JOINT SPRING SYMPOSIUM 2010

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

Abstracts

KEYNOTE LECTURES

EVOLUTIONARY MEDICINE: QUESTIONS ABOUT MALARIA AND MOSQUITOES

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I will discuss two aspects of evolutionary medicine, focusing on the evolutionary ecology of malaria-mosquito interactions.
First, I will use evolutionary ideas to better understand epidemiology. Starting with a discussion on the evolution of the parasite's virulence, I will discuss how evolutionary pressures influence the parasite's developmental period of malaria in mosquitoes, which is the epidemiologically most important parameter of malaria. Second, based on these ideas, I will discuss predictions for malaria control with three questions. How does evolution make insecticide-treated bed-nets our best weapon for malaria control? How rapidly will insecticide-resistance spread? How can we overcome resistance?

CO-EVOLUTION OF HOST IMMUNE RESPONSES AND PARASITES

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Responses to invading elements have been a basic and decisive ability of all unicellular and multicellular organisms throughout evolution. Many effector mechanisms in these processes, originating in the earliest life stages on earth, are preserved in highly developed vertebrates including humans. Both invertebrates and vertebrates activate a long range of innate responses when confronted with pathogens. Membrane bound pattern-recognition receptors interacting with PAMPS activate NFκB which leads to activation of immune relevant genes resulting in the production of a series of functional effector molecules (eg. antimicrobial peptides, lysozyme, acute phase reactants, complement factors, lectins). Toll-like receptors represent one the most important receptor-classes demonstrated by the presence of 222 TLR genes in the sea urchin genome alone. However, vertebrates play on additional strings as well. Adaptive responses appeared with cartilaginous and bony fish 500-600 million years ago. Thus,

immunoglobulins, T-cell receptors and MHC play a major role in fish, amphibians, reptiles, birds and mammals. Despite the development of complex defense systems in hosts, many parasite species have been able to resist, at least partly, the responses by applying a series of immune evasion mechanisms. This selective force may be one of the factors driving evolution of parasites which has resulted in the existence of a vast amount of parasitic species. Thus, it has been estimated that at least 75% of all species existing may be parasitic. This is based on the fact that all free-living species are associated with one or more specific parasite species. The evolution of effector molecules in hosts may show some correlation to the evolution of their associated parasites but the connection is not straight forward. Host switching through evolution has been a common event. Further, parasite type, invasion mechanism and life cycle strategy are of high importance in this co-evolutionary process.

SUBMITTED SCIENTIFIC ABSTRACTS

P = POSTER PRESENTATION

O = ORAL PRESENTATION

*** = YOUNG SCIENTIST**

1. P. EXPRESSION OF TWO DIFFERENT PFEMP1 ANTIGENS ON *P. FALCIPARUM* INFECTED ERYTHROCYTES SHOW INCREASED BINDING TO ENDOTHELIAL CELLS

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Abstract

The *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) antigens play a major role in antigenic variation, cytoadhesion of infected erythrocytes (IE) and immunity to malaria. The current consensus on control of variant surface antigen expression is that only one PfEMP1 is expressed per cell at a time. However, recent results have shown one selected parasite sub-line ($3D7_{PFD1235/PF11\_0008}$) simultaneously expressed two different PfEMP1 as surface antigens, on single infected erythrocytes. This study compared *in vitro* binding of the "double" PfEMP1 expressing $3D7_{PFD1235/PF11\_0008}$ sub-line and binding of IE expressing either PFD1235w or PF11_0008 to Human Umbilical Vein Endothelial Cells (HUVEC) and to Transformed Human Bone Marrow Endothelial Cells (TrHBMEC) in a static adhesion assay. In the static assay, approximately 50% of $3D7_{PFD1235/PF11\_0008}$ IE and 25% of $3D7_{PFD1235}$ and $3D7_{PF11\_0008}$ IE were shown to bind HUVEC with all three parasite sub-lines showing less binding to TrHBMEC. Preliminary data using a Cellix VenaFlux assay showed a significantly higher proportion of $3D7_{PFD1235/PF11\_0008}$ IE to roll on recombinant ICAM-1 as compared to the $3D7_{PF11\_0008}$ sub-line. These data identifies ICAM-1 as a potential receptor for the PFD1235w PfEMP1 and suggest double PfEMP1 expressing parasites to show an increased ability to bind human endothelial cells. We speculate that if more than one PfEMP1 antigen can be expressed on individual IE in *in vivo* infections it would probably occur to ensure the highest avidity binding interaction possible and rapid sequestration during the immediately post-hepatocytic establishment phase.

2. O. ESTABLISHMENT OF CHRONIC INFECTION WITH *PLASMODIUM BERGHEI* IN MICE REQUIRES EXPRESSION OF VARIANT SURFACE ANTIGENS

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Abstract

It is generally accepted that variant surface antigens (VSA) of parasite origin expressed on the surface of the host erythrocyte is important for establishment of *Plasmodium falciparum* malaria in humans, and that different types of VSA are associated with different organ-specific disease manifestations, such as cerebral (CM) and placental malaria (PM). VSA can be detected and typed using a flow cytometric assay that determines the binding of IgG from hyper-immune individuals on the surface of parasite infected RBC. Another species of *Plasmodium*, *P. berghei* can cause both CM and PM-like syndromes in laboratory mice. In spite of the apparent similarity in disease manifestation and pathology the model is not commonly used for studies of the relation between VSA-expression and disease. One major reason for this is that rodent parasites appear to express little if any VSA. In laboratories *P. berghei* is maintained by frequent passages between naïve host-animals. If the infection is allowed to continue for more than a few days, the parasiteamia will decrease or reach a plateau. After this a second steep rise in parasiteamia will occur. We have used the above-mentioned assay to compare the expression of VSA in the first and second wave of parasiteamia in naïve BALB/c mice. In two independent experiments we saw very little binding of hyper-immune IgG to parasite isolates obtained during the first peak parasiteamia, whereas all parasites from second peak were strongly recognised. It thus appears that in order to establish infections of longer duration in the same host *P. berghei* parasites express significant amounts of VSA on the surface of the host erythrocyte. Our results also imply that the usual lack of VSA expression in experimental *P. berghei* infections may be an artifact resulting from the frequent passage in naïve hosts. This may contribute to our understanding of the evolution of VSA. Furthermore, our findings lend support to the usefulness of this animal model.

3. O. *REVISING A DOGMA:* SIMULTANEOUS SURFACE EXPRESSION OF TWO DIFFERENT PfEMP1 ANTIGENS ON SINGLE *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES

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Abstract

The *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) antigens play a major role in antigenic variation, cytoadhesion of infected erythrocytes (IE) and immunity to malaria. The current consensus on control of variant surface antigen expression is that only one PfEMP1 is expressed per cell at a time. We measured *var* mRNA transcript levels by real-time Q-PCR and directly compared these with PfEMP1 antigen surface expression in three different antibody-selected *P. falciparum* 3D7 sub-lines using confocal microscopy and flow cytometry. Contradicting the ‘mutually

exclusive expression' paradigm for the control of PfEMP1 gene expression, we found one selected parasite sub-line simultaneously expressed two different *var* genes as surface antigens, on single infected erythrocytes. These results provide new insights into PfEMP1 antigen expression and call for revision of the accepted paradigm of absolutely mutually exclusive *var* gene transcription.

4. O. ANALYSIS OF POLYMORPHISMS IN THE MEROZOITE SURFACE PROTEIN-3α GENE AND TWO MICROSATELLITE LOCI IN SRI LANKAN PLASMODIUM VIVAX: EVIDENCE OF POPULATION SUB-STRUCTURE IN SRI LANKA

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Abstract

Malaria is a serious public health problem in the tropical and sub-tropical regions of the world. Four human malaria parasite species exist, but attention is commonly focused on the most virulent and lethal malaria parasite species infecting humans, *Plasmodium falciparum.* However, in recent years studies of *P. vivax* have shown that this species is the most prevalent malaria parasite outside Africa and is the cause of significant morbidity and relapse of malaria Development of suitable and effective control strategies against the *Plasmodium* species, requires knowledge of local transmission dynamics and whether local parasite populations are interconnected or isolated. In this study, the geographical distribution of genetic variation in *Plasmodium vivax* samples (n=386) from nine districts across Sri Lanka is described. We calculated statistical indices of genetic diversity and population structure using three markers; the *P. vivax merozoite surface protein-3α* (*Pvmsp-3α*) gene, and microsatellites located on chromosome 1 (m1501) and chromosome 3 (m3502). A high level of allelic diversity was present in all loci. At *Pvmsp-3α*, 11 alleles were found with an expected heterozygosity (H_e) of 0.80, while at m1501 and m3502, 24 alleles (H_e=0.84) and 8 alleles (H_e=0.74) were detected, respectively. Overall, 95 unique alleles were detected among the 280 samples positive at all three loci, with a high overall H_e of 0.95, but theses alleles were not uniformly distributed among the districts. Pairwise fixation index (F_{ST}) revealed statistically significant population structure. Furthermore, the presence of identical 2-loci genotypes in a large number of samples revealed clusters of closely related isolates, which contributed to strong linkage disequilibrium between marker alleles. These results from Sri Lanka show evidence of high genetic diversity and population sub-structure of *P. vivax* populations on the island.

5. P. MOLECULAR AND PARASITOLOGICAL TOOLS FOR THE STUDY OF *ASCARIDIA GALLI* POPULATION DYNAMICS IN CHICKENS

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Abstract
Experiments were first conducted to compare and evaluate different methods of *Ascaridia galli* larval recovery from the chicken intestine. The number of larvae recovered from the intestinal wall of chickens infected with 1000 embryonated *A. galli* eggs and killed 15 days post infection (p.i.) by three methods (EDTA, pepsin digestion and scraping) were compared. The EDTA and pepsin digestion were found to be the most efficient methods with no significant difference ($P > 0.05$) in number of recovered larvae between the two. Subsequently, three different *A. galli* cohorts were established using the polymerase chain reaction linked restriction fragment length polymorphism (PCR-RFLP) technique. A 533 bp long region of the cytochrome c oxidase subunit 1 (*cox*1) gene of the mitochondrial DNA (mtDNA) was targeted and 22 *A. galli* females were allocated in to three different haplotypes. The four females with the highest embryonation rate from each halpotype group (total 12 females) were selected and used to inoculate each of 12 chickens with a dose of 1000 embryonated eggs. The chickens were killed 15 days p.i. and *A. galli* larvae were recovered from the small intestinal wall by the EDTA method and by sieving the lumen content on a 90 μm sieve. DNA of 40 larvae from each of the three different haplotypes was extracted using a worm lysis buffer (WLB) and PCR-RFLP analysis of these larvae revealed same haplotype as that of their maternal parent. The identification of distinguishable cohorts may be a powerful tool in population studies of parasite turnover within the animal host.

6. O. HUMAN MONOCLONAL ANTIBODIES CAN INHIBIT ROSETTE FORMATION OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES

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Abstract
P. falciparum-infected erythrocytes (IEs) have antigens on their surface with affinity for uninfected erythrocytes. This leads to formation of so-called rosettes, where a central IE is surrounded by few to many uninfected erythrocytes. The interaction between IEs and uninfected erythrocytes appears to be mediated by the N-terminal DBL1-α domain of several members of the *P. falciparum* erythrocyte membrane protein 1 family of adhesion proteins. The ability to form rosettes has repeatedly been associated with severe malaria in Africa. Immunity to this phenotype develops during childhood, and serum from immune adults living in endemic areas contains antibodies that can inhibit the formation of rosettes. We have used the rosetting *P. falciparum* lines PAR+ and R29 to identify B-cell lines and clones producing antibodies that can inhibit rosette formation *in vitro*. To date, we have been able to identify four B-cell clones producing anti-rosetting antibodies.

Two produce IgM, one produces IgG3, whereas the isotype of the remaining one has not yet been determined. We will present results regarding the ability of the monoclonal IgG3 antibody AR03.1 to inhibit formation of rosettes in different *P. falciparum* lines, which express different PfEMP1 variants, and the reactivity of AR03.1 with recombinant DBL1-α domains implicated in rosetting.

7. O. ATOMIC FORCE MICROSCOPY ANALYSIS OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES

Jonas Bruun,[1,2] Jorid Soerli,[1] Lea Barfod,[1] Tue Hassenkam[2] and Lars Hviid[1]. [1]Centre for Medical Parasitology, University of Copenhagen and Rigshospitalet, and [2]Nano-Sceince Center, University of Copenhagen

Abstract

Standard bright field microscopy cannot resolve objects smaller than about 250 nanometres (nm), mainly due to optical diffraction, and therefore cannot be used for analysis of the so-called knob protrusions (diameter: 70-130 nm) on the surface of *P. falciparum*-infected erythrocytes (IEs). However, such analysis is feasible by atomic force microscopy (AFM), which offers a >1,000x better resolution. By AFM it is possible to measure the interacting forces between the microscope chip and single atoms on the sample surface, and to generate a topographic representation of the measured surface at nm-scale. AFM thus makes it possible to map small antigenic clusters on the sample surface without the need of labelling and the occurrence of antigens can independently be correlated with antibody recognition. We are using AFM to study *P. falciparum*-IE surface knobs, which are considered to be of key importance in the pathogenesis of malaria and to be major targets of naturally acquired protective immunity. Members of the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family are expressed on the knobs and mediate IE adhesion to a range of host vascular receptors. We are comparing the density of knobs on IEs selected for specific adhesive phenotypes and expression of particular PfEMP1 variants. Data will be presented regarding our analysis of IEs showing the so-called rosetting phenotype that has been repeatedly associated with severe childhood malaria.

8. P. FUNCTIONAL CHARACTERIZATION OF HUMAN MONOCLONAL ANTIBODIES SPECIFIC FOR PLACENTAL MALARIA-TYPE *PLASMODIUM FALCIPARUM* VARIANT SURFACE ANTIGENS

Irene S Hansen, Tina Dobrilovic, Lars Hviid and Lea Barfod

Abstract

People living in malaria-endemic areas acquire protective immunity over several years. This naturally acquired immunity is mediated by antibodies to asexual blood-stage antigens, in particular to members of the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family of adhesins. PfEMP1 variants are expressed on the surface of infected erythrocytes (IEs), where they mediate adhesion to several different host vascular receptors. The PfEMP1 variant VAR2CSA is exclusively expressed on IEs adhering to chondroitin sulphate A (CSA) in the placenta of pregnant women. Primigravidae are consequently susceptible to infection by VAR2CSA-expressing parasites, while protection against placental malaria (PM) follows acquisition of VAR2CSA-specific antibodies. We have used recombinant antigen constructs corresponding to VAR2CSA

Duffy-binding-like (DBL) domains to identify four human monoclonal antibodies with specificity for either the DBL3-X or the DBL5-e domain of VAR2CSA. Flow cytometry analysis revealed high reactivity of one antibody with antigens on the surface of *P. falciparum*-infected erythrocytes selected *in vitro* for high levels of adhesion to CSA. However, all four antibodies appear to recognize surface-expressed epitopes, as they opsonized IEs for phagocytosis by THP-1 cells.

9. P. A NEW METHOD TO GENERATE HUMAN MONOCLONAL ANTIBODIES

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Abstract

Plasmodium falciparum malaria continues to be one of the most important human health problems in the developing world both in terms of mortality, morbidity and socio-economic impact. In *P. falciparum* endemic areas clinical immunity does develop and anti-malaria parasite antibodies have clearly been demonstrated to play a major role in this immunity. However, the epitopes mediating protection remain unknown. Acquiring information on which malaria parasite epitopes that are involved in the acquisition of protective immunity towards severe malaria is crucial in the development of anti-malaria vaccines. Generation of *P. falciparum*-specific monoclonal antibodies (mAbs) corresponding to those acquired by clinically immune individuals would be an important tool in epitope identification. In collaboration with the Danish biotech company Symphogen, we are developing a novel technology that allows this in a high-through-put system. Memory B cells with specificity for recombinant *P. falciparum* antigens are collected from malaria-exposed donors, and stimulated *in vitro* to differentiate into antibody-secreting plasma cells. These are single-cell sorted, and a multiplex RT-PCR is performed that results in the amplification of the two expressed variable antibody genes from the light and the heavy chains (V_L,V_H). A second PCR fuses the V_L and V_H gene segments, thus preserving the natural affinity maturated light-heavy chain pairing. The paired V_L-V_H gene segments are subsequently bulk-cloned into an expression vector fusing the V_L-V_H gene segment to an IgG1 Fc gene segment. This construct is finally expressed in human HEK293 cells growing in suspension and the secreted mAbs purified. The obtained mAbs are tested in a multiplexed Luminex system containing the recombinant *P. falciparum* proteins used for the memory B cell labeling and further tested for surface reactivity in FACS with *P. falciparum* infected erythrocytes from malaria patients with characterized clinical syndromes.

10. P. ERYTHROPOIETIN STIMULATES DIFFERENTIATION AND MIGRATION OF NEURAL STEM CELLS IN AN EXPERIMENTAL CEREBRAL MALARIA MODEL

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Abstract

Cerebral malaria (CM) has a high mortality despite effective anti-parasitic treatment. There is a need for a neuroprotective treatments aiming at ameliorating acute symptoms such as seizures and coma as well as to improve survival. Survivors frequently suffer from neurological sequelae, which are mostly of a reversible nature. Erythropoietin (EPO) reduces brain pathology and improves survival in experimental CM. Since neural stem cells express a functional EPO receptor, we wanted to assess neurogenesis in this experimental model. Using the *Plasmodium berghei* model of CM and life-saving treatment with EPO, we investigated if the neuroprotective effects included regeneration by neural stem cells. We used immunohistochemical methods to assess the changes *in situ* within specific stem cell niches of the murine brain. Using markers of different maturation stages of the neural stem cells, we showed an increased number of nestin$^+$ cells in the dentate gyrus and in the sub-ventricular zone in EPO-treated compared with saline-treated mice. A significant fraction of the EPO-treated CM mice also had migrating nestin$^+$ stem cells moving from the sub-ventricular zone along the rostral migratory stream compared to control mice. The number of PSA-NCAM$^+$ neuronal progenitor cells was increased in the sub-ventricular zone in EPO-treated, CM mice. Moreover, neurite growth in the dentate gyrus was visualised by α-internexin, but was not markedly changed. These results indicate a rapid, EPO-dependent activation of neural stem cells during CM pathology.

11. O. A MULTIPLEX PCR SYSTEM FOR TAENIID INFECTIONS IN DEFINITIVE AND INTERMEDIATE HOSTS

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Abstract:

The family taeniidae includes several species of veterinary and public health importance, but diagnosis of the etiological agent in definitive and intermediate hosts often relies on labor intensive and less specific morphometric criteria. In the present study, a new multiplex PCR system, based on five primers targeting the 18S rDNA and ITS2 sequences, produced unequivocal banding pattern for a range of taeniids. Identification at the species level by the multiplex PCR was evaluated in comparison to classical morphological identification and sequencing. Compared to sequencing, the multiplex PCR identified *Taenia* to species in 36 out of 43 infections in carnivores (83.7%, 69 - 93; 95% CI), while only 20 cases were identified by morphology (46.5%, 31 - 62; 95% CI). Among rodent infections, the multiplex PCR identified *Taenia* to species in 85 out of 94 infections (90.4%, 83 – 96; 95% CI), whereas only 55 cases were identified by morphology (58.5%, 48 – 69; 95% CI). In foxes, *T. polyacantha* was more prevalent than *T. crassiceps* and *T. taeniaeformis* was detected in five cats, while *T. martis* and *T. mustelae* were identified in a stone marten and a pole cat, respectively. In rodents, *T. mustelae* and *T. polyacantha* were most prevalent in rural forests, whereas *T.*

taeniaeformis was the dominant species in urban forests and the sole species found in domestic gardens. Analysis of sequence variation and a phylogenetic tree based on sequenced of *cox1* gene were performed. The multiplex PCR system proved its superiority to the classical morphological detection and could be applied in epidemiological studies and clinical diagnosis.

12. O. PERFORMANCE OF CONVENTIONAL PCR FOR SCREENING FOR *STRONGYLUS VULGARIS* ON HORSE FARMS

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Abstract

Strongyle parasites are ubiquitous in grazing horses. Of these, the bloodworm *Strongylus vulgaris* is regarded most pathogenic. Increasing levels of anthelmintic resistance in strongyle parasites has lead to recommendations of decreased treatment intensities, and there is now a pronounced need for reliable tools for detection of parasite burdens in general and *S. vulgaris* in particular. The only method currently available is the larval culture, which is laborious and time-consuming, so veterinary practitioners most often pool samples from several horses together in one culture to save time. This raises doubts about the reliability of the method. Recently, molecular tools have been developed to detect *S. vulgaris* in fecal samples. The aim of this study was to compare the performance of a conventional polymerase chain reaction (PCR) assay with the traditional larval culture and furthermore test the performance of pooled versus individual PCR for farm screening purposes. Fecal samples were obtained from 331 horses on 18 different farms. Farm size ranged from 6 to 56 horses, and horses aged between 2 months and 31 years. Larval cultures and individual PCRs were performed from all horses. In addition, PCR was performed on 66 fecal pools representing 5 horses each. PCR primers previously developed for a real-time PCR assay were used for the PCR reaction. Results showed that the PCR and larval culture detected *S. vulgaris* in 12.1 and 4.5 % of horses, respectively. Eight farms tested positive with the larval culture, while 12 and ten farms were positive with the individual and pooled PCRs, respectively. The individual PCR method was statistically superior to the larval culture, while no statistical difference could be detected between pooled and individual PCR for farm screening. In conclusion, pooled PCR appears to be a reliable tool for farm screening for *S. vulgaris.*

13. P. EVALUATION OF ANTHELMINTIC RESISTANCE IN LIVESTOCK PARASITES USING OBSERVATIONAL DATA AND HIERARCHICAL MODELS

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Abstract

Anthelmintic resistance is an increasing challenge in the control of parasites in livestock. The fecal egg count reduction test (FECRT) is the practical gold standard method for evaluating resistance, but the interpretation is complicated due to high levels of variability. Several factors contribute to cause this high variability and these must be taken into account to accurately identify a reduction in anthelmintic efficacy. To address this problem, we developed a hierarchical statistical model for analysis of FECRT data from multiple farms. The model includes animal effect and farm clusters as random effects. Resistance classifications are based on model adjusted lower confidence limit (LCL) values of predicted mean efficacies on each farm. The model was used to evaluate the efficacy of pyrantel embonate paste from 64 Danish horse farms. On these farms 614 out of 1644 horses had egg counts ≥ 200 eggs per gram (EPG) and were treated. Post treatment samples and information on age, gender and farm zip code were collected for each horse. In addition, individual coprocultureswere performed on all pretreatment fecal samples to determine the presence of *Strongylus vulgaris*, with 31farms (48.4 %) testing positive. The efficacy of pyrantel was unaffected by the presence of *S. vulgaris* on the farm. The cutoff LCL values used for classifying farms as pyrantel resistant were: >92%: no resistance, 88-92%: suspect resistance, and <88%: resistance. Using model-adjusted LCLs, we classified 7 (10.93 %) farms as pyrantel resistant, 5 (7.81 %) as suspect resistant and the remainder of farms (81.25%) as not resistant. In comparison with unadjusted LCLs, the model provided a more stable classification of farms with a 1.1 % false discovery rate. The statistical model presented here can be adapted to handle FECRT data obtained from other livestock species, drug types, and parasite species.

14. O. MOLECULAR EPIDEMIOLOGY OF ASCARIASIS

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Abstract

We are using molecular epidemiology techniques to study the population structure of *Ascaris* obtained from humans and pigs. Worms were obtained from human hosts on Zanzibar and in Uganda, Bangladesh, Guatemala and Nepal and *Ascaris* from pigs were collected from in Uganda, Tanzania, Denmark, Guatemala and the Philippines. Genomic DNA was extracted from each worm and a 450 base pair fragment of the mitochondrial cytochrome oxidase gene subunit 1 (COI) was PCR amplified. The products were sequenced from both strands and sequences were manually edited. Fifty different *Ascaris* CO1 haplotypes were identified in the 200+ worms included. Haplotypes 1 and 3 were common in *Ascaris* from human hosts and haplotypes 7, 33 and 36 was frequently found in worms obtained from pigs. Even though near complete segregation of *Ascaris* haplotypes between humans and pigs were seen, shared haplotypes were also observed suggesting retention of ancestral polymorphisme or cases of cross-infections. In addition, data suggests that speciation may have been driven not only by host affiliation but also by e.g. geography.

15. P. MOLECULAR EVIDENCE FOR SUSTAINED TRANSMISSION OF ZOONOTIC *ASCARIS SUUM* AMONG ZOO CHIMPANZEES (*PAN TROGLODYTES*)

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Abstract

Chimpanzees in the Copenhagen Zoo frequently excrete ascarid worms onto the cage floor inspite of a regular anthelmintic treatment program. Previously it had been shown that the source of the infections was of pig origin. However, it was unknown whether the recurrence of the infection was due to reintroduction of eggs from an external source or to a sustained transmission cycle within the Zoo. We found that isolated eggs were able to embryonate into the infective stage and PCR-RFLP analysis on the ITS region amplified from single embryonated eggs suggest these to be *Ascaris suum*. In addition, sequence analysis of the *cox*1 gene ('barcoding') on expelled worms followed by cluster analysis revealed that the chimpanzees are infected with pig *A. suum* which now, in spite of control efforts, has stabilized into a permanent transmission cycle in the Zoo's chimpanzee troop.

16. P. IDENTIFICATION OF THREE QTLS WITH INFLUENCE ON SUSCEPTIBILITY TO HELMINTH INFECTIONS IN PIGS

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Abstract

Intestinal helminth infections are causing health and welfare problems in both human and animal populations. A family, in which susceptibility towards *Ascaris* (large round worm) and *Trichuris* (whipworm) infections are segregating, was constructed. Our data demonstrate that genetic components are responsible for approximately 45% and 70% of the variation in *Ascaris* and *Trichuris* parasite loads, respectively. A genome scan using the Ilummina 7K SNP-chip has been performed in order to locate genomic regions controlling this susceptibility. A total of 194 pigs from 19 full-sib litters have been genotyped and 3955 informative SNPs were used to perform genotype association analysis. For *Trichuris* parasite load (faecal egg excretion) four SNPs in a 2 Mb region on SSC13 and 4 SNPs in a 7 Mb region on SSC11 have been identified, whereas 7 SNPs located within a 6 Mb region on SSC4 were associated with *Ascaris* egg excretion and

worm load. The proportion of phenotypic variance accounted for by a SNP's genotype ranged from 0.08-0.14 and 0.06-0.12 for *Ascaris* and *Trichuris,* respectively. The identified QTLs will be verified in unrelated pig material and we are currently collecting samples for that purpose. Re-sequencing technology will be applied in candidate regions on pigs with deviating phenotypes, which will provide information on all genetic differences in the genomic regions.

17. O. EFFICACY OF SINGLE AND DOUBLE DOSES OF ALBENDAZOLE AND MEBENDAZOLE ALONE AND IN COMBINATION IN THE TREATMENT OF *TRICHURIS TRICHIURA* INFECTION IN SCHOOL AGED CHILDREN IN UGANDA.

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Abstract
Studies indicate that albendazole and mebendazole show low cure rates and egg reduction rates when given against *Trichuris trichiura*. In a randomized controlled clinical trial *T. trichiura* infected pupils were divided into six groups of 100 pupils. Two groups received a single dose of either albendazole, 400 mg (A) or mebendazole, 500 mg (M), while two groups received a double dose (given 6-8 hours apart) of either albendazole (A-A) or mebendazole (M-M). The last two groups received a combination of albendazole and mebendazole given as a single dose (AM) or with 6-8 hours apart (AM-AM). Egg counts were performed at baseline and at 7, 14, 21 and 28 days after treatment. Preliminary results showed that the prevalence and intensity (geometric mean of all investigated) of *T. trichiura* in children who received a single and a double dose of the combination (AM and AM-AM) were similar, but significantly lower than that of the other treatment arms. The study indicates that although albendazole and mebendazole are both benzimidazoles and their mode of actions are believed to be similar, the use of a combination of the two drugs seemed to be much more effective than using the single drug therapy.

18. O. STUDY ON THE WINTER SURVIVAL OF *HAEMONCHUS CONTORTUS* ON PASTURE

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Abstract
The objective of this study was to investigate the presence of overwintering *Haemonchus contortus* on Danish pasture. Three different methods were applied i.e. concentration McMaster, larval culture and peanut agglutination method (PNA). The study also included the validity of PNA method. A farm was selected which had had a problem with haemonchosis for the last few years. Faecal egg counts (FEC) of strongyle eggs including *H. contortus* were high at first test prior to turned-out. The presence of *H. contortus* was shown by both PNA and larval culture. From PNA, we found that at least 63% of s*trongyle* eggs were *H. contortus* and likewise, the majority of isolated larvae (from 44 to 98%) were *H. contortus.* After treating all the ewes with Closantel, no *H. contortus* eggs

or larvae were found by PNA and larval culture respectively. The absence of *H. contortus* eggs after the treatment of Closantel indicates that it was almost 100% effective against the *Haemonchus*. From our results, we can conclude that L3 of *H. contortus* did not survive overwinter on pasture under the conditions prevailing on the Danish farm. However, they had survived inside the ewes by undergoing hyobiosis during the winter. Lastly, PNA is a rapid and effective method to diagnose the *H. contortus* eggs in faecal samples and can be used as routine diagnostic test.

19. P. THE EFFECT OF TEMPERATURE AND AGE ON THE INFECTIVITY AND DEVELOPMENT OF *ANGIOSTRONGYLUS VASORUM* IN THE SLUG *ARION LUSITANICUS*

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Abstract

Experimental infection with *Angiostrongylus vasorum* was conducted in Iberian slugs *Arion lusitanicus.* Initially, different size/age groups of juvenile slugs (small: <0.5g and medium: 0.5-1g) were exposed to freshly isolated first stage parasitic larvae (L1) of *A. vasorum*. The slugs were subsequently incubated at 5ºC, 10ºC and 15ºC for 6 weeks. Larval development within the slugs differed significantly with temperature. At 15ºC all larvae developed into the third larval stage (L3), at 10ºC into the second stage (L2), whereas no development was observed at 5ºC. The mean larval burdens were highest in the largest group of slugs and tended to increase with higher temperature. In a second experiment isolated L1 were incubated at 5ºC, 10ºC and 15ºC for 3 and 7 days prior to infection of slugs, which then were kept for 6 weeks at 15ºC. The infectivity decreased significantly with the larval storage time and the mean larval burden per slug was lower at higher incubating temperature. However, all established larvae developed into infective L3. Temperature has an effect on the development of the larvae and thus an impact on transmission of the parasite as only L3 are infective to the definitive canid hosts.

20. P. PCR DETECTION OF *ANGIOSTRONGYLUS VASORUM* IN FAECAL SAMPLES OF DOGS AND FOXES

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21. P. PCR DETECTION OF *ANGIOSTRONGYLUS VASORUM* IN FAECAL SAMPLES OF DOGS AND FOXES

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Abstract:

The cardiovascular nematode *Angiostrongylus vasorum* is spreading in fox and dog populations of northern Europe. A specific diagnosis is crucial for prevention of often severe clinical manifestation in dogs, and for the risk assessment of prevalence in foxes. In the present study, faecal samples from foxes and domestic dogs were tested by a new PCR targeting the ITS2 region. Initial isolation of faecal larvae by sieving facilitated processing of larger sample volume, reduced the level of inhibitory substance, and allowed for recovery of dead larvae from frozen samples. The sieve-PCR system, which detected down to 1 larva per sample and did not cross react any of a range of canine helminths, presents a non-invasive tool for wildlife surveillance and for confirmative diagnosis in dogs.

22. P. ETIOLOGICAL UNRAVELLING OF A WASTING SYNDROME IN DANISH ROE DEER (*Capreolus capreolus*) FROM FUNEN: PARASITOLOGICAL ASPECTS

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Abstract

Increased mortality was observed among roe deer on Funen since 2004. Symptoms included diarrhoea and wasting. This study compared the nematode worm burdens in the abomasum + the first half of the small intestine among roe dee belonging to one sick and two healthy populations. The study is still ongoing, so a qualitative evaluation will eventually be undertaken. The study was conducted in eighteen randomly selected roe deer allocated into three groups: Group SF comprising sick roe deer from Funen (n = 6), Group RF comprising healthy roe deer from Funen (n = 6) and Group RB comprising healthy roe deer from Bornholm (n = 6). The animals were collected from May 12th to August 18th 2009. The criterion for categorising an animal as sick was signs of diarrhoea, either by thin faeces in the hindgut or by dirty fur. Animals without signs of diarrhoea were categorised as healthy. The animals were all adults, including both sexes. The abomasum contents were mixed with water to a total volume of five litres from which a

5% subsample was washed through a 212µm sieve. From the material left in the sieve parasites were collected and counted under microscope. The same procedure has been conducted for the first half of the small intestine, after which the two counts were summarized. The results showed that group SF had a mean burden of 25745 worms (SD = 17021,55), whereas the healthy groups, RF and RB, had means of 5043,33 (SD = 1711,87) and 5811,33 worms (= 2755,39) respectively. Thus the sick group had significantly larger ($p<0.05$) gastrointestinal worm burdens than the two healthy groups, indicating a correlation between diarrhoea and a heavy worm burdens.

23. P. A MOLECULAR METHOD TO DIFFERENTIATE BETWEEN THREE GENOTYPES OF *TRICHURIS* RECOVERED FROM HUMANS AND PIGS

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Abstract

The whipworms *Trichuris trichiura* and *T. suis* in humans and pigs, respectively, are believed to be closely related but different species. Despite several attempts no reliable morphological characters have been identified which can discriminate worms from the two hosts. Recent sequence analysis has suggested that 3 major ITS-2 genotypes are present in *Trichuris* obtained from human and pigs; namely a type 1 and 2 and a heterozygote. We have used this information to develop a PCR-linked Restriction Fragment Length Polymorphism (PCR-RFLP) that can identify the three genotypes. The method was applied on worms obtained from pigs collected in Uganda, USA, Tanzania, Jamaica and Denmark and worms obtained from humans collected in Uganda, Jamaica and China. The distribution of the three genotypes (type 1, type 2 and heterozygote) was 2, 0 and 57, respectively, in 59 worms recovered from pigs and 26, 2 and 1, respectively in 29 worms recovered from human. The results clearly suggest that *Trichuris* in humans and pigs belong to two different populations ($\chi^2_2 = 49.5$; $P < 0.001$). However, we have also found evidence for a few cases of cross-infections and the existence of heterozygote worms, the latter suggesting cases of hybridization or retention of ancestral polymorphism.