ABSTRACTS (* Indicates candidate for Young Scientist Award)

THE ROLE OF MALARIA RAPID DIAGNOSTIC TESTS (RDTS) IN AFRICA

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The availability of rapid diagnostic tests for malaria based on antigen detection makes it possible to envisage major improvements of malaria control and curative services in malaria endemic areas. Most studies have found these tests to be less sensitive than microscopy, but there are few examples of sustained provision of malaria microscopy of high quality in rural health services. Thus, RDTs should make it possible to diagnose malaria with higher specificity than is currently the case in peripheral health services, reducing the costs, side effects and adherence problems associated with antimalarial therapy. This is of particular importance, as relatively expensive combination treatments need to be used in most areas endemic for falciparum malaria as a result of resistance. Also, by identifying patients who do not have malaria, RDTs should increase the chance that the clinician will look for a possible alternative, treatable cause. Through these advantages, RDTs could make health services more attractive to the population. The results can also be used in malaria surveillance, with advantages for early detection of epidemics, and monitoring and evaluation.
There are however, numerous caveats: There are many brands of RDTs, but no scheme to ensure compliance with good manufacturing practice or agreed performance criteria. Sensitivity and specificity vary for reasons, which are not clearly understood, but may in some cases be related to antigen variation. The tests are heat sensitive. There is little data on the degree of reliance placed on the test results by service providers and patients. There are so far no tests, which are sufficiently sensitive for vivax malaria to be fully acceptable for clinical use. Finally, at a cost of USD 0.60 - 0.70 for the cheapest tests, there is still an affordability problem. Finally, in areas of intense, endemic malaria transmission, as is seen in most rural areas in tropical Africa, most young children are parasitaemic, and it is doubtful, whether in such circumstances, the benefits of relying on laboratory-based diagnosis outweigh the risks and the costs.
There is an urgent need for various kinds of operational research in this area as well as for quality assurance at production and service levels.

CURRENT DIAGNOSES OF FISH BORNE TREMATODES IN SOUTHEAST ASIA

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Fish borne trematodes (FBT) cause significant public health problem in many endemic countries particularly in Southeast Asia. Under the situation when control measures or chemotherapy is non existent or inadequate in some areas, the conventional diagnostic methods, mostly parasitological diagnosis, are sufficiently reliable and accurate. However, in the present circumstance in which chemotherapy is widely used for parasite control in various parts of the endemic area, the frequency of moderate and heavy infection is therefore pushed towards a light infection category. In order to obtain accurate diagnosis we need not only a more specific but also a more sensitive diagnostic method. In this presentation, an overview of the liver fluke, *Opisthorchis viverrini* in Thailand and the diagnostic problems of will be addressed. *O. viverrini* is known as the major risk factor for cholangiocarcinoma (CCA) and hence it was classified as a carcinogenic parasite. One of the approaches to reduce the incidence of CCA is through control of the liver fluke. Therefore accurate and reliable diagnoses for various life stages the in the present situation are essential for success of the comprehensive control program. Alternative methods for diagnosis of liver fluke infection other than the conventional method including faecal antigen detection as well as DNA-based method in man as well as the intermediate hosts will be described. Further development of selected diagnostic methods to make them simple and practical to use in the field condition clearly deserves attention.

RECENT ADVANCES IN DIAGNOSIS OF ACQUIRED AND CONGENITAL TOXOPLASMOSIS

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The protozoan parasite *Toxoplasma gondii* is capable of infecting a variety of mammalian hosts. In humans primary infections in most cases only causes mild to unnoted symptoms. If women experience primary *T. gondii* infections during pregnancies there is a risk of congenital infection of the fetus. It is suggested that early treatment of the congenital infected newborn can reduce the possible severity of the infection. In this aspect it is crucial to obtain a quick and reliable diagnosis of the congenital infection. For all practical use it is not possible to detect the antigen it self in these newborns i.e. by PCR, instead indirect measurements like detection of an antibody response is the only possibility. Since maternal IgG can be transferred to the fetus it will in some cases demand the option to distinguish between maternal and the newborn *T. gondii* specific IgG response to confirm the diagnosis. During our study we evaluated the possibility to include 2 dimensional immune blotting techniques to obtain a higher resolution of the individual *T. gondii* specific antibody response pattern. By the 2-D IB technique it was possible to detect neosynthesis of *T. gondii* specific IgG antibodies in the sera from congenital infected newborn, that otherwise was not detected by the comparative Western blotting technique (Nielsen HV et al. JCM. 2005).

In this presentation data from studies performed with this technique will be presented and compared with other know methodologies.

* COMPARATIVE COPRO-DIAGNOSIS OF *ECHINOCOCCUS MULTILOCULARIS* IN EXPERIMENTALLY INFECTED FOXES

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Faecal samples originating from fifteen foxes experimentally infected with *Echinococcus multilocularis*, (0-90 days post inoculation (dpi) were examined by means of coproantigen-ELISA, egg isolation by sieving, PCR on DNA extracted from sieved material (sieve-PCR) and PCR on DNA directly extracted from faeces (direct-PCR). A new egg counting system was developed by combining steps from sieving and McMaster techniques. In comparison with the classical McMaster technique, the new modified system had 32% higher counts in samples from the high patent period, and was also able to detected eggs in samples below the threshold of the classical McMaster method. The egg isolation method detected 83% of patent infections (95% CI: 69-93). The detection level of the coproantigen-ELISA test during the pre-patent period was 63% (95%, CI: 41-81), while it was 47% (95%, CI: 30-65) in the whole patent period. The highest copro-antigen levels were observed at the late pre-patent and early patent periods, declining from mid-patency, with increasing numbers of negative samples towards the end of patency. The direct-PCR method developed in this study detected 16% (95%, CI: 5-36) of samples from pre-patent period and 56% (95%, CI: 38-72) of samples from patent periods. Although addition of BSA improved the detection level of the direct-PCR in the low patent period from 40% to 47%, still prominent inhibition was observed. The detection level of the sieve-PCR test in the whole patent period was 85% (95%, CI: 70-94). This method enabled for the first time the detection of *E. multilocularis* DNA in the pre-patent period but with low sensitivity (19%). DNA from samples with one or even no eggs in 2g of faeces from the patent period was also amplified. The sieve-PCR proved to be the most sensitive method when applied on samples from any period of the infection. This method can be used as a screening method in large-scale epidemiological surveys in low or non-endemic areas and also as a one-step diagnostic test in individual cases.

*** COMPARISON OF THREE ANTIMALARIAL PREVENTIVE REGIMENS ON MATERNAL PARASITAEMIA, ANAEMIA AND DELIVERY OUTCOMES IN OUAGADOUGOU (BURKINA FASO)**

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Burkina Faso is endemic for malaria and is facing increasing resistance to chloroquine (CQ) which has been used as first line drug for treatment and chemoprophylaxis in pregnancy for a long time. Intermittent Preventive Treatment (IPT) with Sulphadoxine-Pyrimethamine (SP) has been shown to be effective during pregnancy for prevention of malaria ill effects on mother and child and therefore we aimed at evaluating the efficacy of IPT/SP compared to CQ in pregnant women to contribute data to inform the process of developing new treatment guidelines in a setting where malaria transmission is moderate and seasonal. The objective was to compare the effect of IPT/SP, IPT/CQ and weekly CQ (WCQ) chemoprophylaxis on maternal parasitaemia, anaemia and birth weight. The study was conducted from August 2003 to March 2005 in a peripheral district hospital of Ouagadougou. CQ was still used for chemoprophylaxis in pregnant women, while SP was rarely used. The study was designed as a randomized controlled trial. Primigravidae and secundigravidae (gestational age 13 to 34 weeks) were enrolled, randomly assigned to one of the intervention groups and followed up to delivery. At delivery, peripheral and placental parasitaemia, child birth weight, and haemoglobin level were measured. Comparisons were done using chi square test or Fisher's exact test for proportions, and t test or ANOVA analysis of variance for means. A written informed consent was obtained from each participant. The study protocol was reviewed and approved by institutional ethical committees in Denmark and Burkina Faso, and authorized by the ministry of health in Burkina Faso. 614 women were enrolled. 405 of them delivered at the health centre. They were composed of 55.1% primigravidae and 44.9% secundigravidae. The participants were mostly young (median age: 21, range: 16-33), married housewives, and illiterate. The 3 groups were composed as following: IPT/SP (160), IPT/CQ (129), and WCQ (116). Increase in Hb was observed in all three treatment groups (1.1 – 1.4 g/dL, $P < 10^{-5}$) Prevalence of maternal peripheral parasitaemia fell significantly in the three treatment groups. IPT/SP was found to have the largest effect on maternal peripheral parasitaemia (P= 0.04). IPT/SP also had larger effects on placental parasiteamia, Hb level, and birth weight than IPT/CQ and WCQ, however these differences were not significant. IPT/SP could be a very effective strategy for preventing malaria ill effects on mother and child.

DIAGNOSIS OF ANTHELMINTIC RESISTANCE

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Anthelmintic resistance in helminth parasites is recognized as a growing concern for animal and human health. In many areas of the world multiple-drug resistant worms threaten the viability of small ruminant production, and have caused an important rise in parasite-related disease in horses. The problem is less severe in cattle, but the numbers of reports of anthelmintic resistance continue to increase and it is likely that the cattle industry will soon suffer similar problems to that seen in the sheep and goat industries. Furthermore, anthelmintic resistance looms as a growing concern in mass drug administration campaigns for the elimination of lymphatic filariasis and onchocerciasis in humans. Diagnosis of anthelmintic resistance is hampered largely by a lack of detailed knowledge of the biochemical/molecular mechanisms of resistance. Consequently, *in vivo* and *in vitro* testing remain the only means to detect resistance for all of the commonly used anthelmintics except for the benzimidazoles. *In vivo* testing systems include the fecal egg count reduction test and the critical test, which can be used for pre-mortem diagnosis, and the controlled efficacy test which requires necropsy of infected animals. *In vitro* testing systems include the egg hatch, larval development and larval motility assays. Unfortunately, these phenotypic-based tests are very poorly sensitive – it is estimated that about 25% of parasites in the population must possess resistance alleles before resistance can be detected with these methods. Therefore, developing molecular-based assays for diagnosing resistance remains a high priority. Such assays could detect resistance long before reduced drug efficacy is observed, thereby permitting improved management of drug treatment programs, and detection during quarantine in order to reduce the spread of resistance into new areas.

PROTOZOAN PARASITES IN VIETNAM – STUDIES IN WASTEWATER-IRRIGATED FIELDS, VEGETABLE MARKETS AND HOSPITALS

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The presence of protozoan parasites was studied in vegetables produced in peri-urban Hanoi where either untreated wastewater or rainwater and pig manure was used for irrigation and fertilizing. The vegetable, morning glory, was sampled during wet and dry season along the chain from fields until arrival at the markets. Vegetables and herbs were collected from a small peri-urban market and a large market in central Hanoi. Samples were analysed for *Giardia*, *Cryptosporidium*, *Cyclospora*, helminth eggs and presumptive thermotolerant coliforms. Faecal samples from children below the age of 5 years, hospitalized with diarrhoea, were examined for *Giardia*, *Cryptosporidium* and *Cyclospora*.

The highest prevalence in vegetable samples was found at the wastewater site during dry season (*Giardia*: 45.8% ; *Cryptosporidium*: 33.3%). The prevalence of *Cyclospora* appeared not to differ between seasons or sites (range: 0 – 6.7%). At markets, the prevalence of *Giardia* was highest during wet season (37.5%) whereas for *Cryptosporidium* it was highest during dry season (20.8 – 33.3%). The overall prevalence in faecal samples was 7.6% for *Giardia* and 11.4% for *Cryptosporidium*.

The prevalence of protozoan parasites on vegetables for human consumption and in human faecal samples was found to be comparable to those reported for other developing countries. This study is the first of its kind to study the prevalence of protozoan parasites in the vegetable production, at markets and in human cases of diarrhoea in Vietnam.

* *TRICHURIS SUIS* EXCRETORY/SECRETORY ANTIGEN SPECIFIC ANTIBODIES IN SERUM OF SINGLE INOCULATED PIGS

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An enzyme-linked immunosorbent assay (ELISA) was developed to test the serum levels of *T. suis* excretory/secretory (E/S) specific IgG1, IgG2, IgM and IgA antibodies. E/S antigen was obtained from culture fluids of adult *T. suis*. Forty pigs were inoculated once with 5,000 *Trichuris suis* eggs and at 1, 3, 5, 7 and 9 weeks post infection (pi) 6 pigs were necropsied. Serum was collected from all pigs at day 0 and at the time of necropsy. The 10 remaining pigs were necropsied 11 weeks pi and from these pigs serum was collected 0, 1, 3, 5, 7 and 9 weeks pi. as well as at the time of necropsy. The worm burden of each pig was determined at necropsy. The majority of pigs single inoculated with *T. suis* eggs expel their worms around 9 weeks pi.. The average E/S specific IgA, IgG1 and IgG2 levels for all pigs started to increase 5 weeks pi and peaked at 9 weeks pi., whereas the average E/S specific IgM level increased already from 1 week pi. and remained elevated by 11 weeks pi.. There is only a significant ($P<0.05$) positive linear association between IgG1 as well as IgG2 levels and worm burden at 11 weeks pi.. Whether the antibody levels are merely a consequence of the infection or they are important for the expulsion of worms has yet to be determined and further research is required to determine the future use of this ELISA for immunodiagnosis of *T. suis*.

* VIABILITY OF *ISOSPORA SUIS* OOCYSTS UNDER VARIOUS ENVIRONMENTAL CONDITIONS

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A method involving a dye exclusion test for studying *Isospora suis* oocyst viability under various environmental conditions, characterized by defined combinations of temperature and relative humidity, is described.
Faecal samples were collected at a farm with a history of *I. suis* infection and no treatment of neonatal piglets with coccidiostatica. Faecal smears were made to identify positive samples and oocysts were subsequently purified by filtration and centrifugation.
Four replicates of 50 µL oocyst suspension were exposed to a given combination of relative humidity and temperature, and studied over time using fluorescence- and light microscopy until <5% of the oocysts were considered to be viable. Viability was determined based on morphology and fluorescent properties of the oocyst as well as on the permeability of the oocyst wall characterized by inclusion/exclusion of the fluorescent dye propidium iodide.
Results show a rapid reduction in viability of the oocysts when exposed to quick desiccation characterized by high temperatures (25°C & 30°C) in combination with low relative humidities (53% rh. & 62% rh.). Viability is prolonged when oocysts are exposed to higher values of relative humidity (75% rh. & 95% rh.) as well as a lower temperature (20°C).
The results are promising as they indicate that it might be possible to break the transmission of *I. suis* in modern sow herds by changing the microclimate of the farrowing pens and thereby increase animal welfare without the use of routine medication.
Viability determined by this dye exclusion test needs to be verified. This may be done by development of an efficient and reliable excystation method specific for *I. suis*.

HELMINTH INFECTIONS IN PIGS: POSSIBLE EFFECTS OF HOST GENOTYPE

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An extremely aggregated distribution of parasites within the host population is a characteristic of natural and experimental infections of many parasites, which may suggest an effect of host genetics on resistance. The aim of the present study was to examine the association between host genotype and resistance to parasite infections in pigs. A total of 197 piglets were produced after artificial insemination of 19 sows (Danish Landrace-Yorkshire crossbreds) with semen selected from 13 individual Duroc boars (mean litter size: 10.4; range: 5-15). At 10 weeks of age pigs were trickle infection with *Trichuris suis* and *Ascaris suum* eggs (5 and 25 eggs/kg/day, respectively) in the feed for 14 weeks until necropsy and worm counts (*Ascaris* only). Faecal samples were taken at regular intervals during patency and examined for eggs by a modified McMaster technique. Preliminary analysis of variance was performed. Maximum faecal egg counts for *Trichuris* were observed week 8 (prevalence: 54%) followed by a decline and by week 14 only 16 piglets were positive. The mean faecal egg counts (geometric) of litters at week 8 p.i. ranged from 3 to 680 epg for *Trichuris* (median: 110 epg). The effects of litters and sow nested within boar were highly significant ($P<0,001$ and $P<0,001$, respectively). The prevalence for *Ascaris* reached 59% at week 8 and 62% by week 14. At week 8 p.i., the mean faecal egg counts (geometric) of litters ranged from 210 to 6224 epg for *Ascaris* (median: 1415) and the effects of litters and sow nested within boar were significant ($P<0,001$ and $P<0,05$, respectively). Litters that were high and low ranking with respect to *Ascaris* tended also to have the similar ranking for *Trichuris*. Our results indicate that there are clear differences in parasite load, as measured by epg, between litters, which most likely is related to host genotype. The material will be further analysed by means of a genome scan using microsatellite markers and SNPs in candidate genes in order to assess associations between specific genes and worm burden.

* SPECIES-SPECIFIC DETECTION OF EQUINE LARGE STRONGYLE DNA BY REAL-TIME PCR

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Equine large strongyles (*Strongylus vulgaris, S. edentatus* and *S. equinus*) are important nematode pathogens of horses with high pathogenic potential, but many years of frequent anthelmintic use in horses has greatly decreased the prevalence of these parasites. However, new national policies in Denmark aimed at reducing the problems of drug resistance in equine small strongyle (cyathostomin) parasites may inadvertently lead to higher levels of large strongyle infections. The only means of differentiation between strongyle species is a two-week larval culture with subsequent identification of third stage (L3) larvae by a trained technician. But even with a trained technician, sensitivity is poor when *Strongylus* spp. L3 are present in low numbers. The aim of the present study is to overcome this diagnostic problem by developing a real-time Polymerase Chain Reaction (PCR) assay capable of specific-specific identification of these three *Strongylus* species. Species specific primers and TaqMan® probes were designed by alignment of published ribosomal DNA sequences of the second internal transcribed spacer of cyathostomin and *Strongylus* spp. nematodes. Sensitivity and specificity of the primers were tested using traditional PCR. Primer optimization was then performed using primer matrices with real-time PCR and SYBR Green detection. Probe matrices were performed real-time to determine optimum concentrations of the probes. The present assay can reliably and specifically detect minute quantities of DNA of each of the three *Strongylus* species whether alone or mixed in the same sample. The current assay can be used for qualitative detection of *Strongylus* spp. eggs in faecal samples, but with further development it has potential for species-specific relative quantification of eggs within a sample.

*** FIELD TESTING OF THE WHO "DOSE POLE" IN ADMINISTRATING PRAZIQUANTEL FOR TREMATODE TREATMENT IN LAO PDR**

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In 2001, the World Health Organization developed a dose pole, which employs height measurements rather than weight measurements for administration of praziquantel. In the present study, height interval dosing using a dose pole was compared with the performance of a weight interval scale using a bathroom scale in estimating dosages of praziquantel in two studies. The first study was based on 313 individuals and the second study on 232 individuals in a Lao village. The first study and second study showed that weight interval dosing performed significantly better than height interval dosing (84.7% vs. 38.3%) and (73.6% vs. 43.1%), respectively, in delivering dosages of 40-50 mg/kg for treatment of opisthorchiasis and Asian schistosomiasis, as well as below and above 40-50 mg/kg. It was concluded that weight interval dosing is recommended for future administration of praziquantel in Lao PDR.

MICROSATELLITES DISTINGUISH *SCHISTOSOMA JAPONICUM* POPULATIONS IN DIFFERENT DEFINITIVE HOST SPECIES

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Multi-host parasites, those capable of infecting more than one species of host, are responsible for the majority of all zoonotic, emerging or persistent human and animal diseases and are considered one of the major challenges of the biomedical sciences in the 21st century. We characterised the population structure of the multi-host parasite *Schistosoma japonicum* in relation to its definitive host species by genotyping miracidia collected from humans and domestic animals across five villages around the Yangtze River in Anhui province, Mainland China, using 11 microsatellite markers. High levels of polymorphisms were observed and two main genetic clusters were identified which separated water buffalo, cattle and humans from goats, pigs, dogs and cats. We thereby present the first evidence of definitive host based genetic variation in *S. japonicum* which has important epidemiological, evolutionary and medical implications.