretion of RNA-containing extracellular vesicles by the porcine whipworm, *Trichuris suis*

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Trichuris suis is a common parasitic helminth of pigs. As with many other parasites, *T. suis* ensures its own survival by evading host immune responses, but little is known about how this is achieved. Micro RNAs (miRNA) have been shown to be involved in various immunological processes by post-transcriptional regulation of specific genes, and the potential of using these molecules as biomarkers of disease is currently being examined. It has recently been shown that parasites may secrete extracellular structures such as exosomes and microvesicles, containing proteins and miRNA. The fusion of these structures with host cells has been demonstrated and a role of exosome-derived miRNA in host gene regulation has been suggested. In the present study, we show that exosome- and microvesicular-like structures are secreted by *T. suis* L1 larvae, and also demonstrate the presence of RNA of miRNA-size inside these structures. A potential role of these molecules in host-parasite interactions is suggested. In addition, an electron-dense layer covering the surface of the larvae was observed, which may play a function in host immune evasion.

Paired analysis for documenting host responses to parasite infection in natural free ranging mammals

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Studying physiological reactions to parasite infection in free ranging wildlife can be difficult because seasonal variation in host attributes co-vary with seasonal patterns of parasite infection. Documenting that host responses are present cannot be done by simple sampling, but requires stratified sampling procedures. From a biobank of bank voles (*Myodes glareolus*) collected in different months and locations in Denmark in 2007, two sets of paired samples were chosen to analyze the effect of *Hydatigena taeniaeformis* on the level of corticosterone in hair. This was done selecting paired samples (infected and non-infected liver) in two different seasons (late spring and winter). This type of study was chosen because of the parasite´s life-cycle coincide with seasonal variation in the hormone levels (corticosterone is high in summer and low during the winter). The analyses show that corticosterone is elevated in *M. glareoulus* infected with *H. taeniaeformis*, but not significantly. Also, the higher level of corticosterone is less than in unspecific infections causing liver-spots – which by PCR was confirmed not to be *H. taeniaeformis*. With this double paired data the variability is reduced, allowing us to conclude that there is no association of this parasite on the corticosterone levels in this rodent, and hence other factors must be responsible for the observed differences. This illustrates the importance of a good study design for a good data analysis.

Swedish wolves are not endangered,- by parasites

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The gray wolf inhabits various regions in the northern hemisphere. They are generally opportunistic feeders and prey on large and small mammals, birds and vegetables but generally more than 90% of the wolves' feed derives from moose, which may shape the parasite fauna of the wolves. The population of gray wolves in Scandinavia is small and protected; therefore it was rarely subject to systematic studies on its disease biology, especially gastrointestinal parasites. Twenty wolves in Sweden were hunted, on a limited license as a part of a wildlife management program in 2010, and the prevalence of gastrointestinal helminths were investigated after post mortem by macroscopy (PM) and coprology. Six helminth species were recovered by PM from 18 wolves (90%): *Uncinaria stenocephala* (90%), *Taenia spp*. (45%), *Alaria alata* (25%), and *Mesocestoides spp*. (5%). Of the *taeniid* specimens typed by multiplex PCR and sequencing of the cox1 gene, 25% belonged to *Taenia hydatigena* and 25% to *Taenia krabbei*. This parasite diversity is lower than what was found in other population of wolves in the northern hemisphere. Previously, Capillarid eggs in wloves' feces were not diagnosed to the species level, but in this study we report the first finding of eggs of *Eucoleus boehmi* in 12 wolves (60%). Metastrongylid larvae resembling *Angiostrongylus vasorum* were recovered by coprology, but molecular analysis suggested infection, or passant, of other metastrongylids. Despite being parasitized, the wolves were generally in good body condition; therefore the current parasite infestation had no indications of clinical implication or reduced fitness of the hosts. In general, the wide scale ranging capability of wolves and the relative long life span and being protected, wolves can act as reservoir and facilitate transmission of parasites of zoonotic and/or veterinary impact. However, the currently reported parasite fauna of the Swedish wolves have no significant impact for humans or domestic animals.