Occurence of Cryptosporidium spp. in low quality water and on vegetables in Kumasi, Ghana

Petersen, T. B.; Petersen, H. H.; Abaidoo, R. C.; Enemark, Heidi; Dalsgaard, A. D.

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Frederiksberg Campus, Lecture room 1-01 (Festauditoriet),
Bülowsvej 17, 1870 Frederiksberg C

ORAL PRESENTATIONS – KEY NOTES

Mark Booth mark.booth@durham.ac.uk

Environmental change and the future transmission potential of neglected tropical diseases

Mark Booth
Durham University

Neglected Tropical Diseases are a class of globally important infection that have been historically neglected in terms of control and research. Recent efforts at control have been partially successful but could be improved by developing a more holistic knowledge base of all factors that cause variation in transmission and disease risk. One important component of this knowledge base will be an understanding of the relationship between environmental change and transmission potential. Many NTDs are susceptible to environmental (ecological) factors due to the presence of free-living life stages. Several infections have life-stages that infect vectors and intermediate host which themselves are susceptible to environmental factors. This presentation will demonstrate how the environment (including human behaviour and residential location) affects the transmission of several NTDs within and between populations, over time and space in the African context. Attention will be given to describing efforts to understand the impact of environmental change on one parasite in particular – schistosomiasis – from the effects of El Nino on intermediate host snails in a Kenya river system, via investigations of varying lake conditions and snail survival in Uganda, to 30 year projections of schistosome transmission potential across East Africa under different scenarios of global warming

Kim Nørgaard Mouritsen kim.mouritsen@biology.au.dk

Parasitism, climate change and the structure of coastal communities

Kim Nørgaard Mouritsen
Department of Bioscience at Aarhus University

Trematodes are widespread metazoan parasites in coastal animal communities, where they typically infect shorebirds as definitive host, snails or bivalves as first intermediate host, and - depending on the trematode species in question – a range of e.g. polychaetes, bivalves, crustaceans and fish as second intermediate host. The trematodes affect their intermediate hosts in several ways, including changed behavior, growth, reproduction and mortality rates. The latter is often parasite intensity-dependent, and because the transmission of larval trematodes is strongly temperature-dependent, these parasites impact on intermediate host populations is likely affected by climate changes, including anthropogenic global warming and natural climate fluctuations.
By using coastal amphipods and their microphallid trematodes as model-system, I will here through field studies, out-door mesocosm experiments and laboratory experiments demonstrate that temperature increases in lieu of e.g. global warming can have severe repercussions for the amphipod hosts, ultimately leading to their local extinction. In turn, the parasite-induced regulation of amphipod populations can have significant ramifications to also the surrounding non-host community of plants and animals in the coastal ecosystem.

CONTRIBUTED ORAL PRESENTATIONS

Ulrik Pedersen ubop@sund.ku.dk Young Scientist Award candidate (**YSAc**)

Climate Change Impact Assessment of the Geographical distribution of trematode schistosomes by modelling the ecological landscape of the intermediate snail host

Ulrik Pedersen (1), Anna-Sofie Stensgaard (1,2) Birgitte J. Vennervald (1), and Thomas K. Kristensen (1)

(1) Department of Veterinary Disease Biology, University of Copenhagen;
(2) Centre for Macroecology, Evolution and Climate, Natural History Museum of Denmark, University of Copenhagen

Schistosoma haematobium and *S. mansoni* are trematode helminths causing urinary and intestinal schistosomiasis, respectively. These blood flukes reside in the veins of the urinary system and intestines where they undergo sexual reproduction. Eggs are excreted via urine/faeces and after asexual reproduction in the intermediate fresh water snail host, humans are infected when coming into contact with the free swimming cercaria.
Both parasite and host snail are sensitive to climate and the over-all geographical distribution can be modelled through information on e.g. temperature and precipitation at know snail presence locations. The resulting maps can serve as information to health planners on efficient resource allocation.
Systematically collected snail occurrence data, from all regions of Zimbabwe were used in a maximum entropy statistical algorithm (MaxEnt) to predict current snail habitat suitability on a 10km resolution. The model was parameterised with a regional climate model (ECHAM/HIRHAM).
The aim of this work was to identify areas of possible high endemicity of urinary and intestinal schistosomiasis in Zimbabwe, based on snail presence data and to forecast the schistosomiasis distribution in a future climate.
Prediction of current distribution of *Bulinus globosus*, (the *S. haematobium* host) identifies hotspots in the northern highveld but with large areas in the southern lowveld also holding suitable habitats. *Biomphalaria pfeifferi* (the *S. mansoni* host) has a somewhat more narrow distribution with large areas in the highveld holding suitable habitats but with a more distinct delimitation. Suitable habitats are predicted to be reduced in the predicted warmer and drier climate of 2090 with distributions only close to their present core distribution area.
On the basis of this modelling work, it is concluded that the distribution of schistosomiasis is likely to be reduced in the areas that presently holds the least suitable snail habitats.

Anna-Sofie Stensgaard asstensgaard@snm.ku.dk **YSAc**

Potential effects of climate change on schistosomiasis: predicting current and future distributions of *S. mansoni* and intermediate host snails in Africa.

Anna-Sofie Stensgaard (1, 2) and Thomas K. Kristensen (2)

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Climate is considered to be the predominant range-determining mechanism for most species at large geographical scales, but a species' ranges may be controlled by other environmental or non-environmental factors. Like any other species, many infectious disease agents have natural habitats: they are found in focal areas where the spatial distribution of the parasite, host, vector and required environmental (e.g. climate) conditions coincide. This is also the case for snail-borne schistosomiasis, where eg. the rate of development and survival for both parasite and snail is highly temperature dependent. Hence, it is not difficult to imagine that the on-going and predicted global climatic changes are likely to alter the distribution and transmission patterns of this neglected tropical disease, and appraisal of both present and future impact is a pressing public health issue. However, the interactions between climate, schistosomes and the multiple species of intermediate host snails are complex, and challenging to account for in climate change impact models. Together with a paucity of accurate data to parameterize models, this probably remains the main reason for the relatively few studies that have attempted to forecast future impacts of climate change on schistosomiasis. Here, I highlight a few of these studies, including examples of recent work exploring the large-scale environmental drivers of the distribution of several intermediate host snail species (*Biomphalaria* spp.), which has provided new insights into the eco-epidemiology of intestinal schistosmiasis in Africa. We suggest that a combination of climate-based statistical distribution modelling with process-based models of parasite development, and not least a solid knowledge of snail ecology, as a promising way forward in the challenging field of predicting impacts of climate change on schistosomiasis.

Nao Takeuchi-Storm naonow_t@hotmail.com **YSAc**

Climate matters? - Assessment of host and environmental factors on *Echinococcus multilocularis* prevalence by a meta-analysis

Nao Takeuchi-Storm (1), Ian David Woolsey (1), Per Moestrup Jensen (1), Brian Lund Fredensborg (1), Christian Bressen Pipper (2), Christian Moliin Outzen Kapel (1)

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Echinococcus multilocularis is a zoonotic cestode and accidental ingestion of its eggs excreted via faeces of carnivores may cause severe and eventually fatal disease, alveolar echinococcosis (AE), in humans. The adult intestinal tapeworm establish in carnivore hosts (mainly foxes), and tissue cysts develop in rodent intermediate hosts via ingestion of eggs deposited on vegetation by the definitive hosts. The parasite is widely distributed in the northern hemisphere, with some endemic regions in central Europe, most of northern and central Eurasia, and part of North America. It appears to have expanded its geographical distribution over the past decades, apparent from new observations in e.g. Holland, Denmark, and Sweden. It is therefore important to identify contributing factors to such spread, in order to assess potential changes in spatial distribution, identify high-risk areas, and evaluate eventual measures for prevention and control of AE. A meta-analysis was conducted on 174 documents, by a generalised estimation equation approach (GEE) to assess the effect of taxonomic, environmental and diagnostic variables on *E. multilocularis* prevalence in definitive and intermediate hosts. In red fox (*Vulpes vulpes*) populations, the prevalence was approximately 10 times higher than among domestic dogs (*Canis lupus familiaris*); the diagnostic method and its interaction with species playing an important factor in determining prevalence of the parasite. For intermediate hosts, taxonomy was an important factor, although the most susceptible rodent genera could not be determined. Although a particular environmental factor could not be identified as a significant determinant for the parasite prevalence, temperature and precipitation appear to influence the transmission of *E. multilocularis*.

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Kinetics of B-cell responses to *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) in Ghanaian women naturally exposed to malaria parasites

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Naturally acquired protective immunity to *Plasmodium falciparum* malaria takes years to develop. It relies mainly on antibodies, particularly IgG specific for PfEMP1 proteins on the infected erythrocyte surface. It is only partially understood why acquisition of clinical protection takes years to develop, but it probably involves a range of immune-evasive parasite features, not least PfEMP1 polymorphism and clonal variation. Parasite-induced subversion of immunological memory and expansion of “atypical” memory B cells may also contribute. In this first longitudinal study of its kind, we measured B-cell subset composition, as well as PfEMP1-specific antibody levels and memory B-cell frequencies in Ghanaian women followed from early pregnancy up to one year after delivery. Cell phenotypes and antigen-specific B-cell function were assessed three times during and after pregnancy. Levels of IgG specific for pregnancy-restricted VAR2CSA-type PfEMP1 increased markedly during pregnancy and declined after delivery, whereas IgG levels specific for two PfEMP1 proteins not restricted to pregnancy did not. Changes in VAR2CSA-specific memory B-cell frequencies showed typical primary memory induction among primigravidae and recall expansion among multigravidae, followed by contraction post-partum in all. No systematic changes in the frequencies of memory B cells specific for the two other PfEMP1 proteins were identified. The B-cell subset analysis confirmed earlier reports of high atypical memory B-cell frequencies among residents of *P. falciparum*-endemic areas, and indicated an additional effect of pregnancy. Our study provides new knowledge regarding immunity to *P. falciparum* malaria, and underpins efforts to develop PfEMP1-based vaccines against this disease.

Rebecca Wendelboe Olsen rwo@hotmail.com **YSAc**

Mapping the Binding Site of a Cross-reactive *Plasmodium falciparum* PfEMP1 mAb Inhibitory of ICAM-1 Binding

Rebecca W. Olsen (1), Frank Lennartz (2), Anja Bengtsson (1), Louise Joergensen (1), Eric Forest, Alan Brown (3), Lea K. Barfod (1), Matthew K. Higgins (2), Anja T. R. Jensen (1)

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The virulence of *Plasmodium falciparum* is linked to the ability of infected erythrocytes (IE) to adhere to vascular endothelium mediated by surface expressed *P. falciparum* erythrocyte membrane protein 1 (PfEMP1). In this study, we report for the first time the functional characterization of a mAb recognizing a panel of PfEMP1s and inhibiting their ICAM-1 binding. The 24E9 mouse mAb was produced against PFD1235w DBLβ3_D4, a group A PfEMP1 associated with severe malaria. 24E9 recognizes native PfEMP1 expressed on the IE surface and show cross-reactivity with and cross-inhibition of the ICAM-1 binding capacity of domain cassette 4 (DC4) PfEMP1s. Surface plasmon resonance (SPR) experiments show that the 24E9 Fab fragment binds DBLβ3_D4 with nanomolar affinity. The antigenic regions targeted by the 24E9 Fab were mapped by hydrogen-deuterium exchange mass spectrometry (HDX-MS) and revealed three discreet sites which are strongly solvent-protected in the complex. When mapped onto a homology model of DBLβ3_D4, these sites cluster to a defined, surface-exposed region and make up a conformational epitope. This region lies on the convex surface of DBLβ3_D4, previously identified as the potential ICAM-1 binding site of DBLβ domains. This observation is further supported by small angle X-ray scattering (SAXS) analysis of the DBLβ3_D4::24E9 Fab complex. These findings show that 24E9 inhibits ICAM-1 binding by blocking the previously predicted ICAM-1 binding site on DBLβ3_D4, and support the potential of mAbs, such as 24E9, as a therapeutic tool to inhibit ICAM-1-specific adhesion of group A PfEMP1 expressed by *P. falciparum* IE during severe malaria.

Ramesh Subramani ramesh@sund.ku.dk **YSAc**

Mechanical measurements on *Plasmodium falciparum* infected erythrocytes by Atomic Force Microscopy

Ramesh Subramani (1), Zhang Shuai (2), Mingdong Dong (2), Lars Hviid (1), Lea Barfod (1)

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When *Plasmodium falciparum* invades the human red blood cells, several modifications occur in the infected erythrocyte (IE) membrane surface. The important modifications are progressive changes in mechanical properties of the cell and the formation of nanoscale protrusions, which are known as 'knobs' on the erythrocyte membrane. These characteristic changes in the erythrocytes develop the properties of cyto-adhesion and rosetting. Therefore, a detailed knowledge of structural and mechanical properties of infected cell is required to understand how these changes regulate the cyto-adhesion and rosetting. Atomic force microscopy (AFM) enables to measure the mechanical properties of living cells by measuring local and overall properties of individual cells under physiological conditions. In this work, by using recently developed PeakForce-Quantitative Nanomechanical Mapping technique, we are measuring structural and mechanical properties of the late stage IE's and comparing with the non-infected erythrocytes.

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Combining parasitology and ecotoxicology, and using a parasite-host model to assess the effects of parasitism and insecticide exposure.

Fredrik Samuelsson (1), Nina Cedergreen (1), Nicolai Vitt Meyling (1), Brian Lund Fredensborg (1)

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There is a knowledge gap regarding the impact parasites have on host populations, the impact pollution have on host populations; and the impact on populations when exposed to multiple stressors such as insecticide residues and parasitic infections. The recent losses of managed honey bees (*Apis mellifera*) have raised the attention to the environmental effects from pesticide residues. As a result a restriction of Imidacloprid and two other neonicotinoid insecticides, has been implemented in the EU. However, this group of insecticides alone does not seem to carry the responsibility of the decline in bee populations, and a combined effect of mite infestations, virus infections, parasites, and insecticides is suggested to be the cause of the bee colony collapse. In this study we used the established parasite *Hymenolepis diminuta* – host *Tenebrio molitor* model to evaluate combined effects of a parasitic infection and exposure to sub-lethal doses of insecticides. Beetles were infected with *H. diminuta* and exposed to a sub-lethal dose of Lambda-Cyhalothrin. No effect of insecticide exposure to the host was found on the parasites establishment success. The effect of insecticide exposure and infection led to a higher decrease in beetle fecundity compared to insecticide exposure solely. In order to make theoretical models regarding the effects on populations exposed to multiple stressors and to pinpoint what factors are leading to the declining populations of honey bees, there is a need for baseline data on parasites, pollutants and environmental changes. The extensive research that already has been conducted on tenebrionid beetles and *Hymenolepis* spp. regarding physiology, reproduction and their parasite-host relationship; and the cosmopolitan distribution of both genera, makes this constellation a flexible and relatively low cost model continuous research.

Nethe Eva Touborg Bune nethebune@gmail.com **YSAc**

Metacestode establishment, immune response and corticosterone in the intermediate host *M. agrestis*, following oral infection with different doses of *Echinococcus multilocularis* eggs

Ian Woolsey (1), Nethe Eva Touborg Bune (1), Per Moestrup Jensen (1)

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Transmission of the zoonotic parasite *Echinococcus multilocularis* and infection of the intermediate host is dependent on the host immune response. Dose of antigen and glucocorticoid endocrine hormones have been shown to influence the immune response. The aim of this study was to perform comparative analysis of metacestode establishment, splenocyte proliferation, nitric oxide (NO) and tumor necrosis factor (TNF) production in response to *E. multilocularis* antigen, and to quantify corticosterone (CORT) concentration in *Microtus agrestis* infected with 100, 500 or 1000 *E. multilocularis* eggs. As control, C57BL/6 mice were infected with 1000 eggs. There was no effect of sex or weight on host variables. There was effect of species on TNF production, with significantly higher TNF production in C57BL/6 mice and low TNF production in *M. agrestis*. We found significant lower CORT in the 100-dose group. Correlation analysis of metacestode establishment demonstrated significant effect of dose. Furthermore, metacestode establishment was significantly correlated with CORT concentration, TNF concentration, splenocyte proliferation and female animals. The dynamics of metacestode establishment is affected by dose of eggs and correlated with host physiological and immunological response interactions, which remained unexplained in this study. As we were unable to exclude adverse physiological and immunological effects in the 500 and 1000-dose groups, an infection dose of 100 *E. multilocularis* eggs is considered as the optimal dose for experimental infections.

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Co-prevalence of Schistosomiasis and *Taenia solium Taeniosis*/*Cysticercosis* in Sub-Saharan Africa? Implications for Mass Drug Administration against Schistosomiasis

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The World Health Organisation (WHO) is aiming for control by year 2020 of two neglected tropical diseases: schistosomiasis and *Taenia solium taeniosis*/*cysticercosis*. As vaccines against these diseases are not yet available, WHO's strategy for achieving the goal is preventive chemotherapy by mass drug administration (MDA). The preferred anthelminthic drug used in MDA against schistosomiasis is praziquantel (PZQ) mainly because of the ease of administration. However, the recommended PZQ dosage used (40mg/kg) is suspected to aggravate the symptoms of the neural form of cysticercosis (NCC) which are headaches, seizures and in rare occasions even death. In sub-Saharan Africa schistosomiasis is widespread, in some countries the prevalence of the disease lies above 50%. At the same time pig farming is getting more popular, for instance it has been quoted as the fastest growing livestock sector in Uganda. There is limited knowledge on the presence and prevalence of *T. solium*, whether it is porcine cysticercosis, taeniosis or human cysticercosis/NCC. It is suspected though, that both diseases co-exist in many rural areas, where the conditions for maintaining the life cycle of both diseases are favourable. Based on a review of the literature we have investigated the current co-endemicity of schistosomiasis and *T. solium taeniosis*/cysticercosis. The geographic location of either type of cysticercosis manifestation was georeferenced using an online gazzetteer. The location was then entered into a level 2 district map of sub-Saharan Africa, resulting in a cysticercosis presence map on district basis for each country. Cysticercosis was found in 29 countries of sub-Saharan Africa. All are targeted for MDA against schistosomiasis. Based on the widespread co-endemicity, integrated intervention approaches for both diseases should be considered. Furthermore, increased emphasis should be given to investigating the possible adverse effects caused by the PZQ treatment.

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Independent emergence of the super resistance-conferring mutation at dhps codon 581 in East African *P. falciparum* populations.

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The emergence of super resistant falciparum malaria threatens the efficacy of sulphadoxine-pyrimethamine in intermittent preventive treatment in pregnancy and is characterized by the appearance of the A581G Pfdhps mutation on a background of the double mutant Pfdhps and the triple mutant Pfdhfr. We investigated the evolutionary origin of the A581G mutation by characterizing microsatellite diversity flanking Pfdhps triple mutant (437G+540E+581G) alleles from 3 localities and comparing it with double mutant (437G+540E) alleles from the same area. In Ethiopia both alleles were derived from a single lineage which was distinct from those in Uganda and Tanzania. Ugandan and Tanzanian triple mutants were derived from the Southeast African double mutant lineage. We conclude that the Pfdhps A581G mutation occurred multiple times on local Pfdhps double mutants, however a novel microsatellite allele incorporated into the Tanzanian haplotype since 2004 illustrates the local expansion of emergent triple mutant lineages.

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Investigating anti-parasitic effects of plant secondary metabolites: effects on swine nematodes

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(3) University of Reading

Organic and outdoor animal production presents challenges to animal health and productivity. In organic pig production, animals must have access to outdoor pastures which increases exposure to pathogens such as gastrointestinal nematodes. Moreover, the routine use of synthetic anti-parasitic drugs is not allowed. Thus, novel parasite-control options are required. We present here results from a comprehensive in vitro screen of plant secondary metabolites (PSM) from diverse plant sources on the economically important pig parasites *Ascaris suum* and *Oesophagostomum dentatum*. We focused on two classes of PSM commonly found in natural dietary sources – condensed tannins (CT) and sesquiterpene lactones (SL). Different CT were purified from a range of different plant sources to reflect the diversity of this group of PSM; SL were purified from forage chicory. The purified compounds were then tested in assays that measured inhibition of worm motility and migratory ability.
Condensed tannins had potent activity against *A. suum*, with substantial inhibition of migratory ability of in vitro hatched larvae (EC50 values ranging from 40 to 120 µg/mL). In contrast, migratory ability of *O. dentatum* larvae was not significantly affected. However, the motility of adult *O. dentatum* recovered from pigs was reduced after in vitro incubation with CT. The purified chicory extract showed potent inhibition of *A. suum* larval migration (EC50 value of 42 µg/mL) and was also active against larval and adult stages of *O. denatum*. Electron microscopy confirmed direct structural damage in nematodes exposed to the purified molecules. Therefore, plants rich in PSM such as CT and SL show promise as natural anthelmintics against two highly prevalent swine parasites. Experiments to determine in vivo efficacy and the mechanisms of the nematocidal action are ongoing.

POSTER PRESENTATIONS

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First report of Spirocerca sp. in Denmark – a tumor-inducing parasite in carnivores

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During routine health surveillance of wild carnivores in Denmark, several tumors, measuring up to 3.0 x 4.5 x 2.5 cm, were detected in the stomach and the omentum of an autopsied red fox (*Vulpes vulpes*). The fox was hunted in the Hanstholm Nature Reserve, which is 230 km from the closest mainland borders. The tumors had a thick layer of fibrous tissue in which adult worms of *Spirocerca* sp. were detected. Despite egg excretion by female worms (identified by histology and examination of female worms), no eggs were detected in feces by sedimentation, floatation with saturated sugar solution or sieving. Partial sequencing of two segments of the mitochondrial cox1 gene revealed unique sequences that were distinct from known isolates of *S. lupi* from Europe, Asia and Africa. Phylogenetic analysis supported the later finding by grouping Danish isolates in one separate node which was distant from other nodes including *S. lupi* from other countries. It is not known whether this case was an autochthonous infection or whether it was introduced by migrating paratenic or definitive hosts. This is the first report of *Spirocerca* sp. in Denmark. Additional molecular and/or biological studies are warranted to further characterize the isolated *Spirocerca* species.

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Parasites from the past

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(3) Centre for GeoGenetics, Natural History Museum, University of Copenhagen

Helminth infections used to be common in the humans in Denmark. *Trichuris* (whipworm) and *Ascaris* (round worm) produce very hard-shelled eggs which allow them to be recovered in the environment after extended periods of time. Notable findings include *Trichuris* eggs in the mummified remains of the Tollund Man (210. B.C) and the Grauballe Man (3-400 A.D.), as well as *Ascaris* eggs found in the intestinal remains of King Richard III (died 1485 A.D.).
In this project we will investigate how the diversity of food-borne parasitic infections has changed with cultural and dietary habits, hunting practice and intensity of animal husbandry. This is done by isolating and typing ancient DNA remains from parasite eggs found in archeological samples from across Denmark. Initial focus is on samples from Viking settlements near Viborg Søndersø (1018-1030 A.D.) and from the graveyard of the medieval church Sankt Clemens. This church was in operation from 1192-1536 A.D. and is situated under Strøget in the heart of Copenhagen.
The project is a collaborative effort between leading researchers at Copenhagen University in parasitology, ancient DNA and archeology. It is financed through the 'KU2016 Initiative' on excellence in research and is an integrated part of the multidisciplinary project, 'The Genomic History of Denmark'.

Tobias Petersen tib3s0@hotmail.com **YSAc**

Occurrence of *Cryptosporidium*spp. oocysts in low quality water and on vegetables in Kumasi, Ghana.

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Protozoan parasites belonging to the genus *Cryptosporidium* are transmitted e.g. by food and water and may cause severe diarrhoea, dehydration, weight loss and malnutrition. Ingestion of 10 oocysts can lead to infection and pathogenic symptoms.
Thus, to characterize *Cryptosporidium* spp. contamination level of river water, irrigation water and lettuce, 10L of water and 16 lettuce samples were collected four times in the period of, September – October 2013, with weekly intervals from six sample sites in and around Kumasi, Ghana. Oocysts were purified from water by sedimentation for 2 x 48 hours or pulsifying of lettuce followed by immunomagnetic separation and quantification by immunofluorescence microscopy, with sensitivities of 2 and 9%, respectively. After approximately six weeks of storage at 4C°, analysis and additional storage on slides, oocysts were washed off the slides and attempts to characterize Cryptosporidium spp. positive samples were done by PCR amplification and sequencing of the SSU rRNA, the HSP70 and the GP60 genes after.
Cryptosporidium oocysts were found in 75% of the water samples and on 43% of the lettuce with concentrations of 53 – 3268 per 10 L water and 11 – 118 oocyst per 15 g of lettuce.
Positive water samples on one or more occasions were demonstrated in all water and farm sites while all farms had positive lettuce samples on all occasions. Rainfall seemingly lowered the concentration of oocysts in water but not on lettuce. Molecular characterization of *Cryptosporidium* positive samples was unsuccessful, thus no conclusions can be drawn concerning sources of contamination. Nevertheless, the detection of high prevalence and concentration levels of *Cryptosporidium* oocysts on vegetables consumed raw and in water with direct contact to humans entails a potential risk of infection in humans. Implementation of preventive measures based on this study should be considered and actions taken accordingly.

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Detection of *Trichobilharzia* sp. infected snails in freshwater lakes around Copenhagen

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Cercarial dermatitis (also known as swimmer's itch) is a globally distributed skin disease commonly caused by avian schistosomes belonging to a number of genera. In Europe, infections of humans are most often caused by the larval stages, cercariae, of a number of species within the genus *Trichobilharzia* and probably also the genus *Bilharzialla*. Studies have shown that some species can penetrate skin and migrate through mammalian tissue to the visceral organs, and in the case of *T. regenti* to the central nervous system, suggesting that other symptoms than dermatitis might occur in humans accidentally infected. A series of avian species act as final hosts, freshwater snails are intermediate hosts and humans may be infected if they are exposed to water contaminated with cercariae. *Trichobilharzia* sp. and *Bilharziella* sp. have been detected in several European countries, including Denmark, and outbreaks of cercarial dermatitis are increasingly being reported, emphasizing the need for surveillance and mapping of parasite distribution. The objective of this study was therefore to investigate the current occurrence of avian schistosomes in selected lakes around Copenhagen and to identify the found species using PCR and sequencing. Furthermore, we aim to test a more sensitive and specific PCR method to diagnose infected snails. Pulmonate freshwater snails were collected from mid-August to mid-October 2013 from several lakes and ponds around Copenhagen and investigated for avian schistosomes by shedding and subsequent morphological investigation of the released cercariae. Positive snails were found in Furesø, in a small pond close to Furesø and in Søndersø, and the overall prevalence was 0.5% (N=212) in *Lymnaea stagnalis* and 1.7% (N=116) in *Radix* sp. Using PCR and sequencing, the cercariae were identified as *T. franki* and *T. regenti*. The implications of these findings as well as further studies into surveillance methodology are discussed.

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Transfected HEK293 Cells Expressing Functional Recombinant Intercellular Adhesion Molecule 1 (ICAM-1) – A Receptor Associated with Severe *Plasmodium falciparum* Malaria

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Intercellular adhesion molecule 1 (ICAM-1) is a membrane-bound glycoprotein expressed on endothelial cells and cells of the immune system. Human ICAM-1 mediates adhesion and migration of leucocytes, and is implicated in inflammatory pathologies, autoimmune diseases and in many cancer processes. Additionally, ICAM-1 acts as receptor for pathogens like human rhinovirus and *Plasmodium falciparum* malaria parasites. A group of related *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) domains, the DBLβ, mediates ICAM-1 binding of *P. falciparum*-infected erythrocytes. This ICAM‑1-binding phenotype has been suggested to be involved in the development of cerebral malaria. However, more studies identifying cross-reactive antibody and ICAM-1-binding epitopes and the establishment of a clinical link between DBLβ expression and e.g. cerebral malaria are needed before the DBLβ domains can be put forward as vaccine candidates and go into clinical trials. Such studies require availability of functional recombinant ICAM-1 in large quantities. In this study, we compared recombinant ICAM-1 expressed in HEK293 and COS-7 cells with mouse myeloma NS0 ICAM-1 purchased from a commercial vendor in terms of protein purity, yield, fold, ability to bind DBLβ, and relative cost. We present a HEK293 cell-based, high-yield expression and purification scheme for producing inexpensive, functional ICAM‑1. ICAM-1 expressed in HEK293 is applicable to malaria research and can also be useful in other research fields.

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Discovery of novel non-immune IgM binding PfEMP1 variants in *Plasmodium falciparum* line NF54

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The antigen family *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) mediates binding of infected erythrocytes (IEs) to a variety of human receptors. The IE binding phenotype depends on which PfEMP1 variant is being expressed. Each parasite genome contains about 60 var genes encoding different PfEMP1 variants. This and the substantial interclonal var gene diversity together make the repertoire of PfEMP1 variants immense. PfEMP1 variants involved in placental malaria (VAR2CSA) and some variants displaying the rosetting phenotype (shown to be correlated with severe childhood malaria) are able to bind non-immune IgM. We have previously shown that this binding results in masking of protective IgG epitopes on VAR2CSA-positive IEs. In order to determine if this apparent immune evasion is a general property of IEs expressing IgM-binding PfEMP1 variants, and to map the IgM binding epitope, we set out to identify new IgM-binding PfEMP1 variants in the genetically well-characterized *P. falciparum* line NF54.
Switching among transcription of different var genes rarely occurs in long-term in vitro cultures, probably as a reflection of epigenetic memory. Therefore, the selection of IEs expressing particular PfEMP1 variants can be troublesome. We overcame this problem by establishing a novel method based on the NF54 clone G6 that had its epigenetic memory deleted by the plasmid VBH. This clone was used in flow cytometry assisted single-cell sorting experiments to select for IEs binding non-immune IgM.
So far, we have identified four additional PfEMP1 variants in NF54 that bind non-immune IgM. Furthermore, we have further defined the binding site for IgM to the C-terminal part of IgM-binding PfEMP1 variants. These results pave the way for a more comprehensive analysis of the functional significance of the binding of non-immune IgM that is characteristic of some, but by far all, PfEMP1 variants.

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Ex vivo dual placental perfusion – a novel model of placental malaria

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In placental malaria (PM) *P. falciparum* infected erythrocytes (IE) sequester in the placenta through specific binding of the *P. falciparum* VAR2CSA antigen to Chondroitinsulfate A (CSA). Anti-PM vaccine development is focused on hindering the placental parasite accumulation by identification of sub-units of VAR2CSA that induce antibodies inhibiting the binding of VAR2CSA expressing IE to CSA. Binding inhibition is studied in in vitro models. The ability of these models to reflect in vivo events is unknown and we currently lack more physiologically relevant models to study placental malaria. In this study the ex vivo dual placental perfusion model was implemented to study adhesion of IE in placental tissue.
Placentas are obtained from healthy pregnant women immediately after delivery. The fetal and maternal circulation of a cotyledon is reestablished and the cotyledon is placed in a heated chamber. IE are added to the maternal circulation and perfusate is collected at regular time intervals to measure the parasitemia. At the end of the experiment perfused tissue is collected for histological examination. The binding characteristics of parasites expressing VAR2CSA versus other PfEMP1s, and the specificity of the binding, are investigated.
Results show that erythrocytes infected with parasites expressing VAR2CSA accumulate in the ex vivo perfused placenta. The accumulation of VAR2CSA expressing parasites can be inhibited by soluble CSA. Histological examination of perfused tissue show accumulation of IE on the syncytiotrophoblast and in the intervillous space, similar to in vivo PM. Furthermore, parasite adhesion in the ex vivo perfusion model is investigated by transmission electron microscopy and compared to in vitro adhesion to BeWo cells. Experiments to study binding inhibition by anti-VAR2CSA antibodies are ongoing.

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Prevalence of pfmdr1N86Y (SNP) in *P.falciparum* isolated from the vicinity of Muzaffargarh Pakistan

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Plasmodium falciparum has developed resistance to nearly all classes of anti-malarial drugs available, leading to a resurgence of malaria mortality and morbidity in most of 3rd world countries including Pakistan. In a wild type parasite strain (Dd2), the replacement of only single amino acids can change it in its mutant type e.g. substitution of asparagines at position 86 in wild type for tyrosine found to be associated with emerging resistant against quinine, chloroquine (CQ) and amodiaquine (AQ). On the basis of high endemicity the area of Muzaffargarh, Pakistan was selected to find out the prevalence of pfmdr1N86Y (SNP) in *P.falciparum*. Blood films of suspected malarial patients (10,372) were examined microscopically from November 2008 to November 2010. *P.falciparum* positive samples were used for DNA extraction. Nested PCR was used to amplify pfmdr1 gene fragment of 330 base pairs. Frequency of chloroquine resistant pfmdr1 N86Y (SNP) was found by DNA sequencing method. Association of mutant (pfmdr1Y86) and wild type (pfmdr1N86) with different months, seasons, genders, ages, socioeconomic status, disease symptoms and *Plasmodium* stages was also found. Over all slide positivity rate (SPR), *Plasmodium vivax* and *P.falciparum* positivity rate was 21.40%, 19.37% and 2.03% respectively. The highly significant ($\chi 2=1456$; $p<0.001$) difference was observed between *P.vivax* (90.49%) and *P.falciparum* infection (9.51%). Existence of wild type pfmdr1 N86 (TAT) and mutant type pfmdr1 Y86 (AAT) was 33% and 84.30% respectively. No significant ($p>0.05$) association of mutant (pfmdr1 Y86) and wild type (pfmdr1N86) was found with different months, seasons, genders, ages, socioeconomic status, disease symptoms and Plasmodium stages. The high prevalence of pfmdr1 86Y depicts that Plasmodium circulating in Muzaffargarh has been selected by long use of CQ and AQ. So AQ is not recommended to be used as a partner drug in ACT in this locality.

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Parasitic fauna of the gyrfalcon (*Falco rusticolus*) in Iceland

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The Icelandic gyrfalcon (*Falco rusticolus*) population is small and vulnerable, under protection by Icelandic law and red-listed. As survival and health condition of birds of prey can be affected by a wide range of parasites. The present study investigates the occurrence, intensity and composition of the parasitic fauna of gyrfalcons from Iceland. In total, 31 gyrfalcons carcasses were examined. The samples were analysed and the cestode Mesocestoides sp., the nematode Serratospiculum sp., and six ectoparasites; the mallophagan *Degeeriella rufa*, the flea *Ceratophyllus vagabundus*, the louse fly *Ornithomya chloropus*, the ixodid mite *Ixodes caledonicus*, the astigmatan mite *Dubininia accipitrona* and a mesostigmatan rhynonyssid mite of an unknown taxonomical status were detected. These species have previously been identified parasitizing gyrfalcons. In addition, the mallophagan, *Nosopon lucidum*, a previously unknown gyrfalcon parasite, was identified. Furthermore, a number of helminth species were assigned as accidentally ingested by the falcons when eating prey. At least six distinct digeneans and five cestodes were found in the small intestines and five distinct nematode species were detected in the upper alimentary tract. Some of these species possibly represents rare host-specific gyrfalcon parasites.
No relationship was found between body condition of the gyrfalcons and the total parasite burden. However, one parasite showed a relation with the body condition: *Capillaria contorta*. This was further emphasized by the presence of frounce disease in these infected birds. Birds with frounce had a significantly lower body condition index than birds with clean mouth, suggesting higher vulnerability of infected birds compared to non-infected. The occurrence of frounce appears to be age related, with a peak in impacted birds about the age of 2 years. This finding suggests that birds are able to regain immunity when exposed to the parasite over a longer period.

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The Vicious Worm – A cysticercosis advocacy information tool

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The aim of the project is to create a computer-based information tool advocating the prevention and control of *Taenia solium* cysticercosis – "The Vicious Worm Disease". Cysticercosis caused by *T. solium*, is a zoonosis transmitted between humans and pigs and is widespread in many low and moderate income countries. In sub-Saharan Africa it affects millions of people and is a serious public health risk. The disease is emerging because: the number of pigs and people is rising, free roaming pigs are the norm, the demand for pork is increasing, meat inspection is inappropriate or lacking, open defecation is highly prevalent, personal hygiene is poor and knowledge regarding the disease is almost non-existing. The computer-based information tool will target three levels of stakeholders, and be displayed using an interactive map showing a village, a town and a city. At each of the three levels information on the transmission, diagnosis, treatment and prevention of *T. solium* cysticercosis/taeniosis in pigs and/or humans is shown. The information tool comprises a range of different education materials including cartoons, pictures, videos, and scientific and political texts designed for the different stakeholders. At the village level, information about The Vicious Worm will be provided to laymen. At the town level, detailed information about diagnosis, treatment and prevention of *T. solium* cysticercosis/taeniosis will be available for practitioners such as: medical doctors, veterinarians, meat inspectors and agricultural extension officers. At the city level, a policy brief and an information sheet about the disease for decision makers at national ministries can be found. Additionally a library with relevant internet links and central references will be available. The information tool will be distributed on a USB flash drive and made as a free shareware. The project is part of an EU-7th framework funded programme on Integrated Control of Neglected Zoonoses in Africa (ICONZ).

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Antimicrobial intake and risk of *Dientamoeba fragilis* infection

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Objectives: Associations between antimicrobial use and risk of enteric infection with intestinal protozoa are scarcely studied. The aim of this study was to quantify the risk of *D. fragilis* infection conferred by exposure to antimicrobials.
Methods: We conducted i) a registry-based retrospective cohort study of 9,945 Danish patients investigated for *D. fragilis* infection between 2008 and 2011, using the result of each patient's initial investigation for D. fragilis, either positive or negative, as outcome. We then captured data on patients' medicinal prescriptions from the Danish Register of Medicinal Product Statistics, calculating relative risks (RR) for *D. fragilis* infection by stratified binary regression. Furthermore, we conducted ii) a population based case-control study using controls sampled from the Danish Civil Registration System, individually matched (1:20) on sex, age (+/- 90 days), time of index date and geography (municipality), for each of the cases identified in the cohort study (i), and calculated Hazard ratios (HR) for *D. fragilis* infection by conditional logistic regression.
Results: Analysis showed that within time window of 91 days - 5 years prior to test, exposure to metronidazole, commonly used to treat *D. fragilis*, conferred a decreased risk of infection of *D. fragilis* (RR 0.85 (0.76, 0.96)). However, similar associations was found for antimicrobials not commonly used to treat *D. fragilis*, such as fluoroquinolones (RR 0.66 (0.55, 0.79)) and macrolides (RR 0.87 (0.82, 0.91)). In contrast, mebendazole exposure was associated with increased risk (RR 1.11 (1.06, 1.16)).
Conclusions: Our findings suggest a general detrimental effect of antibiotic intake on risk of *D. fragilis* infection, and also further support the hypothesis of *D. fragilis* transmission by pinworm vector.

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Effect of disinfectants on viability of *Ascaris suum* and *Ascaridia galli* eggs

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Ascaris suum and *Ascaridia galli*, the large round worms of pigs and poultry, respectively, persist even in intensive management systems. It is necessary to control these helminths to minimize production losses and improve animal welfare. Commercial disinfectants such as Virkon S® (potassium peroxymonosulfate, sodium dodecylbenzenesulphonate, sulfamic acid), FL-des GA® (glutaraldehyde, benzyl-C12-16-alkyldimethylammoniumchloride, didecyldimethylammoniumchloride) typically claim efficacy against viruses, bacteria and some fungi, but their efficacy against the thick-shelled nematode eggs has not been sufficiently documented. FL-des Allround® (hydroxybiphenyl 1-2 fatty acid, peracetic acid, hydrogen peroxide and acetic acid) also claims to eliminate ascarid eggs and coccidial oocysts. In the current study, unembryonated *A. suum* and *A. galli* eggs were exposed to Virkon S® (1 or 10%), FL-des GA® (1 or 10%) or FL-Des Allround® (0.3 or 3%) for 2 hr or 3 days in plastic tubes. Eggs were then washed with deionized water, incubated at 22–25°C and the viability (ability to embryonate) of 40-200 eggs from each tube was examined after 6 days. Compared to control eggs (deionized water), FL-Des Allround® killed 99% of *A. suum* eggs at the recommended concentration and time (3% for 2 hr), but killed only 14% of A. galli eggs though 3 days exposure killed all eggs. Virkon S®, at the recommended concentration and exposure time (1% for 2 hr), had no effect although exposure to 10% for 3 days disrupted the integrity of the eggs. FL-des GA® forte was ineffective irrespective of the concentration and exposure. The overall results indicate that A. galli eggs can be more resistant than *A. suum* eggs, suggesting some underlying structural differences between the two egg types. Furthermore, FL-des Allround® could potentially be used in intensive pig farms to control *A. suum*, though an appropriate application method has to be developed.

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Profiling gene expression in mesenteric lymph nodes in pigs with different levels of resistance to *Ascaris suum*

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A single nucleotide polymorphism on chromosome 4 (SNP TXNIP) has been reported to be associated with roundworm (*Ascaris suum*) burden in pigs. The objective of the present study was to profile the immune response mounted by pigs with two SNP TXNIP genotypes following an *A. suum* infection. We selected pigs with genotypes AA (n=24) and AB (n=23) and trickle-infected them with *A. suum* from eight weeks of age until necropsy eight weeks later. An uninfected control group (AA; n=5 and AB; n=5) was also included. At post mortem, we collected mesenteric lymph nodes and measured the expression of 28 selected genes. Recordings of worm burdens confirmed our previous results that pigs of the AA genotype were more resistant to infection than AB pigs. By estimating the genotype difference in relative expression levels in infected and uninfected animals, we found that IL-13 levels tended to change with genotype (P=0.077); specifically, pigs of the AA genotype had increased IL-13 expression following A. suum infection but IL-13 expression was unchanged in AB pigs. Furthermore, IL-13 expression tended to be associated with total worm burden in AB pigs (P=0.07). The expression of chemokine ligand 17 (CCL17) was up-regulated in AA pigs (P<0.05) but not in AB pigs following A. suum infection. Pigs of genotype AB had higher expression of the high-affinity IgG receptor (FCGR1A) than AA pigs in both infected and non-infected animals (P=1.85*10-11). In conclusion, our results suggest the two genotypes differ in the magnitude of their Th2-type response following *A. suum* infection.

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Worm expulsion dynamics of *Trichuris trichiura* after mebendazole treatment

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Together with *Ascaris lumbricoides* and hookworms, *Trichuris trichiura* is one of the 3 most important geohelminths, and human *trichuriasis* is a common disease in developing countries where poor sanitation, geophagy and use of night soil as fertilizer for vegetables are important sources of infection. Mebendazole and albendazole have a high efficacy against *A. lumbricoides*, a moderate efficacy against hookworms but a low cure rate for human *trichuriasis* when given as single dose. In this study a healthy individual was self-infected with *T. trichiura* by ingestion of 600 embryonated eggs. Twenty-three weeks post infection a single dose of 500 mg mebendazole was taken and expelled worms isolated for 14 days post treatment. The total number of worms expelled was 422, and were found between days 2-13 peaking at days 5-7 post treatment. Faecal egg count was 5500 eggs per gram of feces 4 weeks post treatment. A similar study was conducted with the same person and same infection dose but where 2 times 100 mg mebendazole was taken daily for five consecutive days. Here *T. trichiura* were recovered from days 2-9 post treatment with most worms expelled between days 4-7. A total of 101 *T. trichiura* were recovered and faecal egg counts went to nil. The worm expulsion pattern observed in these studies differ from what has previously been reported, having a higher recovery of worms and a longer expulsion period.

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Development of ribosomal small subunit amplicon based phylogenetic interrogation of the complete microbiome and eukarytome in human clinical samples using next-generation sequencing.

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The identification and differentiation of pathogens have relied on single targeted analysis, based on medical expertise and a most-likely-infection approach, which is laborious, expensive, time consuming and requires skilled personnel, especially with regard to differential diagnostic considerations. The small subunit (SSU) rRNA (16S or 18S) gene is a widely used target for molecular characterization and identification of microbes. Four different primer sets were designed for detection of parasitic helminths and protists and other non-human eukaryote species in human fecal samples plus one primer set for universal detection of bacteria. Each primer set targets groups of organisms, based on highly conserved regions spanning hyper-variable regions of the SSU rRNA gene, and found to target approximately 20,000 eukaryotic and 11,000 prokaryotic species by in silico analysis. PCR products are subsequently processed and pooled for multiplex purposes and sequenced on the Illumina MiSeq platform. Additionally, software for species annotation is currently being developed in collaboration with Danish Genome Institute (DGI, Aarhus, Denmark), which is able to use next-generation sequencing data for species annotation at a very rapid rate (hours). The output is a complete list of both the pro- and eukaryotic composition of any given sample. Data from a synthetic sequencing, 24 human fecal samples and an artificially constructed sample containing several fungi, parasites and bacteria will be presented at the meeting.

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Development and evaluation of a novel conventional PCR method for the detection of *Sarcocystis* in faecal samples

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Sarcocystis species are apicomplexan parasites with a two host life cycle including an intermediate host (prey) and definitive host (predator). Humans can be definitive hosts and may experience intestinal sarcocystosis. At least two species with humans as the definitive host exist including *Sarcocystis hominis* and *Sarcocystis suihominis* with cattle and pigs as intermediate hosts, respectively. Natural infections with S. hominis and S. suihominis are mostly described as asymptomatic, but experimental infections have been associated with gastroenteritis and eosinophilia. The prevalence of intestinal sarcocystosis has been estimated in cohorts of 362-3,500 individuals from Europe and Asia. The prevalence has been estimated to 1.1-10.4% in Europe and 7.9-23.2% in Asia. All studies used flotation and sedimentation of faecal samples combined with microscopy to detect oocysts/sporocysts. The prevalence of intestinal sarcocystosis in Denmark is unknown and there is no nucleic acid-based method available to diagnose intestinal *Sarcocystis* infections.
A conventional PCR was developed to detect DNA of Sarcocystis oocysts and sporocysts from faecal genomic DNA. Primers were designed to bind to a conserved region of the Sarcocystis 18S gene. The primers were evaluated on positive control material from *Sarcocystis rangiferi* and enabled amplification of the expected gene. Specificity testing included DNA from *Entamoeba histolytica*, *Giardia intestinalis*, *Blastocystis*, *Cryptosporidium*, microsporidia and *Dientamoeba fragilis* positive samples. The specificity is assumed to be high due to the absence of non-specific DNA amplification. We plan to test the sensitivity by spiking human faecal samples with oocysts purified from infected carnivores. After evaluation of the method the goal is to screen Danish and African human faecal DNA samples to get an overview of the current prevalence of intestinal *Sarcocystis* infection in these two study groups.

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Molecular Typing of *Trichuris* spp. Recovered From Pigs, Baboons and Humans

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The whipworms *Trichuris trichiura* and *T. suis* infecting humans and pigs, respectively, are expected to be separate but closely related species. Whipworms in non-human primates are historically believed to be T. trichiura. However, species delimitation based on morphological characters is not possible and recent analysis of the Internal Transcribed Spacer-2 (ITS-2) region suggested that there are several genotypes that infect human and non-human primates. Herein, we applied PCR linked Restriction Fragment Length Polymorphism (PCR-RFLP) on different nuclear regions (18S (rDNA), ITS-2 and beta-tubulin) of *Trichuris* spp. recovered from pigs, baboons, and humans. Worms were obtained from humans in Uganda (n=32), baboons kept in captivity in USA and Denmark (n=74) and pigs in Uganda, Denmark and USA (n=68). PCR-RFLP on the 18S region and the beta-tubulin gene showed identical gel band pattern for worms obtained from humans and baboons, suggesting that these are the same species (i.e. *T. trichiura*), and were distinctly different from worms of pigs. Similar finding was obtained for the ITS-2 regions except for 5 human-derived worms which had a “heterozygote” genotype, i.e. had a band pattern of both primate and pig worms. The latter observation may be due to lineage sorting or retention of ancestral polymorphism as this ”heterozygosity” was not observed for the two other markers. In conclusion, we found that the PCR-RFLP on the 18S and beta-tubulin gene was able to allocate worms into two groups, namely primate and pig derived but the 18S region was easier to amplify. In addition, our results suggest that baboon and humans share the same *Trichuris* species which have importance for control measures.

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Correlation between real-time qPCR and development of strongyle eggs from cattle

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Differentiation of veterinary important parasitic strongyle eggs is time-consuming, because morphologically distinct third-stage larvae (L3) must be cultured for species/genus identification. A recently published qPCR technique provides a non-labour intensive method for detection and quantification of the two most important nematode eggs in cattle faeces. However, as quantification correlates with DNA content, quantification of copy numbers of the second internal transcribed spacer (ITS2) region is problematic as DNA content increases during egg development.
The aim of this study was to assess the impact of oxygen availability and temperature on the multiplication of ITS2 copy numbers in *O. ostertagi* eggs. Fresh eggs were recovered from cattle faeces by sieving, flotation and entrapment in nylon-mesh filters and subsequently deposited in aliquots (n=18) of 5 ml distilled water with air circulation. To test the effect of oxygen deprivation, fresh faecal samples (n=18) were vacuum packed. A total of 36 aliquots were stored at temperatures of 4°C or 25°C for up to 336 hours. Morphological changes were observed, and DNA content was measured at nine time points throughout the study period. Preliminary morphological analysis demonstrated developmental progression. In water-deposited eggs, first-stage larvae (L1) were observed after 336 hours at 4°C and after 24 hours at 25°C. An additional 24 hour study showed formation of L1 already after 12 hours incubation at 25°C. In oxygen-deprived eggs, no development was observed neither at 4°C nor at 25°C throughout the 336 hours study. Thus, the importance of oxygen and temperature as regulators of egg development was verified.
Ongoing studies will quantify ITS2 copy numbers by qPCR. The results will allow us to correlate observed developmental progression with ITS2 copy numbers, and thereby provide knowledge on optimal storage conditions and sources of errors for data interpretation of this diagnostic molecular technique.

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Detection of non-human eukaryotic 18S DNA in otherwise sterile human clinical samples.

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The identification and differentiation of parasites has principally relied on phenotypic features, which is laborious and time consuming. The small subunit rRNA (18S) gene is a widely used target for molecular diagnosis. At the Department of Microbiology & Infection Control, Statens Serum Institut, Denmark, we have developed a PCR based method for detecting non-human eukaryotic 18S DNA in otherwise sterile human clinical samples. Three different primer sets were designed for detection of parasites and fungi based on highly conserved regions of the 18S gene. A series of parasite-positive clinical samples (*Babesia* sp., *Schistosoma mansoni*, *Toxoplasma gondii*, and *Plasmodium* spp.) was tested by PCR (the three primer sets used in each PCR analysis), and amplicons were sequenced and annotated to species level by BLAST, to evaluate the ability of the primers to amplify the target region. For clinical validation, 78 genomic DNAs from otherwise sterile, human clinical samples (bronchoalveolar lavage, pleura exudate, blood, urine, and spinal fluid) were collected from 64 patients with suspected parasitic infections but without a positive result by conventional tests. The clinical validation resulted in 4/64 patients (6 %) being positive for parasites (*Toxoplasma*, *Fasciola*, and *Onchocerca*); the remaining 94 % represented negative, fungal and, in a few cases, human DNA or sequences that could not be identified mostly due to mixed sequences or failure to match with data in public databases. In conclusion, this broad spectrum PCR enables detection of parasites and other eukaryotes in human clinical samples containing high amounts of human DNA, in cases where the applicability and diagnostic efficiency of other methods is limited. Preliminary results suggest that this approach is compatible with NGS technology, gaining high resolution and simultaneous detection and annotation of multiple microorganisms.