World Association for the Advancement of Veterinary Parasitology

2009

Abstract Volume

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Plenary Presentations

PL1 - Plenary 1

Sunday, August, 09, 2009

PL1.1

The Arctic as a Model for Anticipating, Preventing, and Mitigating Climate Change Impacts on Host–Parasite Interactions

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Climate change is influencing the structure and function of natural ecosystems around the world, including host–parasite interactions and disease emergence. Understanding the influence of climate change on infectious disease at temperate and tropical latitudes can be challenging because of numerous complicating biological, social, and political factors. Arctic and Subarctic regionsmay be particularly good models for unraveling the impacts of climate change on parasite ecology because they are relatively simple systems with low biological diversity and few other complicating anthropogenic factors. We examine some changing dynamics of host–parasite interactions at high latitudes and use these to illustrate a framework for approaching understanding, preventing, andmitigating climate change impacts on infectious disease, including zoonoses, in wildlife.

PL2 - Plenary 2

Monday, August, 10, 2009

PL2.1

Canada and Veterinary Parasitology

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A World Association for the Advancement of Veterinary Parasitology tradition for its conference is to present some highlights of the country hosting the event, and with an emphasis on the history of, and research in, veterinary parasitology. A review of Canada's peoples, physiography, climate, natural resources, agriculture, animal populations, pioneers in veterinary parasitology, research accomplishments by other veterinary parasitologists, centres for research in veterinary parasitology, and major current research had been presented at a World Association for the Advancement of Veterinary Parasitology Conference in Canada in 1987, and was published. The present paper updates the information on the above topics for the 22 years since this conference was last held in Canada.

PL2.2

Parasite Control in the Age of Drug Resistance and Changing Agricultural Practices

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The benefits of using antiparasitic drugs in farm animals are unquestionable. However, despite anthelmintic use as the predominant control strategy, extreme parasite infection cases are appearing in sheep and goat production; these impact productivity and have show mortality rates reaching pre-drug use levels. This was a predictable situation resulting from the loss of efficacy by all available products, particularly when some products were used as the sole intervention. The concepts of agroecology and holistic agriculture, which advocate the use of integrated management strategies, such as target selected treatment, herbal medicine, and the application of other parasite control alternatives, are not completely new, but are undergoing a resurgence because of their more sustainable appeal. The objective of this review article is to examine the problem of parasite control in the face of parasite drug resistance and to outline some strategies that may be used in parasite control programmes. Before they are accepted and recommended by the WAAVP, agroecological methods such as those listed above and described in detail herein should be validated based on scientific evidence of

their efficacy for parasite control and should be tested for both host and environmental safety.

PL3 - Plenary 3

Tuesday, August, 11, 2009

PL3.1

Future of the Animal Health Industry at a Time of Food Crisis

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It is popular in some quarters to say that there is no food crisis; that there is food aplenty; and that the problemis one of distribution or other over-arching technical difficulty. To the starving, however, there is a food crisis; and it neither speaks well nor bodes well for humanity if we dismiss their plight so glibly. The United Nations has called for a large and rapid increase in food production. Veterinary parasitologists and industry leaders can contribute to the production of healthier livestock and the expansion of aquaculture, but enhanced production and better delivery of plant foods may provide faster relief. Although livestock farming is not the most energy-efficient way of producing food,meat will remain a significant component of the global diet for the foreseeable future. New measures for parasite control will be needed, and we must improve our methods of inventing them. They need not act directly against the parasite. In the distant future lie other threats to the inhabitants of planet Earth, and here we must acknowledge the cogency of the no-foodcrisis argument. In the long term, the production of animal foods and animal feeds will be revamped in ways that depend on how (or whether) we solve the energy crisis, the environmental crisis, the increasingly dire regional population crises, and the current world financial crisis. Throughout the 20th century, the animal health industry had to adapt to industrialization and expansive agribusiness. It will have to adapt to even greater changes in the 21st century and beyond.

PL3.2

Vectors in a Changing Environment

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The evolution of pathogens is *driven* by intrinsic biological barriers, but is *directed* and *constrained* by extrinsic environmental factors. Once evolved, their arrival, establishment and spread in a geographical sense can be investigated, understood and predicted by contrasting, but complementary, analytical methods.

This will be illustrated by a variety of vector-borne disease systems that are biologically complex and highly sensitive to environmental conditions.

Arrival is now commonly recognized as due to human trade and travel, resulting in much more rapid, long-distance dispersal than can be achieved by the vectors' or hosts' own mobility. Viruses that cause Dengue, Chikungunya, West Nile fever and Blue Tongue are prime examples.

Establishment depends on the introduced pathogen finding environmental conditions that are both abiotically and biotically permissive for on-going transmission equal to or above the critical threshold level, that is defined by the simple condition of the basic reproduction number (R_0) = 1. Predictions of the global distribution of such conditions allow forewarning and therefore forearming.

Spread and increased incidence ('emergence') may depend as much on human factors as biological dynamics, as is well illustrated by recent abrupt upsurges in tick-borne infections in Europe and Eurasia over recent decades.

My research is supported by the Wellcome Trust (070696/Z/03/Z), the UK Natural Environment Research Council (NER/K/S/1999/00142), and EU-Fr6 grant GOCE-2003-010284 EDEN

PL4 - Plenary 4

Wednesday, August, 12, 2009

PL4.1

Selected Parasitosis in Cultured and Wild Fish

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While intensive aquaculture has and will continue to supply the ever growing population with highly nutritious protein, it also comes with problems which include more frequent

outbreaks of diseases in fish farms and transmission of diseases between farmed and wild fish. We have selected four Phyla of economically important fish parasites for our present discussion—a haemoflagellate (Cryptobia salmositica), a microsporidian, (Loma salmonae), a monogenean (Gyrodactylus salaries) and two copepods (Lepeophtheirus salmonis, Caligus rogercresseyi). This review consists of two parts with a brief description of each parasite and its biology related to transmission, followed by discussions on epizootic outbreaks in both wild and farmed fish, interactions between wild and farmed fish, and disease prevention and control.

PL5 - Plenary 5

Thursday, August, 13, 2009

PL5.1

From Parasite Genomes to One Healthy World; Are We Having Fun Yet?

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In 1990, the Human Genome Sequencing Project was established. This laid the ground work for an explosion of sequence data that has since followed. As a result of this effort, the first complete genome of an animal, *Caenorhabditis elegans* was published in 1998. The sequence of *Drosophila melanogaster* was made available in March, 2000 and in the following year, working drafts of the human genome were generated with the completed sequence (92%) being released in 2003. Recent advancements and next-generation technologies have made sequencing common place and have infiltrated every aspect of biological research including parasitism. To date, sequencing of 32 apicomplexa and 24 nematode genomes are either in progress or near completion, and over 600K nematode EST and 200K apicomplexa EST submissions fill the databases. However, the winds have shifted and efforts are now refocusing on how best to store, mine and apply this data to problem solving. Herein we tend not to summarize existing X-omics datasets or present new technological advances that promise future benefits. Rather, the information to follow condenses up-to-date-applications of existing technologies to problem solving as it relates to parasite research. Advancements in non-parasite systems are also presented with the proviso that applications to parasite research are in the making.

PL5.2

Emerging Food-borne Parasites

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Parasitic food-borne diseases are generally underrecognised, however they are becoming more common. Globalization of the food supply, increased international travel, increase of the population of highly susceptible persons, change in culinary habits, but also improved diagnostic tools and communication are some factors associated with the increased diagnosis of food-borne parasitic diseases worldwide. This paper reviews the most important emerging food-borne parasites, with emphasis on transmission routes. In a first part, waterborne parasites transmitted by contaminated food such as Cyclospora cayetanensis, Cryptosporidium and Giardia are discussed. Also human fasciolosis, of which the importance has only been recognised in the last decades, with total numbers of reported cases increasing from less than 3000 to 17 million, is looked at. Furthermore, fasciolopsiosis, an intestinal trematode of humans and pigs belongs to the waterborne parasites as well. A few parasites that may be transmitted through faecal contamination of foods and that have received renewed attention, such as Toxoplasma gondii, or that are (re-) emerging, such as Trypanosoma cruzi and Echinococcus spp., are briefly reviewed. In a second part, meat-borne parasite infections are reviewed. Humans get infected by eating raw or undercooked meat infected with cyst stages of these parasites. Meat inspection is the principal method applied in the control of Taenia spp. and Trichinella spp. However, it is often not very sensitive, frequently not practised, and not done for T. gondii and Sarcocystis spp. Meat of reptiles, amphibians and fish can be infected with a variety of parasites, including trematodes (Opisthorchis spp., Clonorchis sinensis, minute intestinal flukes), cestodes (Diphyllobothrium spp., Spirometra), nematodes (Gnathostoma, spp., anisakine parasites), and pentastomids that can cause zoonotic infections in humans when consumed raw or not properly cooked. Another important zoonotic food-borne trematode is the lungfluke (Paragonimus spp.). Traditionally, these parasitic zoonoses are most common in Asia because of the particular food practices and the importance of aquaculture. However, some of these parasites may emerge in other continents through aquaculture and improved transportation and distribution systems. Because of inadequate systems for routine diagnosis and monitoring or reporting for many of the zoonotic parasites, the incidence of human disease and parasite occurrence in food is underestimated. Of particular concern in industrialised countries are the highly resistant waterborne protozoal infections as well as the increased travel and immigration, which increase the exposure to exotic diseases. The increased demand for animal proteins in developing countries will lead to an intensification of the production systems in which the risk of

zoonotic infections needs to be assessed. Overall, there is an urgent need for better monitoring and control of food-borne parasites using new technologies.

WAAVP Symposium
One World, One Health: Parasites in a Chalanging Landscape

Sunday, August, 09, 2009

SY1.1

Parasites and People in a Changing Landscape

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The environmental and ecological impacts of climate change are likely to affect many human parasitic diseases. Malaria, with the highest global burden of disease for any parasite, has emerged as the 'pin-up' for climate change and parasitic disease, with research being conducted worldwide on modelling the potential effects, and more funding than ever for research into vaccines and cures. Yet there are numerous neglected tropical diseases that may be affected by climate change. Schistosomiasis, ascariasis, hookworm and filariasis already collectively affect approximately two billion people, mainly in developing countries. Unfortunately, these diseases have also been truly neglected in research and scientific assessments in relation to health impacts of climate change. The IPCC's Fourth Assessment Report, while having comprehensive information on risk and vulnerability to climate change of malaria for example, neglected to mention the major helminth diseases, save for several mentions of schistosomiasis. To further complicate an already extremely complex situation, other social and economic impacts of climate change (e.g., poverty, education, agriculture, food and water availability etc.) are likely to exacerbate helminth infections, while at the same time pushing them further down the list of priorities. The ecological disturbance caused by climate change is also likely to similarly alter distribution and infection rates of protozoan parasites worldwide, not the least through changes in movement of populations (of both humans and animals). It is essential that the multi-disciplinary nature of these problems is acknowledged, to ensure that the appropriate spread of expertise is assembled to tackle the increasing problem of parasitic diseases and climate change.

SY1.2

Parasites and Domestic Animals in a Changing Landscape

Morgan, Eric R.
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Environmental change affects not only the abiotic factors acting on parasite transmission and population dynamics, but also the availability of intermediate and reservoir hosts, and the management of domestic animals themselves. Net effects on parasitic disease can therefore be complex, especially when the nature and extent of landscape and environmental change varies so much around the world. Thus, expansion of farming into wilderness, and attempts to regenerate natural habitats in intensively farmed regions, although very different processes, potentially expose livestock to wildlife parasites and to microhabitats suitable for intermediate hosts. Consequences include, for example, re-emergence of liver fluke in ruminants in restored wetlands. Elsewhere, overgrazing and drought can alter infection pressure, but also the condition and susceptibility of livestock hosts. Companion animals also face altered parasite threats from changes to the natural environment, and from the redistribution of parasites through movement of pets. In attempting to predict and mitigate parasitic risks to domestic animal health through landscape change, it is important to focus on the interactions between animal management and parasite transmission. The most realistic biological models show that the effects of climate change on parasite development and population growth can be minor in relation to those of even small changes in management. This emphasises that when addressing parasite control in a changing environment, due attention must be paid to factors outside the traditional realm of parasitology. This is a challenge for us all.

SY1.3

From Climate to Molecules, Who Does Matter the Most? The Dynamics of Host-Parasite Interaction in Wildlife Systems

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Climate change has been suggested to have a major effect on infectious disease dynamics and yet, unequivocal evidence of casual mechanisms is weak and often burred by other ecological factors. So a number of questions arise: Why is the effect of climatic variables on parasite dynamics lost? When is this effect lost? Are there alternative and more important factors driving infectious diseases? Does the host matter the most?

I shall address these questions using two long term studies, the red grouse-*T. tenuis* and the rabbit-*T. retortaeformis/G. strigosum* systems. I shall examine the interactions between

the host and its parasites and highlight how unexpected climatic events, seasonality, co-infections and host characteristics affect parasite dynamics. I shall explain that host immunity plays a fundamental role in driving parasite transmission and consequently long term infection dynamics. Finally, I shall highlight that we need a better understanding of within host mechanisms of infection to understand large scale parasite persistence.

SY1.4

Parasites in a Changing Landscape: A Global Perspective on History, Connectivity and Consequences

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Humans are integral components of a complex biosphere where our influence and impacts have deepened and accelerated over time. During much of human history, we existed in a "large and slow world" where relatively isolated populations occupied disparate landscapes dominated by local effects, and as intimate players in a broader and interconnected ecological arena. Historically, exploitation of diverse habitats and resources, concurrent with expansion of agriculture and animal husbandry, has driven environmental modification and landscape fragmentation. Subsequent exploration and industrialization has engendered burgeoning human populations, urbanization, globalization and a transformation to a "small and rapid world" characterized by the breakdown of ecological isolation. Across landscape, regional and global scales, we have disrupted the biosphere. New waves of extinction threaten biological diversity and ecological continuity in terrestrial, aquatic and marine systems. Landscapes in transition influence the proximity of wildlife, domestic animals and people, and ecotone-effects at borderlands determine transmission, emergence and resurgence of pathogens. In the current regime of accelerating change, 'One World- One Health' is a synergy emphasizing connectivity and consequences, recognizing that our interface with the environment has profound implications for the health and vitality of human and ecological communities. We have at our disposal, and should use, powerful tools in describing and countering these threats. These include: the means to understand ecological and epidemiological changes and to define underlying evolutionary processes; the capacity to document and archive past and present environments; and the opportunity to predict future conditions based on foreseeable scenarios. Heightened collaboration and communication across disciplines and borders, and a sense of urgency, will be necessary to effectively address this global challenge.

Oral Presentations

CS1-Drug Resistance and Production

Monday, August, 10, 2009

CS1.1

Control of Resistant Parasites on a Commercial Stocker Operation

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Necropsy data collected on a commercial stocker ranch in both 2003 and 2004 showed that Haemonchus placei, H. contortus, Cooperia punctata, and C. oncophora were resistant to macrocyclic lactones, while H. contortus and C. punctata also were resistant to benzimidazoles. In 2005, animals maintained on these same pastures were dewormed with macrocyclic lactone plus levamisole at turnout and again at approximately days 35 and 70. A fourth and final deworming was carried out with fenbendazole in the feed and seasonal helminth control was obtained. In 2006 and 2007, all animals on these pastures were dewormed at turnout and again at approximately day 35 with a macrocyclic lactone plus levamisole. A third deworming was carried out with fenbendazole in the feed. Similar to 2005, seasonal control of all helminth species was accomplished. However, fecal egg counts were generally higher than those recorded in previous years prior to discovering resistance on this property. In 2008, animals were dewormed with an injectable macrocyclic lactone plus levamisole at turnout and were not dewormed again until they exhibited a positive fecal egg count. Once a positive fecal egg count was established, animals on two pastures were dewormed once with an injectable macrocyclic lactone and then with fenbendazole in the feed (third deworming). At days 15 and 52 after the third deworming with fenbendazole, all fecal samples showed 0 EPG. Animals on a third pasture were dewormed with an injectable macrocyclic lactone plus levamisole two additional times (second and third deworming). At day 19, after the third deworming, fecal samples showed 0 EPG. Thus, using a single-active dewormer for the second and third treatments, and only treating after the appearance of positive fecal egg counts, provided a similar efficacy to that obtained with a combination dewormer. These data raise questions regarding the effect of using cost-effective single injectable dewormers on the current and future state of redeveloping nematode resistance on these pastures.

CS1.2

Prevalence of Parasitism in Treated and Non-Treated Cattle as Determined by Fecal Egg Counts Within Specific Age Groups and Designated Management Systems in the US

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Introduction: The prevalence of parasitism in thousands of treated and non-treated cattle was determined from fecal samples collected throughout the United States. It includes beef and dairy cows/bulls, sucking calves, background calves, heifers, stockers and feeders.

Methods: Fecal samples were collected and submitted to one of four parasitology laboratories for analysis using the Modified Wisconsin Sugar Fecal Flotation Method. A form accompanied the samples to identify collection date, owner, cattle age group and management system. Treatment history was not revealed until the laboratory analysis was completed. The results were submitted to a national database supported by Intervet/Schering Plough Animal Health.

Results: A total of 24,239 samples from 1,219 operations have been recorded (12,597 samples from non-treated cattle and 11,642 from treated cattle). Of the non-treated cattle, 6,109 yearling cattle from 357 operations were shedding eggs with an average count of 52.7 eggs/3gm. The average egg count for 5,314 yearling cattle from 282 operations treated with macrocyclic lactone (ML) pour-ons was 27.8 eggs/3g. The average egg count for 2,475 yearling cattle from 109 operations receiving injectable ML was 30.1 eggs/3g. The average egg count for 3,581 yearling cattle from 175 operations treated with fenbendazole medicated oral suspension, paste or a feed-grade formulation was 0.1 eggs/3g.

Significance: Producers should be made aware of the effect of parasitism on the immune system and profitability if they are not treating cattle. Counts following treatment can be used to evaluate deworming practices.

CS1.3

Documentation and Prevalence of Parasite Resistance in U.S. Cattle Using the Fecal Egg Count Reduction Test According to a Scientific Panel Consensus Protocol Presented at the 2007 AAVP Meeting

Bliss, Donald H.[2]; Newcomb, Harold[3]; Moore, Robert D.[4]; Kvasnicka, William G.[1]

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Introduction: The prevalence of parasite resistance in cattle throughout the United States was determined using the 2007 AAVP consensus Fecal Egg Count Reduction Test (FECRT) protocol. A total of 176 FECRT trials throughout 20 key livestock states were conducted in cooperation with 103 veterinary clinics or consulting veterinarians.

Methods: Target animals used for all trials were naturally parasitized yearling cattle where sufficient animals were available in each trial to sample a minimum of 18 animals at the time of treatment and again 14-days post-treatment. All samples were analyzed using the Modified Wisconsin Sugar Fecal Flotation Method. The consulting parasitologist remained masked to treatment until the analysis was completed. All samples were submitted to a national database supported by Intervet/Schering Plough Animal Health and the University of Nevada-Reno.

Results: A total of 6,896 samples were analyzed (3,467 samples collected prior to treatment and 3,429 samples collected post-treatment). The mean efficacy of macrocyclic lactone (ivermectin, dormectin, eprinomectin and moxidectin) pour-ons (83 trials; 3,313 samples) was 61.3%, the mean efficacy of the macrocyclic lactone injectables (31 trials; 1,203 samples) was 65.3%, the benzimidazoles tested (six different fenbendazole formulations) in 46 trials (1,806 samples) demonstrated an efficacy of 99.2% while a combination fenbendazole plus a macrocyclic lactone (pour-on or injectable) in 16 trials (526 samples) demonstrated an efficacy of 99.9%. These results were generated under field use conditions with the supervision of the veterinary service provided by each participating clinic.

CS1.4

Parasite Control Practices on Sheep Farms in the UK and Ireland

Coles, Gerald C.[1]; Burston, Sarah[1]; Carder, Kathryn[1]; Pritchard, Lee[1]; Whitmarsh, Amber[1]; Morgan, Eric R.[1]; Hosking, Barry C.[2]

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Parasite control practices on sheep farms are coming under increasing scrutiny as the problem of drug resistance takes hold and seriously threatens to undermine the sustainability of the industry. Efforts to change parasite control practices are hindered by limited published data on current behaviour. A telephone survey was conducted of >600 farms in the UK and Republic of Ireland in 2008. Only 9 % of farmers perceived a resistance problem in nematodes, with 23 % unsure and 68 % reporting that drugs continued to work well. This is at odds with the known high prevalence of anthelmintic resistance in the study area, and suggests widespread complacency when resistance is present but at levels as yet too low to cause clinical disease. The median number of annual treatments against nematodes was 2 in ewes and 3 in lambs. However, the number of lamb treatments was strongly influenced by region and farming system, with an average of 5.6 treatments per year in south-west England compared with 2.5 in Wales, Scotland and northern England. 46 % of farmers considered tapeworms to be a problem, and 76 % treated against them. The criterion for assessing anti-parasitic treatment efficacy was almost universally lack of clinical or production problems following treatment. The present study suggests that efforts to warn farmers of the dangers of resistance and to persuade them to monitor drug efficacy should be strengthened. Supported by Novartis Animal Health.

CS1.5

Variation in the Effectiveness of Different Classes of Anthelmintics on Fecal Egg Counts in Cattle Entering the West Virginia Southern Bull and Replacement Heifer Test Program

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Each year, the West Virginia University Extension Service, the West Virginia Department of Agriculture, and the West Virginia Cattleman's Association conduct the Southern Bull and Replacement Heifer Test Program. In 2006, researchers observed, that in spite of a strict vaccination program on arrival, test cattle developed more health problems than expected. Fecal samples were found to have high egg counts in many cases, even though the animals had been treated multiple times with cattle wormers. In 2007, fecal samples were collected from the rectum from a total of 86 heifers from 9 different producers and 103 bulls from 13 producers on delivery to the test station. At this time, the bulls were treated with an oral benzimidazole (fenbendazole) and the heifers with a pour-on endectocide (ivermectin). Fecal samples were taken from the animals for a second time, 14 days after treatment. Initial egg counts did not differ between the bulls and heifers (49.4 and 42.4 respectively). In contrast, endectocide treatment reduced fecal eggs counts by only 45 %, whereas treatment with an oral benzimidazole reduced the mean fecal egg count by 95% (26.9 and 2.2 respectively). In the heifers the effectiveness of the treatment varied based upon the source of the animals (range of 83 to 26% reduction). These results indicate that the resistance to endectocides can be demonstrated in cattle operations in West Virginia, and that the effectiveness of endectocides can vary markedly from cattle operation to cattle operation. Producers should be carefully monitoring the effectiveness of their anthelmintic treatments.

CS1.6

Verminous gastroenteritis, Measured by EPG, Reduced Milk Yield in a Grazing System in Argentina's Humid Pampa. (Hormonal Control of Milk Production in Bovines. Effect of Verminous nematodiasis During the Cow's Development and Reproductive Stages Part 1: Diagnosis of Parasitism on Adult Cows and Effect on Milk Production – ANPCYT- PICT04 21-20294)

Mejía, Miguel E.[2]; Perri, Adrián F.[1]; Licoff, Nicolás[2]; Lazaro, Luciana[2]; Fernandez-Igartua, Belisario M.[2]; Miglierina, Martin M.[3]; Becú-Villalobos, Damasia[1]; Lacau-Mengido, Isabel M.[1]

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Gastrointestinal parasitism is a major constraint in grazing livestock production systems. Most studies on the effects of these parasites in bovines have been performed on beef herds. In dairy systems the importance of verminous gastroenteritis is well documented in growing animals, but there are few studies on adult dairy cows, with confusing results. As a part of a multi-disciplinary study, milk production of each cow (n=200) was daily measured from a dairy herd in a grazing system in the province of Buenos Aires. Parasitism was evaluated through monthly EPG of every animal within the system, and larval cultures were performed to determine the genera involved. Also grass forage samples were taken every 14 days from every paddock where each herd grazed, in order to obtain, identify and count infective larvae.

Cows with positive EPG, whether from the pre-calving sample (29.21 %), the first post-calving sample (33.33 %) or both (14.60 %) showed a decrease in milk production of 2.38 l/d, 0.93 l/d and 2.41 l/d respectively, throughout lactation. The difference was pronounced during the first five months of lactation (2.98 l/d, 2.23 l/d and 3.88 l/d). Nematodes of the genera Ostertagia, Cooperia, Haemonchus and Trichostrongylus were identified in fecal egg cultures and grass samples, showing regional normal prevalence curves and seasonal variability.

These data show that gastrointestinal nematodiasis dramatically affects milk production and suggests that EPG can be a useful diagnostic tool. Further studies are being performed with treated and untreated cows with anthelmintic drugs.

CS2 - Pharmacology and Pharmacokinetics

Monday, August, 10, 2009

CS2.1

Disposition of Eprinomectin in P-gp Deficient Mice: a Comparison with Ivermectin and Moxidectin

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Antiparasitic macrocyclic lactones (MLs) are good substrate of P-glycoprotein (P-gp), an ABC transporter involved in the drug efflux out of host body and target parasites. We have studied the contribution of P-gp in the in vivo behaviours of three structurally different MLs with different P-gp affinities. Plasma kinetics, brain concentration and intestinal excretion of ivermectin, eprinomectin and moxidectin were compared in control and mdr1ab-/- mice.

Animals were orally or subcutaneously administered with each drug (0.2 mg/kg) and plasma and brain concentrations were measured by HPLC. The MLs intestinal excretion rates were measured at different small intestine level by using in situ open intestinal perfusion model.

P-gp deficiency induced a significant increase of the area under the plasma concentration-time curve (AUC) of ivermectin (1.5 fold) and eprinomectin (3.3 fold) while moxidectin AUC was unchanged. The three drugs accumulated in mdr1ab-/- brain but eprinomectin concentrations were lower when compared to the two other drugs in both groups. Interestingly, ivermectin and to a higher extend eprinomectin were both excreted by the duodenum, jejunum and ileon via a P-gp dependent pathway while moxidectin excretion was weaker and mostly independent of P-gp.

A relationship exists between plasma kinetic, brain accumulation and intestinal excretion of the three drugs tested. Eprinomectin disposition in mice is strongly affected by P-gp deficiency more than ivermectin, while moxidectin appeared to be poorly concerned by the absence of P-gp. We propose a correlation between the disposition of these three drugs in mice and their relative affinity for P-gp.

CS2.2

The Influence of Ketoconazole and Pluronic 85 on the Efficacy and Pharmacokinetics of Ivermectin in Lambs Infected with Anthelmintic Resistant Haemonchus contortus

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Non-specific mechanisms of resistance such as the ATP binding cassette proteins play an important role in xenobiotic clearance in ovine gastro-intestinal nematodes. The aim of this trial was to assess the possibility of increasing drug bioavailability in the host whilst reducing drug clearance in anthelmintic resistant nematodes, thereby improving treatment efficacy. Thirty-six lambs were infected with 5000 multiple resistant Haemonchus contortus L3 and separated in to six groups (n=6); ivermectin alone (IVM; 0.2mg/kg bodyweight), ketoconazole alone (KET; 10mg/kg BW), pluronic 85 alone (P85; 4mg/kg BW), IVM+KET, IVM+P85 or untreated control. Ivermectin treatments were single oral administrations on day 28 post-infection (PI) for all appropriate groups, whereas the KET and P85 treatments were both administered orally as five separate doses on days 26-30 PI inclusively.

Concomitant administration of KET or P85 with IVM induced increases in plasma and tissue concentrations of IVM in treated animals, resulting in a two-fold increase in the area under the time-concentration curve (AUC, $p<0.05$). Faecal egg counts and worm burdens of the IVM+KET and IVM+P85 groups were lower than the untreated, KET and P85 control animals.

The findings of increased IVM bioavailability within the host with no increase in treatment efficacy requires further investigation, though the possibility of the parasites having upregulated specific or alternate non specific mechanisms for effectively handling anthelmintic treatment exist and merits further examination.

CS2.3

Measurement of Ivermectin Concentrations in the Host Gastrointestinal Tissues and in Haemonchus contortus Recovered from Infected Lambs. Comparison Between Subcutaneous and Oral Treatments

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An improved efficacy against gastrointestinal resistant nematodes in sheep and goats has been documented when the macrocyclic lactones are administered orally compared to their subcutaneous injection. This work aimed to assess the influence of the IVM administration route on the relationship among concentration profiles achieved in the bloodstream, the gastrointestinal fluid/tissues and in a target abomasal parasite (H. contortus) in lambs naturally infected. Twenty (20) parasitized lambs were assigned into two experimental groups treated with IVM either intraruminally or subcutaneously at 0.2 mg/kg. Blood samples were collected from six animals in each treated group between 0 and 15 days post-treatment (plasma disposition study). Four animals from each group were sacrificed at day 3 post-treatment. Mucosa and content samples from abomasum and small intestine were collected. Adult specimens of H. contortus were recovered from the abomasum. Drug concentrations were measured by HPLC. The IVM plasma availability was higher ($P<0.05$) after the subcutaneous administration. However, IVM concentrations recovered in the gastrointestinal contents were higher in lambs treated by the intraruminal route. The ratio between IVM concentrations measured in the abomasal contents and mucosa were 0.07 (subcutaneous) and 2.97 (intraruminal). The content/mucosa ratios in the small intestine were 1.39 (subcutaneous) and 1.99 (intraruminal). IVM concentrations were 15-fold higher in H. contortus recovered from intraruminally treated lambs. The higher IVM concentrations achieved in the digestive tract shortly after the oral treatment may account to the observed enhanced efficacy compared to the parenteral administration against parasites of reduced susceptibility.

CS2.4

The Anthelmintic Ivermectin: a Substrate of Breast-Cancer Resistant Protein (BCRP)

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The massive usage of the parasiticide ivermectin in both livestock and humans dictates that a better knowledge is urgently needed on the determinants that control the drug behaviour in the organism. The ABC transporter P-glycoprotein (P-gp/MDR1A, ABCB1) plays a central role in ivermectin pharmacokinetic. The present study aimed at thoroughly characterizing the interaction of ivermectin with breast cancer resistant protein BCRP (ABCG2).

We have evaluated the capacities of ivermectin (i) to modulate ATPase activity in BCRP membrane vesicles (ii) to inhibit the transport activity in cells overexpressing BCRP (iii) to be actively transported through Caco-2 cells by BCRP and

in cells overexpressing BCRP. Furthermore, the influence of BCRP on ivermectin availability was explored in mdr1ab-/- mice treated with BCRP inhibitor.

Ivermectin inhibited the basal and the activated BCRP ATPase in a concentration dependent manner (IC50 of 1-2 µM). In cells overexpressing BCRP, ivermectin inhibited the transport of BCRP substrates. Furthermore, the reference BCRP inhibitors fumitremorginC or Ko143 both induced the accumulation of the fluorescent conjugate bodipy-ivermectin. In Caco-2 cell monolayers, BCRP inhibitors increased ivermectin apparent basolateral-apical permeability (Papp). In mdr1ab-/- mice, the area under the ivermectin concentration-time curve (AUC) was significantly increased and Ko134 led to further increases in AUC and brain concentrations.

Our data demonstrate a clear interaction of ivermectin with BCRP. BCRP participates to ivermectin efflux out of the cells and the host organism. This study points to P-gp and BCRP being important players in the modulation of ivermectin pharmacokinetic parameters.

CS2.5

Pharmacokinetics of Amino-Acetonitrile Derivatives in Sheep with a Special Emphasis on Monepantel

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The Amino-Acetonitrile Derivatives (AADs) are a new chemical class of synthetic anthelmintic compounds, demonstrating a wide spectrum of activity against parasitic gastro-intestinal nematodes in ruminants, including multi-drug resistant isolates. The AADs possess a chiral center and therefore exist in two enantiomeric forms of which only one is biologically active.

In many cases highly enantioselective pharmacokinetics could be demonstrated. Furthermore, it was shown that the sulfur-containing compounds were transformed into sulfoxides that were readily metabolized to sulfones. This presentation focuses on the pharmacokinetics of monepantel, which is the active enantiomer of a potent AAD analogue, developed for oral anthelmintic treatment of sheep (ZOLVIX®).

Comparison of blood concentrations of the sulfone metabolite after intravenous and oral application of monepantel revealed good oral absorption of monepantel. Within a few hours after oral application, the sulfone metabolite already reached about twice the concentration of the parent. This phenomenon was attributed to a strong hepatic first pass effect. As the metabolite has similar anthelmintic potency compared to the parent drug, the in vivo efficacy of monepantel is attributed to the sum of the parent drug and the sulfone metabolite. Further pharmacokinetic characteristics of monepantel sulfone are a high volume of distribution and a low clearance. It can be concluded that the oral application of monepantel is a very efficient route of administration leading to considerable efficacy against a wide spectrum of gastro-intestinal nematodes in sheep.

ZOLVIX and monepantel are not registered or available for sale in Canada.

CS2.6

The Influence of Short-Term Food Removal and Route of Administration on the Efficacy of Monepantel Against Gastro-Intestinal Nematodes of Sheep

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Studies were conducted to measure the effect of (1) short-term pre-treatment removal of food and (2) route of administration on the efficacy of monepantel (an Amino-Acetonitrile Derivative).

In study 1, lambs were randomly allocated to three groups (n=6/group). Group 1 was not fed for 24 hours prior to treatment, group 2 was fed 2 hours before treatment and group 3 was managed like group 2 but without anthelmintic. The sheep were infected with nematodes and treated when infections were at the fourth larval stage. Efficacy was determined from worm burdens. The removal of food had no significant effects on the efficacy of monepantel.

In study 2, lambs were randomly assigned to four groups (n=5/group). When nematode infections were at the fourth larval stage, the animals were treated at 0.8 mg monepantel/kg by oral, intra-ruminal or intra-abomasal routes. Efficacy was based on worm burdens (cf. controls). There was a trend for oral treatment to be best, then intra-ruminal and intra-abomasal. Differences were not always statistically significant. There appears to be a slight risk for poorer efficacy through rumen bypass with some nematode species. However, in the field, should the rumen bypass be activated, there should be minimal if any, effect on overall efficacy in younger lambs. To prevent rumen bypass, farmers should be encouraged to adopt a drenching technique where the anthelmintic is administered over the back of the tongue, directly into the esophagus. A low volume anthelmintic could be beneficial.

Monepantel is not registered or available for sale in Canada.

CS3 - Diagnosis

Monday, August, 10, 2009

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CS3.1

Serological Diagnosis of Bovine Besnoitiosis: Development of an Indirect-ELISA and a Comparative Study with a Commercial ELISA

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Bovine besnoitiosis is an emergent parasitic disease with an important economic impact in affected herds. The causative agent is the cyst-forming coccidian *Besnoitia besnoiti,* which is predominantly localized in subcutaneous tissue-cysts in the skin, mucosal membranes and scleral conjunctiva. Apparent cases of besnoitiosis are easily diagnosed by a conjunction of clinical signs and the identification of tissue cysts. However, many infected animals remain asymptomatic. Thus accurate serological tools are needed to early diagnose *B. besnoiti* infections with perspectives for control measures. Additionally, well validated tests are required in order to have comparable data among the different diagnostic laboratories. According to these requirements, an indirect enzyme-linked immunosorbent assay (ELISA) has been developed and standardized with 46 reference positive and negative bovine sera. As gold standards IFAT and western blot were employed. After TG-ROC analysis, high precision and perfect diagnostic performance (Se and Sp=100%) were obtained. Secondly validation was extended to a target population composed of 333 sera from 2 herds with besnoitiosis and diagnostic performance was compared with the only commercial ELISA available up to date. The commercial ELISA test showed high Se once the cut-off had been recalculated. Consequently, comparable results were obtained since similar apparent and true prevalence rates were obtained with both tests. However, the employment of the new cut-off may involve loss of Sp of the commercial ELISA. Additionally, discrepancies still exist, which may be problematic at the individual level. The implications of discrepancies and cross reactions together with recommendations on serological diagnosis will be further discussed.

CS3.2

First in-vitro Isolation of *Besnoitia besnoiti* from Chronically Infected Cattle in Germany

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Besnoitia besnoiti was isolated during the first recorded outbreak of bovine besnoitiosis in Germany. Molecular characterization of the new isolate, named Bb-GER1, revealed almost 100% identity with other *B. besnoiti* isolates obtained in Portugal, Spain, Israel or South Africa, when partial sequences of the 18S ribosomal RNA gene, of the internal transcribed spacer 1 and of the 5.8S RNA gene were compared. Cystozoites obtained from skin tissue of one bull were infectious for -interferon knockout (GKO) mice by intraperitoneal (ip) inoculation. Tachyzoites were detected in the peritoneal cavity, spleen, liver and lung of the mice 5 days post infection. The parasite could be maintained in GKO mice by ip inoculation for at least 5 passages. Peritoneal washings containing tachyzoites were obtained from infected mice and used to infect five cell lines (Vero, MARC-145, NA42/13, BHK21, KH-R). The best growth of tachyzoites was observed in BHK21 cells, but replication occurred to a smaller extent also in MARC-145, NA42/13 and KH-R cells. Subsequent comparative analyses revealed that after direct infection of these cell lines with cystozoites derived from bovine skin, the growth was best in NA42/13 cells. Considerable replication was also observed in the BHK21 and KH-R cell lines. Our observations on the growth characteristics of Bb-GER1 partially contrast those for other isolates. The preferential growth in particular cell lines may be characteristic for particular *B. besnoiti* isolates. A potential association between growth properties and differences in virulence remains to be established.

CS3.3

Novel 18S rRNA Sequence of *Hepatozoon* Species in Wildlife from the Southern United States

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Hepatozoon species have been documented in a variety of invertebrate and vertebrate hosts world wide. Although approximately 150 18S rDNA sequences of *Hepatozoon* spp. have been deposited in databases, only a limited number are reported from North America, and all of those obtained prior to 2007 were from domestic dogs. In this study, we compiled published *Hepatozoon* 18S rRNA sequences available from North America, collected novel sequences from previously unrecognized vertebrate hosts of *Hepatozoon* spp., and examined the phylogenetic relationships between the different *Hepatozoon* organisms found cycling in nature in the United States. An approximately 500 bp fragment of 18S rDNA common to *Hepatozoon* spp. and some other apicomplexans was amplified and sequenced from the tissues or blood of a variety of vertebrate hosts from the southern United States, including domestic dogs, coyotes, bobcats, raccoons, rabbits, several rodents (n=8 species), a gray fox, an opossum, and a snake. Phylogenetic analysis and comparison with sequences in the existing database revealed distinct taxonomic groups of *Hepatozoon* spp., with clusters formed by sequences obtained from canids/felids/raccoons; rodents/opossum; and snakes. Surprisingly, *Hepatozoon* spp. sequences from wild rabbits clustered with those obtained from carnivores (canid/felid/raccoon), and one sequence (gray fox) was most closely related to *H. canis*, a *Hepatozoon* sp. only recently recognized in North America.

CS3.4

Modification of a Commercial *Toxoplasma gondii* I IgG ELISA for Use in Multiple Animal Species

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Toxoplasma gondii is a coccidial organism that can cause disease in both its definitive and intermediate hosts. Toxoplasmosis has been described from humans and numerous species of wild and domestic animals, including feline, canine, ruminant, rodent and sea mammals, and birds. Though many testing methodologies are commercially available for diagnosis in humans, few serological tests are appropriate and easily adaptable for use in the broad range of animals at risk of infection. The aim of this study was to develop a serological test for detection of anti-*T. gondii* antibodies that would allow rapid evaluation of multiple animal species. A commercially available kit for the detection of anti-*T. gondii* IgG in human serum samples was modified by the replacement of kit supplied conjugate with protein A or protein G horseradish peroxidase conjugate. Serum that had been submitted to New York State's Animal Health Diagnostic Center and previously tested for *T. gondii* by indirect hemagglutination was retested using these modified kits. The choice of protein A or protein G conjugate type was guided by specific binding profiles provided by Molecular Probes product information. Agreement between the two testing methods analyzed by Kappa value ranged from good (canine samples using protein A) to excellent (caprine and ovine samples using protein G). This newly described strategy will allow rapid throughput testing of animal samples for evidence of infection with *T. gondii*, aiding in accurate diagnosis of individual animals and allowing a more enlightened portrayal of disease prevalence across multiple animal species.

CS3.5

Quantitative Detection of *Sarcocystis aucheniae* in Naturally Infected Alpacas by Real Time PCR

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Sarcocystiosis in south american camelids is produced by two cyst-forming coccidian parasite species, *Sarcocystis aucheniae* which produces macrocysts in the skeletal muscles and *Sarcocystis lamacanis* which produces microscopic cysts either in skeletal or heart muscles. Diagnosis of these are made at the abattoir because of their chronic and asymptomatic presentation and there is no data on their vertical transmission to offspring so far. Lately, a PCR was developed for detection in live animals and to monitor infection kinetics.

We report the development of a real-time PCR assay for the detection of *Sarcocystis aucheniae* in skeletal muscle cysts and blood of naturally infected alpacas and aborted fetuses. This assay used a double-strand DNA-binding dye FAM and the oligonucleotide primers Sa-1 (1473-1992) and Sa-2 (1552-1534) were designed to produce an 80 bp amplicon corresponding to a partial sequence of the 18S ribosomal RNA gene (GenBank accession no AY840990).

DNA was isolated from blood and bradyzoites of dissected macroscopic cysts of alpacas and llamas, and brain, heart and skeletal muscles of aborted fetuses. This real-time PCR successfully detected *S. aucheniae* in the clinical samples from adult alpacas and llamas but failed to detect it in tissues of aborted fetuses suggesting that in south american camelids vertical transmission is unlikely. Detection limit of this real-time PCR was determined by assaying serial dilutions of parasite DNA equivalent to 10 to 10^4 bradyzoites. PCR ampli-

cons were visualized on 2% agarose gels, and the expected amplicon size of 80 bp was registered.

CS4 - Equine Parasitism

Monday, August, 10, 2009

CS4.1

Fatal Halicephalobus gingivalis (Nematoda) Brain Infection in an Icelandic Horse

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Introduction: Infections with the soil nematode Halicephalobus gingivalis (Rhabditida) are infrequently reported but the cases are widespread geographically. There are only about 40-50 published cases in horses worldwide, one in zebra, two in ruminants, and three in humans, and infection is apparently always fatal. The nematodes are believed to penetrate wounds and subsequently reproduce within the host tissues. We report a fatal case in a horse in Iceland.

Material and Methods: Following an accident in a stallion of the Icelandic breed, the horse sustained injuries to the mouth. After a few months, the stallion developed severe neurological signs and had to be euthanatized. The horse was autopsied and samples from the brain, liver, heart and kidney were examined histologically.

Results: Histological examination of the brain (cerebellum) revealed severe multifocal malacia, with numerous intralesional nematodes and mild inflammation. Mature nematodes, larvae and eggs were present in the perivascular space. There was a moderate, mononuclear perivascular cuffing and infiltration of mononuclear inflammatory cells in the meningis. The nematode was confirmed as being the species Halicephalobus gingivalis based on its morphological features. Adult worms were 250 - 300 m long and 15 m wide, eggs measured 45x15 m. Nematodes were not detected in other organs, but hemorrhages were seen in the liver and kidneys.

Discussion: The worms had presumably penetrated the wounds in the mouth of the horse as a result of the accident. This is first case of Halicephalobus gingivalis infection reported from Iceland.

CS4.2

The Grazing Horse Gets the Worm: the Use of Coprological and Coproantigen ELISA Analyses for Diagnosing *Anoplocephala perfoliata* Infection in Southern Alberta Horses

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Anoplocephala perfoliata infection in horses has been found to cause gastrointestinal disease and it has become apparent a more accurate diagnostic method is needed. Risk factors contributing to transmission of this tapeworm also need to be identified. Prevalence and distribution of this tapeworm in western Canada is not well described. Faecal samples were collected from privately owned horses in realistic management settings and evaluated using standard centrifugation flotation. A subset of samples was further evaluated using a coproantigen ELISA. Samples collected at a local abattoir served as the gold standard for evaluating diagnostic techniques. A positive correlation was found between tapeworm antigen and infection intensity using the coproantigen ELISA. Spatial and temporal prevalence of *A. perfoliata* in faecal samples varied among seasons from 18% in the winter and 8% in the summer based on coprological analysis. Significantly more pastured horses were infected than non-pasture horses during both sampling seasons. Upon further examination of pastured horses with the coproantigen ELISA, a significantly higher proportion of horses were found to be infected during each sampling season. Prevalence was found to range from 25 to 62% over the sampling seasons from winter 2006 to summer 2007.

CS4.3

Inaccurate Diagnosis Between Anoplocephala perfoliata and A. magna in Naturally Infected Horses

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In Spain, the prevalence of the infection by horse tapeworms is 30% ca. (A. perfoliata, 40% and A. magna, 20%), with low to moderate tapeworm burdens. The probability of infection by Anoplocephala magna is higher in young than in adult animals, and some data supports the absence of A. magna infection in immune competent adult horses. Data are presented to support the postulate that the actual debate on the role of A. perfoliata in colic in horses must be due to the yet unreliable tools for in vivo differentiating accurately the presence of each tapeworm species.

It has been stated that the possibility to detect tapeworm infections by faecal diagnosis is much higher when animals

harbour A. magna, either in single or mixed infections, than when only A. perfoliata is present. Antibody levels are routinely being use to establish the relationship between A. perfoliata infection and clinical disease due to the relationship between circulating IgG antibodies levels and high burden of tapeworms. However, data about the sensitivity and specificity of ELISA tests to identify A. perfoliata or A, magna infected horses resulted unreliable (Sensitivity: 75% and 43%; specificity: 60% and 100%, respectively). ELISA test has been extensively used to diagnose A. perfoliata as the cause of equine colics as well as studies on prevalence to define the extent of equine tapeworm infection, in spite of the moderate sensitivity and low specificity to differentiate between A. perfoliata and A .magna as well as other factors (individual variability or previous anthelmintic treatments) that avoid correct diagnosis. The nested PCR developed to detect and amplify A. perfoliata DNA equine faecal samples artificially contaminated with parasite material is a valid method also from faecal samples collected in the field. This internal primer set has been evaluated against A. magna DNA and Anoplocephaloides mamillana with specific positive PCR results. A recent comparison between techniques strongly indicates that the nested PCR approach for the specific detection of A. perfoliata DNA could provide a useful tool for clinical studies, diagnosis and research being an alternative to classical methods.

CS4.4

Parasitological and Serological Diagnosis of Trypanosoma evansi in Experimentally Inoculated Donkeys

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Six donkeys (one year old) free from T.evansi or its antibodies were reared conventionally during the experimental period of 50 days. The donkeys were assigned into: 5 experimental and one control non-inoculated donkey. The T.evansi strain (Egypt, CH1) used for the experiment was isolated from a naturally infected horse in Cairo city, Egypt and propagated by sub-passaging in mice. The five experimental donkeys (A,B,C,D and E) were inoculated each with 350.000, 500.000, 250.000, 50.000 or 100.000 T.evansi respectively. From each experimental and control donkey, two blood samples (3ml each) one on heparin (obtained daily) and one plain blood (collected every 4 days) until the end of the experiment (50 days post-infection [DPI]). Blood was examined parasitologically daily. Serum was prepared from plain blood and two fold serial dilutions (1 : 4 to 1 : 128) were made for testing by using Card agglutination test (CATT, T. evansi).

The prepatent period of T. evansi in the inoculated donkeys varied from 1 – 4 DPI. Among the experimental donkeys only donkey A could survive until the end of the experiment. The other 4 donkeys died after 8DPI (No. C), 28DPI (No. B) 36DPI (No. E) and 40DPI (No.D). The patent period varied from 7 to 48 DPI with some negative days in between. The number of T.evansi in the inoculated donkeys varied from 2-2360 per 50 high power fields (x400). The first detection of antibodies occurred on the 4th DPI (No.D), 8th DPI (No.B), 12th DPI (No.A) and 16th DPI (No.E). The end point titer in these donkeys varied from 1:4 – 1:32. The control donkey was parasitologically and serologically negative during the experimental period.

In conclusion, CATT was more easy and reliable than parasitological diagnosis of T. evansi in donkeys.

CS5 - Trichinella and Food Safety Symposium

Monday, August, 10, 2009

CS5.1

Animal Production Food Safety: Trichinella Certification as a Model for On-Farm Programs

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It is widely accepted that an effective food safety strategy, with respect to meat products, must incorporate participation along the entire food chain, including animal production, transport, slaughter, processing and distribution. While a number of research studies have demonstrated opportunities for positively effecting food safety on the farm, the use of this information in organized programs for reducing pathogen contamination in livestock has not been pursued. Recently, the European Union (EU) and the United States (US) launched programs to certify pork as free from Trichinella by documenting adherence to production practices which reduce or eliminate risk of infection. Good production practices in the Trichinella-free certification programs are monitored by on-farm audits conducted by trained veterinarians, overseen by competent veterinary authorities, and, in the initial phases, absence of infection in certified animals is verified by post-slaughter testing. The Trichinella-free pork certification programs provide a framework which could be adapted to risk reduction programs for other pathogens associated with pork (such as Toxoplasma and Salmonella) as well as zoonotic pathogens associated with other commodity meats. Certification of animal production systems adhering to programs of reduced risk management for foodborne pathogens could be used to market premium products as well as to improve

consumer confidence in the safety of commodity meats. In this symposium, speakers will describe and discuss the Trichinella-free certification programs as legislated in the EU and the US, and, using knowledge regarding transmission of other foodborne pathogens in livestock, propose ways in which reduced risk management systems could be incorporated into certification programs for these pathogens.

CS6 - Non-Pharma Control

Monday, August, 10, 2009

CS6.1

Effects of Incremental Protein Supply on Lactational Performance and Resistance to *Nippostrongylus Brasiliensis* in Lactating Rats

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Background: Reduced periparturient resistance to parasites may arise from a prioritisation of scarce protein allocation to reproductive functions over immune functions. Here, we tested in our *Nippostrongylus brasiliensis* re-infection lactating rat model, whether increments of scarce protein supply indeed increase lactational performance without improving resistance.

Methods: Rats were infected with 1600 *N. brasiliensis* larvae before mating and re-infected with the same dose on day 2 of lactation. During lactation, rats were daily fed the same amount of non-protein energy, and one of 6 equidistant amounts of protein (*n*=7), ranging from 1.75 to 6.75 g. The first four levels of protein supply were calculated to be scarce, and the last two to be more than adequate. This resulted in foods with increasing protein to energy (P:E) ratios. Litter size was standardised to 9 pups. Dams and litters were weighed daily until day 11 of lactation when worm burdens were taken.

Results: Unexpectedly, food refusals were observed at the two highest P:E diets. Consequently, achieved protein intake did not increase further after the third increment of protein offered. Feeding treatments affected both lactational performance ($P<0.01$) and resistance ($P<0.05$). The first three increments of protein offered improved dam and litter weight gain, and reduced worm burdens. Further increments did not further improve these performance and resistance traits.

Conclusions: Our results suggest that increments of scarce protein supply are allocated towards both reproductive and immune functions. However, whether such nutritional sensitivity of periparturient resistance is consistent across parasite species remains to be elucidated.

CS6.2

Nutritional Sensitivity of Periparturient Resistance to Parasites in Sheep May Differ Between Parasite Species

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Background: A reduced periparturient resistance to parasites may arise from preferential scarce metabolizable protein (MP) allocation to reproduction over expression of immunity. In addition, periparturient resistance may be higher for small-intestinal than for abomasal nematode species. Here, we propose that such differences may arise from variation in scarce MP allocation to reproductive and immune functions.

Methods: Two experiments were carried out, using identical nutritional and parasitological protocols. Twin-rearing sheep were trickle infected during the periparturient period with either 5000 L3 *Trichostrongylus colubriformis* (Exp.1) or 10,000 L3 *Teladorsagia circumcincta* (Exp. 2). Ewes were given the same amount of metabolizable energy during lactation, whilst their one of five levels of MP supply incrementally ranged from ~65% to ~125% times their estimated MP requirements. Lambs were weighed twice weekly from parturition onwards to estimate milk production. Worm burdens were assessed on day28 of lactation.

Results: In both experiments, estimated milk production increased supply over the first two increments of MP supply before reaching a plateau. *T. colubriformis* worm burdens were reduced from the second MP increment onwards, whereas *T. circumcincta* were reduced only at the highest level of MP supply.

Conclusions: Although combining results across experiments should be done with caution, even when using identical methodologies, the contrast between effects of MP supply on resistance to *T. colubriformis* and *T. circumcincta* supports the view that periparturient immunity to small intestinal nematodes may be less sensitive to MP scarcity than periparturient immunity to abomasal nematodes. This may suggest that different parasite species impose different nutritional penalties on their host.

CS6.3

Consequences of Ewe Protein Nutrition and Grazing on Chicory (*Cichorium intybus*) on Animal Performance and Parasitism

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Background: Periparturient protein supplementation and grazing the bioactive forage chicory are being developed independently as two non-chemical worm control strategies for sheep. Here, we investigated their interactive effects, and hypothesized that both strategies decrease ewe and lamb faecal egg count (FEC) and improve lamb performance, but that benefits arising from chicory grazing are more pronounced in unsupplemented ewes.

Methods: Thirty-six twin-rearing Greyface ewes, trickle infected whilst housed with 10,000 L3 *Teladorsagia circumcincta* from day-42 to turn-out on day32 (day0 is parturition), were either not supplemented or supplemented with protein (n=18) from day-21 to weaning at day102. Sheep were turned-out onto either grass/clover or chicory plots (n=6 with 3 ewes each) that were grazed previously by *T. circumcincta* infected sheep. Lambs were drenched whenever they showed diarrhoea, weight loss and/or high FEC.

Results: Pre-turn-out protein supplementation reduced ewe FEC by 60% (P<0.001) and increased lamb weight gain by 164±19.2 g/day (P<0.001). Post turn-out, feeding treatment did not interact and also did not affect ewe and lamb FEC. However, protein supplementation and chicory grazing independently reduced lamb drench requirement (by 31% and 40%, respectively, P<0.05) and increased lamb weight gain (by 20.0±9.3 g/day, P=0.072; and 67.3±9.4 g/day, P<0.001 respectively). Together, periparturient protein supplementation and chicory grazing improved terminal lamb body weight at day159 by 5.9±0.96 kg and 6.9±0.96 kg, respectively (P<0.05).

Conclusion: Ewe protein supplementation and chicory grazing independently improved sheep performance and reduced parasitism. The reduced drench requirement arising from both nutritional strategies may assist in reducing the rate of anthelmintic resistance development.

CS6.4

New Zealand Native Plants for Parasite Control

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The first Maori arrived in New Zealand around 1000 years ago with dogs and the small Polynesian rat on board their canoes. In 1773 James Cook was the first to introduce small numbers of pigs, poultry and two sheep. Therefore there is not a strong ethnopharmocological tradition with respect to livestock in New Zealand. There is however an increasing interest in alternative methods of worm control, particularly plant based ones.

The search for indigenous anthelminthic plants began with Maori literature, but mentions of species with anti-parasitical properties are sparse. New Zealand however has a good botanical relationship with South America for which there is a database of plants with recorded anthelminthic activity available. The database was searched at the family level to find groups of species with a reputation for anthelminthic activity and New Zealand relatives were identified.

In order to evaluate the likely activities of short listed plants a screening assay using *Dictyocaulus* spp. was developed and extracts of each plant tested. Forty plants were then selected for further testing. Of these, five were used in an indoor trial in which they were fed to previously parasite free red deer (*Cervus elaphus*). Once the animals had accepted the browse as part of their diet they were given a single dose of *Dictyocaulus* spp. and monitored. Individual intakes of each browse were measured and animals were sampled daily for larval output then slaughtered after 30 days. One native species was found to have significant anthelminthic activity and a further three had some activity.

CS6.5

Evaluation of Three in Vitro Bioassays for Measuring the Anthelmintic Activity of a Condensed Tannin Extract from Sericea Lespedeza

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Certain forages high in condensed tannins (CT) demonstrate anthelmintic activity, and appear to be a useful non-chemical adjunct to parasite control in small ruminants. In addition to feeding trials, in vitro bioassays have been used to evaluate this antiparasitic effect against several species of trichostrongyle nematodes. However, it remains unclear which in vitro assay is the most appropriate evaluation tool. The goal of this research is to evaluate the repeatability and sigmoidal dose response characteristics of different in vitro methods, and determine which are most suitable for measuring the effective concentration (EC_{50}) of these extracts. In this project, 3 in vitro methods (larval migration inhibition assay (LMIA), egg hatch assay (EHA), and larval development assay (LDA) were performed using the CT extract of sericea lespedeza (*Lespedeza cuneata*) with *Haemonchus contortus* eggs or larvae. The concentration ranges used were 1.5 - 1560 µg/ml. The LMIA was repeated 11 times in triplicate, whereas the EHA and LDA were repeated 14 times in triplicate. The LMIA

yielded inconsistent values for EC_{50}, ranging from 9.3 - 470.4 µg/ml. In contrast, the LDA yielded much more consistent results, with EC_{50} ranging from 26.5 - 66.2 µg/ml. The EC_{50} for the EHA was > 1560 µg/ml in all cases, thus could not be measured. Our results indicate that the LDA yields the most consistent and repeatable EC_{50} and dose response curves. Therefore, the LDA appears to be the most appropriate bioassay for measuring the antiparasitic activity of CT plant extracts in trichostrongyle nematodes of small ruminants.

CS6.6

Exploring the Potential for Australian Native Perennial Shrubs to Have an Anthelmintic Role in Livestock Grazing Systems in Southern Australia

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The 'Enrich' project is exploring the use of forage shrubs in a mixed feedbase for grazing enterprises in the low-medium rainfall zones of southern Australia. Forage shrubs are being assessed for their ease of establishment, growth performance and nutritive value, as well as their ability to contribute to gut health through reduced methane emissions and effects on gastrointestinal worm infections. We are using nematode larval development assays with Haemonchus contortus in order to identify plants which show anthelmintic properties. A number of plants have shown significant activity in this assay system (IC50 values down to 60 µg extracted solids per ml). We have further investigated activity in some species by looking at plants collected from different positions within single paddocks, and from different regions, as well as plant samples collected from the same plants at different times. This has revealed significant variations in activity over space and time. Our initial work to identify the nature of the anthelmintic compounds has shown that while tannins are almost wholly responsible for activity in some cases, other plants show activity which is not due to tannins. We have also observed significant toxicity towards adult H. contortus worms in some extracts in in vitro assays. Our study indicates that there is potential for Australian native shrubs to play an anthelmintic role in grazing systems in southern Australia, however, in vivo experiments with plant species showing the most promising levels of in vitro activity will be needed to confirm this potential.

CS7 - Cryptosporidium / Giardia

Monday, August, 10, 2009

CS7.1

Occurrence and Molecular Characterization of *Cryptosporidium parvum* from European Hedgehogs (*Erinaceus europaeus*)

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European hedgehog is often brought to hedgehog feeding stations for overwintering, in case of illness or abnormal behaviour. To determine the occurrence of *C. parvum* infections and to estimate the zoonotical potential of shed oocysts the faecal samples from geographically distinct stations were examined by ELISA and staining techniques for the presence of developmental antigen and oocysts, respectively. A part of positive samples was subjected to PCR-RFLP as well as sequencing on 18S rRNA, actin gene, 70 kDa heat shock protein gene (HSP70) and 60 kDa glycoprotein gene (GP60).

Forty-nine (24.5 %) of 200 submitted samples were positive for both antigen and oocysts. While 35 (27.7 %, n=126) samples were positive from newly found hedgehogs, 14 (18.9%, n=74) samples were positive from animals after several month stay on the station.

Thirteen samples subjected to PCR-RFLP on 18S rRNA locus suggested *C. parvum*. The subtyping on GP60 locus revealed three different subtype families: IIa (n=1, IIaA19G1RI), IIc (n=5, IIcA5G3) and a new IIk subtype family (n=6, subtypes IIkA19R12, IIkA19R11, IIkA21R10, IIkA19R11, IIkA21R11, IIkA26R4). One sample was positive for both IIcA5G3 and IIkA22R11 subtypes. The multilocus sequence analysis (18S rRNA, Actin, HSP70) on 12 samples belonging to IIa, IIc and IIk subtype families proposed that IIk subtype is probably a hedgehog-specific *C. parvum* genotype. Hedgehogs shedding *C. parvum* oocysts have a potential infection risk for humans and the anthroponotic nature of IIc subtype family should be reviewed.

CS7.2

Prevalence of Giardia and Cryptosporidium on Dairy Farms and in Water Bodies Surrounding the Farms in Prince Edward Island, Canada

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Giardia and Cryptosporidium are major causes of waterborne gastrointestinal disease in humans. Livestock have been suggested as sources for water contamination. The prevalence of Giardia and Cryptosporidium was determined in dairy cattle and the water sources within the vicinity of the dairy farms. Fecal samples were collected from 20 adult cattle (>6 months) and 20 calves (<6 months) from each of 20 farms. Ground (wells) and surface water samples in and around each farm were also collected. Giardia cysts and Cryptosporidium oocysts were isolated from fecal samples using a sucrose flotation gradient method and from surface and ground water samples using the Environmental Protection Agency Method 1623. An immunofluorescence antibody assay was used to determine positive samples for further genotyping. Herd prevalence for Giardia was 100% while Cryptosporidium was 55%. Within the positive herds Giardia was present in 75% of adults and 100% of calves. Cryptosporidium was detected in 45% of adults and 55% of calves. Giardia was detected in 9% of surface water samples with cyst concentrations ranging from 0.1 to 0.20 cysts/L. Giardia cysts were not detected in ground water. Cryptosporidium was detected in 61% of surface water and one ground water sample. Cryptosporidium oocysts concentrations from positive surface water samples ranged from 0.05 to 12.4 oocysts/L while the ground water sample contained 8.0 oocysts/L. These study findings suggest that Giardia and Cryptosporidium are highly prevalent in dairy farms in PEI and potentially can contaminate ground and surface water. Genotyping is being performed on positive cattle and water samples to determine if cattle are the source of water contamination as well as to determine the zoonotic potential of the isolated parasites

CS7.3

Cryptosporidiosis (and giardiasis) in Peri-Urban Zambia - Preliminary Status of an Ongoing Study

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Introduction: Gastro-enteritis caused by *Cryptosporidium* is widespread in humans/animals but the consequences are particularly severe in poor communities with inadequate sanitation and lack of clean, safe drinking water. Therefore, a study was initiated with the objectives of determining prevalence/intensity of helminths and protozoa in pre-school children, incidence/seasonal variation of cryptosporidiosis, risk factors, sources, and genetic diversity of *Cryptosporidium* from children, domestic animals and water.

Methods: Cross-sectional study: Single stool samples from 403 children from 10 pre-schools in Kafue district, Zambia: duplicate Kato-Katz thick smears and immunofluorescence (Merifluor ® *Cryptosporidium/Giardia*) + questionnaire survey.

Longitudinal study: Monthly stool samples from children + questionnaire survey. Quarterly stool samples from animals (148 pigs, 20 dogs, 17 goats, 30 ducks, 13 chicken, 7 pigeons): immunofluorescence. Water samples every 2 month: centrifugation + immunofluroescence. Molecular methods: QIAamp ® DNA Stool Mini Kit + PCR/sequencing of 18S rDNA and HSP70.

Preliminary Results: Prevalence of helminths in children was 15.6% with *Ascaris lumbricoides* most common (10.7%). Prevalence of *Cryptosporidium/Giardia* was 28.0% [15.2 – 54.2%] and 29.0% [17.4 – 38.5%] respectively. Mixed infection was found in 22.2%. All children were asymptomatic. Association was found for *A. lumbricoides* and hookworm (P=0.001), *A. lumbricoides* and *Cryptosporidium* (P=0.033), *Cryptosporidium* and *Giardia* (P<0.001). So far 6 pigs and 2 chicken were positive for *Cryptosporidium*. Results from water analyses/ molecular typing are not yet available.

Preliminary Conclusions: Prevalence of *Cryptosporidium/ Giardia* in pre-school children in Zambia is very high with potential severe consequences. This study will reveal important information concerning transmission routes, genetic diversity and risk factors.

CS7.4

A Novel Intestinal Epithelial Cell Line to Evaluate the Infectivity of *Cryptosporidium parvum*

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Cryptosporidium spp. are Apicomplexa protozoan parasites that infect the microvillus border of the gastrointestinal and respiratory epithelium of a wide range of vertebrate hosts including humans. Because of the robust structure of oocyst, there are a few effective methods inactivating *Cryptosporidium* oocysts. We investigated disinfection of *C. parvum* oo-

cysts by photocatalytic fibers incorporating titanium dioxide (TiO_2) excited by UV light and nano-bubble ozone generated by a new apparatus (Nature's Co., Japan). We evaluated the effectiveness by using mouse infection model and a novel cell culture method. One liter suspension of *C. parvum* HNJ-1 was exposed at 500 mL/min flow rate in a 1L reactor containing 8W low-pressure UV lamp and TiO_2 photocatalytic fibers (UBE Industries, Japan). The mouse model demonstrated that the reactor decreased approximately $4\log_{10}$ of *C. parvum* oocysts infectivity. We developed an *in vitro* culture method of *C. parvum* by using a novel murine intestinal epithelial cell line (MIE) derived from the C57BL/6 mouse. *C. parvum* oocysts were exposed by 50mL of 20ppm ozone for 30 min at 25 °C. The cell culture method demonstrated that ozone decreased approximately $2.7\log_{10}$ of oocyst infectivity which was slightly underestimated compared to $3.8\log_{10}$ demonstrated by the mouse infection model.

CS7.5

Giardia in Dogs – Treatment with Drontal® Plus flavour Tablets

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Introduction: The administration of three consecutive daily doses of the recommended 1X dose of Drontal® Plus flavour tablets (Bayer) was examined for its effect on *Giardia* cyst shedding in 7 treated and 7 untreated random-source dogs. **Methods:** Dogs were treated on study Days 0, 1, and 2. Cysts were quantified using direct immuno-fluorescent labeling on Days -7, -5, -3 and -2 and daily from Day 1 through 11.

Results: Three treated dogs never shed cysts again during the study, one shed again only on Day 4, and the remaining three dogs started to shed again on Days 8, 9, and 11. The mean numbers of cysts per gram in the feces of the treated dogs were significantly reduced (t-tests using Log10(counts)) on Days 1 and 2 (Geometric means: controls = 447,000; treated = 1,050, $p = 0.004$) and Days 3 to 8 (Geometric means: controls = 23,400; treated 5.0; $p < 0.001$). Four controls that had been consistently positive changed to negative status on Day 11, and thus, on the final day of the Trial, there were only three positive control and three positive treated dogs.

Discussion: Three consecutive days of treatment with Drontal® Plus flavour tablets halted *Giardia* cyst shedding by dogs. But starting six days after treatment's end, some of the dogs started shedding cysts again. Since the prepatent period of *Giardia* can be as short as 4 days, shedding of *Giardia* cysts 6 days after treatment could be caused by a reinfection.

CS7.6

Survival Kinetics of *Cryptosporidium* in Swine Facility Wastes in the Southern Piedmont and Coastal Plain Watersheds

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Introduction: The objective of this study is to minimize the impact of swine rearing facilities on watersheds by abrogating or reducing the number of oocysts of *Cryptosporidium* species leaving the facilities in their waste streams and to examine oocyst inactivation as a surrogate for other less environmentally resistant organisms present in these lagoons. Specific objectives: (1) Determine the viability of oocysts within swine lagoons and material leaving the lagoons for land application. (2) Determine the effects of lagoon storage on the inactivation kinetics of oocysts placed within the lagoons in sentinel chambers. (3) Determine the inactivation kinetics of oocysts that have been land applied to forage crops after their treatment within a swine lagoon.

Methods: Between June 1, 2007 and May 31, 2008, monthly samples were collected from each of 10 swine waste lagoons in the Southern Piedmont and Coastal Plain. Oocysts were purified from samples using immunomagnetic beads, viability determined using a fluorescent dye exclusion assay, and genetic assemblage of the recovered oocysts determined with an 18S SSU rRNA PCR protocol and sequencing.

Results: The majority of oocysts recovered from the 380 samples were nonviable, and almost all recovered oocysts were *Cryptosporidium suis* (also a few Pig Genotype 2 and occasional rat, mouse, cat, and wildlife genotypes).

Discussion: Work will next examine lagoon storage on the viability of oocysts added in chambers. This research is supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2006-35102-17191

CS8- Pharmacology and Physiology

Monday, August, 10, 2009

CS8.1

Identification of Monepantel as the First Anthelmintic Drug Development Candidate from the Amino-Acetonitrile Derivatives (AADs)

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The recently discovered Amino-Acetonitrile Derivatives (AADs) offer a new class of synthetic chemicals with anthelmintic activity. The evaluation of AADs was pursued applying in vitro assays, and efficacy and tolerability studies in rodents, sheep and cattle. Amongst various suitable compounds, monepantel (AAD 1566) eliminated many tested pathogenic nematode species, both at larval and adult stages, at a dose of 2.5 mg/kg bodyweight in sheep and 5.0 mg/kg bodyweight in cattle. The same doses were sufficient to cure animals infected with resistant or multi-drug resistant nematode isolates. These findings, complemented by the good tolerability and low toxicity to mammals, suggested that monepantel would be a suitable anthelmintic drug development candidate.

Monepantel is not registered or available for sale in Canada.

CS8.2

Haemonchus contortus Acetylcholine Receptors of the DEG-3 Subfamily and Their Role in Sensitivity to Monepantel

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Gastro-intestinal nematodes in ruminants are a global threat to farming. Resistance to the currently available classes of broad-spectrum anthelmintics stresses the need for new anthelmintics against gastro-intestinal nematodes.

A novel anthelmintic class, the Amino-Acetonitrile Derivatives (AADs), was recently discovered and the drug candidate AAD-1566 was chosen for development.

Studies with Caenorhabditis elegans suggested that the AADs act via nicotinic acetylcholine receptors (nAChR) of the nematode-specific DEG-3 subfamily. The role of nAChR genes of the DEG-3 subfamily from Haemonchus contortus in AAD sensitivity was investigated. Using a novel in vitro selection procedure, mutant H. contortus populations of reduced sensitivity to AAD-1566 (monepantel) were obtained. Sequencing of full-length nAChR coding sequences from AAD-susceptible H. contortus and their AAD-1566-mutant progeny revealed two genes were affected. In the gene monepantel-1 (Hco-mptl-1, formerly named Hc-acr-23H), a panel of mutations was observed exclusively in the AAD-mutant nematodes, including deletions at intron-exon boundaries that resulted in mis-spliced transcripts and premature stop codons. In the gene Hco-des-2H, the same 135 bp insertion in the 5' UTR created additional, out of frame start codons in two independent H. contortus AAD-mutants. Furthermore, the AAD mutants exhibited altered expression levels of the DEG-3 subfamily nAChR genes Hco-mptl-1, Hco-des-2H and Hco-deg-3H as quantified by real-time PCR. These results indicate that Hco-MPTL-1 and other nAChR subunits of the DEG-3 subfamily constitute a target for AAD action against H. contortus and that loss-of-function mutations in the corresponding genes may reduce the sensitivity to AADs.

Monepantel is not registered or available for sale in Canada.

CS8.3

HcGGR3: a Dopamine-Gated Ion Channel Subunit in Haemonchus contortus

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HcGGR3 is a gene which encodes a novel ligand-gated ion channel subunit. Protein sequence analysis indicates that this channel is anion selective. Analysis of the cDNA sequence shows putative microRNA interaction site which could be important in relation to developmental expression of this subunit. qRT-PCR analysis of HcGGR3 shows that it is differentially expressed among the various life-stages and the rank order of expression was eggs > adult female > larvae > adult male. In addition, HcGGR3 is significantly down regulated in macrocyclic lactone (ML) selected laboratory strains of H. contortus. We also found a single nucleotide polymorphism in the 3' UTR that appears to be associated with ML selection. Immunolocalization of this subunit in adult worms has revealed that in females, the localization is distinctly punctate around the cervical papillae (deirids) in socket cells and in males, expression was observed around the deirid socket and possibly some sheath cells. Electrophysiological analysis of this subunit expressed in Xenopus laevis oocytes showed

that it forms a homomeric channel that responds mainly to dopamine. This subunit could have a possible role in mechanosensation.

CS8.4

Isolation and Electrophysiological Characterization of Two GABA Receptor Subunits from the Parasitic Nematode *Haemonchus Contortus*

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Haemonchus contortus is a gastrointestinal parasitic nematode, which infects cattle and sheep worldwide. This parasite is controlled by nematocides; many which target receptors of the inhibitory nervous system called ligand-gated chloride channels (LGCCs). Research on the model free-living nematode *Caenorhabditis elegans* has identified several different types of LGCCs. One example is a set of GABA receptors called UNC-49 that play a role in locomotion (Bamber et al 1999). Our aim was to determine whether there is structural and functional similarity between UNC-49 receptors in *H. contortus* compared to what has been reported for *C. elegans*. We have identified two *H. contortus* genes *HcUNC-49B* and *C* that, like *C. elegans*, appear to be generated from alternative splicing of the same *UNC-49* gene. Electrophysiological analysis show HcUNC-49B is able to form a functional homomeric channel in *Xenopus laevis* oocytes that produces a robust dose dependant response to GABA and is highly sensitive to picrotoxin. In contrast, HcUNC-49C alone did not form a functional channel. When both HcUNC-49B and C are expressed in combination, the HcUNC-49B/C heteromer appears to be preferentially formed, evident by its increased sensitivity to GABA and lower sensitivity to picrotoxin. While the EC_{50} of GABA for the HcUNC-49B channel is similar to that reported for the same channel in *C. elegans*, the HcUNC-49B/C channel is 3x more sensitive to GABA compared to the *C. elegans* heteromeric channel. These results suggest that there may be differences in the function of the UNC-49 GABA receptors between the two nematode species.

CS8.5

Measurement of Cytosolic Calcium in Ascaris Suum Muscle During Maintained Levamisole Application and Desensitization

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Introduction: Resistance to the nicotinic anthelmintics like levamisole is now significant and limits the usefulness of these drugs. Levamisole is a selective cholinergic anthelmintic that produces spastic paralysis in nematode parasites but much remains to be determined about its mode of action. One problem is that some parasites recover (desensitize) despite the maintained presence of the anthelmintic.

Methods: To study effects of levamisole on cytosolic calcium we adapted a ratio-metric method for use in somatic muscle of Ascaris suum. Pressure injection of the potassium salt of fura-red through a micropipette into the bag region of somatic muscle cells was used. The fura-red was excited using 440 and 490 nM light and emission monitored at >510 nM. We used the longer wave length fura-red for calcium measurement because of the auto-fluorescence that is present in A. suum. The 440/490 ratio was taken as a measure of the intracellular calcium.

Results: We found that maintained application of levamisole was associated with a transient increase in intracellular calcium followed by a return towards resting levels over a period of about 10 minutes.

Discussion: We do not yet know the factors that cause the calcium concentration to return to resting levels in the maintained presence of levamisole. However, we are now able to study these factors which may underlie the ability of parasites to desensitize and recover from the effects of nicotinic anthelmintics. These factors may also be involved in the development of resistance.

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CS9 - Non-Pharma Control

Monday, August, 10, 2009

CS9.1

The Potential of Copper Oxide Wire Particles to Control Haemonchus contortus in Indigenous Goats Owned by Small-Scale Farmers in South Africa

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The control of haemonchosis, the most economically important gastrointestinal disease of small ruminants in the Tropics and Subtropics, has been compromised by widespread anthelmintic resistance. Copper oxide wire particles (COWP) have been shown to have an anthelmintic effect against Hae-

monchus contortus, but their field efficacy in South Africa requires evaluation. As such, COWP efficacy was evaluated in indigenous goats raised by small-scale farmers in Bergville, KwaZulu-Natal Province.

Individual female goats owned by 15 farmers (15 herds) were monitored for faecal egg counts (FECs) at 4-weekly intervals from the start of the summer rainfall season (October 2007). In January 2008, when FECs were sufficiently high for an FEC reduction test to be carried out, half the goats within each herd were treated with 4g COWP or not. FECs were determined on the day of treatment and 2 weeks later. Mean pre- and post-treatment FECs for the COWP-treated group (n = 73) were 2347 epg and 264 epg, respectively, resulting in a reduction of FEC of 89%. The corresponding FEC values for the untreated controls (n = 66) were 2652 epg and 2709 epg. Pre- and post-treatment faecal cultures showed a prevalence of 72 % and 46 %, respectively, for Haemonchus spp. larvae. The FECs of the COWP-treated goats were similar to the untreated animals 4 weeks after treatment.

The authors propose that COWP may be used for tactical anthelmintic treatment to reduce the expected late-summer peak in FECs in goats raised by these small-scale farmers.

CS9.2

Efficacy of Copper Oxide Wire Particles Against Gastrointestinal Nematodes in Sheep and Goats

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Economic sheep and goat production in the USA is severely hampered by gastrointestinal nematode (GIN) parasitism, particularly Haemonchus contortus. Copper oxide wire particles (COWP) have anti-parasitic properties in the diet of small ruminants, but efficacy of COWP may differ between sheep and goats. In a trial with weaned kids (Kiko x Spanish cross, 6 months old) and lambs (Katahdin or Dorper x Blackface crosses, 5 months old) grazing the same pasture in Central Georgia, half the animals for each species were given 2 g of COWP in a gel capsule, while the other half were given no COWP. Fecal and blood samples were taken from individual animals weekly to determine GIN eggs per gram (EPG) and blood packed cell volume (PCV). Half the animals were slaughtered 28 days post-treatment and adult GIN recovered from the abomasum and small intestines for counting and identification to species. Remaining animals were allowed to graze for an additional 14 d (42 d total). For both sheep and goats, COWP treatment reduced EPG ($P<0.05$), increased PCV ($P<0.05$), and lowered abomasal GIN numbers ($P<0.05$). Reductions in EPG ranged from 75-91% for goats and 83-95% for sheep from days 7-42 of the trial, while numbers of adult H. contortus were reduced by 67 and 86% for COWP-treated sheep and goats, respectively. The COWP treatment was equally efficacious against GIN infection in sheep and goats and is an effective method for controlling these parasites in small ruminants.

CS9.3

Evaluation of Copper Oxide Wire Particles in a Feed Pellet to Control Gastrointestinal Nematodes in Sheep and Goats

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Copper oxide wire particles (COWP) can effectively reduce gastrointestinal nematode (GIN) infection in small ruminants (sheep and goats) when administered in a gel capsule down the throat, but this can be challenging. As an alternative delivery system, COWP were milled into feed pellets and fed to parasitized goats (Kiko x Spanish cross, 15-18 months old), and sheep (Katahdin or Dorper x Blackface crosses, 14 months old) grazing the same pasture in Central Georgia, USA, during spring (Trial 1) and summer (Trial 2), 2008. Only the goats were used in Trial 2 because of low parasite egg counts in the sheep. Half the sheep (Trial 1; n = 8) and goats (Trials 1 and 2; n = 8) received the equivalent of 2 g COWP in supplemental feed over a 24-h period at the start of each trial, while the other half received feed pellets with no COWP. In both experiments, fecal and blood samples were taken weekly for 28 d following treatment to determine GIN eggs per gram (EPG) and blood packed cell volume (PCV). Goats were slaughtered after the second trial and adult GIN recovered from the abomasum and small intestines for counting and identification to species. In Trial 1, COWP treatment reduced EPG ($P<0.05$) in both sheep and goats, with a greater effect in goats, and increased PCV ($P<0.05$) in the goats only. In the second trial with goats only, COWP treatment reduced FEC ($P<0.05$) and total worm count ($P<0.05$), but had no effect on PCV values. Milling COWP into feed pellets made treatment easier and may be an effective alternative delivery method for use of this GIN control technique with small ruminants.

CS9.4

The Effect of Dietary Inclusion of Dried Chicory Roots on *Oesophagostomum* spp. Infections in Naturally Infected Sows

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Introduction: Chicory roots containing easily fermentable dietary carbohydrates (fructans) have been shown to have a negative impact on experimental *Oesophagostomum dentatum* infections in pigs, but chicory roots have never been tested on-farm.

Methods: Two trials (spring and autumn) were carried out in an organic herd targeting naturally infected sows. The spring trial included 1 experimental (n=9) and 2 control (n=7, n=8) groups whereas the autumn trial included 1 experimental (n=10) and 1 control (n=10) group. The experimental groups were given a feed with 35% dried milled chicory roots by substituting part of the cereals days 0-14 and were then returned to the normal (control) feed days 14-41. Strongyle faecal egg counts were monitored regularly. In the autumn, individual larval cultures were set up day 0 for larval differentiation. In addition, faeces from control sows were pooled day 34 for culturing infective larvae that were used to inoculate 2 uninfected pigs that were slaughtered for species differentiation.

Results: The strongyle population consisted entirely of *O. dentatum* (47%) and *O. quadrispinulatum* (54%). Faecal egg excretion was almost completely stopped within 2-6 days on the chicory diet. Return to the control feed resulted in a resumed egg excretion in the experimental groups but overall egg excretion remained significantly lower than for the control groups in both trials.

Conclusions: For the first time a negative effect on *O. quadrispinulatum* by chicory roots has been demonstrated. Chicory may potentially be used strategically to reduce *Oesophagostomum* spp. infections in organic sow herds.

CS9.5

Counterintuitive Temperature-Driven Effects of Climate Change on Gastrointestinal Nematodes

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The first papers on likely effects of temperature increases on parasite epidemiology have been published. Reports indicate increases in development rates as well as extensions of windows of opportunity for parasite transmission. However, higher temperatures are likely to also impact negatively on parasite survival. This may not only lead to unexpected seasonal alterations in parasite epidemiology but also to shifts in the relative importance of economically important parasite species.

Here we explore likely effects of climate change on the epidemiology of Teladorsagia cirumcincta, Trichostrongylus colubriformis and Haemonchus contortus in temperate regions in a simple, temperature-driven, R0-based model. In the absence of host immunity, the model predicts the expected pattern of increased overall abundance and expansion of transmission windows for H. contortus. However, for T. circumcincta and T. colubriformis, a more unexpected seasonality change, a delayed start to higher levels of predicted transmission success in spring and early summer and a more 'peaked' parasite abundance in late summer and spring, is predicted. A validation process, comparing model output to a 30-year UK surveillance data set, shows that the model reflects recently observed trends very accurately. This underlines the potential of such simple models for the study of the effects of climate change on parasites. The results suggest that immune systems of hosts may not necessarily nullify an increased force of infection at pasture. Changes in seasonality of these parasites are likely to lead to adaptations of their over-winter strategies.

CS10 - Genomics / Functional Genomics

Monday, August, 10, 2009

CS10.1

Molecular Characterization of Theileria Species of the Africa Buffalo (Syncerus caffer) by 18S rRNA Gene Sequence Analysis

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The African buffalo (Syncerus caffer) is the natural reservoir host of both pathogenic and non-pathogenic Theileria species. Corridor disease, caused by Theileria parva, is a controlled disease in South Africa. Theileria parasites usually occur as mixed infections in infected animals, and although the non-pathogenic forms do not have any significant economic importance, their presence interferes with the diagnosis of T. parva. In this study, the phylogenetic relationship of pathogenic and non-pathogenic Theileria species obtained from buffalo blood samples originating from different geographical regions in South Africa were investigated using 18S rRNA gene sequences analysis. DNA was extracted, the V4 hypervariable region of the 18S rRNA gene was amplified and subjected to the Reverse Line Blot (RLB) hybridization assay using Babesia and Theileria genus- and species-specific probes. Results of the RLB revealed the presence of the pathogenic T. parva, benign T. mutans, and the non-pathogenic T. velifera, T. buffeli and Theileria sp. (buffalo). In some samples, the PCR products hybridized only with the genus-specific probes, and not with any of the species-specific

probes, suggesting the presence of novel species or genotypes. The full length 18S rRNA gene of selected samples was amplified, cloned and the recombinants sequenced. Sequence and phylogenetic analyses indicated that novel T. mutans, T. velifera and Theileria sp. (buffalo) genotypes occur in buffalo. This could have serious implications, since such sequence variants could compromise the specificity of the real-time PCR test currently used to detect T. parva infections in buffalo and cattle in South Africa.

CS10.2

The Detection of *Babesia spp*. in Domestic Felids Using DNA Probes and Phylogenetic Analysis

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Babesia is an intracellular erythrocytic haemoprotozoan of mammals and it has also been reported in reptiles and birds. The two most frequently reported *Babesia* species in felids are *B. felis*, which causes clinical babesiosis in domestic cats, and *B. leo*, primarily reported from asymptomatic lions. In this study, DNA was extracted from blood collected from 480 domestic cats (*Felis domesticus*) and the hypervariable region of the 18S rRNA gene was amplified. The PCR products were analysed using the Reverse Line Blot (RLB) hybridization assay, a technique that simultaneously detects and differentiates between *Babesia* and *Theileria* spp. RLB probes to detect *B. felis*, *B. leo* and *Babesia* sp. (cheetah) were designed, using the 18S rRNA gene sequence data, and used to screen samples collected from domestic cats. Results showed that *B. felis*, *B. leo* and *Babesia* sp. (cheetah) occur in domestic cats either as single or as mixed infections. However, some samples tested positive only with the genus-specific *Babesia* and or *Theileria* probe. This suggested the presence of a novel species or variant of a species. The full-length 18S rRNA gene of these unknown samples was subsequently amplified, cloned and sequenced. Sequence and phylogenetic analysis confirmed that a novel *Babesia* spp. was present.

CS10.3

The Obligate Intracellular Parasite Toxoplasma gondii Secretes a Soluble Phosphatidylserine Decarboxylase

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Introduction: Toxoplasma gondii is an obligate intracellular parasite that causes fatal infections in immunocompromised individuals and fetuses. Our research focuses on investigating the dependence of Toxoplasma on its host cell regarding its phospholipid biogenesis.

Methods: The host-free parasites were prepared by their in vitro culture in human fibroblasts. Fresh Parasites were incubated in intracellular-type media to test for their PtdSer metabolism by PtdSer decarboxylase (PSD) using 14CO2-trap assays.

Results: The host-free T. gondii secretes a novel soluble PSD that can decarboxylates exogenous liposomal PtdSer to PtdEtn. Quantitatively, extracellular T. gondii can secrete up to 20% of its total PSD pool in 2-hrs at 37°C. Either depletion of parasite ATP or reduction in incubation temperature inhibits PSD secretion by ~90%. The intracellular calcium chelator BAPTA-AM can also partially (~40%) reduce the PSD secretion, suggesting calcium dependence of the process. TgPSD cDNA encodes a 337-aa protein with a putative 22-aa secretory-signal peptide at its N-terminus. TgPSD is 40% identical to P. falciparum PSD and 36% identical to H. sapiens PSD. Protein contains an LGST motif, the site of auto-proteolytic processing of the PSD pro-enzyme, constituting the enzymatic active site. Processing of the TgPSD results in an alpha-subunit of 5.63-kDa at the C-terminus and a beta-subunit of 32.54-kDa at the N-terminus. TgPSD gene can also functionally complement an S. cerevisiae mutant devoid of PSD activity.

Conclusion: These findings demonstrate extremely novel features of the parasite enzyme, since neither soluble nor secreted forms of PSD have been previously described for any organism.

CS10.4

Characterization of the Thrombospondin Related Anonymous Protein 2 (TRAP 2)) of the Protozoan *Neospora caninum* in the Cell Invasion Process

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Neospora caninum is responsible for infecting a wide range of animals, inducing abortions in bovines and, as every Apicomplexan protozoan the invasion step is crucial for its survival. One important group of proteins for the invasion process is the Thrombospondin Related Anonymous Protein (TRAP) family. *N. caninum* has one NcTRAP-1 described and our group has focused on the second and undescribed TRAP of *N. caninum*, here named NcTRAP-2. The aim of this work was cloning of the NcTRAP-2 full-length sequence, production of recombinant antisera, localization of native form by 2D western blot and by confocal microscopy, and also functional evaluation through an in vitro invasion inhibition assay. The full-length gene was obtained through a combination of RLM-RACE (RNA ligase mediated Rapid Amplification of cDNA ends) technique and PCRs based on contigs from the *N. caninum* genome project website. The predicted protein sequence has 38% of identity and 52% of similarity with its homologues of *Toxoplasma*

gondii (TgMIC-2); 39% and 53% with NcTRAP-1. The TRAP homologues have a signal peptide, two adhesive domains (an integrin-like domain and one or more thrombospondin type I repeats) and a transmembrane region. Two recombinant fragments (fragments 1 and 2, both without signal peptide and transmembrane region) of NcTRAP-2 were generated (pET28 vector), with MW of 50 and 78 kDa (fragment 2 is 163 aa longer towards the C-terminal end). Antisera localized an 80 kDa NcTRAP 2 native form, and its soluble version in ESA (Excreted/Secreted Antigen) with 70 kDa through 2D western blot. The serum against recombinant 1 had the ability to inhibit the invasive process from 53 to 61%, depending on the experimental method used (manual counting or real time PCR). NcTRAP 2 was localized at the apical complex of the parasite of the tachyzoites by confocal immunofluorescence, pointing towards its micronemal localization.

CS10.5

In Silico Analysis of the Cyclophilin Repertoire of Apicomplexan Parasites

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Cyclophilins are peptidyl cis/trans isomerases, implicated in diverse processes such as protein folding, signal transduction, and RNA processing. They are also well-known as drug targets, in particular for the immunosuppressant cyclosporine A. In addition, cyclosporine is known to exhibit anti-parasitic effects on a wide range of organisms including several apicomplexa. In order to obtain new non-immunosuppressive drugs targeting apicomplexan cyclophilins, a profound knowledge of the cyclophilin repertoire of this phylum would be necessary.

BLAST and maximum likelihood analyses identified 16 different cyclophilin subfamilies within the genomes of Cryptosporidium hominis, Toxoplasma gondii, Plasmodium falciparum, Theileria annulata, Theileria parva, and Babesia bovis. In addition to good statistical support from the phylogenetic analysis, these subfamilies are also confirmed by comparison of cyclophilin domain architecture. Within an individual genome, the number of different cyclophilin genes varies between 7-9 for Cryptosporidia and 14 for T. gondii. Many of the apicomplexan cyclophilins are predicted to be nuclear proteins, most of them presumably involved in RNA processing.

The genomes of apicomplexa harbor a cyclophilin repertoire that is at least as complex as that of most fungi. The identification of cyclophilin subfamilies which are specific for lower eukaryotes, apicomplexa, or even the genus Plasmodium is of particular interest since these subfamilies are not present in host cells and might therefore represent attractive drug targets.

CS11 - Trichinella – Epidemiology

Monday, August, 10, 2009

CS11.1

Survey on Porcine Trichinellosis in Nepal Diagnosed by ELISA and Pepsin Digestion Methods

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A study on porcine trichinellosis was conducted in 8 districts of Nepal from September 2007 to October 2008. A total of 645 blood samples were collected at slaughter for serological analysis from local indigenous and crossbred pigs reared in traditional farms practicing free ranging. In addition, muscles samples (diaphragm, shoulder, foreleg, abdomen, hind leg and intercostal muscles) from 298 randomly selected pigs out of these 645 animals were collected for parasitological examination. Serum samples were screened by Enzyme Linked Immunosorbent Assay (ELISA) using excretory/secretory antigens from L1 larvae. A minimum of 10 g meat samples was processed from each individual in pooled artificial HCL-Pepsin digestions. Out of the 645 serum samples 165 (25.58%) were found positive for trichinellosis by Ab-ELISA test. However, only 19 serum samples (2.95%) gave a strong positive reaction in ELISA; the other 146 samples had an OD value close to the cut off and should be considered doubtful. Seroprevalence increased with the age of the pigs and more male than female animals had a positive result in ELISA. No larvae were recovered by digestion from the 298 meat samples. These results suggest that Trichinella spp are circulating among domestic pigs in Nepal. Further studies on a larger number of animals and using confirmatory techniques should be conducted to confirm these findings and assess the public health risks.

CS11.2

A Survey on Trichinella Infection of Wild and Domestic Animals in Switzerland

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Trichinella spp. have not been detected anymore in Swiss pigs, horses or wild boar for many decades, although the parasite (T. britovi) was repeatedly isolated from foxes (Vulpes vulpes) and lynxes (Lynx lynx). In order to provide actual data on the prevalence of Trichinella spp. in Switzerland, a basic requirement to intensify the Trichinella control in domestic pigs, a respective survey was carried out. The study included (1) red foxes (the main reservoir host of T. britovi) and Eurasian lynx (a good indicator species), (2) wild boars (Sus scrofa) (a source of infection for humans) and (3) domestic pigs of different housing systems and age, especially including free-ranging pigs kept on pasture.

Results: (1) Muscle tissue samples from 1'298 foxes and from 55 lynxes were analyzed using a standardized artificial digestion method. Trichinella britovi larvae, as specified by multiplex PCR, were found in 21 (1.6%) foxes and in 15 (27.3%) lynxes. (2 & 3) Muscle tissue samples from 1'458 wild boars, 7'412 adult pigs, 9'973 conventional finishing pigs and 2'779 free-ranging pigs were examined with both parasitological and serological methods. Trichinella-larvae could be recovered from none of the porcine animals. Although some meat juice samples were antibody-positive in the initial E/S-Ag-ELISA, none of the domestic pigs and only three wild boars remained seropositive in the confirmatory Western blot (seroprevalence in wild boars: 0.2%). In conclusion, the results show that T. britovi is present in Swiss carnivorous wildlife and that some wild boars were in contact with the parasite. The results further demonstrated the negligible risk of Trichinella infection in pigs in Switzerland, irrespective of the production system.

CS11.3

Surveillance of Trichinella Infection in Wild Boar in the United States

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Based on data from national surveys of domestic pigs conducted over the past 20 years, Trichinella infection is absent or occurs at extremely low levels in domestic pigs raised in the United States. In conventional, confinement management systems, there is no risk of infection. However, for pigs that are raised outdoors, Trichinella may still pose a risk. To assess the presence of Trichinella in specific geographic regions, recent legislation in the European Union endorses surveys of wildlife or indicators populations. Knowledge of Trichinella prevalence in indicators animals in the U.S. would help to assess infection risk to exposed populations such as outdoor pigs. In an effort to determine if a surveillance program might be useful in monitoring Trichinella infection in the U.S., we undertook a serological study of wild boar collected during an annual national survey conducted by the U.S. Department of Agriculture. Approximately 2000 samples per year were tested beginning in 2007 to the present. Samples were tested using a commercial ELISA kit (SafePath Trichinae Immunoassay Kit) according to the manufacturer's instructions. The location of positive animals was determined and spatially plotted using the longitude and latitude coordinates recorded for each collected sample. Results of this study indicate that Trichinella infection does exist in wild boar in the U.S. and these infections are clustered in specific geographic regions. Raising domestic pigs outdoors in these areas could pose a risk for exposure to infection through reservoir or intermediate hosts.

CS11.4

Cessation of Trichinella spiralis Transmission Among Scavenging Mammals After the Removal of Infected Pigs from a Poorly Managed Farm

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Pigs infected with Trichinella spiralis were detected on a farm in Maryland during an animal welfare investigation. Sera and/or tissues were collected from 49 pigs and 3 pig carcasses. Tissues were tested for the presence of T. spiralis muscle larvae by tissue digestion, and the sera were tested for the presence of anti-Trichinella antibodies by ELISA. Seventeen of 50 (34%) pigs were infected with T. spiralis based on tissue digestion. Of these 17 pigs, sera were collected from 16; 9 were serologically positive, 3 had elevated OD results, and 4 were negative (suggesting that they had become infected within a few weeks of testing). All pigs which tested negative by tissue digestion for muscle larvae were also ELISA negative.

The farm was subsequently depopulated of pigs. Six months later, testing of trapped scavenging mammals in the farm environment demonstrated that 41% were infected with T. spiralis. After 12 months, 10% of trapped animals were T. spiralis positive, and after 18 months, T. spiralis could not be detected in the scavenging mammal population surrounding the farm. Results of the study suggest that infected swine on poorly managed swine farms act as reservoirs of infection for peridomestic scavenging mammal populations; elimination of infected swine and carcasses halts transmission of T. spiralis in these scavenging mammals. In the absence of a significant source of T. spiralis-infected swine in the U.S., the risk of infection to wildlife hosts and the development of an independent sylvatic transmission cycle of T. spiralis is minimal.

CS11.5

Towards a Risk-Based Trichinella Surveillance in Denmark

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In its directive on Trichinella control, the EU has opened up for the possibility of countries being recognized as areas with negligible Trichinella prevalence in pigs for slaughter. In Denmark, Trichinella spp. have not been detected in domestic pigs for more than 70 years, and repeated surveys in the Danish fox population have shown a very low prevalence (< 0.1%) of Trichinella. Therefore, Denmark has applied to the EU to become recognized as an area with negligible Trichinella prevalence (< 1 per million). It is proposed that future Trichinella surveillance in Denmark will be risk-based, i.e. only testing of high-risk subpopulations such as all sows and boars and all outdoor reared pigs. A model called Discounting Historical Evidence, which incorporates several years of surveillance data, was used to calculate the probability that the national pig herd is free from Trichinella. The model results showed that the estimated risk of not detecting Trichinella in domestic pigs in a risk-based surveillance system is negligible. The risk-based program will include annual monitoring of red foxes and other wildlife for Trichinella with special focus on the region along the German border and areas with previous findings of Trichinella in foxes. The first results of wildlife testing are available and will be presented. Contingency plans in case of Trichinella suspicion or detection in pigs or foxes have been developed. Quality assurance programs for all laboratories performing Trichinella testing are being implemented. In 2007, the EU granted Denmark status as a region with negligible Trichinella prevalence.

CS12 - Ectoparasites

Monday, August, 10, 2009

CS12.1

Developmental Stages of Fleas on Resting Places of Cats and Dogs

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Cats and dogs are often infested with *Ctenocephalides felis* (the cat flea) and *C. canis* (the dog flea). These ectoparasites can cause flea-allergy-dermatitis and transmit flea-borne pathogens. Elimination of fleas is difficult as reinfestation can occur in the animal household if an in-house developmental cycle is established.

This study aimed to monitor adult fleas on primary hosts and to evaluate the occurrence of developmental stages in the animals own habitat.

From 2003 to 2004, a total of 1909 dogs and 1848 cats were surveyed for fleas during examination in small-animal practices in three regions of Germany.

Altogether 167 dogs and 301 cats were positive for fleas or flea-feaces. The owners of 20 cats and 12 dogs in 31 private households agreed to have the animals' resting places and vicinity probed for flea development. Each resting place and one square meter of its adjacent floor space was individually vacuum cleaned for 1 minute. The contents of each vacuum cleaner bag were deep-frozen until examination for flea developmental stages and faeces.

All homes were positive for flea developmental stages: Adult cat fleas were found in 11 cases; larvae or eggs were present in resting places (10) and direct vicinity (24), and flea faeces in > 90% of cases.

These results clearly demonstrate the need to establish an integrated flea control program to treat simultaneously the animals and their resting places, in order to break the in-house developmental cycles of fleas, resulting in an effective control of the flea population on animals.

CS12.2

Determining the Susceptibility of Cat Flea Populations to Insecticides

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The methodology selected for testing the susceptibility of insects to insecticides frequently depends upon factors such as the availability of certain stages for testing, the stage of the insect that is targeted for treatment, and the types of insecticides tested. Most on-animal treatments target the adult flea. However, the major disadvantage to testing adult fleas is that populations must be maintained on separate hosts. The advantages in testing larval stages is the possibility of isolates been shipped easily as eggs from clinics and tested without having to maintain populations on adult hosts. A flea larval bioassay has been used to monitor the susceptibility of field-collected fleas (Ctenocephalides felis) to imidacloprid (Advantage®, Bayer AG) for the last 8 years. Flea eggs are exposed to larval rearing media treated with serial dilutions of imidacloprid ranging from 0.05 to 30 ppm. The LD50s ranged from 0.14 to 1.52 ppm and a diagnostic dose of 3 ppm was established. Topical applications were applied to adult flea isolates to determine amount of imidacloprid required to kill adults. Imidacloprid is extremely active on contact with adult fleas, the LD50s ranging from 0.02 to 0.2 ng/flea. The level of variability in the larval and adult bioassays to imidacloprid in the field and laboratory isolates is similar providing additional support for using the larval bioassay.

CS12.3

Cat Flea Susceptibility to Imidacloprid: Review of an 8-Year Monitoring Initiative

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A flea larval bioassay was developed to monitor the susceptibility of fleas (Ctenocephalides felis) to imidacloprid (Advantage®, Bayer AG). Flea eggs representing different field isolates of C. felis were collected by veterinarians in the United States, the United Kingdom, and Germany. From 2001-2008, 1,356 isolates were received; 1,014 were placed in the assay. Host species was recorded for 1,342 isolates - 1,006 isolates were from cats; 336 from dogs. Percent of isolates received by month were: January - 2.53%; February – 2.16%, March – 1.42%, April – 2.83%, May – 6.26%, June – 9.39%, July – 13.11%, August – 14.38%, September – 16.02%, October – 15.35%, November – 10.36%, December – 5.51% (not data for 0.67%). Flea eggs collected by month were highest from July-October (63.13% of eggs collected). Veterinarians assessed the level of adult flea infestation as high, medium or low. High levels were reported with greatest frequency from June-October. Low infestation levels were reported with greatest frequency from November – May. Validity of the test results, based on the acquisition of reliable data after completion of the assay, could be confirmed for 668 of 851 isolates submitted during years 2002-2008 (validity data was available for years 2002-2008 only). Numbers of valid assays (parentheses) by year were: 2002 (106), 2003 (106), 2004 (171), 2005 (130), 2006 (51), 2007 (34), 2008 (70). Numerous challenges were encountered during specimen collection, conduct of the assays, and interpretation of results. Challenges and solutions will be discussed.

CS12.4

Skin Distribution of Imidacloprid by Microautoradiography Following Topical Administration to Beagle Dogs

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To investigate the cutaneous distribution, localization, and persistence of imidacloprid in the dog, [14C]imidacloprid was admixed with Advantage® Topical Solution (9.1% w/w or 10% w/v, imidacloprid) and topically applied to two adult beagle dogs (8.5 and 12.3 kg) at the therapeutic dose rate associated with the recommended tube size (11.8 and 20.3 mg/kg, respectively). At several post-treatment intervals between 7 and 56 days, hair, skin surface stripping and skin biopsies were collected from the application sites and/or distal body regions (scapula, thorax, lumbar, and hip) of the dogs for radioactivity determination. The radioactivity recovered from the various body regions demonstrated the migration of the imidacloprid-derived radioactivity from the application site(s) to the distal areas on the canine coat and skin.

Microautoradiography of the skin samples taken at 7, 14, 21, 28, and 56 days post-treatment intervals showed radioactivity (as exposed silver grains) diffusely distributed throughout

the epidermis and dermis with focal concentrations of the radioactivity being in the superficial epidermis, hair follicles, and sebaceous glands. The distribution of the radioactivity changed with time and sampling area and the silver grain intensity appeared to diminish slowly but steadily over the 56-day post-treatment period. The localization and persistence of imidacloprid within hair follicles and sebaceous glands, followed by its re-excretion onto the skin surface, is believed to represent an important mechanism by which imidacloprid efficacy against fleas on dogs and cats is maintained despite post-treatment bathing, shampooing, and/or swimming.

CS12.5

Evaluation of Larvicidal Activity of Eight Essential Oils Against Stomoxys calcitrans in La Réunion Island (Indian Ocean)

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Introduction and Objectives: The stable fly, Stomoxys calcitrans, is a haematophagous and aggressive fly, causing painful bites and significant blood loss to their cattle host. In addition, S. calcitrans adult flies may mechanically transmit various pathogens. Different methods of fly control are used: chemical compounds, biological control and control of larval habitat. The objectives of this study were to assess the larvicidal activity of eight essential oils (Ocimum basilicum, Cymbopogon nardus, Eucalyptus citriodora, Geranium rosat, Lavandula officinalis, Moringa oleifera, Azadirachta indica and Melaleuca alternifolia). Eggs of an endemic population of S. calcitrans from La Réunion Island were produced in the laboratory of the Groupement Régional de Défense Sanitaire des Bovins à la Réunion. Essential oils were diluted in Polysorbate 80 and applied on the larval development medium.

Results: Emergence of flies was significantly reduced after application of O. basilicum (no emergence), M. aternifolia (- 91%), C. nardus or G. rosat (-80%) when essential oils are applied at a similar (0.5%) concentration. Five independent repetitions were done with highly similar results.

Conclusion: These data suggest that the application of essential oils on larval habitat in farms could be an alternative control of stable flies.

CS13 - Modeling

Monday, August, 10, 2009

CS13.1

***In silico* Exploration of Gastro-Intestinal Parasite Induced Anorexia in Sheep and its Impacts on Infestation Levels and Lamb Growth Rate**

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Gastro-intestinal parasitism is a major challenge to the health and welfare of sheep and a significant cause of production loss for the sheep industry, with losses resulting from parasite-induced reductions in feed intake, nutrient loss due to digestive tract damage, and an increase in the protein requirement of immunologically naïve animals. *In silico* mathematical predictive models, based on biological principles and validated against available experimental data, can assist the understanding of host-parasite interactions and provide insights in the evolution and control of disease.

Previously we have developed an *in silico* simulation model of *Teladorsagia circumcincta* infections, which describes nutrient utilisation, host-parasite interactions and the development of immunity. This has been used to predict the joint effects of host nutrition and genotype on the progression of gastro-intestinal infections. Model inputs include expected growth attributes of the animal, feed quality, various parasitological parameters and daily larval intake. Outputs include feed intake, growth rate and body composition, as well as worm burden and faecal egg counts.

This paper explores alternative methods of describing anorexia in this model, including a reduction in food intake or growth rate as a function of worm mass, and a reduction in food intake or growth rate as a function of immunity, e.g. anorexogenic cytokine levels. The impacts of these alternative approaches for describing anorexia on growth rate, body composition, feed intake, worm burden and faecal egg counts are quantified and evaluated. This represents a step towards a fuller description of the impacts of parasite infections on lamb growth.

CS13.2

A Risk Management Approach to Using a New Anthelmintic in Australian Grazing Management Systems

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The recent launch of an Amino-Acetonitrile Derivative (AAD) anthelmintic in New Zealand (ZOLVIX®, 25 g/L monepantel) brings the opportunity to not only preserve the effective life of the new anthelmintic class but also to use it to slow the further development of resistance to the older classes.

In Australia, the use of mathematical models has enhanced our understanding of resistance management, so a simulation study was done to examine the risks of selecting nematodes resistant to monepantel in various grazing management systems.

We examined the effect of treatment programs on populations of Haemonchus, Teladorsagia and small intestinal Trichostrongylus. The modeled programs either reflected what is scientifically desirable or what is currently happening on commercial farms. We determined optimal and high-risk use patterns of strategically timed treatments of monepantel and existing anthelmintics, i.e. moxidectin and a triple combination containing a benzimidazole, levamisole and abamectin.

The success of each programme was measured using the relative time to drug resistance and the level of worm control.

ZOLVIX and monepantel are not registered or available for sale in Canada.

CS13.3

Managing a New Sheep Anthelmintic in New Zealand

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In recent years, considerable advances have been made in our understanding of the factors that contribute to the development of anthelmintic resistance in nematode parasites of sheep. Moreover, empirical evidence from field studies has been accumulating to support this knowledge. This should allow advisors and farmers to have more confidence when planning nematode control programmes.

The recent registration in New Zealand of ZOLVIX® (25 g/L monepantel), the first Amino-Acetonitrile Derivative (AAD) to be approved for use in sheep, presents an opportune time to review management practices associated with the development of anthelmintic resistance and to assess whether we have sufficient knowledge to effectively manage this new drug class in such a way as to provide effective parasite control while minimizing the selection pressure for resistance.

The evidence suggests that we do have the knowledge to manage the AADs better than other new anthelmintic classes have been managed historically. However, a key requirement will be industry buy-in to the changes in parasite control that will be necessary. If this can be achieved, the programmed use of ZOLVIX might also be able to minimize the further development of resistance in New Zealand to the existing anthelmintic families.

ZOLVIX and monepantel are not registered or available for sale in Canada.

CS13.4

Evaluation of a Predictive Computer Model for Use in Canadian Sheep Flocks

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With increasing levels of anthelmintic resistance worldwide and a growing demand to produce more organic products, utilisation of control strategies for gastrointestinal nematodes (GIN) that minimise the use of anthelmintics becomes even more important. This study evaluated the farm-level performance of an existing predictive sheep parasite model from the United Kingdom (UK), using Canadian data. The UK model, first introduced at WAAVP in 2005 by Taylor et al., simulates the epidemiology of three major GIN species of interest (Telodorsagia sp., Haemonchus sp. and Trichostrongylus spp.) and provides a prediction about seasonal parasite levels. Model inputs were generated by using data from the first year of a three year (2006-2008) study which examined the epidemiology of GIN parasitism in organic sheep flocks located in Ontario and Quebec. Required input data included ewe parasite egg output, pasture-related information, and management dynamics. Farm visits in 2006 provided relevant data that were collected monthly, on six occasions during the grazing season, from 10 ewes and 10 lambs on each farm. These values were compared against the model output and assessed using regression analysis (R^2>50%) to determine fit. Results for 2006 indicated that for 32 farms with available data, 10 had suitable data to run in the model. The number of unsuitable farm data can be explained by the fact that the Canadian study was not specifically designed with the model in mind. However, of these 10 suitable farms, approximately five showed reasonable fit within model. Required model modifications focused on accommodating the differences

between UK and Canadian management styles; specifically the practice of bringing lambs indoors for weaning, which was occasionally used on Canadian farms.

CS13.5

Characterization of *Rickettsia* spp. in *Amblyomma americanum*

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The lone star tick (LST), *Amblyomma americanu*m, is commonly reported from people and animals throughout the eastern U. S. and is associated with transmission of diseases, including ehrlichioses caused by *E. chaffeensis* and *E. ewingii*, and southern tick-associated rash illness, which has an unknown etiology. To better define the microbial communities within LSTs, 16S rDNA-wide PCR followed by sequencing of individual clones (n=358) was used to identify the most common bacterial operational taxonomic units (OTUs) present within colony-reared and wild LSTs. The colony-reared ticks contained primarily sequence affiliated with members of the genus *Coxiella* (89%; 81/91), common endosymbionts of ticks, and *Brevibacterium* (11%; 10/91). Similarly, analysis of clones from unfed wild LSTs revealed that 96.7% (89/92) of all the OTUs identified were affiliated with *Coxiella*-like endosymbionts, as compared to only 5.1%-11.7% (5/98 – 9/77) of those identified from fed wild LSTs. In contrast, the proportion of OTUs identified as *Rickettsia* sp. in wild caught ticks increased from 2.2% (2/92) before feeding to as high as 46.8% (36/77) after feeding; all *Rickettsia* spp. sequences recovered were most similar to those described from the spotted fever group *Rickettsia*, specifically *R. amblyommii* and *R. massiliae*. Additional characterization of the *Rickettsia* spp. present by PCR of 17kDa and GltA genes confirmed these initial findings and suggested that novel *Rickettsia* spp. are likely also present in these ticks. These data provide insight into the rickettsial community of wild lone star ticks and may ultimately lead to identification of novel pathogens transmitted by *A. americanum*.

CS14 - Neospora

Tuesday, August, 11, 2009

CS14.1

Microsatellite Analysis of *Neospora Caninum* from Bovine Foetuses and Dogs in Germany

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Neospora caninum infection is an important cause of bovine abortion. The aim of the present study was to compare *N. caninum* DNA derived from aborted bovine foetuses and from oocysts from naturally infected dogs using a microsatellite-based typing technique. Nested-PCR techniques were developed for the sensitive and specific amplification of regions in the *N. caninum* genome which contain microsatellites. Amplification products were analysed by length determination using capillary electrophoresis or by direct sequencing. Substantial genetic diversity was observed and in most cases individual microsatellite patterns were present. However, identical microsatellite patterns were observed among foetuses collected during epidemic abortion outbreaks and in foetuses of the same herd in consecutive years. All canine *N. caninum* oocyst isolates had individual microsatellite patterns except those of two dogs. Microsatellite analysis may allow the typing of *N. caninum* from clinical samples without need of culturing the parasite. The technique may prove useful for molecular-epidemiological studies.

CS14.2

Molecular Evidence of *Neospora caninum* In Arctic Fox (*Vulpes lagopus*) from Alaska

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Little is known about the sylvatic cycle of *Neospora caninum* in arctic and subarctic ecosystems. Serosurveys have shown exposure to the organism in a variety of arctic and subarctic mammals, including caribou (*Rangifer tarandus*), musk-ox (*Ovibos moschatus*), and gray wolves (*Canis lupus*), but the definitive hosts for N. caninum in these ecosystems have not yet been identified. Domestic dogs and coyotes (*Canis latrans*) are the only confirmed definitive hosts for Neospora

caninum, although there is reason to speculate that red foxes (*Vulpes vulpes*) and gray wolves may also be definitive hosts. Given this, coupled with the close taxonomic relationship between red foxes and arctic foxes, it is possible that arctic foxes could serve as definitive hosts for *N. caninum*. In this study, gastrointestinal tracts from arctic foxes (n=120) were opportunistically collected and frozen at -80°C until processing. Feces were recovered and analyzed using a nested polymerase chain reaction (PCR) protocol with the previously described primers Np6+/Np21+ and Np6/Np7, which targets the Nc5 region. PCR analysis was followed by the sequencing of amplicons. Identity was confirmed through comparisons with known isolates on GenBank. *Neospora caninum* DNA was detected in the feces of arctic foxes, suggesting that these animals are exposed to *N. caninum* through hunting or scavenging or, possibly, coprophagy. Further research will address the intermediate host status of arctic canid prey species and continue surveys of arctic fox feces for evidence of natural infections. Partial funding provided by the Colorado State University's College of Veterinary Medicine and Biomedical Sciences Research Council and by the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH).

CS14.3

Seroprevalence of Neospora caninum in Grey Wolves in Sweden

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Transmission of the protozoan parasite *Neospora caninum* between wild and domestic animals has gained some interest during recent years. Because of the close relationship between Grey wolf (*Canis lupus*) and dog it has been suggested that Grey wolf is a definitive host for the parasite. The aim of this study was to estimate the national seroprevalence of *N. caninum* in Grey wolves in Sweden and to investigate any geographical patterns of the infection. The investigation was based on blood samples collected from 97 wolves from 1998 to 2007 within the Scandinavian wolf project Skandulv. The samples were analysed by *N. caninum* iscom ELISA and those with absorbance values exceeding 0.20 were also analysed by immunoblotting. Samples that were positive in both tests were deemed positive. Five (5%) of the investigated wolves had antibodies to *N. caninum* and antibodies were found in both females and males. Three samples collected over 7 years were available from one of the seropositive animals. This animal was seronegative at 6 month of age and clearly positive in both ELISA and immunoblot the two following samplings. Presence of clustering as well as spatial associations between infected animals in different animal populations will be investigated. To our knowledge, this is the first report of *Neospora* infection in Grey wolf in Europe.

CS14.4

Analysis of BoLA Alleles by PCRand Sequencing in Serum Samples from Quebec Holstein Cattle with Known Neospora caninum Infection Status and Reproductive Outcome

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The BoLA DRB3 and DQA1 genes are part of the major histocompatibility complex (MHC) class II in cattle. These genes are highly polymorphic and have been associated with resistance to several diseases, such as mastitis, Bovine Leukemia Virus (BLV) and dermatophilosis. Sequence-based typing of these genes has been carried out extensively from blood samples; however it is often impractical or expensive to obtain such samples. Repositories of well-characterized serum from cattle are readily available in many veterinary research facilities. This report describes a retrospective analysis of BoLA class II genotypes obtained from stored serum samples from Holstein cattle from Québec dairy farms, which were obtained as part of a previous study on bovine neosporosis. Using a PCR technique to amplify DNA from serum, it was possible to genotype 56 cattle with known infection status for Neospora caninum. We identified 14 different DRB3 and 10 different DQA1 alleles in this population. The allele frequency distribution was consistent with previously studied cattle populations, and alleles known to be associated with BLV and mastitis were present. No association was found between allele frequency distribution of DRB3 or DQA genes and infection with N. caninum. However, an association of allele DRB3*1001 and allele DRB3*2703 with resistance and susceptibility to pregnancy loss, irrespective of infection status, was identified. The ability to obtain retrospective DNA sequences from stored serum samples may find applications to many retrospective studies of infection biology.

CS15 - Ectoparasites

Tuesday, August, 11, 2009

CS15.1

Identification of Pro-Inflammatory Factors from the Sheep Scab Mite, *Psoroptes Ovis*

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Psoroptes ovis (P. ovis) is the causative ectoparasite of sheep scab, a highly contagious and debilitating disease of sheep. This non-burrowing mite resides on the skin surface and infection results in severe pruritis and loss of condition. The inflammatory reaction caused by the mite results in release of serous exudate which forms a crusty lesion, and develops into a scab. As the mite appears to feed on the exudate produced, this inflammatory reaction may be crucial for establishment and maintenance of infection.

We utilised primary ovine keratinoctye cultures to investigate potential pro-inflammatory effects of whole mite antigen and excretory/secretory products on keratinocytes, the initial cell type exposed to *P. ovis*. Quantitative real-time PCR has demonstrated that keratinocytes exposed to mite wash show increased IL-8 and TNF expression within 1 hour. Mite wash treatment resulted in a higher fold increase in expression of these cytokines than observed with whole mite antigen treatment, suggesting that secreted/excreted factors are crucial. Fractionation of mite wash proteins has identified a distinct fraction that retains the bio-active component, with a profound increase in IL-8 and TNF expression. This fraction is currently being analysed to identify the potent pro-inflammatory factor(s) which may be crucial in the initiation of the lesion and thus establishment and maintenance of mite infection.

CS15.2

Sheep Scab - an Integrated Genomic Approach to the Host Parasite Interaction

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Sheep scab is a highly contagious ectoparasitic disease of sheep caused by the mite *Psoroptes ovis* (*P. ovis*). It is an important welfare issue due to the disease symptoms which include intense pruritis and severe exudative dermatitis. Host response to infection is directed against mite secretory/excretory products and is typical of an immediate hypersensitivity reaction. Sheep scab is controlled by chemical intervention however, there are concerns over chemical residues in meat and their effect on human health and the environment, there is also an emerging problem of drug resistance. Vaccine candidates have been identified by fractionating mite protein extracts however, isolation of individual protective proteins has proven difficult. To further improve development of a sheep scab vaccine we must gain a better understanding of the host-parasite relationship. In order to interrogate the host-parasite interaction in sheep scab we are utilising an integrated genomic approach, combining a *P.ovis* cDNA microarray to interrogate parasite-specific responses, with ruminant-specific microarrays to analyse host responses to infection, both at the local site of infection (skin biopsies) and systemically in whole blood. Initial data analysis confirms an early host systemic response to infection, indicating that sheep scab is likely to be regulated at both local and systemic levels. In addition, the importance of a number of host pro-inflammatory factors has been highlighted in development of the early scab lesion. We believe that these resources will increase our understanding of the host-parasite relationship and will potentially lead to the identification of vaccine candidates and biomarkers of disease.

CS15.3

Comparison of Microscopical Examination of Skin Scrapings and ELISA for the Diagnosis of Sheep Scab (Psoroptes ovis)

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Diagnosis of sheep scab is traditionally based on a combination of clinical observation and detection of Psoroptes ovis in skin scrapings. Clinical signs may not be pathognomic and taking skin scrapings relies on finding sub-clinical lesions and can therefore be time consuming. The quality of the scraping is also important. The cryptic sites (external auditory canal, infra-orbital fossae and inguinal pouches) also have to be examined, thus increasing the examination time. In this study an improved ELISA was compared to the microscopical examination of 297 skin scrapings submitted by UK Government Veterinary Officers, from sheep presenting with clinical signs of possible P.ovis infestation. P.ovis was confirmed in 49.8% of the scrapings, with 98.6% also confirmed positive by ELISA. In contrast, 50.2% of scrapings were reported negative for P.ovis, with 54.4% also confirmed negative by ELISA. Bovicola bovis were found in seven scrapings, two of which (28.6%) demonstrated weak ELISA reactions. Eleven scrapings reported exclusive infestation by blowfly larvae (Lucilia spp), seven of which (63.6%) demonstrated positive ELISA reactions. Two scrapings demonstrating exclusive infestations of non-parasitic mites were both negative by ELISA. The improved ELISA may be more sensitive, identifying more P.ovis infested sheep compared to traditional skin scraping, the active lesions undetected by the Veterinary Officer. False positives may occur through detection of residual anti – P.ovis antibody titres from previously cured P.ovis infestations and/or concurrent blowfly larvae or B.bovis infestations.

These investigations were funded by Defra Project: OD 0548

CS15.4

Flyboss: a New Blowfly Strike Management Website

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Prevention of blowfly strike is a major priority for Australian wool producers. Provision of up to date, best-practice advice and information must be in a user-friendly format to assist the phase-out of mulesing and to see the adoption of optimal insecticide use strategies. FlyBoss aims to fit that niche, and in particular will address the need to effectively treat existing flystrikes and to plan efficient preventative programs. Computer model based decision aids based on local weather data and sheep susceptibility factors will assist sheep farmers to optimise sheep management, chemical treatments and non-chemical options to minimise the flystrike risk. FlyBoss will also provide a comprehensive reference for fly biology (especially for the major species, Lucilia cuprina), sheep and environmental factors associated with flystrike, and information to facilitate the appropriate choice of chemicals for different situations. Though the national sheep research organisation, the Cooperative Research Centre for Sheep Industry Innovation, FlyBoss joins WormBoss and LiceBoss in utilising expertise from a number of institutions to provide the sheep industries with easily-accessed, current and locally-targeted information to facilitate effective blowfly strike management.

CS16 - Workshop - Resistance and Control

Tuesday, August, 11, 2009

CS16.1

Nematode Control in Farm Situations. Evolution of Control, and the Challenge of Resistance. A Practitioner's View

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Parasite control has evolved over the last 25 years in Argentina. In the 80s, productivity impulsed nematode control and many systems were developed: Tactic systems based on fixed treatments, Systematic (falsely called "0" risk) models proposed to cut the cycle, and Integrated control as the most rational. Ivermectin appeared with great impact in production and in "apparent" control of worms.

In the 90s, epidemiological-based models proposed the "Strategic" system based in the effect of some strategic treatments on larval availability and the consequent infection. Then, a pronounced decrease in drug prices also took place, and it became easier to deworm with long acting drugs, than to sample, pay for assessment, or think; every-30-days deworming was established.

In this decade resistance appeared and control systems proposed by scientists are more rational, with rotation of drugs and less use of them, and an epidemiological base for the control. But farmers continue to deworm without assessment, and long acting products are invading the market. Resistance is not known, nor believed to exist in many cases.

We will discuss the real problems a vet has when he/she has to deal with parasites, resistance, farmers, publicity, drug quality, and production system variability. We will show many examples of everyday problematic decisions, and try to give a "practical" view on EPG interpretation in relation with resistance; on reduction tests in farm situations, and the "real"? significance of those results. We will also analyze the evolution of resistance in some farms and the concept of practical reversion.

CS17 - Companion Animal Symposium

Tuesday, August, 11, 2009

CS17.1

ESCCAP: the Role of an INGO (International Non-Governmental Organisation) in Contributing to the Understanding and Control of Companion Animal Parasites in a Changing Landscape

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European Scientific Counsel Companion Animal Parasites, Malvern, United Kingdom

ESCCAP (European Scientific Counsel Companion Animal Parasites) is an independent, not-for-profit, international non-govermental organisation that develops guidelines and promotes good practice for the control and treatment of parasites in companion animals within Europe. ESCCAP believes that with effectively communicated proper advice the risk of diseases and parasite transmission between animals and humans can be minimised. ESCCAP aspires to see a Eur-

ope where companion animal parasites no longer threaten the health and wellbeing of animals and humans.

Despite many parts of Europe being united within the European Union, linguistic and other national differences has led ESCCAP to adopt a central structure affiliated to national associations in each of the countries or regions where it is active. Legislative individualities in some countries, such as the interpretation of medicine regulations in Denmark and the border parasiticide treatments in the UK, require special consideration. In contrast, climate, travelling pets, wild animals and parasite species are not constrained by legislative or national boundaries. ESCCAP is faced with a situation where current evidence suggests that the potential for the extension of the endemic areas of various zoonotic and non-zoonotic parasites including tick-borne diseases, leishmaniosis and Echinococcus multilocularis is becoming a reality.

The symposium will consist of an introduction presenting an update on ESCCAP and its activities, individual presentations to illustrate the challenges facing a European organisation committed to the reduction of the impact of companion animal parasites, and the ways in which ESCCAP has addressed this to date and will in future. Members of CAPC (Companion Animal Parasite Council) from the United States of America, will be invited to join the discussion which will focus on how non-governmental organisations like as CAPC and ESCCAP can contribute effectively to controlling parasites in a changing landscape.

CS18 - Equine Parasites

Tuesday, August, 11, 2009

CS18.1

Donkeys Parasites in the UK; Infection Levels, Treatment Intervals and Anthelmintic Use

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Donkeys are often perceived as important parasite reservoirs, often blamed for being asymptomatic carriers of lungworm and carriers of drug resistant cyathostomes. Little is known about parasite infection levels in donkeys in the UK or anthelmintics used for donkeys and their treatment intervals. The aim of this study was to determine levels of parasite infection in donkeys new to The Donkey Sanctuary, UK over a 4 year period (2004-08) using coprological techniques. A questionnaire survey of previous anthelmintic use was carried out to determine drug choice and frequency of treatment. A study of all new relinquishments (n=735) showed that 73% of donkeys had an identifiable strongyle infection, 4% liver fluke, 3% tapeworm and 4% lungworm infection. The median interval since last anthelmintic treatment was 3 months (range 0-48 months). Notably 13.6% (n=73) of responders (n=542) indicated that their donkeys had never been treated for parasites. Owners only identified the anthelmintic product used in 62% of cases, ivermectin was the most commonly used (41%) followed by benzimidazoles (21%), moxidectin (13%), pyrantel embonate (12%), ivermectin + praziquantel (11%) and moxidectin + praziquantel (2%). Donkeys in the UK clearly carry a range of parasites; levels of infection with lungworm in particular appear much lower than that which is regularly quoted. Donkeys obviously will act as parasite reservoirs but they may not deserve their reputation as a significant parasite reservoir within the UK. Treatment intervals are similar to those seen in other equids in the UK with most (74%) anthelmintics administered being specifically licensed for donkeys.

CS18.2

Gasterophilus Nasalis (Diptera: Ostridae): a Major Cause of Rectal Prolapse in Working Donkeys in Ethiopia

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Gasterophilus larvae are common obligate parasites in the gastrointestinal tract of equids. Although the current concept is that they are considered to be well tolerated by their host, they have been incriminated in inducing gastric erosion, ulcers and abscesses. A recent retrospective study made in Ethiopia showed that Gasterophilus nasalis was the major cause of rectal prolapse in working donkeys. Data obtained from the Donkey Health and Welfare Project (DHWP) clinic in Ethiopia from 1995 to 2004 revealed 83.7% (n=177) of rectal prolapse cases were due to G. nasalis. The larvae re-attach temporarily to the rectal mucosa after passing through the digestive tract. This may stimulate an increased frequency of defaecation, irritation, inflammation and intense tenesmus, leading to mucosal prolapse. Previous studies have reported that this re-attachment at the rectal mucosa is the main behaviour of G. haemorrhoidalis. The current result showed that G. nasalis also follows similar behavior, at least in donkeys. The average and median numbers of G. nasalis per infected donkey were 66 and 64, respectively, with a range of 2-195. Over 100 G. nasalis were recovered from the rectum of over 21% of donkeys presented with rectal prolapse. Cases of rectal prolapse and the number of larvae recovered were higher during the wet and cool season compared to the dry and warm season. A significant decrease of rectal prolapse was noted since the DHWP, under the auspices of the Donkey

Sanctuary, launched its strategic anthelmintic treatment programme.

CS18.3

Structure of Strongylid Communities of Wild and Domestic Equids in Ukraine and Modern Methods of Parasite Control

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The aim of the present work was to examine the structure of strongylid communities of 5 equid species and analyze the current methods of parasite control in Ukraine. Totally 156 animals were involved into study: 84 horses, 30 donkeys, 22 Przewalski's horses, 11 zebras and 9 Turkmenian kulans. Animals were treated with "Univerm" (0.2% aversectin, Russia). Faecal sampling (200 g each) was performed at 24, 36, 48 and 60 hours after treatment; all nematodes expelled (76,054 specimens) were collected and identified. Totally 37 strongylid species were found. In horses, 26 strongylid species were found; the number of species per host was from 7 to 18 (aver. 10.2 ± 3.4). In Przewalski's horses, 31 species were found; from 10 to 18 species (14.6 ± 2.3) per host. In donkeys, 24 species were found; from 6 to 15 species (9.6 ± 2.9) per host. In zebras, 17 species were found; from 3 to 13 species (7 ± 3.6) per host. In kulans, 24 species were found; from 10 to 16 species (13.2 ± 2.6) per host. The shape of the prevalence frequency distribution of strongylid species was bimodal in horses and donkeys and multimodal in Przewalski horses, zebras and kulans. Bray-Curtic cluster analysis revealed similarity of strongylid communities of donkeys and kulans comparing with others equids. Frequent anthelmintic treatments lead to strongylid community structure destruction and cause the development of resistance in nematodes. Integrated control has to be prospective approach for long-term parasite control in various types of horse-keeping systems in Ukraine.

CS18.4

Seasonal Variation in Development and Survival of *Parascaris equorum* Eggs in Pasture or on Gravel Surface

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It has been suggested that a major source of *Parascaris equorum* infection in foals is from overwintered eggs deposited by the previous generation of foals. To study the transmission in different environments an experimental study was initiated in August 2008, when standardised faecal pats with a known number of *P. equorum* eggs were deposited in plots with either short grass or gravel. A second deposition was also conducted in late October. The aim is to quantify the number of surviving eggs and their developmental stages in faeces and at the ground level in both environments until April 2010. Triplicate samples will be analysed at 1, 2, 4, 8, 16, 32, 52 och 84 weeks after deposition. Air and ground temperatures and relative humidity's will also been constantly recorded near the faecal pats.

Eight weeks after the summer deposition the number of eggs in the faecal pats had decreased by approximately 50% on gravel and by as much as 90% on grass, which was partly due to the fact that the faecal pats disappeared quicker on grass. In contrast, eight weeks after the autumn deposition the number of eggs in the faecal pats had decreased by approximately 10% on both gravel and grass.

The difference was related to a higher activity in the pats of dung degrading organisms after deposition in the summer compared to the autumn.

There were about 10-200 times more eggs in the soil samples from the grass surface than from the gravel.

CS19 - Epidemiology and Control

Tuesday, August, 11, 2009

CS19.1

Effect of Maize Supplementation and Natural Nematode Infection on Fodder Selection and Consumption by Hair Sheep Lambs Under a Mixed Grass/Browse Pasture

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Universidad Autonoma de Yucatan, Merida, Mexico

Introduction: Energy supplementation improves resilience of sheep and goats browsing tropical forest. Nutrient manipulation and infection might induce consumption of legume trees, containing protein and plant secondary metabolites such as tannins. The aim was to evaluate the effect of maize supplement and natural gastrointestinal nematode (GIN) infection on the feeding behavior of sheep fed in a mixed grass/browse pasture.

Methods: Twenty nine hair sheep lambs, raised nematode free, were allocated to four groups: Infected, non-supple-

mented (I-NS, n=8), infected, supplemented at 1.5% LW (I-S1.5%, n=7), treated against GIN and non-supplemented (T-NS, n=7), and treated against GIN, supplemented at 1.5% LW (T-S1.5%, n=7). Animals browsed for 70 days. Intake, forage selection, LWG, blood red cells (RBC), hemoglobin (Hb), hematocrit (PCV) and eggs per gram of feces (EPG) were measured every 14 days. Metabolizable energy (ME) and protein (MP) supply from feeds were estimated.

Results: All groups showed low dry matter intake (DMI) (<2% LW). The T-S1.5 and I-S1.5% groups had higher DMI (fodder + maize) (P<0.05) resulting in higher ME and MP intake. All groups had similar fodder selection patterns. No correlation was found between type of fodder selected and EPG. The T-S1.5%, I-S1.5% and T-NS groups had higher LWG, RBC, Hb and PCV than I-NS. No difference was found on EPG between the I-NS and I-S1.5% groups (P>0.05). The metabolic cost of GIN was lower in the I-S1.5% (ME 0.70 MJ/d and MP 9.2g/d) than I-NS (ME 1.46 MJ/d and MP 12.71g/d).

Conclusion: In this trial, energy supplementation and natural infection did not increase consumption of legume trees in growing hair sheep.

CS19.2

Genes that Affect Faecal Egg Count (FEC) for Gastro-Intestinal Parasites in Lambs

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Lamb resistance to GI parasites, as assessed by Faecal Egg Count (FEC), has been associated with variation in genes encoding components of the immune system. In this study we assessed the role of variation in the ovine immunoglobulin heavy constant alpha-chain gene (IGHA), Toll-like receptor 4 gene (TLR4), MHC-DQA1 and MHC-DQA2 on FEC in lambs subjected to "mixed field-challenges" by the Nematodirus and Strongyle species present on farms in the South Island, NZ. The lambs were of the Merino, Polwarth and Corriedale breeds. Faecal samples (at 4 and 9 months) were collected from over 7000 male lambs, on 37 farms and over two years, and Nematodirus sp. and Strongyle genera FEC was calculated. Variation in the genes was assessed using published methods and Strongyle genus/species identifications were performed on a pooled faecal sample from each farm by larval culture. For each gene, General Linear Mixed Effects Models (GLMMs) were used to analyse the impact of allele presence and genotype on each of the measures of FEC (i.e. Nematodirus sp., Strongyle genera and total FEC). When the data was split at the level of farm by predominant parasite type in the pooled samples, some associations were observed between genetic variation and the different measures of FEC, but there were few overall effects detected. Typically the variation in FEC that could be predicted was small, and we conclude that the genes would not be that effective as gene-markers for resistance and/or would only be useful for specific parasite challenges at a particular time during lamb growth.

CS19.3

Epidemiology of Cryptosporidium Isolates from Swedish Dairy Cattle

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What was previously defined as Cryptosporidium parvum based on morphology at microscopy has recently been separated into several species based on a combination of morphology, infection pattern and DNA-analysis. Cryptosporidium-positive samples from cows, young stock and preweaned calves in 70 dairy herds were analysed by DNA sequencing of the 18S rRNA-gene to determine species identity. Cryptosporidium bovis was the most common species, detected in 98 of 139 (71%) calves, 51 of 63 (81%) young stock and 10 of 12 (83%) cows. Cryptosporidium bovis showed some age related distribution but we did not see the total C. parvum dominance in preweaned calves that has previously been reported. In addition, C. bovis was detected from seven days of age, showing that the prepatent period in Cryptosporidium-naïve calves is shorter than the previously stated 10 days. Cryptosporidium ryanae was detected in calves (9%) and young stock (16%), whereas C. andersoni was found in young stock (3%) and cows (17%). Cryptosporidium ryanae became gradually more common with age in calves. Cryptosporidium parvum was found in 21% of calves and showed an age-related pattern as 90% of these calves were <1 month old, in comparison to 56% of C. bovis-positive and 42% of C. ryanae-positive calves. Diarrhoea was more common in calves with C. parvum, and these calves were also younger than diarrhoeal calves with C. bovis.

The GP60 gene was sequenced from C. parvum-positive samples (n=29). Nine subtypes from subtype families IIa and IId were detected. Only one subtype was present in any herd. Six of the subtypes have been described and three were novel ones. Five of the six previously described subtypes identified have been isolated from humans. Whether there is a zoonotic potential or not in the three new subtypes remains to be revealed.

CS19.4

Evaluation of Efficacy of Baycox® (toltrazuril) for Improvement of Post-Weaning Gut Health in a European Pig Herd

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Clinical studies of possible post-weaning benefits of toltrazuril for neonatal coccidial control have not been comprehensive. This study evaluated the efficacy of toltrazuril (Baycox ® 5% oral suspension) for the improvement of gut health in a typical EU grower-finisher pig herd. The herd had a long-standing embedded Isospora coccidial infection. Over 200 Group A pigs received Baycox® at day 4 of age, and over 200 Group B pigs received placebo. Pigs were weaned at day 21 and subsequent gut health monitored up to day 105. No major enteric or other disease outbreaks were recorded during the trial. The results showed markedly improved feed conversion in grower-finisher pigs given Baycox®, particularly in the mid to late finisher period. Serology results suggested an early (before day 56) and mild ongoing exposure to Lawsonia. The titres in Group B are higher, with fewer negatives throughout, suggesting that Baycox has protected pigs from developing pathogenic Lawsonia infections. Three putative positive results for Brachyspira hyodysenteriae (swine dysentery) were clearly identified from Group B between days 84 and 105. Salmonella, PCV2, Brachyspira pilosicoli, E coli and Clostridia results were not noteworthy. There was more diarrhoea noted ($p<0.001$) in the piglets around weaning age in group B (untreated), by blinded investigators. These results confirm the protective effect of Baycox® on the gut health of young pigs around weaning age. They also suggest that valuable effects on feed conversion can be due to protection against damages caused by coccidiosis and both B hyodysenteriae and Lawsonia infections.

CS20 - Besnoitia Symposium

Tuesday, August, 11, 2009

CS20.1

Bovine Besnoitiosis: Emerging in Europe

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In Europe, the historically known areas of bovine besnoitiosis included Southern France, Spain and Portugal, where the disease actually received very little attention until the past 5 years, since when its prevalence seemed to be dramatically increasing in France, reaching the near borders of Switzerland, Germany and Italy. Consequently, first cases/outbreaks have been recently recorded in neighboring countries of France, such as Italy and Germany. Respective data and information will be provided with two presentations, one by [Jacquiet P, Alzieu JP, Grisez C, Prevot F, Lienard E, Salem A, Lagalisse Y, Buholzer P and Gottstein B] ("Emergence of bovine besnoitiosis in France between 2001-2009 and recent epidemiological surveys") and the other by [Gollnick NS, Rostaher A, Majzoub M, Basso W and Schares G] ("Besnoitiosis - a new emerging cattle disease in Germany?"). In the presence of disease on a herd level, or by the time the option is on buying animals for reproduction purposes, it is important to avoid acquirement of or to eliminate the presence of infected animals. Clinically manifest cases, due to the occurrence of typical signs, are easily detectable. Subclinically infected animals, however – more difficult to be diagnosed – play an important role, as the parasite may be inapparently transmitted either iatrogenically or by insect vectors. Histopathology, due to the very high number of cyst on the skin of sick animals is a good method to diagnose acute disease, but not for the detection of chronic or subclinical infection, where the number of cyst on an overall cattle may still be high, but scarce on a histological skin slide. In this field, extensive epidemiological, clinical and diagnostic experiences will be provided by two respective presentations, one by Cortes et al. ("Experiences with bovine besnoitiosis in Portugal), and another by [Aguado-Martínez A, Alvarez Garcia G and Ortega-Mora LM] ("Present situation of bovine besnoitiosis in Spain"). Recently, new diagnostic tools (serology and PCR) have been developed, which for the first time will allow to carry out reliable large scale epidemiological surveys. These tools will be presented by [Gottstein B, Cortes H, Jacquiet P, Frey C and Mueller N] ("laboratory diagnosis of bovine besnoitiosis"). Conclusively, bovine besnoitiosis is emergingly affecting cattle herds of Western and central Europe, thus veterinary public health authorities need to become sensitized on the problem. Due to the lack of knowledge on many aspects of epidemiology and transmission, respective research needs to be urgently developed.

CS21 - Drug Resistance

Tuesday, August, 11, 2009

CS21.1

Ivermectin Resistance in Cyathostomes in Four Donkeys Herds at the Donkey Sanctuary, UK

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The Donkey Sanctuary has 2500 donkeys resident on its nine farms in the south west of the UK. In 2005 we reported reduced egg reappearance periods (ERP) for moxidectin in our donkey herds. Since 2005 we have been monitoring faecal egg counts in our herds on a four weekly basis and subsequently identified a reduction in ERP for the macrocyclic lactone ivermectin. To determine efficacy of the drug in four of our geographically separate locations we carried out faecal egg count reduction (FECR) tests 14 days post-deworming with an ivermectin equine paste formulation (Eraquell™). FECR tests showed mean reductions of 91% (range 0 – 100%), 97% (range 50 – 100%), 84% (range 0-100%) and 94% (range 33 – 100%) in locations A, B, C and D respectively. Post-dosing samples were positive in 25% of donkeys in location A, 10% of donkeys in location B, 39% donkeys in location C and 17% in location D. Under field conditions there was a lack of efficacy of ivermectin which, on the basis of both the shortened ERP and the FECR test results, was attributed to ivermectin resistance of cyathostomes. This result is perhaps unsurprising as parasite populations at location A and B have previously been demonstrated to have reduced susceptibility to the related macrocyclic lactone moxidectin.

CS21.2

Phenotypic and Genotypic Characterization of Benzimidazole Susceptible and Resistant Isolates of Haemonchus Contortus

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The study was designed to compare the in vitro (Egg hatch test - EHT) and molecular (Allele-specific PCR – AS-PCR, Pyrosequencing) methods as tools for detection of benzimidazole resistance in Haemonchus contortus, a nematode parasite of small ruminants. Comparisons were made during the course of experimental infection and changes in EHT and Pyrosequencing test were monitored to measure the correlation between in vitro and molecular tests. EHT and AS-PCR test were performed according to WAAVP recommendations. Both molecular tests have been used to discriminate of the TAC/TTC polymorphism in the beta-tubulin 200 codon of nine (four resistant and five susceptible) isolates of Haemonchus contortus. Using DNA from 100 third-stage larvae (AS-PCR) or from 1000 L3s (Pyrosequencing) the PCR based analysis revealed a decrease of the homozygous TTC/TTC genotype and an increase in heterozygous TTC/TAC and homozygous TAC/TAC individuals in all resistant isolates. Both methods showed comparable and reliable results with regard to detection of benzimidazole resistance. The molecular tests have an advantage over the EHT because of its higher sensitivity. On the other hand, EHT is less time-consuming, allowed reliable detection of <10 % resistance allele frequency and is fairly reliable for detection of benzimidazole resistance under field conditions.

The study was supported by PARASOL (FOOD-CT-2005-022851) project of EU 6th Framework Programme.

CS21.3

Standardisation, Evaluation and Field Use of the LMIT for Cattle Parasitic Nematodes

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Gastrointestinal parasites of livestock cause serious economic losses on farms with intensive grazing. Currently many cattle and sheep industries rely heavily on chemically based parasite control. Resistance against all classes of commercially available anthelmintics is an increasing problem in sheep and emerging resistance in cattle nematode populations has been reported in several countries worldwide. To be able to determine the extent of resistance in nematodes of farm animals and to monitor the success of any resistance management requires reliable tests for the detection of anthelmintic resistance.

Various in vitro detection methods such as the Egg Hatch Test, Larval Development Test and Larval Migration Inhibition Test (LMIT) have been developed for sheep parasitic nematodes. This work describes the development and standardization of a LMIT for two cattle nematodes, Cooperia oncophora and Ostertagia ostertagi. Subsequently, the LMIT has been evaluated by ring testing susceptible and ivermectin (IVM) resistant isolate (C. oncophora only) in five different laboratories and EC50-values were found to be highly reproducible. Screening of field isolates revealed higher EC50-values for

IVM in the LMIT before treatment on those farms where insufficient reduction of egg counts in the FECRT were observed, compared with those farms, where complete FECR was recorded. This demonstrates that the established LMIT might be suitable for the detection of emerging resistance. The prevalence of C. oncophora present after treatment on more than 70% of the tested farms in Germany suggests that reduced efficacy of IVM might be a still underestimated problem in European cattle stocks.

CS21.4

Faecal Egg Count Reduction Test (FECRT): Arithmetic or Geometric Means?

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The process of conducting a faecal egg count reduction test was simulated to examine whether arithmetic or geometric means offer the best estimate of efficacy. By assuming a known drug efficacy, distribution and mean of worm counts we drew random samples from the hypothetical population, to estimate efficacy using arithmetic or geometric means for comparison with the known efficacy. Two components of sample variation were simulated: the counts of individual hosts selected from the general population was modelled by the negative binomial distribution with varying degrees of aggregation; for eggs found in an aliquot of faeces used to estimate the worm egg count a Poisson distribution was assumed. To determine the geometric mean a constant(C) must be added to all counts if the dataset contains zeros but different Cs can provide substantially different efficacy estimates, C was set to 25, 12 or 1. Ten thousand separate efficacy trials to estimate mean efficacy and its 95% confidence limits based on arithmetic or geometric means were simulated. Arithmetic means best estimated efficacy for all different levels of worm aggregation in the host population. For moderate levels of aggregation and with C=1 the geometric mean substantially overestimated efficacy. The bias was reduced if C was increased to 25 but the results were no better than those based on arithmetic means. For very high levels of aggregation (over-dispersed populations) the geometric mean underestimated efficacy regardless of the size of C. Arithmetic means offered the simplest and least biased way to estimate efficacy.

CS21.5

Use of Box-Cox Transformation Technique for Fitting Fecal Egg Count Observations in a Cattle Population

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Fecal egg count (FEC) is used to identify and quantify gastrointestinal parasite infestations. FEC values are not "normally" distributed and a small percentage of the herd is responsible for the majority of parasite transmission. In an attempt to bring FEC distribution close to normality, logarithmic transformations have been used for these data. Unfortunately, normalization of the distribution is not achieved in the majority of the cases, resulting in the necessity of using less sensitive non-parametric statistics analyses, and the inability to fit the data into more complex genetic analyses. In this study we compared an extension of the Box-Cox transformation to determine the efficiency of this transformation compared to log transformation. Between 1992 and 2006, 12,450 observations of FEC were collected from 498 animals (males and females) from the BARC Angus herd. Contemporary group was defined as animals entering in experiment together. There were 19 contemporary groups in the study. The statistical model used in ANOVA included the contemporary groups, sex of the animal and age at test, as fixed effect, and error, as random effect. Results show that Box-Cox transformation reduced coefficients of asymmetry in all the variables studied. Plotting the residuals (stem-leaf and Normal plot graphs for normal distribution and box-plot for homogeneity of variance) for each trait and checking the parameters of the normal distribution and homogeneity of variance, Box-Cox was always superior to log. As the log transformation is a special case of the Box-Cox, this transformation will be, at least, equal to log, never worse.

CS21.6

Monitoring the Efficacy of Anthelmintic Drugs in Small Ruminants in the State of São Paulo, Brazil: Preliminary Results

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The sheep and goat industry in the state of São Paulo (SP) is relatively new with animals, and their parasites, coming from many parts of Brazil without previous drug history. Producers have focus on meat producing animals with large investment. Helminths, mainly Haemonchus sp., Trichostrongylus

sp. and Cooperia sp. maybe found throughout the year due to SP's subtropical climate (hot and dry to mild and humid areas). The objective of this 2-year project is to determine the efficacy of ivermectin, moxidectin, albendazole, levamisole and closantel in small ruminants by faecal egg count reduction test (FECRT) and coproculture in 32 farms. The DNA from larvae and adults will be collected for -tubulin determination. Although there is no data regarding previous drug testing the actual management will be investigated. Comparison will be made using RESO 2.0. So far, 08 farms were tested and the results showed a disturbing situation to all compounds with high efficacies reaching less then 80%. The most prevalent genus found was Haemonchus sp.. Taken these first results one may presume that the sheep and goat industry will suffer to have a positive balance for investments if no interventions are made towards the introduction of a new set of sustainable parasite control strategies. These must include the introduction of parasite-tolerant breeds, FAMACHA, herbal therapy and the introduction of susceptible parasite isolates. Results will be present at the individual level.

CS22 - Pharmacology

Tuesday, August, 11, 2009

CS22.1

Use of a Rodent Model for the Optimization and Characterization of a Novel Class of Anthelmintics

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Amino-Acetonitrile Derivatives (AADs) are a recently described chemical class with high activity against gastrointestinal nematodes in sheep and cattle. The chemical optimization leading to the discovery of drug development candidates was pursued in gerbils (Meriones unguiculatus) simultaneously infected with sheep derived Haemonchus contortus (Hc) and Trichostrongylus colubriformis (Tc). This model proved to be ideal for structure–activity relationship analysis. Other advantages were a short experimental time, the limited quantity of active required, and the economy of space and resources.

The first tested AADs, active orally in gerbils at 10 mg/kg against only Hc, were chemically optimized to obtain molecules able to eliminate Hc and Tc at doses of 1 mg/kg orally and 3.2 mg/kg subcutaneously. These results correlated with those obtained in sheep, leading to a reduction of the minimum effective dose and to a wider activity spectrum. Tolerability, enantiospecific activity and activity against nematode isolates resistant to known anthelmintic classes could also be evaluated in gerbils. The best analogues in the gerbil model were used to identify drug development candidates, including monepantel (ZOLVIX®), in sheep.

ZOLVIX and monepantel are not registered or available for sale in Canada.

CS22.2

The Safety of ZOLVIX® (Monepantel), an Amino-Acetonitrile Derivative, when Administered to Sheep

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The novel chemical class of synthetic anthelmintics, the Amino-Acetonitrile Derivatives (AADs) has recently been brought to the market in New Zealand as an oral anthelmintic for sheep (ZOLVIX®; 25 g/L monepantel). As a new chemical class with a novel mode of action, the tolerability, margin of safety and potential adverse effects on reproduction were investigated in a preliminary study with a development formulation and three comprehensive studies with the commercial formulation. These were conducted in compliance with applicable guidelines for target animal safety studies.

Safety was evaluated on identification of potential adverse effects from clinical observations, food and water consumption, veterinary examinations, body weight, clinical pathological variables, and gross and microscopic pathological variables. Potential adverse effects on reproductive indices were also determined in a specific study covering all phases of the reproductive process.

There were no adverse events related to treatment and monepantel was well tolerated at x33 the maximum recommended dose (MRD). ZOLVIX was also well tolerated by sheep. No differences in responses to treatment were noted in 2–4 week old lambs at x10 MRD. Repeated administration to weaned lambs (eight treatments at 3-week intervals at x1, x3 and x5 MRD) did not induce any relevant adverse effects. There is no risk for the use of ZOLVIX in breeding sheep of both genders.

The margin of safety for ZOLVIX is at least x10 MRD. The AADs are well tolerated and of low toxicity to sheep.

ZOLVIX and monepantel are not registered or available for sale in Canada.

CS22.3

Efficacy and Safety of ZOLVIX® (Monepantel) in Sheep: A European Field Study

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The Amino-Acetonitrile Derivatives (AADs) are a recently discovered class of anthelmintic with a novel mode of action. ZOLVIX® (monepantel) is the first product from the class to be developed for nematode control in livestock.

During 2007, a field study was conducted at 12 sites across Scotland, England and France, to assess the efficacy and safety of ZOLVIX when administered to sheep maintained at pasture in a range of commercial production systems. A total of 1200 sheep representing a wide range of breeds, ages and bodyweights was recruited to the efficacy phase of the study and a further 1100 were treated and added to the safety phase. ZOLVIX was administered as an oral treatment at a minimum dose of 2.5 mg/kg bodyweight for the control of gastro-intestinal nematodes.

Efficacy against mixed-genus natural field infections was measured by fecal egg count (FEC) reduction, 7 days after treatment. The combined primary efficacy was very high, >99% for both strongylate and Nematodirus eggs and site-specific efficacy was uniformly high, >98% at all sites (geometric means).

There were no treatment-related adverse events during the study, which included the use of a range of concomitant treatments.

This high level of efficacy was in agreement with large-scale field studies conducted in Australia and New Zealand, which also demonstrated efficacy against nematodes resistant to other anthelmintic classes.

ZOLVIX and monepantel are not registered or available for sale in Canada.

CS22.4

Potentiation of the Levamisole Depolarization by the Neuropeptide AF2 in Muscle of *A. suum*

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Introduction: Use of the anthelmintic levamisole is now hindered by the development of resistance. We are interested in understanding the mode of action of this drug and how its effects may be modulated to increase potency. Our aim is to identify cellular principles that could be used to counteract the development of resistance.

Methods: We used electrophysiological techniques, specifically current-clamp, to study the action of levamisole on *A. suum* somatic muscle.

Results: We found that the depolarization produced by levamisole (1 µM) was potentiated for ≥20 minutes by a brief pretreatment with the FMRFamide related neuropeptide, AF2 (KHEYLRFamide). The levamisole response consisted of a primary depolarization followed by a slower secondary depolarization. Pretreatment with AF2 (1 µM, 2 min) significantly increased the duration of the secondary depolarization. We investigated the ionic basis of the potentiation and found that external calcium contributed significantly. Potentiation was also inhibited by 100 nM ryanodine, indicating a role for the release of intracellular calcium. Removal of external chloride inhibited potentiation suggesting a role for a calcium-activated anion channel present in the membrane.

Conclusion: A model that can explain our observations was developed and will be presented. Although the potentiation by AF2 was complex, our studies offer some optimism that we can find pharmacological approaches for potentiating the actions of levamisole and that we may be able to develop pharmacological methods to overcome anthelmintic resistance.

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CS22.5

Cyclooctadepsipetides – Tracking the Mode of Action

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In the early 1990s the cyclooctadepsipetide PF1022A was shown to have strong anthelmintic activity. Its semi-synthetic derivative emodepside has not only a wide range of efficacy but is also effective against multidrug-resistant nematodes. It is commercially available as a spot-on product in combination with the cestocide praziquantel for cats since October 2005. The basis of its molecular mode of action is not known in detail yet, but some putative target proteins have been investigated already. For the G-Protein coupled receptor Hc 110-R of the sheep nematode *Haemonchus contortus* and its orthologue, the latrophilin-like receptor 1 (LAT-1) of *Caenorhabditis elegans*, binding to emodepside could be shown. *C. elegans lat-1* knockout mutants were less susceptible for the paralysing effects of emodepside on the pharynx. For mediating the effects on locomotion, the calcium activated potassium channel SLO-1 of *C. elegans* was shown to play a key role. In this respect SLO-1 loss-of-function mutants

are highly resistant to emodepside. We expressed the *slo-1* coding sequences of *Ancylostoma caninum* and *Cooperia oncophora* in the *C. elegans* null mutant *slo-1(js379)*. Heterologous expression of parasitic nematode *slo-1* in *C. elegans slo-1* loss-of-function mutants driven by the endogenous *C. elegans slo-1* promoter completely rescued the sensitivity to emodepside. Continuing experiments compared heterologous expression of parasitic *slo-1* from the *C. elegans slo-1* promoter with expression from the homologous parasitic *slo-1* promoter.

Therefore, the conserved functionality of SLO-1 in *C. elegans* and parasitic nematodes was confirmed. Whether emodepside acts directly through SLO-1 and in which way LAT-1 or other proteins are involved as mediating factors remains to be investigated.

CS22.6

Meta-Analyses of the Effects of Halofuginone Against Calf Cryptosporidiosis

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Cryptosporidium parvum is a cause of neonatal calf diarrhoea; and. halofuginone seems to have positive effects on calves affected by cryptosporidiosis. In many clinical trials, a limited number of study objects are used, thus lowering predictability and statistical power. Meta-analysis increases statistical power because a number of trials are evaluated together, increasing the effective sample size and possibility of finding true differences. To develop more precise estimates on the effects of halofuginone on calf cryptosporidosis, meta-analyses were performed, including clinical trials on prophylactic and therapeutic treatment. Effects on infection prevalence, diarrhoeal prevalence and mortality were investigated. Other variables e.g. mean oocyst output and weight gain could not be investigated. Effects in favour of the treated group were found day 4 and 7 of prophylactic treatment for infection and diarrhoeal prevalence, and in favour of the control group day 21 for infection prevalence. Prophylactic treatment thus delays oocyst output but provides no complete cure. Infection pressure was reduced to some extent by lowered infection prevalence. There was no effect of prophylactic treatment on mortality at natural infection. A shortage of studies made interpretations for therapeutic treatment uncertain, and we could not determine if treatment benefited calves. Based on these results and the fact that halofuginone is quite toxic, we believe that halofuginone should not be seen as a solution for cryptosporidiosis, but be used only with severe C. parvum-associated diarrhoeal problems in combination with other measures taken to lower infection pressure.

CS23 - Parasite Control

Tuesday, August, 11, 2009

CS23.1

Epidemiology of Ovine coccidia Under Conventional Sheep Rearing Conditions and Control by Toltrazuril (Baycox®) - a Multicentre Study in Central Europe

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The study was conducted as a multi-centre field study with 118 male lambs on three farms (conventional lamb-fattening farms A, B, C) in Saxony-Anhalt, Germany. On each farm, animals were equally and randomly divided into two treatment groups: one group treated with 20mg toltrazuril per kg body weight (Baycox®) on study day 12, and an untreated control group. A total of 2,190 faecal samples were taken during a period from about two to seven weeks after birth; the *Eimeria* species involved were determined in 274 of the samples taken from the untreated and in 39 of the samples from the toltrazuril-treated animals.

Coccidiosis was observed on all the farms. Regarding the control group, the cumulative incidence reached 100% during the study. Twelve *Eimeria* species were identified at farms A and C: *E. ahsata, E. bakuensis, E. crandallis, E. faurei, E. granulosa, E. intricata, E. marsica, E. ovinoidalis, E. pallida, E. parva, E. punctata* and *E. weybridgensis*. The same species were also detected at farm B, with the exception of *E. granulosa, E. intricata* and *E. punctata*. To our knowledge this is the first reported occurrence of *E. punctata* in Germany. *E. ovinoidalis* predominated in the faecal samples which underwent oocyst differentiation (> 90 % positive samples on each farm). Specimens with 3, 4 and 5 *Eimeria* spp. were the most common. The occurrence of diarrhoea correlated with the excretion of *Eimeria* spp. oocysts over all farms. Oocyst excretion, also in regard of *E. ovinoidalis*, was significantly reduced in the toltrazuril-treated group. Coccidia-related diarrhoea also was significantly reduced in the toltrazuril-treated group compared to the untreated control.

The results of this study confirm that lamb coccidiosis is a common problem on lamb-rearing farms in central Germany and is controlled by a single preventive toltrazuril treatment.

CS23.2

Efficacy of Toltrazuril and Diclazuril Against Ovine coccidiosis in Housed Lambs

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The objective of the study was to evaluate the efficacy of toltrazuril and diclazuril regarding the control of naturally occurring coccidial infections in housed lambs. The investigation was conducted at a farm in central Germany (Saxony-Anhalt) with previously reported coccidiosis and a high prevalence of *Eimeria ovinoidalis*.

Single treatments each with toltrazuril (Baycox® 5% suspension; 20 mg/kg b.w.) or diclazuril (Vecoxan® oral suspension, 2.5 mg/ml; 1 mg/kg b.w.) were either administered metaphylactically (study day 12 = SD 12) or therapeutically (after onset of coccidiosis) and the results were compared with untreated controls. 145 male lambs (German Mutton Merino x Suffolk cross-breed) aged 1 to 5 days at the start of the study were included. Faecal consistency and oocyst excretion were determined in faecal samples taken individually by faeces collection bags at two-day intervals from SD 13 to 49, i.e. 19 times throughout the study. The assessment of efficacy was based mainly on total oocyst excretion and the amount of *E. crandallis* and *E. ovinoidalis* oocysts (opg) shed throughout the study.

Coccidial infections mainly due to *E. ovinoidalis* and also including *E. crandallis* were observed in all groups. However, no severe clinical coccidiosis developed. The course of the oocyst excretion in the control group started 15 to 30 days after birth with highest intensities occurring between SD 19 and SD 23. Oocyst excretion was reduced significantly in both toltrazuril treated groups compared with the control group and both diclazuril treated groups. The faecal consistency was influenced by other factors but most prevalent and most severe diarrhoea was observed in the untreated control group.

CS23.3

Toltrazuril Treatment of Congenitally Acquired Neospora Caninum-Infection in Newborn Mice

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C57BL/6-mice were infected with N. caninum tachyzoites during pregnancy, yielding a transplacental infection of developing foetuses. Subsequently, congenitally infected newborn mice were treated either once or three-times with toltrazuril (or placebo) at a concentration of 31.25 mg compound per kg body weight. Both toltrazuril and placebo treatment had no negative effect on newborns, as non-infected treated pups developed normally without differences in mortality and morbidity to matching non-treated control animals. Already one application of toltrazuril was significantly ($p < 0.01$) able to delay the outbreak of neosporosis in newborn mice, when compared to placebo-treated infected controls. We found significantly higher proportion of surviving newborns in one-time and three-time-toltrazuril-treated groups (34%; 54%) when compared to one-time- and three-time-placebo-treated groups (14%; 30%), respectively. There was no significant difference ($p = 0.2$) in the proportion of surviving pups between one- and three-time-toltrazuril treatment. However, the number of diseased and Neospora-positive pups (46% and 47%, respectively), was markedly reduced after three-time-toltrazuril-treatment compared to all other groups. Three-time-treatment also resulted in the highest antibody (IgG, IgG2a) response. Pharmacokinetic analyses using individual serum samples revealed that, although toltrazuril was absorbed and metabolized to toltrazuril-sulfone by newborn mice, medicated animals exhibited an unexpected rapid turn-over (half-life-time) of the compound. Toltrazuril and the metabolite were also found in brain tissues, indicating that passage of the blood-brain-barrier occured.

In conclusion, we could show that three-times treatment with toltrazuril had a high impact on the course of infection in congenitally N. caninum-infected newborn mice.

CS23.4

Comparative Efficacy of Toltrazuril and Diclazuril Against Pasture Coccidiosis in Lambs in Norway

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In Norway, coccidiosis (eimeriosis) is an important disease in young lambs 2-3 weeks after turnout on pasture in spring.

To evaluate the efficacy of suspensions of toltrazuril (Baycox©) and diclazuril (Vecoxan©) in preventing coccidiosis, a multicentric field study was conducted, using 790 lambs on six farms (2 in south-western and 4 in south-eastern Norway). In each herd, 4 groups were treated orally with either diclazuril (1 mg/kg b.w.) or toltrazuril (20 mg/kg b.w.), either on the day of turnout on pasture (day 0) or on day 7. A fifth group remained untreated. The treatments effects were evaluated by comparing oocyst counts, faecal consistency scores, frequency of soft faeces, frequency of treatments of diarrhoea, and weight gains.

Coccidial infections developed on all farms. For, as yet, undetermined reasons there were no treatment effects of either toltrazuril or diclazuril as regards reducing oocyst shedding and preventing diarrhoea in the two herds in south-western Norway. However, treatments with toltrazuril on either day 0 or 7 significantly reduced the oocyst shedding on day 21 in all herds in south-eastern Norway, whereas diclazuril caused a significant reduction only in three of these herds and only when administered on day 7. Toltrazuril was superior to diclazuril in preventing development of soft faeces. Toltrazuril gave a satisfactory prevention of clinical coccidiosis even when administered at turnout. Lambs treated with either toltrazuril or diclazuril had a better weight gain from turnout till autumn than untreated lambs, and those treated with toltrazuril gained more than those treated with diclazuril.

CS23.5

Potential of Controlling *Henneguya zschokkei* (Myxozoa) in Cultivated Whitefish

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Based on the present-day knowledge of the life-history of myxozoan parasites of fish, with annelids generally included in the transmission, it is of considerable interest to look for novel control strategies in specific cases. In a project aiming at establishing the identity, extent of infection and strategies for preventing the infection of *Henneguya zschokkei* sensu lato in farmed whitefish *Coregonus lavaretus* both from fresh and brackish water fish farms in Finland were studied. The prevalence of the infection ranged from 0% to as high as 55% (in one sub-population in a brackish water fish farm). In addition, we could establish that in one case the origin of the infection in whitefish most probably was to be found in the fresh water area from which the fish had been brought for further growth into a brackish water site. By having defined the likely origin of the infection in the fish control of the infection can be considered. The importance of developing control methods is evident as farming of whitefish recently has increased and infected fish are unsuitable for human consumption because of hygienic and esthetic reasons.

CS23.6

Evaluation of the Efficacy of Vectra 3D Following Topical Administration to Dogs Between 21-55 Pounds of Body Weight Infested with Adult Lone Star Ticks (Amblyomma americanum)

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The objective was to determine the efficacy of Vectra 3D topical administration to dogs of 21-55 pounds of body weight infested with adult Lone Star ticks (Amblyomma americanum).

Eighteen dogs were allocated to three groups. Group 1 (n=6) and Group 2 (n=6) were treated with Vectra 3D, dose rate 3.6 mL per dog on Study Day 0. Group 3 (n=6) was treated with the control blank, dose rate of 3.6 mL per dog on Study Day 0. The allocation ration of 1:2 was used because 6 treated animals would not permit statistically significant difference in frequency of "tick-free" status if ONE control was tick-free and ONE treated dog wasn't, whereas 6 controls and 12 treated would be significant if 1 control and only 9 treated were tick-free (based on Fisher's exact Test).

Dogs were infested with approximately 40 adult Amblyomma americanum on Study Days, -1, 7, 14, 21 and 28. Live ticks were hand counted on Study Days 1, 8, 15, 22 and 29. Live ticks were comb counted on Study Days 2, 8, 16, 23, and 30.

Dogs treated with Vectra 3D had significantly ($p<0.01$) fewer ticks than the control dogs at all post-treatment examinations except Days 1 and 2. Efficacy was 35%, 81%, 99%, 92% and 91% on Days 1 (24 hours after treatment), 8, 15, 22 and 29 (24 hours after infestation), respectively, and was 6%, 91%, 96%, >99% and 99% on Days 2 (48 hours after treatment), 9, 16, 23 and 30 (48 hours after infestation), respectively.

Significantly ($p<0.01$) more dogs treated with Vectra 3D were tick free than control dogs on Days 15, 16, 23 and 30. On those days, either 10 or 11 of the 12 treated dogs were free of ticks, compared to none of the controls. On the other days, a maximum of 6 treated dogs were free of ticks.

CS24 - Equine Parasites

Tuesday, August, 11, 2009

CS24.1

A Larval Migration Assay for Assessing Macrocyclic lactone Sensitivity in Equine Cyathostomin Populations

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Cyathostomins are common, pathogenic intestinal nematodes of horses. Anthelmintic resistance has been recorded for all three classes of anthelmintic in these nematodes. The recent emergence of resistance against macrocyclic lactones (ML) is of great concern. These are the most commonly used anthelmintics in horses and the ML, moxidectin, is the only

single-dose anthelmintic effective against pathogenic mucosal stages. Traditional in-vivo methods for detecting resistance (for example, the faecal egg count reduction test) lack sensitivity and standardisation. Laboratory based bioassays offer potentially more sensitive and reproducible alternatives to in-vivo analysis. We have developed and optimised an in-vitro larval migration assay (LMA) for cyathostomins. By exploiting the paralysing effects of MLs, the assay measures the effect of a range of drug concentrations on the motility of infective third stage larvae. Cyathostomin populations of differing in-vivo ML sensitivity were subjected to ivermectin-LMA and dose-response curves and LMI50 values generated. These populations displayed significantly different LMI50 values. For example, two populations in-vivo ML-resistant cyathostomins exhibited LMI50 values (2.86 µg/ml and 2.41 µg/ml) which were significantly higher than those derived from two ML-naïve populations (0.15 µg/ml and 0.26 µg/ml) and an ML-sensitive population (0.57 µg/ml). These results indicate that the LMA has potential in screening equine cyathostomin populations for ML sensitivity.

CS24.2

The Effect of Sample Handling and Storage on the Accuracy and Repeatability of Equine Strongyle Fecal Egg Counts

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Faecal Egg Counts (FEC) are increasingly used to diagnose and monitor equine strongyle burdens and evaluate anthelmintic efficacy. In horses, the Faecal Egg Count Reduction Test (FECRT) is the golden standard used to investigate anthelmintic resistance. The purpose was to examine the effect of a combination of storage time and storage temperature as well as airtight versus uncovered storage on the number of strongyle eggs and their degree of embryonation. Faeces were stored airtight at ÷18°C, 4°C, 20°C, and 37°C for a total of 120 hours. Sub-samples were removed for FEC analysis at 0, 3, 6, 12, 24, 48 and 120 hours, using a Stoll technique. In another set of experiments, a pile of faeces was kept uncovered and another airtight on the stall floor for 24 hours. Statistical analyses were performed using linear models. In both studies, each FEC was measured 5 times and experiments were repeated 3 times. Temperature had no influence on the FEC after 12 hours of storage, and storage at 4°C did not reduce FEC for the entire 120 hours. Significant reductions were found after 24 hours at ÷18°C and 37°C, respectively and after 48 hours at 20°C. Availability of air had no significant influence on the FEC or degree of embryonation after 24 hours of storage at 11.8-16.9 °C. Airtight storage of faeces at 4°C preserves FEC for up to 120 hours. Further, faeces may be collected from the stall floor at temperatures in the investigated range for up to 24 hours after defaecation.

CS24.3

Determination of Ivermectin Strongyle Efficacy and Egg Reappearance Period ((ERP) on Danish Horse Farms

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Anthelmintic resistance is widespread in the world. Cyathostomins have developed resistance to several groups of anthelmintics and a number of studies have shown shortened cyathomstomin Egg Reappearance Period (ERP) after treatment with ivermectin. In Denmark, usage of anthelmintics has been restricted by prescription-only for a decade, but current levels of anthelmintic resistance are not known. Efficacy of ivermectin (0.2 mg/kg) was evaluated on a total of 196 horses from farms using selective therapy. Fecal Egg Count Reduction Tests (FECRT) were performed 14 days post treatment. 79 of these horses were infected with Parascaris equorum and 117 horses were infected with strongyles only. Overall efficacy of ivermectin was 96.9% against P. equorum and 100% against strongyles. ERP was investigated on 9 farms with a total of 96 horses, using a selective treatment strategy. 65 untreated horses were investigated from the same farms as controls. All egg counts were determined with the McMaster method. Horses were dosed and treated by the owners, but investigators verified weight estimations by girth tape measurements. Weekly FECRTs were performed from 2 to 6 weeks post treatment. Average efficacy of ivermectin was 96.9% 6 weeks post treatment. One farm had 90% efficacy of treatment at 6 weeks. The large majority of horses were dosed correctly or slightly overdosed, while only 8 horses were marginally underdosed. The untreated group showed a notably increase in Fecal Egg Count (FEC) during the study period. In conclusion this study did not provide evidence of shortened ERP or reduced efficacy of ivermectin.

CS24.4

Prevalence of Strongylus vulgaris and Pyrantel Resistant Cyathostomins on Danish Horse Farms Using Selective Therapy

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Equine cyathostomin nematodes have developed word-wide resistance to several classes of anthelmintics. In Denmark, anthelmintic drugs have been available by prescription-only since 1999, but little is known about current levels of anthelmintic resistance. Individual faecal samples were taken from 1644 horses on 64 farms. Horses with faecal egg counts of 200 or higher were treated with pyrantel embonate paste (19 mg/kg). Each farm had at least six horses receiving the treatment. Larval cultures were performed on all pretreatment samples for identification of Strongylus vulgaris. Faecal egg count reductions and their 95% confidence intervals were calculated using statistical mixed models by taking into account various sources of variability. Robust methods were used to deal with outliers in the data. The lower confidence limits (LCL) were used to define levels of cyathostomin pyrantel resistance as follows: <88%: resistance, 88-92%: suspect resistance, >92%: no evidence for resistance. Pyrantel resistance was determined on 19 farms (30%), suspect resistance on 9 farms (14%) and the remainder of farms (56%) had no evidence for resistance. S. vulgaris was detected in 78 (5%) of individual horses and on 31 (48%) farms. Bayesian and frequentist methods were used for data analysis to detect the influence of S. vulgaris on the efficacy of the drug. Both the methods yielded similar conclusions. S. vulgaris was not associated with pyrantel efficacy; however, lower pretreatment egg counts were associated with its presence. Although overall efficacy of pyrantel was high, this study documented developing pyrantel resistance in cyathostomin nematodes in Danish horse farms.

CS24.5

Parasite Control Programs for Horses: Is an Integrated Approach Feasible?

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Control programs should be individually designed as each horse establishment is unique. Existing problems include decreasing veterinary involvement, inexperienced owners, incorrect use of wormers and confusing / conflicting advice from company brochures and articles in journals. In addition many horses will be kept on premises where their nutrition may be sub-optimal, their grazing is of poor quality and is not correctly managed and they are overstocked. A comprehensive risk assessment needs to be done before instigating a control program. It is important to consider the ages and work schedules of the horses, their diets including pasture quality and management, past wormer usage and health records, especially of colic cases, together with results of any faecal examinations. The challenge for the veterinarian is deciding whether a tapeworm treatment is necessary in the control program as hitherto the diagnostic tests available have not been optimal though the nested PCR test on faecal samples looks more promising. In Denmark in 1999 legislation was brought in making anthelmintics available by prescription only and furthermore, they could not be used routinely for prophylaxis. A survey of Danish equine practices indicates that veterinarians are now playing a central role in parasite control and that this is reducing the number of treatments given. Ideally this approach should be adopted in other EU countries but changes in legislation would be required.

CS24.6

Cyathostome Nematodes (Nematoda-cyathostominae) of Horses: Ecology of Larvae on Grass Brachiaria humidicola in the Seasons in Seropédica, RJ, Brazil

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Climatic conditions of each area influencing the development and survival of pre-parasitic stages of cyathostome, acting directly on the parasite load of animals is an important factor for greater understanding of the epidemiology and host-parasite interaction, which assist in establishing a effective control system. The objective was to evaluate the ecology of cyathostome larvae in Brachiaria humidicola grass in different seasons of the year in Seropédica, RJ. The experiment was developed in E.P.P.W.O. Neitz, the DPA UFRRJ, 2004/September to 2006 october. Feces of naturally infected horses were collected and divided into four samples of 500g each, and deposited on grass B. humidicola at the beginning of each season. From each sample, weekly, collected by a rate (± 2) of feces and grass 8am and 5pm. The samples were weighed, processed by the Baermann technique after processing for recovery of larvae, feces and grass, were kept in oven for 48 hours at 75ºC,to obtain dry matter and the larvae recovered were identified and quantified. The meteorological data of temperature and precipitation were provided by INMET and soil temperature was obtained at the time of collection. In seasons, spring and winter (dry season), the least amount of rain and more mild temperatures and the higher survival and transmission of L3 to the grass, a fact already confirmed in previous work with other grasses for the region. In rainy months, referring to the summer and autumn, there was lower prevalence of L3 in the samples of feces and grass. This study is part of a project entitled "Correlation between survival and migration of cyathostome infective larvae in different types of grasses in the Baixada Fluminense in Rio de Janeiro, Brazil."

CS25 - Food-borne Parasites

Tuesday, August, 11, 2009

CS25.1

Evaluation of Polyclonal Antibodies for the Detection and Isolation of Coccidian oocysts of Public Health Importance

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In recent years, there have been increasing reports of disease outbreaks due to the consumption of food and water contaminated with coccidian oocysts. Although microscopy and molecular methods exist for the detection of some species of coccidian oocysts in environmental and clinical samples, there is an urgent need for efficient and reliable assays to monitor water, fruits and vegetables for other oocysts of public health importance. We generated polyclonal antibodies against intact fixed oocysts of Toxoplasma and Cyclospora and evaluated their use in methods to detect and isolate oocysts of a variety of species in spiked samples. The affinity of these polyclonal antibodies was evaluated by immunolocalisation on oocysts of Toxoplasma, Cyclospora, Eimeria and Cryptosporidium using the indirect fluorescent antibody test (IFAT). Immunoblots using lysates obtained from oocysts of various coccidian species confirmed the IFAT results. Purified IgG from polyclonal antibodies were used in an immunomagnetic separation technique (IMS) to elute coccidian oocysts from spiked water samples. Preliminary trials yielded good isolation of oocysts, indicating promising diagnostic applications using these reagents. Further development and validation research using these antibodies in the IMS and IFA assays for immunolocalisation and isolation of various target species oocysts is underway.

CS25.2

Pathogenesis of Toxoplasmosis and T. gondii *Null Result* Genotype

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Toxoplasma gondii *null result* infections are widely prevalent in humans and animals worldwide, but only a small percentage of exposed hosts develop clinical toxoplasmosis. It is unknown whether the severity of toxoplasmosis in immunocompetent individuals is due to the parasite strain, host variability or other factors. Recently, attention has been focused on the genetic variability among T. gondii *null result* isolates from apparently healthy and sick hosts. T. gondii *null result* isolates have been classified into three archeological genetic Types (I, II, III); Type I isolates are lethal for mice whereas Type II and Type III are less pathogenic for mice. Circumstantial evidence suggests that certain genetic types of T. gondii *null result* may be associated with clinical toxoplasmosis in humans.

Little is known of T. gondii *null result* genetic Type and clinical disease in animals, including wildlife and livestock. Recently, two new genotypes (Types A and X-linked to Type II) of T. gondii *null result* were isolated from naturally-exposed sea otters that died in USA. Whether these new T. gondii *null result* genotypes (Types A and X) are host- or region-specific, and their association with mortality is under investigation. For the past 7 years we have conducted extensive studies on genetic typing of T. gondii *null result* strains prevalent in asymptomatic and symptomatic animals worldwide. We have isolated all genetic types from asymptomatic animals. As yet, Type I T. gondii *null result* has not been isolated from animals that have died of toxoplasmosis. All isolates of T. gondii i*null result* solated from sick animals were Type II or Type III genetic type.

CS25.3

Expulsion and Muscle Larvae Burden of T. nativa in Rats

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Introduction: Previous investigations showed thar rats are quite resistant to T. nativa in the comparison with T. spiralis. In order to define the difference, we investigated in detail the dynamic of worm expulsion, in vitro fecundity of females as the muscle larvae burden in rats infected with each parasite.

Materials and methods: The investigation was performed on Wistar rats of both genders. Totally 78 rats were infected with 1500 muscle larvae of T. nativa or T. spiralis. Each group of rats consisted of equal number of males and females. In order to perform further parasitological analysis rats were sacrified on day 3,5,7,12,19 and 55. The intestine of each rat at each term was incubated in order to collect and estimate the number of adults as collect the females for the estimation of their in vitro fecundity. On the day 55 remaining rats were skinned, eviscerated and ground in order to define the muscle larvae burden.

Results: On day 12 significantly lower numbers of adults were found in both groups of infected rats. Adults in rats from both groups were totally expelled on day 19. Interestingly, more adults of T. nativa were expelled in female rats in comparison with males. No significant differences between differently infected rats were found. According to the results no significant differences between in vitro fecundity of females of T. nativa or T. spiralis collected on day 5 and day 12 were found too. The average number of muscle larvae in T.

nativa infected rats was 30,0 per gram in males and only 2,0 per gram in females.

CS25.4

Assessment of the Economic Damages Caused by Hydatidosis in One-Humped Camels, Iran, Khorasan Province

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One-humped camel is a special breed of camels existing in tropical regions. The population of the Persian Breed also known as Arabian or Swift Breed is about 15 millions in the world and about 300,000 in Iran. Camels have a high tolerance for extremely hot and cold weather. They are resistant against different kinds of diseases. Since an early time, Camels husbandry has always been one of the popular fields in stock breeding.

Camels are bred for different purposes such as meat and milk production, riding and transportation. One of the camel's by-products is its liver that has an average weight of 6-10.5 kg. Liver can be infected by parasitic diseases which will lead to direct economic damages. Liver infections may also cause physiological disorders in camels such as weight loss and decrease in their milk production (indirect economic damages). The purpose of this study was to estimate the direct economic damages caused by Hydatidosis in one-humped camels.

We collected the infected livers by Hydatid Cyst during one year (1386) in Iran, Khorasan province. Then, we arranged them according to the minimum, maximum and average weights of part of the livers which were no longer usable, and considered the price per kilograms of the livers in Rials. According to the results, 112 is the highest number of infected livers in winter and according to our computations, it caused an average of 4,620,000 Rials of direct economic damages. We also did the computation for every season and month in the year of 1386 and got interesting results.

CS25.5

Localization of Host-Protective Onchosphere Antigens of Taenia ovis

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A number of recombinant vaccines have been developed against the veterinary and medically important cestode parasites viz., Taenia ovis, Taenia saginata, Taenia solium and Echinococcus granulosus. These vaccines indicate that it is possible to achieve a reliable, high level of protection against a complex metazoan parasite using the defined recombinant antigens and these are known to be expressed in oncospheres of taeniids. There is little information available about the site of expression of different vaccine antigens in different developmental stages of the oncospheres. The present study presents first report on the localization and colocalization of the vaccine antigens of Taenia ovis in different developmental stages of oncospheres using labelled streptavidin-biotin method, immunofluorescence with confocal microscopy and immunogold labelling with transmission electron microscopy techniques. Results to date suggest that host-protective oncosphere antigens of T. ovis are located inside the oncospheres. These appear to be related either with penetration glands or some embryonic cells of the oncospheres. Immunogold labelling with transmission electron microscope has validated the results of light microscopy that a bipolar staining pattern involving 2-4 cells stain positively for the presence of antigens. Colocalization studies of three different antigens with confocal microscope have revealed different level of expression for different antigens in different developmental stages of oncospheres. It is anticipated that the present study will provide the better understanding of the role of these vaccine antigens in the biology of the taeniids and shed some light on the mechanism through which protective vaccine antigens have their effect on the parasites.

CS25.6

Development of a New Indirect ELISA for the Detection of Trichinella Spiralis in Pigs

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Trichinella spiralis is a worldwide spread nematode capable of infecting carnivores and responsible for trichinellosis, a lifelong disease. Human contamination occurs by ingestion of raw or undercooked meat from pigs, horses or game animals. Inspection of carcasses at slaughterhouses needs to be performed prior to release of meat on the market. The test consists of muscle artificial digestion in order to free larvae and allow for their visual inspection. Unfortunately this direct method is costly, time consuming and needs an heavy quality assurance system to be reliable since it relies on human expertise. We developed an indirect ELISA based on 2 early antigens previously identified, namely Nbl1 and 411; they were expressed as recombinant GST-fusion His-tagged proteins and purified with a double step affinity chromatography. The proteins were tested for their antigenicity by western blot before the development of the serological test. The iELISA performed with proteins alone or pooled was tested with 34 negative sera and 39 positive sera, recovered from

experimentally infected pigs. This iELISA detected infected animals as soon as 20 days post infection, that is an improvement compared to the sensitivity of the current serological test that can only detect infected animals 5 weeks after infection. Moreover the current method, based on excretion/secretion antigens, lacks of specificity for wildlife animals. The two molecules used in this new iELISA could be used in a future approach for early detection and combined with a late antigen allowing thus detection of long-lasting infections.
We acknowledge funding from MedVetNet.

CS26 - Vectors / Ectoparasites

Tuesday, August, 11, 2009

CS26.1

Biting Midges (Ceratopogonidae: culicoides) in Belgium: a Comparison Between Indoor and Outdoor Trapping in Cattle and Sheep Farms

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Introduction: Bluetongue, a vector borne disease of ruminants, was identified for the first time in Northern Europe in 2006. The vectors are insects of the family Ceratopogonidae, genus Culicoides. In Belgium, no recent data were available about the biology of these insects including their feeding habits and behaviour. The present study was carried out in order to evaluate the indoor and outdoor activity of these Diptera in 5 different cattle or sheep farms in 2008.

Methods: Two sheep and 3 cattle farms were selected in the Province of Luxembourg, Belgium. In each farm 2 Onderstepoort Veterinary Institute (OVI) traps were installed respectively inside and outside the animal accommodation. Trapping was carried out twice a week from 17:00 until 24:00. The collecting vials were replaced every hour. A portable suction trap (BackTrap® U.S.A.) was used twice on each visit to collect midges directly on the animals. In each farm the study was carried out for 6 successive weeks, 2 farms being monitored together. The study began on July 28th and ended on November 30th 2008.

Results: A total of 2591 culicoides were trapped. A majority of those (88.76%) were trapped indoors whereas 10.03% were trapped outdoors and 1.21% directly on the animals. The ambient temperature had a marked effect very few culicoides being trapped below 13°C.Three main species or species complex were indentified both indoors and outdoors: C.obsoletus/scoticus, C dewulfi, C.chiopterus. They represented 98.93 and 85.03% indoors and outdoors respectively. On the animals only C.obsoletus/scoticus and C. dewulfi were found.

CS26.2

Detection of Leishmania infantum DNA in Ticks Collected from Brazil and Italy

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Canine leishmaniosis is a widespread disease caused by Leishmania parasites, which are primarily transmitted by phlebotomine sand flies. However, there are some areas where the primary vectors are absent and other arthropods have been suspected to play a role in transmitting the infection. Herewith, we report the detection of Leishmania infantum kDNA in ticks collected from dogs shown to be positive to anti-Leishmania antibodies, living in rural areas in southern Italy (site A) and north-eastern Brazil (site B), where canine leishmaniosis is endemic. Between March and October 2007, ticks were collected from 26 dogs and either placed directly into vials containing 70% ethanol or maintained alive for identification and subsequent dissection. All the 95 ticks collected were morphologically identified as Rhipicephalus sanguineus. After identification, ticks were dissected and genomic DNA was extracted and processed by two different PCR protocols for the detection of L. infantum kDNA. Out of the 73 ticks from site B (tested by a real time PCR) nine (12.3%) were positive, with a parasite load ranging from 0.001 to 0.035/µl. Two pools of salivary glands from ticks (one from five females and other from five males) collected from a seropositive dog (site A) and tested by a conventional PCR were positive. The retrieval of L. infantum kDNA in the salivary glands of Rhipicephalus sanguineus ticks has been here reported for the first time. The present results suggest that new studies are needed to assess the competence of ticks as vectors of Leishmania parasites from dog to dog.

CS26.3

Ticks and the City - the Occurrence of Different Tick Species and their Pathogens in and Around Vienna

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Vienna´s recreational areas are very popular not only with humans and dogs but also with ticks. We conducted a survey in 2006 at selected locations in the city and suburban areas to investigate the distribution of tick species and some of the pathogens they harbour. Of 713 flagged ticks, the most common species was *Ixodes ricinus* (95.6%), followed by *Haemaphysalis concinna* (4%) and *Dermacentor reticulatus* (0.4%). The ticks harboured *Borrelia*, *Babesia* and *Anaplasma* (25.5%, 3.2% and 0.7%, respectively). We also found one probably autochthonous *Rhipicephalus sanguineus*–population in an animal shelter near Vienna, which cares for pets coming from southern Europe. Infections with *Ehrlichia canis* were repeatedly diagnosed in several dogs of this shelter; however, the origin of the infection was unclear. *E. canis* is probably maintained by the indoor population of *R. sanguineus* which became infected by *Ehrlichia*-positive dogs introduced to the shelter from endemic countries. With repeated imports of dogs from southern Europe and the established *Rhipicephalus*-population other non-autochthonous pathogens can probably also be transmitted under such conditions, although in the continental climate conditions in eastern Austria an establishment of a permanent outdoor *R. sanguineus*-population seems unlikely. Warmer weather, however, could provide improved conditions for this tick species. This is supported by the fact that the assumed minimum temperature of 20°C for the development of *R. sanguineus* has to be refuted. In one outdoor trial two fully engorged nymphs molted to females at an average temperature of 9.7°C (min 3.2°C, max. 15.5°C). Further investigations have to be conducted to determine the exact parameters influencing an establishment of this foreign tick species in Austria.

CS26.4

Transmission of Cytauxzoon felis to Domestic Cats by Amblyomma americanum

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Cytauxzoon felis was transmitted to a domestic cat by *Amblyomma americanum*. The infection was produced by the bite of *A. americanum* adults that were acquistion fed as nymphs on a domestic cat that naturally survived infection of *C. felis*. Fever, inappetence, depression, and lethargy were first noted 11 days post infestation (dpi). Pale mucus membranes, splenomegaly, icterus, and dyspnea were also observed during the course of the disease. The body temperature of the experimentally infected *C. felis* cat was subnormal from 16 dpi until 24 dpi when it returned to within normal limits. All clinical signs of cytauxzoonsis began to resolve by 23 dpi when the cat became subclinically infected with *C. felis*. Schizonts of *C. felis* were observed in spleen aspirates of the infected cat at 15 dpi. DNA of *C. felis* was amplified by real-time PCR starting 17 dpi and piroplasms of *C. felis* were first noted by light microscopy 18 dpi. Prior to the present study, only *D. variabilis* had been shown experimentally to transmit infection of *C. felis*. This is the first report of *C. felis* being transmitted by *A. americanum*. Additional studies have been initiated to further test our finding that *C. felis* can be transmitted to domestic cats by *A. americanum*. Preliminary findings suggest *A. americanum* is a more efficient vector of *C. felis* than *D. variabilis*. Results of our additional studies will be presented.

CS26.5

The Real-Time Tracking of Bartonella henselae Through Ctenocephalides felis

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Bartonella henselae, transmitted by Ctenocephalides felis, is the agent of cat-scratch disease. Classed as an emerging infectious disease, it is an ideal model for investigating the real-time tracking of zoonotic bacteria through the flea vector. C. felis were artificially fed canine blood and B. henselae. After 17 hours the blood-meal was changed to uninfected blood. Over ten days, 115 fleas were sampled. A total of 822 organ samples were dissected from the fleas and were treated with EMA to prevent detection of non-viable bacteria. Samples were assayed using C. felis- and B. henselae-specific real-time PCRs and a conventional canine-specific PCR. Ct values were corrected for inter-assay variation and sample size. Positivity rates and Ct values were statistically analysed. Positivity rates for C. felis, B. henselae and canine DNA were 98.3%, 42.2% and 12.4% respectively. Positivity rates for B. henselae was significant against day sampled, with two distinct decreases in positivity, between days one to five and days six to ten ($P<0.001$). Ct values for a number of organ types showed decreasing B. henselae DNA over time, again with two distinct groups ($P<0.001$-0.013). The presence of canine DNA in 2% of organs from outside of the gastro-intestinal tract would suggest some contamination. Although the transit time of a blood-meal through C. felis can take three days, B. henselae is detectable within C. felis for up to ten days, allowing prolonged excretion of the bacterium via faecal material. The

malpighian tubules are a possible site for sequestration of B. henselae.

CS27 - Zoonoses

Tuesday, August, 11, 2009

CS27.1

Environmental Contamination with Toxocara spp.: Towards Quantitative Epidemiology?

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Environmental contamination with Toxocara spp. eggs remains a significant public health concern worldwide. The risk of human infection clearly depends on the number of eggs shed by definitive hosts, their development and survival, and spatial distribution in relation to human contact. In spite of this, there have been few published attempts to quantify these factors, with faecal surveys generally reporting only prevalence, and soil surveys undermined by poor standardization of methods for egg recovery and enumeration. We report ongoing attempts to progress beyond descriptive surveys of egg prevalence, with initial studies in the town of Bristol, UK. Mean egg density in dog faeces was found to vary between 2140 epg (eggs per gram) in pups less than 8 weeks old (prevalence 62 %), to 700 epg in pups 8-12 weeks old (prevalence 15 %), and 4 epg in dogs older than 12 weeks (prevalence 3 %). Foxes were commonly infected, with prevalence around 60 %, but low burdens and population density meant that their predicted contribution to environmental contamination was less than one tenth that of dogs, even without taking into account patterns of habitat use. In public parks, 60 % of soil samples were contaminated, with egg density varying between positive samples from 0.02 to 0.60 epg of soil. The amount of soil sampled from each site and the spatial sampling design strongly influenced results. This study provides the foundation for ongoing attempts to develop an integrated, quantitative framework for the epidemiology of environmental contamination with Toxocara spp. eggs.

CS27.2

Building Community and Laboratory Capacity for Monitoring and Managing Parasitic Zoonoses in Northern Saskatchewan, Canada

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Lack of veterinary and medical infrastructure in remote rural and northern areas in Canada serves to perpetuate disparities in public health in these regions. A recent case of neural cystic hydatid disease in an indigenous community in north-central Saskatchewan, Canada, highlighted a need for better community monitoring and awareness of parasitic zoonoses in general, and hydatid disease in particular, which remains a zoonosis of global concern in developed and developing nations alike. The subsequent joint animal and human health investigation of the prevalence and risk factors for Echinococcus sp. in this community also identified needs for better laboratory infrastructure. This includes detection, genetic characterization, and epidemiological investigation of the strains and species of Echinococcus endemic in northwestern Canada, as well as other potentially zoonotic parasites. Successful management of parasitic zoonoses in northern Canada calls for a "One Health" approach to expand veterinary care for companion animals, to enhance surveillance in wildlife that serve as country food, and to develop and disseminate management recommendations and public health education materials specific to each community and risk group.

CS27.3

Prevalence and Molecular Characterization of Echinococcosis in Stray Dogs in Central Sudan

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Echinococcus granulosus is an important zoonotic infection of dogs. The purpose of this study is to investigate the prevalence of **E.garnulosus** in stray dogs in an area of higher prevalence of Hydatidosis in intermediate hosts and

in most cases the dogs are fed on the offal obtained from the slaughterhouse. 42 dogs shot as a part of the rabies control program in Tamboul and Rofa, central Sudan were autopsied and their intestinal contents were examined for the presence of **E. granulosus** worms. Faecal samples were taken as well for coprodiagnosis. The worm burden in positive dogs was determined using a dilution method and the harvested worms were characterized using G5/6/7 and G1 PCRs. From the 42 euthanized dogs, 12 (28.5%) were harboring **E. granulosu** s worms with a worm burden of 22-80X10 ^{3}. All the DNA samples extracted from the worms were characterized as **E.canadensis** 83.3% (10/12) of the DNA extracted from the faecal samples collected from the 12 positive dogs were found positive with copro- PCR and were identified as **E.canadensis**. Two samples were considered inconclusive as there was no signal in the inhibition test. copro- DNA samples from the 30 samples collected from the dogs which were reported negative at necropsy were negative using copro-diagnostic PCR. This copro-PCR method is used for the first time in such a survey in Sudan and it suggests the predominance of **E.canadensi** s in Sudan.

CS27.4

Taenia Ecology and Competition in People, Pigs and Dogs in Southeast Asia

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Taenia solium is a clinically important zoonotic parasite of significant worldwide public health concern. In Southeast Asia, *T. solium* competes with *T. hydatigena* and *T. asiatica* in the intermediate host and *T. saginata* and *T. asiatica* are competitors in the definitive host. These inter-specific competitive relationships in combination with socio-cultural practices culminate in a complex ecology that shapes *T. solium* transmission dynamics. However, there is a paucity of information relating to *Taenia* ecology and competition and associated risk factors in this region. This paper reports the preliminary results of four studies conducted in four northern provinces of Lao PDR to investigate *Taenia* ecology in people, pigs and dogs. The first study investigated pig production practices and trade relationships between pig producers and consumers. The second study was a slaughter-slab survey to estimate the prevalence of the three *Taenia* species infecting pigs. The third study was a community survey conducted in 332 households in 24 villages to estimate the prevalence of the three *Taenia* species infecting humans and the associated risk factors. A prevalence survey of *T. hydatigena* in dogs was simultaneously carried out in the villages where the community survey was conducted. The results indicate that *T. hydatigena* is the dominant species infecting pigs and that culturally specific behaviour in villages that produce the majority of piglets acts to limit the occurrence of *T. solium* in people and pigs.

CS27.5

Prevalence of Visceral Leishmaniasis in Human Population of Siraha District in Nepal

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In Nepal, Visceral leishmaniasis is a major public health problem in 12 Terai districts of Nepal and some adjoining districts. More than 5.7 million people living in these districts of Nepal are believed to be risk of this disease. Siraha district, the study area are adjoined with the border of Bihar state of India which is endemic for VL. Visceral leishmaniasis (VL) or Kala-azar is a potentially fatal vector-borne zoonotic disease caused by a protozoan parasite, Leishmania donovani. Visceral leishmaniasis affects persons from the lowest socioeconomic strata of the community and the district falls under it, hence the study population are believed to be more prone to having VL. Out of a total of 150 blood samples collected randomly during July to September, 2008 from patients (human) came to district hospital, Siraha and RSUPMS, hospital, Lahan having history of fever for 3-5 days, 22 (14.66%) were found positive reactors for specific and non-specific antibodies against VL with formol-gel test. And at 95% Confidence Interval, the percentage prevalence ranges from 9.56-21.5%. The percentage prevalence of VL in male patients was found higher (17.39) as compared to female patients (10.34). According to the survey report which was conducted for 70 patients, the percentage prevalence of VL in patients having muddy (thatched roof) house group was found higher (24.44) than patients having concrete (pakka) house group (12) and the percentage prevalence of VL in patients not using bed net group was found much higher (25) as compared to patients using bed net group (9.09). Considering the higher prevalence of the disease in the population studied, suitable preventive and control measures including the quality diagnosis and treatment with the emphasis on completion of treatment, monitoring of drug resistance, vector surveillance, transmission interruption through quality Indoor Residual Spray, use of bed net, solving the problems relating to migration and poverty and awareness programs for rural people at all level have been recommended.

CS27.6

Effects of High Temperatures on Sporulation and Infectivity of *Toxoplasma gondii* Oocysts in Water

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The protozoan parasite *Toxoplasma gondii* is increasingly recognized as a waterborne pathogen. The present study was performed to assess the survival of nonsporulated and sporulated *T. gondii* oocysts in water at high temperatures. Oocysts suspended in 200 l of water were heated in the metal block of a thermal DNA cycler. Block temperatures were set from 40 to 70 °C with 5 °C incremental temperatures. The oocysts were exposed to each temperature setting for 1 min or 5 min. The nonsporulated oocyst viability was determined by their ability to sporulate when they were placed at room temperature while the sporulated oocyst viability was tested by mouse bioassay. Serology, squash brain preparations and PCR amplification were used to evaluate mice for infection. Our study finally demonstrated that nonsporulated *T. gondii* oocysts lose the ability to sporulate when heated at 55 °C for 5 min or at 60 °C for 1 min. Our results also indicated that when water containing *T. gondii* oocysts was exposed to the temperature of 55 °C for 5 min or to 60 °C for 1 min, the infectivity of oocysts was lost.

The financial support of the grant project MSM 6215712402 is acknowledged.

CS28 - Drug Resistance

Tuesday, August, 11, 2009

CS28.1

Parasitic Gastrointestinal Nematode Control Practices in US Cow-Calf Operations – Insights from the USDA National Animal Health Monitoring System

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Internal parasites can reduce the reproductive performance of the cow herd, weaning weights of calves, and negatively impact animal health due to respiratory disease and immune suppression. It has long been considered that standard procedure is to treat the beef cow once or twice each year and treat calves at weaning. However, whether these practices have shifted over the past several years is unknown. The opportunity to address this question arose when The USDA National Animal Health Monitoring System (NAHMS) conducted its third study of the cow-calf segment of the beef industry in 2007 and 2008. During the Beef 2007-08 study a stratified random sample of producers in 24 states were selected to be surveyed. These producers represent 79.6 percent of U.S. operations with beef cows and 87.8 percent of U.S. beef cows. To gain information on the cow-calf producers' attitudes and practices towards gastrointestinal parasites and parasite control, 567 respondents were asked questions about deworming practices, parasite evaluations, and sources for information on parasites and anthelmintics. All respondent data were statistically weighted to reflect the population from which they were selected. The inverse of the probability of selection for each operation was the initial selection weight. This selection weight was adjusted for nonresponse within each State and size group to allow for inferences back to the original population from which the sample was selected. The attitudes and practices revealed by this survey and their implications will be discussed.

CS28.2

A Representative Evaluation of the Parasites Present in the US Cow-Calf Population

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During the USDA National Animal Health Monitoring System's (NAHMS) Beef 2007-08 study 567 producers from 24 states were offered the opportunity to collect fecal samples from weaned calves for evaluation of parasite burden by quantifying the parasite eggs per gram of fecal material. Up to 20 fresh fecal samples from weaned beef calves were collected. If the group tested consisted of less than 20 animals, the number of samples collected was equal to the group size. Samples were only accepted where there was at least 45 days since previous anthelmintic treatment of the animals. Golfball sized samples were collected into baggies, cooled over night and then shipped to one of three designated laboratories for evaluation. In the laboratory all samples were processed in a similar manner using the Modified Wisconsin technique and enumeration of strongyle, Nematodirus, and Trichuris eggs, and the notation of the presence or absence of coccidian oocysts and tapeworm eggs. In submissions where the eggs per gram value for strongyle eggs exceeded 30, aliquots from 2-6 animals were pooled for extraction of DNA from the eggs. Extracted DNA was subjected to PCR an-

alysis for the presence of Ostertagia, Cooperia, Haemonchus, and Oesophagostomum. Overall, 102 beef operations from 22 states collected and submitted samples for evaluation. The detailed results of this survey and their implications will be presented.

CS28.3

Effectiveness of Current Anthelmintic Treatment Programs on Reducing Fecal Egg Counts in US Cow-Calf Operations

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During the USDA National Animal Health Monitoring System's (NAHMS) Beef 2007-08 study 567 producers from 24 states were offered the opportunity to collect fecal samples from weaned calves for evaluation of response to treatment with an anthelmintic product. Producers choosing to participate were provided instructions and materials to collect fecal samples at the time of treatment and again approximately 2 weeks after treatment. Samples were only accepted where there was at least a 45 day lapse between initial sampling and any previous anthelmintic treatment of the animals. The choice of treatment was entirely at the discretion of the producer so that the test reflected the current parasite control program of the operation. The protocol required that 20 fresh fecal pats be randomly sampled from the housing area. If the test group consisted of less than 20 animals, the number of samples collected was reduced to equal the group size. Samples were submitted to one of three participating laboratories. Analyses consisted of a double centrifugation floatation followed by enumeration of strongyle, Nematodirus, and Trichuris eggs, and simple notation of the presence or absence of coccidian oocysts and tapeworm eggs. In submissions where strongyle egg per gram exceeded 30, aliquots from 2-6 animals were pooled for extraction of egg DNA. Extracted DNA was subjected to PCR for the presence of Ostertagia, Cooperia, Haemonchus, and Oesophagostomum. A total of 72 producers from 19 States participated in this portion of the survey. Treatment options included oral benzimidazoles, and both injectable and pour-on endectocides. The results of this survey and their implications will be discussed.

CS28.4

Efficacy Determination of Several, Non-generic Cattle Anthelmintics by Way of a Fecal Egg Count Reduction Test Combined with a Control Trial

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In May of 2008, 50 naturally-infected beef-type calves were blocked into comparable groups according to coprological data, and randomly allocated within each block to one of five treatment groups; control, injectable moxidectin at 0.2 mg kg-1BW (MXD), injectable ivermectin at 0.2 mg kg-1BW (IVM), oral oxfendazole at 4.5 mg kg-1BW (OXF) and oral fenbendazole at 5.0 mg kg-1BW (FBZ). Animal confinement onto clean concrete was started on day -7, post-treatment egg counts and coprocultures were conducted on numerous occasions, and animal necropsies by complete replicate were conducted on days 35-39. Fecal egg count reduction test (FECRT) %s were > 90% for all anthelmintics from day 2 to day 28, but by the time of necropsy, FECRT %'s for IVM had declined to 85%. As determined from necropsy nematode counts (geometric means), percent efficacies were as follows: for Ostertagia adults, EL4 and LL4, MXD was 99, 99 and 97%, IVM was 98, 91 and 82%, OXF was 90, 70 and 48%, and FBZ was 73, 0 and 22%, respectively; for adult Haemonchus and Trichostrongylus axei, all anthelmintics were > 95%; for Cooperia oncophora (plus surnabada) and C punctata, MXD was 96 and 98%, IVM was 77 and 85%, OXF was 99 and 98%, and FBZ was 99 and 99%, respectively. FECRT results accurately predicted control trial efficacies for MXD, but failed to reveal two incidences of depressed efficacies: IVM relative to Cooperia spp, and the benzimidazoles relative to most Ostertagia populations.

CS28.5

Field Evaluation of Methods for Detecting Multiple Resistance to Anthelmintics in Sheep Nematodes in England and Wales

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Intensification of animal production systems has led to an increased reliance on effective anthelmintics to control parasitic worms. However, the excessive and continued use of these treatments can lead to high selection pressures and have resulted in increased reports of emerging nematode populations exhibiting resistance to all of the main anthelmintic classes.

Faecal Egg Count Reduction Tests (FECRT) and Larval Development Tests (LDT) were conducted on 6 farms in England and Wales with suspect multiple resistance. The farms

selected were identified from a network of 40 study farms participating in a larger study investigating the implementation of Sustained Control of Parasites in Sheep (SCOPS) worm control principles.

In this study, resistance was indicated as present by FECRT, LDT, or both, to one or more groups of anthelmintics groups on all farms. Comparisons were made between results obtained by the two tests. BZ resistance was identified by FECRT on 5 of the 6 farms; LV resistance on 4 farms and ML-resistance on 5 farms. The LDT identified the presence of BZ-resistance on all 6 farms, and LV-resistance on 5 farms. Generally there was good agreement between the two tests in identifying both BZ and LV resistance. On one farm, the LDT identified the presence of BZ-resistant nematodes, not detected by FECRT, and on 2 farms the presence of LV-resistant nematodes not detected by FECRT.

On two farms "triple" resistance (resistance to benzimidazole (BZ), levamisole (LV) and avermectin (AV)) was identified by FECRT, and on one farm moxidectin resistance in Teladorsagia was suspected based on an early return to egg laying at 28 days post treatment. Resistance was present in one or more genera, but most commonly with Teladorsagia on all of the 6 farms.

CS29 - Vaccines

Tuesday, August, 11, 2009

CS29.1

Vaccination Against *Haemonchus contortus* with a Recombinant Antigen Cocktail

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Haemonchus contortus is globally one of the most pathogenic nematode parasites of sheep. Vaccination offers a promising alternative to anthelmintic treatment, as resistance to *H. contortus* is now widely spread. Substantial protection can be achieved by vaccination with a native glycoprotein complex called H-gal-GP, which is located on the intestinal cell microvillar membranes of the parasite. Fractionation of the dissociated complex has indicated that metallo and aspartyl enzymes are associated with protection.

In this study, three H-gal-GP metalloproteases were recombinantly expressed as soluble proteins in insect cells and one aspartyl protease was expressed in *E. coli*, refolded and solubilised. The four proteins were then combined in QuilA and naïve sheep were immunised with the recombinant cocktail. Sheep were subsequently challenged with 5,000 *H. contortus* larvae. Serology and parasitology indicated that the recombinant cocktail induced a good immune response but did not confer protection against *H. contortus* challenge.

3-D reconstructions of the H-gal-GP complex from electron microscopy data have revealed a novel quaternary structure. It contains an internal chamber in which we speculate haemoglobin and other protein substrates are digested. We hypothesise that protective vaccine antibodies bind around the entrances of this chamber impeding substrate ingress, leading to starvation and death of the parasite. Thus, important conformational epitopes required for protection might not be reproduced when combining individual recombinants of the complex. As it is highly unlikely that the quaternary structure of native H-gal-GP can be reproduced with current recombinant methods, further studies will concentrate on identifying and reproducing protective epitopes.

CS29.2

Twists and Turns En Route to a Vaccine for Haemonchus Contortus

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Substantial protection can be achieved against Haemonchus by vaccination with native H11 or H-gal-GP, two glycoprotein enzyme complexes located on the parasite intestinal membranes. H11 is a family of four aminopeptidases whilst H-gal-GP is composed of four metallo-, two aspartyl, and at least one cysteine protease. All these enzymes are presumed to be involved in the digestion of the parasite blood meal. Neither antigen complex protects well if it is denatured, nor do recombinant versions of any of the subcomponents, even when used as cocktails.

It is highly unlikely that the recently elucidated quaternary structure of native H-gal-GP apparently required for protection can be reproduced with current recombinant DNA methods. However, recent trials indicate that it may in fact be possible to make a commercially viable vaccine from native antigen.

CS29.3

The Effects of a Recombinant Vaccine on Natural Fasciola hepatica Infection in Cattle

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The liver fluke, Fasciola hepatica can cause disease in a wide range of hosts including ruminants and humans. It has a worldwide distribution. Efforts are being made to develop an effective vaccine against Fasciola to reduce our reliance on anthelmintics, particularly in light of increasingly frequent reports of resistance.

In our study, we divided 39 male, castrated Friesian cattle into 3 groups of 13. One group acted as a control, the other two were vaccinated with recombinant Fasciola hepatica Cathepsin L1 and one of two mineral- oil based adjuvants. They were turned out on to fluke-contaminated pastures for 13 weeks. The cattle were then slaughtered and their livers assessed for fluke burdens.

There was a significant reduction in fluke burden in the vaccinated cattle relative to the control group, at $p \leq 0.05$.

The vaccinated groups showed a sharp rise in total IgG levels to Cathepsin L1 post vaccination. This was maintained over the course of infection and the levels were significantly higher that those of the control group

Arginase levels in the macrophages of vaccinated cattle were significantly lower than those of the control cattle, indicating that the parasite-induced alternative activation of the macrophages was altered by vaccination.

This vaccine shows potential as a means of controlling fascioliasis, however further trials, with longer challenge periods would be necessary to establish its efficacy.

CS29.4

Trade-Offs Between Immune Responses, Pathophysiology and Establishment of *Ostertagia ostertagi* in Artificially-Infected Calves

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Twenty-five, castrated male Holstein-cross calves, aged 4-5 months and weighing156.5kg, were allocated to one of five treatment groups on the basis of initial bodyweight. The groups included an ad lib-fed infected (INF) group which received a trickle infection with the equivalent of 10,000 larvae of *Ostertagia ostertagi* per day from Day 0 to Day 56, when the group was treated with eprinomectin; the study ended on Day 77. Parameters measured throughout the study included: liveweight, feed intake, faecal egg counts (FEC); plasma pepsinogen, gastrin, ghrelin and leptin; plasma antibodies to adult *O. ostertagi*. Within the INF group of five calves, three showed the stereotypical pattern of faecal worm egg output, whilst the remaining two animals did not, passing very few or no worm eggs. Further analyses of the data showed that there was a significant sub-group x day interaction for FEC and, in addition, there were significant (P=0.0067) differences between the two sub-groups in blood gastrin response. There were no significant differences between the other parameters. It was also observed that, within the sub-group of three calves with typical FEC patterns, there was a significant (P=0.007) inverse correlation between FEC and *O. ostertagi* antibodies. These results will be considered in terms of resource allocation and differential responses to parasite challenge.

CS29.5

Vaccination Against Liver Fluke in Sheep with Recombinant Leucine Aminopeptidase Induces High Levels of Protection Using Different Adjuvants

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Leucine aminopeptidase (*FhLAP*), an homohexameric Zn++-dependent exopeptidase isolated and purified from *Fasciola hepatica* gastrodermis, showed a promisory potential as vaccine candidate against ruminant fasciolosis. In this work, the recombinant enzyme, a M17 LAP functionally expressed in *Escherichia coli* was tested as vaccine against liver fluke infection in sheep using different adjuvants. Sixty fluke-free Corriedale sheep, 6 -12 months old, were randomly allocated in groups of 10 animals each. Five groups received a subcutaneous injection of 100 ug of *Fh*LAPr mixed in Freund´s (Complete + Incomplete), DEAE-Dextran, Alum, Ribi or Adyuvac® on weeks 0 and 4. The control group received only Freund´s (Complete + Incomplete) adjuvant. Sheep were challenged with 200 metacercariae on week 6 and necropsied on week 18. FhLAP induced a significant protection against liver fluke infection showing high levels of worm reduction in Alum (87%), Freund (84%) and Adyuvac® (81%) groups, and modest levels in Ribi (51%) and DEAE-Dextran (50%) groups compared to controls. Worms recovered from livers showed no significant differences in size (lenght and width) between vaccinated groups or when compared with the control group. All vaccine preparations induced high IgG levels which boosted after the challenge infection but no correlations between antibody titres and worm counts were observed. The results confirms that similarly to the native enzyme, *Fh*LAPr is capable of inducing very high levels of protection against sheep fasciolosis using two different authorized adjuvants, highlighting its potential as a vaccine candidate.

CS30 - Epidemiology

Tuesday, August, 11, 2009

CS30.1

A Survey to Examine the Species of Ovine Nematodes Present on Farms in the UK

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A survey of ovine nematodes present on UK farms was carried out with the long term aim of identifying species present and to examine the genetic diversity of populations from around the UK with regard to the molecular basis of anthelmintic resistance. Around 200 farms from all over the UK were asked to participate by providing 20 faecal samples in the spring from ewes and in the summer from lambs. Faecal samples from individual farms were pooled, with each animal contributing the same unit weight of faeces. Nematode eggs were extracted and cultured to the first larval stage (L1). The L1's were archived in ethanol and DNA was extracted from an aliquot of 1000 L1's from each farm. PCR analysis was carried out to determine the presence of the main gastrointestinal nematode species. Preliminary analyses show that Teladorsagia circumcincta was present on every farm and the Trichostrongylus spp. were found on over 90% of the farms. Haemonchus contortus was found on 53% of farms in England, 38% of farms in Wales and 27% of farms in Scotland. These results have now been expanded using PCR and pyrosequencing techniques on individual first stage larvae to determine the proportion of different gastrointestinal nematode species present on each farm and their genotypes.

CS30.2

Population Genetics of Parasitic Nematodes of UK Sheep

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UK populations of sheep parasites have been sampled to assess species diversity and the genetic differentiation of Haemonchus contortus and Teladorsagia circumcincta. Faeces were collected from ewes and lambs through 2008 present on 100 farms across the UK. Using a species-specific PCR assay based on the ITS gene the prevalence of a range of commonly-found species was assessed. T. circumcincta was present in all samples containing helminth eggs whereas H. contortus was found to be present in 57% of farms, with a bias of these being located in the South of England. The proportion of each species on each farm has been estimated using pyrosequencing technology on individual first stage larvae. The population differentiation of the 2 species was estimated using panels of microsatellites: a panel of 5 T. circumcincta microsatellites and a panel of 15 H. contortus microsatellites. From the trace profiles produced from bulk worm lysates of 1000 worms the allele frequencies were estimated from the area under the allele peaks. Fst analysis using estimated allele frequency data revealed that T. circcumcincta possessed very little genetic differentiation between farms whereas there were high levels of genetic differentiation in H. contortus populations. These results have yet to be confirmed using individual worm genotyping and the production of actual allele frequency data.

CS30.3

Selective Breeding of Scottish Cashmere Goats for Increased Resistance to Gastrointestinal Nematodes

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Genetic selection of animals for increased resistance to parasite infection may result in enhanced animal productivity and reduced disease. Additionally, examining the response of selected animals will provide valuable information on the mechanisms involved in the immune response.

A line of Scottish Cashmere Goat was developed by selectively breeding from sires and dams showing increased responsiveness to infection as measured by low mean ranked Faecal Egg Count (FEC) over 10 generations. Approximately 80 yearlings from the F2 to F10 generations of this line were co-grazed with equal numbers of control animals, and monitored for faecal egg output, productivity and immune markers from the end of the first season prior to weaning through the end of the following grazing season. Data from this was used to select replacement breeding animals.

Data was analysed to examine the effect of selection on faecal egg count and immune markers of the selected offspring. Faecal egg output in the selected yearlings over the whole period was reduced by 24.9% compared with the unselected controls. Circulating eosinophils were elevated in the selected line by 43.1%. IgG and IgE were not found to be significantly different between selected and unselected groups or to have any effect on FEC, but IgA was significantly

lower in selected animals. Plasma pepsinogen levels were not significantly different between selected and control groups.

Selection for responsiveness in cashmere goats based on FEC was found to significantly reduce offspring FEC, potentially as a result of quantitative changes in the immunoglobulin and eosinophil response

CS30.4

Effect of Different Anthelmintic Treatment Startegies on Naturally Acquired Nematodirus Infections in Sheep

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The intestinal parasite Nematodirus poses a threat to lambs in the early grazing season when eggs deposited in the previous season hatch simultaneously. This can lead to extremely high challenges for young naïve lambs resulting in lamb morbidity and mortality. The impact of targeted selective treatments (TST), neo-suppressive treatments(NST, monthly), targeted treatments (TT, as per past farm practice) or metaphylactic/therapeutic treatments (MT, at sign of disease) was investigated in a replicated field trial conducted in lambs for 3 years in central Scotland on Nematodirus populations. NST, TT and TST treatments received an anthelmintic treatment in early May of each year. MT animals were treated when clinical signs of Nematodirus infections were evident, typically one to 2 weeks later except for 2008. Nematodirus egg counts accounted for the majority of the total egg count during the early part of the season in 2007 and 2008 (56-75%, 64-78% respectively). Following primary anthelmintic treatment, Nematodirus FEC decreased to low levels and contributed to 12-20% to the total FEC for the remainder of the season over the 3 years. In 2008 early season small intestine tracer worm burdens were 10900, 32913, 32750 and 39288 for NST, TT, TST and MT treated groups, respectively, while mean Nematodirus larvae recovered from pasture early in the season was 146, 909, 679 and 1319 larvae per kg DM, respectively. NST treatment provided the best control of Nematodirus with no indication of the development of anthelmintic resistance. It is concluded that the timing of anthelmintic treatment early in the grazing season is critical to control Nematodirus populations on pasture.

CS30.5

Period Prevalence and Risk Factors of Bovine Fasciolosis in Five Districts of Punjab Province, Pakistan

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Period prevalence and associated risk factors of bovine fasciolosis was recorded in five districts of Punjab Province viz; Sargodha, Jhang, Muzaffargarh, Lodhran and Layyah. To this end, 80 bovines (cattle and buffaloes of various breeds) were selected from each study district based on proportional allocation and stratified random sampling methods. The faecal samples of selected animals were screened qualitatively and quantitatively through standard parasitological procedures for the presence of eggs of Fasciola species. Of 4800 faecal samples screened, 1222 (25.46%; $P<0.05$) were found positive for fasciolosis. The prevalent species of Fasciola (F.) identified were F. gigantica (22.40%; 1075/4800) and F. hepatica (3.06%; 147/4800). The highest ($P<0.05$) prevalence of both the species was found in winter (39.08%; 469/1200) followed in order by spring (29.50%; 354/1200), autumn (20.33%; 244/1200) and summer (12.92%; 155/1200). Origin had significant association ($P<0.05$) with prevalence of fasciolosis due to different agro-climatic and topographic conditions of the study districts. The prevalence was found higher in buffaloes (30.50%; 732/2400; $P<0.05$) than cattle (20.42%; 490/2400), females (32.88%; 789/2400; $P<0.05$) than males (18.04%; 433/2400), grazing (70.50%; 839/1190; $P<0.05$) than stall-fed (36.79%; 298/810) and mixed farming of small and large ruminants (63.98%;771/1205; $P<0.05$) than isolated large ruminant farming (46.03%; 366/795). Moreover, stagnant pond bathing (OR=2.24) and river/canal bathing (OR=2.06) were found associated with the prevalence of bovine fasciolosis in descending order of their significance. Age of animals was not found as a significant ($P>0.05$) determinant influencing prevalence of fasciolosis in bovines. The results indicated that fasciolosis is an endemic disease in the study districts of Punjab province. However, data on determinants of fasciolosis was reported for the first time in Pakistan that may provide significant data for planning future fasciolosis control programmes.

CS31 - Diagnosis

Tuesday, August, 11, 2009

CS31.1

Digestive Lysozyme Concentrations in Blood: an Alternative to Assay of Serum Pepsinogen?

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Lysozymes are enzymes that disrupt the peptidoglycan molecules of gram positive bacteria. Some foregut-fermenting herbivorous animals, including ruminants, have adapted lysozyme to be a digestive enzyme, helping liberate nutrients from within gut microflora. These digestive lysozymes are adapted to function at a lower pH than normal lysozymes and are more resistant to acidic conditions and the activity of pepsin. It was hypothesised that the same permeability changes in parasitized ruminants that lead to uptake of pepsinogen into the bloodstream would also lead to elevated levels of lysozyme. Lysozyme can be assayed quite simply by incubating samples with lyophilised Micrococcus bacteria and measuring the change in light absorbance over a 30 minute period. Using a buffer pH of 4.5, the assay measures the activity of the digestive form of the enzyme only. Using serum/plasma from a variety of parasitized and non-parasitised cattle and sheep, elevated lysozyme concentrations have been detected in infected animals. When a group of calves were sampled at 5 timepoints (Jan, Feb, Apr, Jun and Oct), highest concentrations ($p<0.001$) of both lysozyme and pepsinogen were seen in June when the number of eggs of Ostertagia spp. shed in faeces also peaked (175epg). Pepsinogen concentrations rose from 4.3iU in January to 17.8iU in June whilst lysozyme concentration increased from 64% to 102% (expressed as a percentage of a lysozyme standard – sheep serum). Although increased lysozyme concentrations are indeed demonstrable in infected animals, at this time, lysozyme assay does not appear to offer any significant advantage over assay of pepsinogen.

CS31.2

Measurement of Ostertagia ostertagi and Fasciola Hepatica-Specific Antibody Levels by ELISA Applied on Meat Juice and Associations with Carcass Parameters Measurement of Ostertagia ostertagi and Fasciola hepatica-Specific Antibody Levels by ELISA Applied on Meat Juice and Associations with Carcass Parameters

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Collection of blood for monitoring infectious diseases is labour-intensive and creates stress for the animals. In dairy cattle, non-invasive monitoring of parasitic infections is possible through the measurement of parasite-specific antibodies in the milk. In order to develop non-invasive diagnostic methods for beef cattle, the objectives of this study were to evaluate antibody-detection ELISAs applied on meat juice samples collected at the abattoir and to investigate the associations between test results and carcass parameters. Preliminary tests were carried out to determine optimal working dilutions. The correlation between test results obtained in serum and meat juice were asessed for 100 animals and were $R= 0.82$ and $R= 0.75$ for Ostertagia ostertagi and Fasciola hepatica, respectively. An abattoir survey was performed, analysing meat juice samples from 790 animals in spring and 796 animals in autumn 2008 and ELISA results were compared with carcass parameters and liver condemnation data. Signifant differences in parasite-specific antibody levels were observed between the seasons and both O. ostertagi and F. hepatica-antibody levels were negatively associated with carcass weight. The results showed that antibody-detection ELISAs applied on meat-juice for monitoring O. ostertagi and F. hepatica infections in beef cattle are feasible and hold potential as a practical diagnostic method and large-scale epidemiological surveys.

CS31.3

Evaluation of Endoscopy as an Alternative to Necropsy for Worm Burden Quantification

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Introduction: Anthelmintic efficacy trials typically involve necropsy of the target animal species to determine worm burdens in treated versus untreated control groups. This results in a large number of animal deaths. The goal of this study is to evaluate endoscopy as a minimally invasive alternative to necropsy for quantification of various intestinal helminth burdens.

Methods: Six 12-week-old beagle puppies were inoculated orally with 100 embryonated *Toxocara canis* eggs. Infection was confirmed by fecal egg shedding. Endoscopy was performed seven weeks post-infection, and video recordings of the procedure were reviewed to enumerate worms. The dogs were treated with Drontal®Plus, and all fecally expelled worms were collected and counted for 7 days. Endoscopy, deworming, and worm collection were then repeated to retrieve any remaining worms. These same dogs were then orally infected with 250 *Ancylostoma caninum* larvae. All the procedures described above for *T. canis* were carried out as before, except that the first endoscopy occurred three weeks post-infection.

Results: For *T. canis*, the endoscopic counts were as follows: 3 out of 5 worms recovered fecally (60%), 4 of 6 (67%), 6 of 48 (13%), 17 of 35 (49%), 1 of 1 (100%), and 9 of 45 (20%). Results for *A. caninum* were not available at time of abstract submission.

Discussion: Endoscopic quantification appears to be more accurate when *T. canis* burden is low, but this may be a function of worm location during endoscopy. Furthermore, the fasting and anesthesia required for endoscopy may affect worm movements and therefore the count accuracy.

CS31.4

Intercalibration of a Concentration Mcmaster Technique Between Eight European Laboratories

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Introduction: Prior to a European prevalence survey of intestinal parasites of organic pig herds it was relevant to introduce one common technique for faecal egg counts and to compare its execution at all involved laboratories to ensure data compatibility.

Methods: Faeces containing *Ascaris*, *Trichuris*, strongyle, and coccidia eggs/oocysts was mixed thoroughly and distributed along with a written description of the selected method to laboratories in Austria, Denmark, Finland, France, Germany, Italy, Sweden, and Switzerland. In each laboratory, 6-10 replicate faecal samples were analysed by one technician using the same concentration McMaster technique. This was followed by distribution of a second batch of faecal material accompanied by key laboratory materials and additional material (films, pictures etc.) on how to apply the technique.

Results: In the first test there was up to a 360-fold variation in egg counts between laboratories. Provision of identical laboratory materials and further instruction was effective as the variation for *Ascaris*, *Trichuris* and strongyles was reduced considerably in the second test. A continued high variability in the coccidia may be attributed to a variation in flotation time. Some variation also remained for all each species individual technicians which may in part reflect some of the constraints inherent to the technique.

Conclusion: Prior to any study of which the outcome depends on comparison of data obtained by one or more persons at the same or different laboratories it is extremely important not only to use identical techniques but also to implement these techniques in exactly the same way.

CS31.5

Detection of the Nematode Angiostrongylus Vasorum in Definitive and Intermediate Hosts Using Real-Time PCR

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The parasitic nematode Angiostrongylus vasorum is an emerging challenge for companion animal and wildlife health, with reported increases in both distribution and incidence in Europe. To facilitate improved detection of this parasite, a SYBR Green real-time polymerase chain reaction (PCR) was developed to amplify a region of the second internal transcribed spacer (ITS-2) of A. vasorum from both definitive and intermediate host samples. The PCR assay was capable of detecting less than four molecules of plasmid DNA containing the entire ITS-2 region, a single first stage larva (L1) in 200 ul canine EDTA blood, a single L1 in 200 mg of canine faeces and a single L3 in 10 mg of Biomphalaria glabrata tissue. The assay also exhibited a high level of specificity to A. vasorum when tested against DNA from a range of host species and other parasitic nematodes. Field evaluation of the PCR assay was conducted by screening canine EDTA blood and faecal samples from suspected cases of A. vasorum infection and compared with Baermann's detection, and also by screening a range of gastropod species from an endemic area. Real-time quantitative PCR offers a more efficient means of detecting A. vasorum infection with a lower limit of detection

than traditional diagnostic tests, and it therefore has important clinical and epidemiological applications.

CS32 - Genomics

Tuesday, August, 11, 2009

CS32.1

Variation in the Genome of Haemonchus contortus

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Parasitic nematodes are thought to contain extensive DNA sequence variation that explains rapid development of anthelmintic resistance. Characterizing this variation could increase our knowledge of its underlying genetic mechanisms. The genome sequence of Haemonchus contortus is derived from a mix of several individual parasites as well as one single male. We have used this resource to investigate the nature and extent of sequence variability in the H. contortus genome. Comparing randomly chosen sequence reads, nucleotide diversity was estimated at 0.022 for both mixed and single worms. The similarity Overlapping BAC sequences of 50 kb to 130 kb found regions of near identity extending up to 6 kb, surrounded by sequence whose divergence ranges from 1% to more than 40%. Variation is found predominantly within predicted non-coding sequence. Insertion-deletions range from single nucleotides up to almost 5 kb. Several insertions have similarity to reverse transcriptase, suggesting these may represent retrotransposons as well as other regions with characteristics of transposons and repetitive sequence. All longer indels lie outside of coding regions, either within introns or in intergenic regions. Overall, the pattern of variation looks quite similar to that seen in Drosophila and other invertebrates.

CS32.2

Exploring the Transcriptome of *Haemonchus contortus* During its Transition to Parasitism Using a Deep-Sequencing Approach

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"New generation" sequencing and bioinformatic technologies provide opportunities to infer developmental processes at the molecular level in whole organisms, tissues and cells. Here, we investigated the transcriptome of *Haemonchus contortus* during the switch from the free-living to the parasitic stage using 454 sequencing combined with detailed bioinformatic analyses, with a view toward exploring the full complement of molecular changes occurring during this transition and the range of molecules predicted to be essential for larval development and survival. Expressed sequence tags (ESTs) (n = 206,506) were determined from cDNA libraries representing each the infective third-stage larvae (=iL3) and the L3 following in vitro-activation (=xL3). Following EST assembly, comparative analysis identified 2975 and 2922 *Caenorhabditis elegans* orthologues in iL3 and xL3, respectively. Of these, 2090 (iL3) and 1936 (xL3) EST were linked to gene knock-down phenotypes, such as 'larval lethal' (n=446) and 'larval arrest' (n=1187). Two-way in silico-subtraction of the datasets revealed 245 and 224 'unique' molecules in iL3s and xL3s, respectively. Based on inference from *C. elegans* data, molecules linked to protein catalysis were "upregulated" in xL3s, whereas protein synthesis dominated for iL3s. Also, transcripts encoding SCP/TAPS proteins were represented in xL3. The depth of the current datasets provides the first comprehensive "snap-shot" of differentially transcribed molecules in *H. contortus* as it undergoes its transition from a free-living to parasitic larva. The key molecules linked to the developmental switch are proteases and SCP/TAPS proteins, which are predicted to relate mainly to host invasion and the parasite-host interplay.

CS32.3

Transcriptional Differences Between Infective Third-Stage Larvae and in vitro Percutaneously Migrated Larvae of *Ancylostoma caninum*

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Infective third-stage larvae (iL3) of *Ancylostoma caninum* are able to infect potential hosts via different infection routes of which the percutaneous infection seems to be the most effective one. For this reason an in vitro model for percutaneous migration was modified and validated and in vitro percutaneously migrated larvae (pmL3) were analysed. A differential transcriptome analysis of iL3 and pmL3 was performed using the Suppression Subtractive Hybridization (SSH) technique. Subtracted cDNA-libraries were created whose transcripts were verified by Differential Screening and Virtual Northern Blot. Obtained ESTs were processed, clustered, and compared with published sequences followed by annotation via gene ontology search, domain/motif search and mapping to biological pathways.

In the iL3 genes involved in osmoregulation, such as p-nitrophenyl-phosphatases and glycerol-3-phosphat-de-hydrogenases, were up-regulated. Furthermore, transcription of genes involved in the homeostasis of different metal ions or in catabolic metabolism was found to be increased. Most pmL3 up-regulated transcripts represent genes known to be essential for host-parasite interactions especially during skin penetration, such as *Ancylostoma*-secreted proteins, aspartyl-, metallo- and cysteine-proteases and various heat shock proteins. Furthermore, genes involved in regulatory processes of the energy metabolism were up-regulated, possibly due to the increased motility of the pmL3 during skin penetration. The results of this work therefore contribute to a further understanding of the skin penetration process on transcript level and might help to further understand the transition from the free-living to the parasitic stage of *A. caninum*.

CS32.4

Genetic and Genomic Approaches to Study Anthelmintic Resistance in H. contortus

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Parasitic nematodes lack many of the functional tools available in other biological systems and there is a need for experimental approaches to keep pace with the rapidly developing genomic resources. We are studying the genetics of the parasitic nematode H. contortus in order to apply genetic analysis alongside genomics to study anthelmintic resistance. We have chosen H. contortus because of its propensity to develop resistance, the availability of characterised strains, high fecundity and the ease of experimental infection. We are undertaking genetic crosses between adult worms of different strains and developing molecular markers to monitor the success of particular crosses. We have successfully achieved, and genetically validated, single pair crosses resulting more highly inbred lines of the reference genome strain MHco3(ISE). We have also undertaken 4 generations of backcrossing for two ivermectin resistant strains - MHco4(WRS) and MHco10(CAVR) –against the susceptible reference genome strain MHco3(ISE). Since these backcrossed strains are phenotypically resistant, they must contain the important resistance genes but now introgressed into the reference genomic background. In the short term these strains are valuable for phenotypic and candidate gene studies as well as genomic analysis. In the longer term we hope the strategies and tools we are developing will ultimately allow genetic mapping and positional cloning of resistance genes.

CS32.5

***C. elegans* as a Model System for Analysing Parasite Gene Function**

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The free-living nematode *C. elegans* provides a useful system for exploring conserved aspects of nematode biology. Here we describe the molecular characterisation of *hsp-90*, a highly conserved essential protein, from three nematodes, the free-living *Caenorhabditis elegans* (Ce) and the parasitic worms *Brugia pahangi* (Bp) and *Haemonchus contortus* (Hc). These molecules were functionally characterised by rescue of a *Ce-daf-21 (hsp-90)*null mutant. Our results show a gradient of rescue: the *C. elegans* endogenous gene provided full rescue of the *daf-21* mutant, while *Hc-hsp-90* provided partial rescue. In contrast, no rescue could be obtained using a variety of *Bp-hsp-90* constructs, despite the fact that *Bp-hsp-90* was transcribed and translated in the mutant worms. *daf-21(RNAi)* experiments were carried out to determine whether knock-down of the endogenous *daf-21* mRNA in N2 worms could be complemented by expression of either parasite gene. However neither parasite gene could rescue the *daf-21(RNAi)* phenotypes. These results indicate that factors other than the level of sequence identity are important for determining whether parasite genes can functionally complement in *C. elegans*.

CS33 - Trichinella / Biology

Tuesday, August, 11, 2009

CS33.1

Freezing Resistance of Trichinella Muscle Larvae in Pork After Long Lasting Infections

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Freeze tolerance of encapsulated *Trichinella* muscle larvae (ML) in pork is influenced by the age of the infection. Indeed, longer is the infection, thicker is the capsule which protects *Trichinella* ML and could thus allows the parasite to be more resistant to freezing processes. This study was focused on freezing resistance of *T. spiralis* and *T. britovi* isolated from long-term infections. Biceps brachii were removed from pig carcasses that were infected with 20,000 ML of *T. spiralis*

(ISS004) for 41 weeks or *T. britovi* (ISS1575) for 14, 27 and 41 weeks. Muscle samples of 70g were stored at –17°C for 12h, 24h and for 1 to 6 weeks. Larvae were recovered by artificial digestion and their mobility was recorded using image analysis softwares. Infecting capacity of larvae was evaluated by experimental mice infections.

Movements of larvae were observed after digestion of samples frozen for 12h and 24h but not for 1 to 6 weeks. Furthermore, mice infected with *T. spiralis* and *T. britovi* larvae from pork frozen for 12h and 24h hosted ML but mice were negative if longer freezing times were applied. Movement of larvae in frozen pork was statistically associated with the presence of ML in mice (Fisher's test, $p<0.0001$) which means that mobility could be a criteria to predict infecting capacity of *Trichinella* . Moreover, freezing treatment at –17°C for 1 week inactivated *T. spiralis* and *T. britovi* larvae even for an infection as old as 41 weeks in pork.

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CS33.2

Freeze Tolerance Characteristics of *Trichinella nativa* (T2) Muscle Larvae from Frozen Black Bear Meat Following Sequential Passages Between Mice and Cats

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Trichinella nativa (T2) survives for more than 5 years at -18C in host carnivore tissues. Passage into non-carnivores, such as mice, results in loss of freeze tolerance, however, re-passage back into foxes restores freeze tolerance. This may be true for other carnivore hosts as well. Current food safety freezing regulations for trichinellosis are based on *Trichinella spiralis* (T1), which is normally cold sensitive, but which exhibits freeze tolerance when passaged into horses. Additional studies are warranted considering that freeze tolerance is poorly understood, human trichinellosis is frequently linked to the consumption of meat from wildlife and geographic ranges of wildlife and domestic animals can overlap. We passaged T2 muscle larvae from a hunter killed black bear into mice, from these mice into cats and then back into mice. Samples of muscle tissues containing encysted larvae from these animals were frozen at -20C for 30, 60 or 100 days. Tissues were thawed and digested using a pepsin/HCl procedure at 37C. Infectivity was determined by oral inoculation into mice. The original larvae from the bear were freeze tolerant, but this was lost following passage into mice. Larvae from these mice regained freeze tolerance when passaged into cats. Freeze tolerance was lost when the larvae were passaged back into mice. These data suggest a predator-prey relationship exists that perpetuates T2 freeze tolerance in carnivore hosts and suggests that laboratory cats may be useful for the production of freeze resistant *Trichinella* larvae for use in proficiency samples to assess *Trichinella* digestion assays.

CS33.3

Trichinella spp. in Terrestrial Arctic Carnivores from Nunavut, Canada

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Carnivores collected in the Canadian Arctic were examined for infection with Trichinella spp. Ninety carcasses harvested around Kugluktuk and Cambridge Bay, Nunavut, from November 2007 to April 2008 were obtained from Inuit hunters and trappers: 49 wolverines (Gulo gulo), 17 red foxes (Vulpes vulpes), 14 wolves (Canis lupus), 5 grizzly bears (Ursus arctos horribilis), 4 arctic foxes (Alopex lagopus), and 1 marten (Martes americana). Tongues were examined by pepsin/HCl artificial tissue digestion for infection with Trichinella spp. Recovered Trichinella spp. larvae were genotyped using multiplex PCR. Of the 90 carcasses tested, 67 (74.4%) were infected with Trichinella spp. Infection of Trichinella spp. was most prevalent in wolverines (87.8%) followed by red foxes (82.4%), grizzly bears (80.0%), arctic foxes (50.0%), and wolves (30.8%). The marten was not infected. Larvae from 23 (18 wolverines, 2 grizzly bears, 2 wolves, 1 red fox) of the 67 infected carcasses have been genotyped and all were Trichinella T6. Genotyping of the additional Trichinella isolates are ongoing and the results will be presented. Previous research on 41 wolverines collected around Kugluktuk, Nunavut in 2006/2007 showed that 39 (95.1%) were infected with Trichinella spp., and genotyping identified Trichinella T6 in 33 (84.6%), T. nativa in 1 (2.5%), and mixed Trichinella T6 and T. nativa infections in 2 (5.1%). Combining the results of the current study with those obtained in 2006/2007 indicate that infection with Trichinella spp. is common in Arctic carnivores from the study area and that Trichinella T6 is the most common genotype in wolverines.

CS33.4

Wildlife Survey for *Trichinella* Species in Mainland Australia

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Trichinella is a globally distributed, zoonotic parasite which has been detected in at least 55 countries on all continents except Antarctica. In the region of Oceania, the encapsulated species *T. spiralis* has been endemic in New Zealand since the introduction of domestic pigs, while sylvatic, zoonotic, non-encapsulated forms of *Trichinella* were discovered relatively recently in the Australian island state of Tasmania as well as in Papua New Guinea. In Tasmania, *T. pseudospiralis* was detected in dasyurids (Tasmanian Devils and Quolls) and migrating, carrion-feeding birds, while in Papua New Guinea, *T. papuae* was found to be widespread in domestic and wild pigs, salt-water crocodiles and humans (serology only). On the continental mainland of Australia, no indigenous case of human or animal trichinellosis has ever been recorded and thus, it has always been regarded as *Trichinella*-free. This status has not been based on any extensive wildlife epidemiological investigations however, and as *Trichinella* spp. may circulate in wildlife independently of domestic cycles, the absence of human and domestic pig infections does not in itself constitute freedom. With risk factors such as the presence of *Trichinella* in sylvatic, vagile animals in Oceania, and the increasing domestic and international markets for game meats such as Australian wild boar and crocodile, wildlife surveys and risk assessment studies will provide more accurate and useful data on the *Trichinella*-status of mainland Australia. The results of a wildlife survey by artificial digestion in *Trichinella* hosts species such as wild boar, foxes, wild dogs, crocodiles, birds and quolls from high-risk regions of mainland Australia will be presented, as well as the results of a serosurvey in wild boars.

CS33.5

Regional Trichinella Infection Foci in Romania: a Permanent Threat to Human Health

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A retrospective analysis on human trichinellosis and animal Trichinella infection in Romania, in the last years, show a decrease for human cases from 3.649 in 1993 to 350 in 2006, and for animal cases from 10.540 in 1993 to 674 in 2006, respectively. In spite of the declining tendency, the evaluation of the epidemiological features of Trichinella infection cases in animals and trichinellosis human cases indicates the persistence of regional foci, mostly in countryside, along with many individual isolated cases, in different parts of Romania. An example of such a regional foci might be an "industrialized" pig farm from Cluj county, with severe lack in hygiene and rodent control, due to an economic breakdown. The prevalence of Trichinella infected animals, determined by ELISA (SafePath Laboratories, USA) was up to 10.1% (18/177). Trichinella positive carcasses originating from the endemic farm had been identified in few abattoirs (6/127). On the other hand, several other pigs, originating from the same farm, home-slaughtered and non-controlled, infected more than 50 persons within Cluj county. All Trichinella isolates were identified as T.spiralis, the worm burden being between 0.01 LPG and 21.8 LPG, with a particular high infestation for the home-slaughtered pigs of as high as 512 LPG. Work founded by the Romanian Education Ministry in part by the "PNII" projects, contract PNII-RU-RP-11/01.10.2007 and "Excelence" project CEEX 99/2006.

CS34 - Modeling

Tuesday, August, 11, 2009

CS34.1

Mapping Risk Foci for Endemic Sheep Scab

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Psoroptic mange in sheep, caused by infestation by the astigmatid mite Psoroptes ovis, is widely prevalent in Europe and other parts of the world. In the UK, psoroptic mange has become increasingly common following the deregulation of the disease in 1992, with the most recent study estimating there to have been 7000 outbreaks in 2004. Concern over growing prevalence has led to calls for a national scab eradication programme. There are, however, many obstacles to success for any such programme. As an alternative to eradication, regional or local scab management programmes that target high-risk areas and aim to maintain the number of outbreaks below an acceptable level may be a more effective use of time and resource. Here, risk foci in the UK are identified using a species distribution model and validated using a questionnaire survey of farmers. The data shows that there are distinct foci of persistent scab associated with upland grazing in Wales, Northern England, South-West England and Scotland. Scab management programmes focussing on these foci have the potential to significantly reduce the prevalence of scab in the UK.

CS34.2

Age Is More Important Than Infection Dose in Determining the Outcome of *Isospora suis* Infections in Suckling Piglets

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Data from 13 trials involving 124 suckling piglets experimentally infected with *Isospora suis* were evaluated for the effects of infection dose and age on the clinical and parasitological outcome of infection in four different models, infections with 1000 oocysts on the 4th day of life (d.o.l.) (model 1; 25 piglets/11 litters), 1000 oocysts on the 1st d.o.l. (model 2; 9 piglets/3 litters), 1500 oocysts on the 4th d.o.l. (model 3; 40piglets/20 litters) and 10000 oocysts on the 4th d.o.l (model 4; 50 animals/10 litters). Weights were determined on the day of birth and in weekly intervals. Faecal consistency and quantitative oocysts excretion were evaluated for 2 weeks starting 4 days after infection (d.p.i.). The weight gain depression was most noticeable in model 2 (infection on the 1st d.o.l.) where animals only gained 2.08x their birth weight until the 22nd d.o.l., compared to 2.31-2.52 in the other groups. This correlated with the occurrence of watery diarrhoea which was found in 37 % of the samples in the acute phase (4-11 d.p.i.) in model 2 but only 12-20 % of the samples in the other models. Median oocyst excretion peaked earlier in the models with higher infection doses but reached the highest values in model 2 (early infection). As in previous studies, this cross-sectional analysis of a larger number of animals confirms the influence of age on the outcome of isosporosis in suckling piglets, stressing the need to control the infection at an early life phase.

CS34.3

Fasciola hepatica and *Galba truncatula* in Ireland

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Between August 2006 and March 2008, studies on prevalence and egg output of *Fasciola hepatica* in lambs as well as population dynamics and infection in *Galba truncatula* snails were performed on the Teagasc Hill Sheep Farm in County Mayo, Ireland. Infection in a cohort of lambs was determined through monthly coprological examination. Snail abundance was determined by sampling at fortnightly intervals in 4 snail habitats on the farm and the prevalence of *F. hepatica* within *G. truncatula* was determined through microscopic examination and PCR. Mean monthly rainfall and temperature were recorded.

Faecal egg output peaked during October in both years of the study. *G. truncatula* was recorded from all 4 habitats. Overall, two peaks in *G. truncatula* abundance were evident, an early spring (March) and an autumn (August – November) peak. The prevalence of *F. hepatica* in *G. truncatula* showed significant seasonal differences and the greatest prevalence of *F. hepatica* in *G. truncatula* was evident during summer (25 %) and autumn (16.2 %) 2007. Two seasonal transmission peaks (i.e. mature infections evident in infected snails) were recorded, one in summer – autumn, and the other in late winter – early spring. Climate (rainfall and temperature) had an influence on snail survival and reproduction with dry weather (as occurred in summer 2006) delaying the peak abundance and the milder wetter winters allowing for continued snail activity.

CS34.4

Interactions Between *Trichuris suis* and *Oesophagostomum dentatum* in Pigs

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The possible interactions between *Trichuris suis* and *Oesophagostomum dentatum* infections in pigs were investigated. Two groups of pigs were trickle inoculated with either 10 *T.suis* eggs/kg/day (Group T) or 20 *O.dentatum* L3/kg/day (Group O). One group (OT) was trickle infected with both 10 *T.suis* eggs/kg/day and 20 *O.dentatum* L3/kg/day. All trickle infections continued until necropsy. In each group, six pigs were necropsied 5 weeks post first inoculation (wpi) and 6 pigs were necropsied 10 wpi. A significantly higher faecal *O.dentatum* egg excretion was seen in Group O compared to Group OT as faecal egg counts remained high in Group O while a marked decrease was seen in Group OT from 4 wpi. The *T.suis* egg excretion was generally higher in Group OT compared to Group T. The *Oesophagostomum* worm burden was significantly higher in Group O compared to Group OT at both necropsies. The *T.suis* worm burdens were non-significantly higher in Group OT compared to Group T. *O.dentatum* was more posteriorly located in the large intestine in Group O compared to Group OT, while *T.suis* was located in the proximal part of the large intestine in both groups. The *O.dentatum* females seemed slightly shorter in Group OT. The results clearly indicate that *Trichuris* may negatively influence *Oesophagostomum* populations in co-infected individuals – we observed a 10x reduction in *Oesophagostomum* worm burdens in these animals. In contrast, the presence of *Oesophagostomum* tended to enhance *Trichuris* populations.

We conclude that co-infections may result in altered parasite population dynamics compared to mono-species infections.

CS35 - Drug Resistance

Wednesday, August, 12, 2009

CS35.1

Assessment of the Impact of Metabolic Inhibitors on the Drug Sensitivity of a Triclabendazole (TCBZ)-Resistant Isolate of Fasciola Hepatica Using TEM

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Recent studies have shown that triclabendazole (TCBZ)-resistant isolates of Fasciola hepatica have an increased capacity to oxidise TCBZ and its sulphoxide metabolite (TCBZ.SO). This could limit the ability of the drug to reach its target within the fluke and exert its fasciolicidal effect. In vitro studies have been carried out on a TCBZ-resistant isolate, using inhibitors of either the flavin mono-oxygenase (FMO) or cytochrome P-450 (CYP) enzymatic systems to assess the effect of metabolic inhibition. The FMO system was inhibited by a 2h pre-incubation of flukes in methimazole (MTZ) (100 m) and the CYP system by a 2h pre-incubation in either piperonyl butoxide (PB) (100 m) or ketaconazole (KTZ) (40 m). Following pre-incubation, the flukes were incubated for 22h in either metabolic inhibitor alone; metabolic inhibitor+NADPH (1nM); metabolic inhibitor+NADPH+TCBZ (15 g/ml); or metabolic inhibitor+NADPH+TCBZ.SO (15 g/ml). Flukes were then processed for TEM. Parallel experiments were carried out with a TCBZ-susceptible isolate. Results indicate that the inhibition of drug metabolism leads to greater internal disruption than that caused by TCBZ or TCBZ.SO alone. Moreover, the disruption is greater in the TCBZ-resistant isolate than the –susceptible isolate. This suggests that inhibiting drug metabolism can alter the drug sensitivity of TCBZ-resistant flukes and so an altered metabolic capacity may contribute to the development of TCBZ resistance.

CS35.2

Do Drug Efflux Pumps Play a Role in the Resistance of Fasciola hepatica to Triclabendazole?

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Triclabendazole (TCBZ) is the drug of choice to treat infections of the liver fluke, Fasciola hepatica. However, resistance to TCBZ has started to spread. Over-expression of P-glycoprotein (Pgp) in nematodes has been linked to resistance to various anthelmintics. It may also contribute to TCBZ resistance in F. hepatica. The current study tested the impact of two P-glycoprotein inhibitors (verapamil and ivermectin) on the action of triclabendazole sulphoxide (TCBZ.SO) against the known TCBZ-resistant Oberon isolate of F. hepatica. Flukes were incubated in vitro for 2h with R(+)-verapamil at a concentration of 1x10-4M and then transferred to a combination of verapamil and TCBZSO (the latter at a concentration of 15µg/ml or 50µg/ml) for a further 22h. A parallel experiment involved a 2h pre-incubation in ivermectin (1µg/ml) followed by a 22h incubation in ivermectin plus TCBZ.SO (15µg/ml). Scanning and transmission electron microscopy was used to assess the effects of drug action and the results were compared to the effect of TCBZ.SO alone on the fluke isolate. The disruption to the flukes was greater following treatment with the Pgp inhibitors. This observation complements that of a previous study which showed that verapamil could increase the susceptibility to TCBZ.SO of another TCBZ-resistant fluke isolate, the Sligo isolate. The results of the two studies suggest that Pgp-linked drug effux pumps may be involved in the resistance mechanism.

CS35.3

Adult Triclabendazole-Resistant Fasciola hepatica: Morphological Responses to in vivo Treatment with the Synthetic 1,2,4-Trioxolane, Oz78 in the Rat Model

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Fascioliasis, the disease resulting from infection by the liver fluke, Fasciola hepatica, is a substantial and growing concern of veterinary and medical importance world-wide. Fluke populations resistant to the primary chemotherapeutic agent, triclabendazole (TCBZ), have been documented in a number of countries and resistance is believed to be underestimated and spreading. Coupled with the pressure of fascioliasis as an emerging or re-emerging disease in many countries, this creates a pressing need for discovery of novel chemotherapeutic agents. The present study examines the drug-induced effects of the 1,2,4-trioxolane OZ78 on mature flukes of the TCBZ-resistant Oberon isolate, following treatment of the rat host with a single oral dose of 100mg/kg bodyweight. Adult flukes were recovered from the bile ducts at necropsy, 48 and 72 h post-dosage. Tegumental surface changes were assessed using scanning electron microscopy while ultrastructural alterations were determined using transmission electron microscopy. Results showed only minor disruption of the fluke surface, with slight swelling and

blebbing of the tegument evident. The disruption increased with time post-treatment and was particularly evident on posterior surfaces and along the lateral margins of the mid-body. By contrast, significant and substantial disruption was observed to internal tissues, with the tegumental syncytium and sub-tegumental cell bodies particularly affected. Both the male and female reproductive tissues showed a high degree of disruption with extensive cellular degeneration evident. The results of this study reaffirm the potential of the 1,2,4-trioxolanes as novel fasciolicides and highlight the need for continuing research with this group of compounds in this field.

CS35.4

SEM Observations on the Impact of Metabolic Inhibitors on Triclabendazole (TCBZ) Action Against a TCBZ-Resistant and –Susceptible Isolate of Fasciola hepatica

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Recent studies have shown that triclabendazole (TCBZ)-resistant isolates of Fasciola hepatica have an increased capacity to oxidise TCBZ and triclabendazole sulphoxide (TCBZ.SO). This could affect the ability of the drug to reach its target within the fluke and exert its fasciolicidal effect. An in vitro study has been carried out on a TCBZ-resistant and a –susceptible isolate, using metabolic inhibitors of either the flavin mono-oxygenase (FMO) or cytochrome P-450 (CYP) enzymatic systems, to assess the effect of metabolic inhibition. The FMO system was inhibited by a 2h pre-incubation in methimazole (MTZ) (100 µm) and the CYP system by a 2h pre-incubation in either piperonyl butoxide (PB) (100 µm) or ketaconazole (KTZ) (40 µm). The flukes were then incubated for 22h in NCTC medium containing metabolic inhibitor; metabolic inhibitor + NADPH (1nM); metabolic inhibitor + NADPH + TCBZ (15 g/ml); or metabolic inhibitor + NADPH + TCBZ.SO (15 g/ml). An in vivo study (rat) has also been carried out using the metabolic inhibitor ketaconazole at a dose of 10mg/kg. Adult flukes were recovered from the bile ducts at necropsy 24, 48, 72 and 96h post-dosage. Flukes from both studies were processed for scanning electron microscopy. Results indicate that the inhibition of drug metabolism leads to more severe surface disruption than that caused by TCBZ or TCBZ.SO alone. Moreover, the disruption is greater in the TCBZ-resistant isolate than in the –susceptible isolate. Therefore, the results suggest that inhibiting drug metabolism can alter the drug sensitivity of TCBZ-resistant flukes.

CS35.5

Multiple Anthelmintic Resistance in Grazing Cattle of Argentina: Revertion Evidences Throughout the Use of a Three Years Rational Control Programme

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The results from a rational control program in fattening cattle naturally exposed to multiple anthelmintic resistant trichostrongyle nematodes infections are described. The program was carried out during three consecutive years in a cattle farm of the Humid Pampa –Argentina- which receive 5000 weaned beef calves every year to initiate a fattening period on improved pastures and strategic supplementations.

At the beginning of 2003 around 140 calves died as a consequence of high worm burdens. During several years a systematic and monthly anthelmintic treatments with ivermectin (IVM) or fenbendazole (FBZ) had been administered to all animals.

The fecal egg count –e.p.g.- reduction test (FECRT) showed an overall clinical efficacy of 73% for IVM (Haemonchus spp. 48% and Cooperia spp.52%), 49.1% for FBZ (Haemonchus spp. 49%, Ostertagia spp. 18% and Cooperia spp. 33%) and 90.4% for levamisole (LVM) (Ostertagia spp. 97% and Cooperia spp. 3%). The animals were necropsied 15 days after treatments to determine the number of worms at the level of abomasum and small intestines. The efficacy of IVM, FBZ and LVM was established comparing treated and non treated control animals. The IVM showed an efficacy of 76.1% and 22.6% against Haemonchus spp. and Cooperia spp. respectively.

The efficacy of FBZ was 0% against Ostertagia spp,. 28.3% for Haemonchus spp. and 24.2% against Cooperia spp. The efficacy of LVM against Ostertagia spp. was 64.9%.

In order to stop mortality and to reduce the impact of subclinical losses, a rational control program based on fecal egg counts, coprocultures and epidemiological back grounds was established. LVM was selected as the anthelmintic to be administered at any recommended time and, a single IVM treatment only once at the end of the spring was applied to eliminate the arrested fourth stage larvae of Ostertagia ostertagi. In 2004, three treatments with LVM (April, July and September), two treatments in 2005 (June and August) and one in 2006 (May) were necessary to reduce mortality and subclinical losses at a minimum and acceptable level.

In 2006 a new evaluation of the efficacy of IVM, FBZ and LVM was performed. The FECRT showed an overall clinical efficacy of 88.3%, 94.5% and 99.7% for IVM, FBZ and LVM respectively. The worm counts showed an efficacy against Haemonchus spp, Ostertagia spp.and Cooperia spp. of 96.4%, 100% and 33.2% for IVM; 96.4%, 78.6% and 85.7% for FBZ and, 100%, 71.4% and 99.3% for LVM.

The results from this three years period of studies strongly suggest that the phenomenon of anthelmintic resistance in cattle might be reverted after the implementation of a rational control program based on e.p.g. countings, coprocultures and epidemiological backgrounds, which allowed the proper anthelmintic compounds selection and mainly, reduce at minimum the number of treatments throughout the grazing period.

CS35.6

Longitudinal Monitoring of Gastrointestinal Parasite Burdens on an Organic Dairy Farm

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Organic farming regulations limit use of anthelmintics in livestock to therapeutic treatments only; in accordance to the organic ethos of reducing reliance on pharmaceuticals. A longitudinal study was conducted to assess the control of gastrointestinal tract (GIT) parasites in livestock on a UK organic dairy farm. The GIT parasite burdens of specific cattle from different age groups on a Scottish organic dairy farm were followed monthly from April 2007 to March 2008. First season grazing stock were subject to significant challenge from Ostertagia ostertagi nematodes: mean (SD) serum pepsinogen concentration increased from 0.98 (0.10) i.u. pre-turnout to 3.2 (1.5) i.u. in October. Additionally, mean (SD) plasma albumin concentration decreased from 31.8 (2.1) g/L pre-turnout to 29.2 (2.9) g/L in October. Faecal egg count data were overdispersed, with mild to moderate increases. Peak indivdual animal faecal egg count from second season grazers was recorded early in 2007 prior to turnout (33% showed >250epg), suggestive of a potential carry-over of Ostertagia spp. infestation ('Type 2') from previous grazing. On the basis of the results of this study, anthelmintic treatment was administered to all first season grazing animals. Adult cow exposure to O. ostertagi. was estimated through the use of individual and bulk milk ELISA testing. The herd exposure level was high (Bulk milk ODR >0.80 for duration of study). Pasture larval counts, fluke faecal egg counts and milk fluke ELISA data were also collected. Despite a low grazing pressure on this farm, a significant challenge to all grazing stock from gastrointestinal parasites was observed. It should be emphasised to veterinary surgeons and farmers that organic livestock should be carefully monitored for parasite burdens with a suitable frequency and type of testing and, that appropriate, judicious use of anthelmintics on organic farms may be justified from animal health, welfare and economic perspectives.

CS36 - Immunology / Vaccines

Wednesday, August, 12, 2009

CS36.1

Monocyte- and Macrophage-Mediated Immune Reactions Against Eimeria bovis

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In bovine coccidiosis little is known on early monocyte/macrophage-mediated responses. We investigated in vivo, in vitro and ex vivo reactions of monocytes and macrophages against Eimeria bovis, one of the most pathogenic species in cattle. Macrophages significantly infiltrated the gut mucosa of E. bovis-infected calves, particularly after challenge infection. Furthermore, peripheral monocytes of infected animals, as precursor cells of macrophages, exhibited enhanced ex vivo phagocytic and oxidative burst activities. Enhanced levels of both activities were found early after infection and towards the end of first merogony. In vitro exposure of macrophages to sporozoites led to phagocytosis of the pathogen, whilst monocytes failed to do so. Phagocytosis occurred independently of the viability of the sporozoites, indicating that active invasion by the parasites was negligible. Phagocytosis occurred already in the absence of immune serum, but could be enhanced by addition of immune serum, suggesting macrophage-derived antibody-dependent cytotoxicity. Co-culture of macrophages with sporozoites and stimulation with merozoite I antigen induced distinct levels of cytokine and chemokine gene transcription. The transcription of genes encoding for IFN-, IL-12, TNF-, IL-6, CXCL1, CXCL8, CXCL10 and COX-2 was up-regulated after sporozoite encounter. In contrast, soluble merozoite I antigen merely induced the gene transcription of IL-6 and IL-12 and failed to up-regulate IFN- and TNF- gene transcripts. In monocytes only IFN- and CXCL10 were found enhanced. Our results strongly suggest that macrophage-mediated, innate immune reactions play an important role in the early immune response to E. bovis infections in calves.

CS36.2

Cloning and Identification of the Trichomonas gallinae ap Gene

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The parasite Trichomonas gallinae lives in the upper gastrointestinal tract of birds, it may cause white plaques and inflammation in the oral cavity. Trichomoniasis occurrence in doves had been reported all the globe. The 1st strand cDNA of adhesion protein (ap) gene was amplified by RT-PCR with total RNA extracted from T. gallinae and was used as template for the amplification of the ap genome, then the amplified product was cloned into the vector pMD18-T. The recombinant plasmid was identified by PCR and restriction endonuclease, the positive clone was sequenced and its sequence was analyzed by comparing the sequence similarity with other sequences in the GenBank. The result showed that the cDNA of the T. gallinae ap gene had a length of 1032 bp, which contained a complete open reading frame (ORF) of nucleotides 930 bp long, coding for 310 amino acids. The sequence analysis revealed that the homology with the genes of T. vaginalis ap33-1, ap33-2, ap33-3 were 94.2, 87.3% and 87.9, respectively. The gene of ap from T. gallinae is cloned successfully, this will provides basis for the expression of ap gene in prokaryotic and eukaryotic cell and the preparation of its recombinant protein. This work was supported by grants from National Natural Science Foundation of China (grant no. 30671577) and Doctor Subject Foundation of Minister of Education, China (grant no. 200805640004).

CS36.3

Role of Regulatory T Cells in Murine *Neospora caninum* Infection

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In addition to inflammatory mechanisms effective in eliminating parasites from the host, an adequate immune response must limit deleterious excessive inflammation. This could be achieved by different regulatory cells such as naturally occurring $CD4^+CD25^+$ T regulatory cells (Treg). We previously reported that *Neospora caninum* -infected mice have increased numbers of splenic Treg. In order to ascertain the role of these cells in the course of experimental neosporosis, wild-type (WT) and lethally susceptible IL-12-deficient C57BL/6 mice were intraperitoneally inoculated with 5 10^5 *N. caninum* tachyzoites (NcT) 24H after treatment with Treg-depleting anti-CD25 antibody. Treg depletion did not worsen inflammation associated with *N. caninum* infection thus excluding a possible role of Treg in preventing an exacerbated immune response. A decrease in inflammatory infiltrates was actually observed in the liver of anti-CD25-treated mice. Furthermore we also demonstrated that Treg are not essential for *N. caninum* persistence in an immunosufficient host as Treg-depleted WT mice had organ parasitic loads similar to those of non-depleted counterparts. Interestingly, we observed in the susceptible mice a significant decrease in the parasitic load upon Treg depletion, suggesting a role of this cell population in parasite persistence in this host. Cell population analysis, cytokine and antibody production was assessed in all groups of mice analyzed.

Overall these results suggest that in NcT-challenged WT mice Treg do not have a major role in either protecting from lesion development or promoting susceptibility to infection while in susceptible mice Treg apparently suppress a protective immune response.

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CS36.4

Development and Evaluation of a live *Salmonella* -Vectored Coccidiosis Vaccine with TRAP+ Upstream and CD 154 (CD 40L) Epitopes in Broiler Chickens

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Coccidiosis, caused by parasites of the genus *Eimeria,* belongs to Phylum Apicomplexa. EmTFP250 is an asexual stage antigen from *Eimeria maxima* (*E.maxima*) strongly associated with maternal immunity. Cloning and sequence analysis predict the antigen to be a novel member of the Thrombospondin-related adhesive protein (TRAP) family of micronemes, associated with host cell invasion and parasite gliding motility. Three novel attenuated *Salmonella enteritidis* strains expressing TRAP oligopeptides in association with a potential immunostimulatory CD154 sequence, on the outer membrane protein lamB, were developed. Broiler chickens were grouped based on treatment and 10^8 cfu/chick of one of three TRAP sequences, or vehicle alone, was orally administered to each group. At 21d of age, all groups were challenged with 10^4 sporulated oocysts of *E. maxima* orally. The mortality at 5dpi was as follows: Control (non-vaccinated) 10/46 (21.7%), TRAP -7/43 (16.3%); TRAP Upstream (US) -1/46* (2.2%) and TRAP Downstream (DS) -6/43 (11%). Similar studies based on the lines of the previous experiment were carried out to evaluate the efficacy of TRAP US as a potential vaccine candidate. Broiler performance increased by ~31% in the vaccinated chicks when compared to the non- vaccinated controls in addition to a decline in mortality upon challenge with *E. maxima* 7dpi in two separate studies. Further experiments will involve the use of challenge with alternate species of *Eimeria* and also the effect of probiotics on intestinal health during a coccidial infection.

CS36.5

Interactive Effects of Protein Nutrition and Breed on Periparturient Immunity to Parasites in Sheep

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Introduction: Periparturient resistance to parasites is sensitive to metabolizable protein (MP) supply and may also differ between breeds. Such differences may partly arise from between-breed differences in MP demand. Here, we compared resistance to parasites in Scottish Blackface (BF) and Greyface (GF) breeds under two MP feeding conditions that accounted for the higher MP demand of the GF breed.

Methods: Twin-rearing BF and GF ewes, trickle infected with 10,000 L3 *Teladorsagia circumcincta* during the periparturient period, were fed at 0.9 times their metabolizable energy requirement and either 0.8 (LP) or 1.3 (HP) times their MP requirement (n=18). Lambs were weighed weekly, and ewe faecal egg counts (FEC) were assessed twice weekly as a proxy for resistance to parasites.

Results: HP litters were heavier at birth than LP litters for GF ewes (P<0.001) but not for BF ewes (P>0.20). Whilst breed and feeding treatment did not interact for litter weight gain, GF litters grew faster than BF litters at 615 vs 444 g/day, and HP litters grew faster than LP litters at 616 vs 453 g/day (s.e.d. 15 g/day; P<0.001). Breed and feeding treatment interacted on FEC during lactation; MP scarcity increased FEC of GF ewes by 60% (P<0.001) but not of BF ewes (P>0.20).

Conclusion: The lower FEC of LP-BF ewes compared to LP-GF ewes suggests that Scottish Blackface ewes could be genetically more resistant to nematode parasite infection than Greyface ewes. Our results also demonstrate that protein nutrition for parasite control may best be targeted at high producing breeds.

CS37 - PARASOL Symposium

Wednesday, August, 12, 2009

CS37.1

Novel Solutions for the Sustainable Control of Nematodes in Ruminants (PARASOL Project)

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The overall objective of the EU-funded PARASOL project was to create low input and sustainable strategies for controlling gastrointestinal nematode infections of ruminants. PARASOL investigated the use of Targeted Selective Treatments (TST), where only animals showing clinical symptoms or reduced productivity are given drugs. Several innovative methods, under various farming conditions, were assessed for identifying animals requiring treatment. Additional objectives included standardising existing in vivo and in vitro tests for detecting AR, developing new tests where required and optimising the efficacy of anthelmintics by modulating parasite P-glycoprotein detoxification systems.

Studies in small ruminants conducted in five European and two African countries investigated regionally-appropriate morbidity/production markers (anaemia (FAMACHA©), liveweight gain, milk production, body condition score and diarrhoea index) and faecal egg count (FEC) as parasitological markers for treatment. Where Haemonchus is prevalent, the FAMACHA© system is the best way of delivering TST. Where Teladorsagia and Trichostrongylus predominate liveweight gain was the most suitable indicator for TST. The TST approach optimised the use of anthelmintics, maintaining both performance and anthelmintic efficacy. Routine monthly treatments were shown to select heavily for resistance. FECs provided a sound basis to target whole flock treatments. Further support for the TST concept comes from Australian

studies, which have concentrated on the development of practicable assessment systems for use in large flocks.

Cattle studies were conducted in Belgium, Germany, Sweden and the UK. Field surveys were conducted in calves on > 900 farms using a serum pepsinogen assay and adult cows on > 3700 farms using a newly developed O. ostertagi antibody detection ELISA applied to bulk-tank milk. Several pasture management practices were negatively associated with the observed levels of exposure and could be proposed as non-chemotherapeutic control measures. Mid-season liveweight gains may be a suitable parameter for selective treatments in first-season grazing calves. Furthermore, an automated body condition scoring system with a digital camera was developed and may have future application in parasite control.

The faecal egg count reduction test was applied to cattle demonstrating macrocyclic lactone failures: this was mostly of Cooperia though some Ostertagia were noted in UK. The egg hatch test has been confirmed by ring testing to work with cattle nematodes for benzimidazole resistance and a Larval Migration Inhibition Test was successfully standardized for ML-susceptibility in C. oncophora, O. ostertagi and H. contortus.

PARASOL results demonstrate that TST approaches are effective, practicable, reduce selection of anthelmintic resistance and are economically competitive. Routine monitoring of anthelmintic efficacy should be promoted.

CS38 - Ectoparasites

Wednesday, August, 12, 2009

CS38.1

Cattle Lice in Iceland; Prevalence and Predilection Sites

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Introduction: One chewing louse (Mallophaga) and at least four sucking lice (Anoplura) species are known to infest cattle. The aim of the present study was to survey which species of lice infest cattle in Iceland and evaluate their prevalence and site preferences.

Material and Methods: The survey included 50 calves (4-15 months old) and 50 dairy cows from 10 farms selected at random from South and West Iceland. Five pre-selected sites on each animal were combed (200-600 cm2 each): Head, ventral neck, dorsolateral trunk, front leg and tail. Lice collected from each site were identified and counted.

Results: Two species were found, the chewing louse Bovicola bovis and the small sucking louse Solenopotes capillatus. Lice were found at 70% of the farms. B. bovis was found at 50% and S. capillatus at 40% of the farms, both species at 20% of the farms. Prevalence of infection was as follows: B. bovis: calves 28%, cows 2%; S. capillatus: calves 16%, cows 2%. Two calves had both lice species. Predilection sites for B. bovis/S. capillatus were as follows (% of infested cattle): head 15/33, neck 23/67, front leg 0/33, trunk 85/17 and tail 23/17. The intensity of infection was low, with a maximum of 20 B. bovis and 11 S. capillatus lice from combed sites per host.

Concluding remarks: Both lice species were much more prevalent on calves than on the dairy cows. The intensities of infections were low for both species. No dermatological signs associated with lice infestations were observed.

CS38.2

Milk Kinetic and Antiparasitic Efficacy of Eprinomectin Against the Louse Haematopinus Tuberculatus in Lactating Water Buffaloes (Bubalus bubalis)

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Water buffalo represent an important economic source in several countries, including some regions of central-southern Italy. Eprinomectin is the only endectocide approved for use during lactation with a zero milk-withdrawal period in cattle. Although pharmacokinetic data from cattle have been reported, these data have not been described in buffalo. The rational use of a drug requires knowledge of basic pharmacokinetic parameters, residue concentrations in edible tissue and withdrawal times. The pharmacokinetics and mammary excretion of eprinomectin were determined in buffaloes following pour-on administration at the cattle dose of 0.5mg/Kg. Following the treatment, plasma and milk concentrations of eprinomectin increased to reach maximal concentrations (Cmax) of 2.74 ± 0.89 and 3.40 ± 1.68 ng ml 1 at Tmax of 1.44 ± 0.20 and 1.33 ± 0.41 days in plasma and milk, respectively. The MRT and the AUC were similar for plasma (3.17 ± 0.41 days and 11.43 ± 4.01 ng day ml 1) and milk (2.70 ± 0.44 days and 8.49 ± 3.33 ng day ml 1). The ratio of AUC milk/plasma was 0.76 ± 0.16. The very low extent of mammary excretion and the milk levels reported lower than the MRL (20 ng ml 1) supports the permitted use of eprinomectin in lactating buffaloes. Furthermore a controlled field trial was conducted to assess the efficacy of eprinomectin pour-on at dose of 0.5mg/Kg against the louse Haematopinus tuberculatus on naturally infested buffaloes. Eprinomectin was highly effective (98.0%) at day 7, and completely effective (100%)

from day 14, until the end of study (day 56). During the trial, eprinomectin was well tolerated by all the animals since there were no adverse reactions following the treatment.

CS38.3

Practical Outputs of the Australian Flystrike Model

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Blowfly strike costs the Australian sheep industry roughly $270m per year of which treatment costs are the major expense.

The choice and timing of several management tools can be altered to optimise the benefits of topical chemical application, minimise costs and avoid overuse of chemo-prophylaxis.

The weather-driven flystrike model (Wardhaugh et al 2007) was based on data collected by CSIRO Entomology with weekly flystrike records over three years from 30-60 properties in three regions, northern NSW, Southern NSW and Flinders Island (Bass Strait), with daily weather records from each region. The program uses long term weather data for each region to estimate the number of struck sheep on a given day, based on the actual weather and relevant management, including shearing, crutching, preventive chemical treatment and mulesing, if used. The program is intended for long-term planning based on historical data, not day-to-day management using current daily weather.

The expected number of struck sheep and the costs of strike are calculated based on the defined management, without preventive chemical treatment. This is compared with the costs of strike if treatment is used and the extra cost of that treatment. The program will calculate optimal treatment methods and dates to minimize the overall costs of flystrike and its control.

Wardhaugh KG, Morton R, Bedo D & Horton BJ (2007) Med. Vet Entomol. 21: 153-167.

CS38.4

Hospesvorare-Derived Sarcoptes Mite in Wild Animals

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Molecular fingerprinting was applied on individual Sarcoptes scabiei, from 16 wild mammalian populations belonging to 11 host species in four European countries, using 10 Sarcoptes mite specific microsatellite markers. Unrooted Dps consensus dendrograms for individual Sarcoptes mites, and the clusters resulted from the multilocus microsatellite clustering analysis, showed that the geographical separations had real biological significance for the definition of mite sub-populations, and that the degree of genetic exchange occurring between mites from different localities was related to the geographical distance between locations.

Wild host-derived mite populations were clustered into three main groups: herbivore-, carnivore- and omnivore-derived Sarcoptes mite populations. Omnivore-derived was halfway between herbivore- and carnivore-derived Sarcoptes mite populations. The difference between the three mite groups (herbivore-, carnivore- and omnivore-derived mite) was more supported than that by the geographical separations; nevertheless a kind of sub-clustering was detected within each group (carnivore-, omnivore- and herbivore-), scattering mite populations up to their geographical localities (countries).

These findings suggest that genetic exchange was occurring within each mite group, but extremely rare among them. Mites from different host groups (herbivore-, carnivore- and omnivore-derived mite) had separate transmission cycles (herbivore-to-herbivore, omnivore-to-omnivore and carnivore-to-carnivore). The lack of gene flow between Sarcoptes populations (carnivore-, herbivore- and omnivore-derived Sarcoptes) might improved parasitic adaptations and led to, what we called, HospesVorare-derived (carnivore host-, herbivore host- and omnivore host-derived) Sarcoptes mite populations in wild animals. HospesVorare effect is stronger than the geographical separation in the definition of mite speciation.

This has important ramifications on the study of population genetic structure, life cycle, diagnosis and monitoring protocols, and could contribute to the better understanding of the epidemiology of the ubiquitous Sarcoptes mite.

CS39 - Future Directions in Veterinary Parasitology

Wednesday, August, 12, 2009

CS39.1

Future Directions in Veterinary Parasitology: Key Research Questions

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Veterinary Parasitology currently faces an unprecedented range of new challenges, associated with factors such as

climate change, economic instability, increased global movement of pathogens, hosts and vectors, changing social habits and perceptions, habitat modification, resistance development and a changing legislative framework associated with concern over the impact of neurotoxic insecticides on environmental and human health. It is essential therefore that veterinary parasitology advances to meet these challenges and research must be clearly focused on areas of key concern and maximum potential gain. Traditional skills must not be forgotten, but future research must embrace the new tools and new approaches that are being developed to contribute to the better understanding and control of parasites; these include new molecular analyses, vaccine development, statistical techniques, modelling and the use of satellite imagery. Botanical repellents, resistant and resilient strains of livestock, biological control, off-host trapping systems and selective treatment systems are also emerging as potential new approaches.

Stimulating and focusing this discussion is one of the primary goals of this symposium. The aim has been to gather together speakers at the forefront of their research areas, with the objective of engendering debate in the veterinary parasitological community about how best to address the future.

CS40 - Trichinella / Diagnosis

Wednesday, August, 12, 2009

CS40.1

Identification of Trichinella Spiralis Early Antigens and their Application in Serological Diagnosis

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Contamination by the parasite Trichinella occurs by consumption of raw or undercooked meat from infected animals. Serological methods such as ELISA, based on excretory–secretory products released from the muscle larvae stage, allow the detection of Trichinella specific antibodies. The use of those tests is still restricted to epidemiological surveillance in animals due to the occurrence of false negative results during the early stages of infection. The objective of this study is to identify intestinal T. spiralis antigens to design an indirect ELISA for an earlier pig trichinellosis diagnosis. Thus, 14 hours post-infection, 20 hpi and 48 hpi T. spiralis cDNA libraries were established and immunoscreened with serum from pigs experimentally infected with T. spiralis. Five nucleotidic sequences were selected and subcloned into an expression vector (PGEX-6P-1). Then, recombinant proteins were subjected to a double purification using nickel and glutathione affinity and were tested for their antigenicity. The fusion protein named Ts20h5, obtained from the 14 hpi and 20 hpi cDNA libraries, allowed detection of the Trichinella infection as soon as 15 days pi by Western blot. The detection was also possible until 60 dpi indicating a persistance of anti-Ts20h5 specific antibodies during at least two months. An indirect ELISA with the Ts20h5 protein will be performed in order to support our findings. This result could constitute an improvement for pig trichinellosis diagnosis since the ELISA currently on the market does not allow detection of positive animals before 25 dpi.

Work funded by the EU Network MedVetNet (FOOD-CT-2004 506122) Trichimed.

CS40.2

Evaluation of Magnetic Stirrer Method for Detection of Trichinella spiralis from Frozen Muscle Samples

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Wild animals such as foxes and raccoon dogs play an important role as reservoir for Trichinella spp. in the sylvatic cycle. In wildlife monitoring, muscle samples from indicator animals are usually stored at freezing temperatures prior examination for Trichinella muscle larvae. The aim of this study was to evaluate the sensitivity of magnetic stirrer method for detection of the cold-sensitive species Trichinella spiralis from frozen muscle samples.

A total of 30 samples from minced musculature each weighting 10 g were spiked with 20 T. spiralis muscle larvae (strain ISS 003). Subsequently, 10 samples (used as controls for fresh muscle) were individually examined for Trichinella larvae (each in a total weight of 100 g) by magnetic stirrer method according to Regulation (EC) No. 2075/2005. Remaining 20 samples were subjected for freezing over 12 days at -20°C. After freezing, 2 x 10 samples (each filled up with fresh minced muscle to 100 g) were examined in the same way by magnetic stirrer method where sedimentation time in the separation funnel/centrifuge tube was 30/10 min and 60/20 min, respectively. Results for recovery rate of muscle larvae were statistically analyzed with respect to influence of freezing treatment (fresh vs. frozen muscle) and influence of sedimentation time in frozen muscle (30 vs. 60 min).

Microscopic examination of digestion fluid revealed coiled larvae in samples from fresh minced muscle. Contrary, larvae from frozen samples showed a curved shape. Number of recovered muscle larvae was significantly lower (p=0.000; 95% CI) in samples from frozen minced muscle (67/200) compared to samples from fresh minced muscle (182/200). Recovery rate for muscle larvae in frozen muscle (95/200) significantly

increased (p=0.037; 95% CI) when sedimentation time in separation funnel/centrifuge tube was prolonged from 30/10 min to 60/20 min.

In conclusion, Trichinella larvae are less detectable in muscle after freezing due to inactivation of nematode which influences larval shape and sedimentation behavior. If samples are subjected for freezing treatment prior examination, sample weight and sedimentation time should be increased in magnetic stirrer method for compensating larval loss.

The study was supported by the 6th Research Framework Programme of the European Commission within the Network of Excellence "Med-Vet-Net" (WP TrichiMed).

CS40.3

Experiences with Techniques for Preparing the Samples to Assess the Efficiency of the Laboratories Performing a *Trichinella* Digestion Assay According to the EU Directive 2075/2005

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Trichinella is an emerging and re-emerging foodborne agent causing trichinellosis in humans. Consequently, all the EU countries perform mandatory official inspection of carcasses of slaughtered domestic pigs, horses, wild boars and other farmed and wild animal species intended for human consumption. The EU regulations now recognize four methods of artificial digestion for the presence of *Trichinella* in meat as valid and recommend pepsin-hydrochloric acid artificial digestion with a magnetic stirrer as the reference method. While this digestion method has been widely applied, specific methods connected with adequate quality assurance measures have been recently applied in some of the EU countries only.

This article describes the practical experiences with a modified method for preparing calibrated meat samples containing known numbers of naked *Trichinella* muscle larvae. A national ring trial was organized to determine the performance of the 128 routine diagnostic laboratories in the Czech Republic using digestion assay according to the EU directive 2075/2005. Each participating laboratory received ten samples of 100 g of ground pork containing 3 larvae (3 samples), 8 larvae (3 samples), 12 larvae (3 samples) and one negative control. The sensitivity was expressed as the percentage of muscle larvae recovered from each proficiency sample.

Proficiency testing is essential for of routine laboratories certification and allows demonstration of required levels of sensitivity and effectiveness of the testing programs. However, as apparent from our results, some questions regarding guidelines for proper and uniform procedures and method optimization for the routine large-scale preparation of samples are still remaining.

The financial support of the grant project QH81069 is acknowledged.

CS40.4

Evaluation of *Trichinella nativa* (T2) in Proficiency Tests for *Trichinella* Digestion Assays

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Proficiency samples using viable encapsulated or released *T. spiralis* (T1) muscle larvae in meat balls are widely used in diagnostic quality assurance programs for training and monitoring the effectiveness of laboratories to perform *Trichinella* digestion tests. Accidental introduction of *T. spiralis* into the environment can present a serious risk to livestock. *Trichinella nativa* (T2) may be a more suitable species for use in proficiency samples as it is freeze tolerant, has a long shelf-life, does not become established in swine, and is endemic in northern wildlife. Encapsulated T2 muscle larvae from guinea pigs (g.p.), and a naturally infected black bear, and encapsulated T1 control larvae from rats were used to prepare proficiency samples. Each proficiency sample contained 25 capsules on an agar disc within a 20g lean ground beef meatball. Samples were stored for various times at 4C or -20C, and a subset of -20C samples were thawed and kept an additional 30 days at 4C before testing to mimic field conditions. All T1 and T2 samples had 95-100% recovery at day 0, and > 80% recovery at day 90 when stored at 4C. By 90 days at -20C, recoveries were lower and more variable for T2 (g.p) and T1. Bear samples frozen for 270d at -20C then held for 30 days at 4C had 88% recovery, whereas g.p. T2 and control T1 samples had 6% and 15% recovery, respectively. This study indicates that T2 is a promising alternative to T1 for proficiency samples.

CS40.5

Evaluation of the Sensitivity of the New Device Gastros® to Detect *Trichinella* Larvae in Pork by the Comparison with the Magnetic Stirrer Method

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The aim of the study was the comparative evaluation of two methods to detect *Trichinella* larvae in meat samples: 1. the magnetic stirrer method according to the EU Regulation #2075/2005 and 2. a new method which use a new apparatus the Gastros® device. The device was designed according to the requirements of the official Russian method to detect

Trichinella larvae in pig muscles. This method differs from that of the EU Regulation for the digestion length and digestion temperature, the mesh size of the sieve, and the sedimentation time. The Gastros® apparatus has been tested in parallel with the magnetic stirrer method of pooled samples according to #2075/2005 which had been previously validated. A known number of alive *Trichinella* larvae, collected after artificial digestion of a mouse carcass, was added in the core of meat ball samples of 50 g each, made with minced pork muscles free of fat and fascia. Some meat ball samples were used to evaluate the amount of undigested material on the sieve. No statistical difference was observed between the number of recovered larvae with Gastros® and those recovered using the validated magnetic stirrer method of pooled samples. Additional experiments are on the way to increase the number of samples tested.

CS41 - Zoonoses

Wednesday, August, 12, 2009

CS41.1

Schistosomes of Birds: New Way to Become Infected

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Recent research in the field of bird schistosomes causing human cercarial dermatitis has led to discovery of new and sometimes highly pathogenic species and genera (e.g. *Allobilharzia visceralis*), and finding that the prevalence of natural bird infections is usually high. Local prevalence of bird schistosomiasis may reach 25-50% (e.g. some localities in Central Europe) or even 70-80% (e.g. Iceland). In Europe the infected birds have been found from south to north, and new schistosome species have been disclosed e.g. in Spain, France, Iceland and Finland. The success of schistosomes as parasites (with their worldwide distribution) is certainly a multifactorial matter, and it is conditioned by host and environmental factors. In addition, from the parasite side, it seems that there are at least two strategies how to infect birds: (a) In the aquatic environment, schistosomes normally penetrate the skin and migrate via blood vessels (or nerves - *Trichobilharzia regenti*) to the target organs of birds. (b) Besides this well known way of infection, our experimental results show that the birds may also be infected perorally by cercariae moving freely in water column or still residing within infected vector snails. In the latter case (b) the parasites penetrate mucosa of the upper part of the digestive tract and continue to migrate to the target organs. This mode of infection (the ability of cercariae to penetrate different tissues) can represent an important adaptation to transmission, and from epizootological viewpoint it probably contributes to circulation of bird schistosomes under e.g. cold climatic conditions.

CS41.2

Fishborne Zoonotic Trematode Infections in Domestic Animals in an Endemic Area of North Vietnam: Prevalence and Species Diversity

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Fishborne zoonotic trematodes (FZT) are endemic in humans and cultured fish in Vietnam but little is known about FZT in domestic animals, which are important reservoir hosts. A study designed to determine FZT prevalence and species diversity, and risk factors for infection, in dogs, cats and pigs in a highly endemic area of northern Vietnam was conducted. Faecal samples from 186 dogs, 94 cats and 168 pigs belonging to 132 households in Nghia Hung district, Nam Dinh province, were examined for small trematode eggs. Prevalence of FZT varied significantly ($p < 0.05$) between cats (70.2%), dogs (56.4%) and pigs (7.8%). Forty nine of the egg-positive animals were necropsied to obtain adult trematodes for identification. The liver fluke, Clonorchis sinensis, and 11 species of intestinal flukes belonging to the families of Heterophyidae, Echinostomatidae and Plagiorchiidae, were recovered from the infected animals. Based on data on animal husbandry practices collected from households, the practice of feeding raw fish to the animals was a significant risk factor for infection; this risk was reduced if the animals were periodically treated with anthelmintics. Based on the high prevalence of FZT and the habit of feeding raw fish to animals, domestic animals are likely to be major contributors of FZT eggs to the environment. Therefore, education of farmers to avoid feeding raw or inadequately heat-treated fish to animals and to perform regular anthelminthic treatment of dogs, cats and pigs are needed in future integrated FZT control programs.

CS41.3

Impact of Super-Shedders of *Cryptosporidium* and *Giardia* on Naïve Pen-Mates and the Feedlot Environment

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Introduction: Livestock feces potentially carries a variety of parasites that can lead to human illness with *Giardia*, *Cryptosporidium* and *E. coli* of primary concern. These organisms may be shed intermittently by healthy cattle and low numbers may cause human illness. It has become apparent that the shedding rate and frequency varies greatly among individual cattle. More recently, it has been theorized that super-shedders (individuals that shed $>10^5$ cfu g^{-1} feces) account for 80% of the transmission of *E.coli* 0157:H7 in feedlots. Consequently, elimination of the top 5% of super-shedders could have a substantial impact on the transmission of pathogens. The objectives of this study were to determine the prevalence of *Giardia* (100 cysts g^{-1} feces) and *Cryptosporidium* (1000 cysts g^{-1} feces) super-shedders within feedlots and to characterize the genotypic nature of these isolates.

Methods: Rectally collected fecal samples (n =1272) from Alberta feedlot cattle were analyzed through sucrose gradient and immunofluorescent staining for the prevalence and intensity of infection of *Giardia* and *Cryptosporidium*. Molecular genotyping was performed.

Results: A total of 22.3 and 2% of individual cattle were infected with *Giardia* and *Cryptosporidium*, respectively. Of the cattle positive 3.5% and 1.6% were considered super-shedders for *Giardia* and *Cryptosporidium*, respectively. Molecular genotyping for *Giardia* revealed that the zoonotic Assemblage A accounted for 7% of the positive samples and the rest were Assemblage E. *C. bovis* and *C. andersoni* accounted for 27.9% and 72.1% of the *Cryptosporidium* positive samples genotyped.

CS41.4

The Potential for Zoonotic Transmission of *Giardia duodenalis* and *Cryptosporidium spp.* in Beef and Dairy Cattle in Ontario, Canada

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The objective of this study was to compare the occurrence and the genotypes and species of *Giardia duodenalis* and *Cryptosporidium* spp. in beef and dairy cattle in one region of Ontario in an effort to determine their potential for zoonotic transmission. Pooled manure samples were collected from farms in the Regional Municipality of Waterloo, Ontario. The presence of *Giardia* cysts and *Cryptosporidium* oocysts was determined by immunofluorescence microscopy. Nested-PCR was run on all samples, and positives were sequenced to determine genotypes and species. A total of 179 pooled dairy cattle manure samples from 45 farms were tested. *Giardia duodenalis* and *Cryptosporidium* spp. were detected in 41% and 8% of these samples, respectively, by microscopy. Most *Giardia* isolates were identified as either the host-adapted "hoofed livestock" genotype *G. duodenalis* Assemblage E (46%) or the zoonotic Assemblage B (43%), while zoonotic Assemblage A, was less common (7%). The zoonotic species *C. parvum* (46%) and the non-zoonotic species *C. andersoni* (42%) were the most frequently identified species in dairy cattle, while the non-zoonotic species *C. bovis* and *C. ryanae* (both 8%) were occasionally found. Of the 102 pooled beef cattle manure samples tested from 30 farms, 67% and 26% were positive for *Giardia* and *Cryptosporidium,* respectively, by microscopy. All *Giardia* isolates in beef cattle were identified as *G. duodenalis* Assemblage E, while all *Cryptosporidium* isolates were identified by sequence analysis as *C. andersoni,* although microscopic analysis suggested the presence of other species. The results of these studies indicate that although *Giardia* and *Cryptosporidium* were identified in a higher percentage of the pooled beef cattle manure samples than in dairy cattle, zoonotic genotypes and species were much more common in dairy cattle than in beef cattle in this region. These results suggest that dairy cattle may pose a greater risk of infection to humans than beef cattle, and they provide preliminary evidence of potential zooanthroponotic transmission (human to animal).

CS41.5

Rats and Parasites: A Long – Term Faunestic Survey in Iran

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This survey has been carried out since 1990 and its main purpose has been to determine the parasito- fauna of Rattus spp. in Iran. During the survey, we were able to isolate 20 genera of endoparasites as well as several ectoparasites from 500 Rats captured from some areas in Tehran, Isfahan, Ahvaz, and Anzali randomly .The rodents were autopsied individually and carefully dissected under unaesthetic using chloroform. Before dissection, the rodents were examined for the presence of ectoparasites and subsequently blood sampling was done via the cardiac puncture. The examination of internal organs revealed the occurrence of 16 species of nematodes

and 4 species of protozoa including Trypanosoma lewisi 125 (25%). The prevalence of ectoparasites in all infested rats was found to be 296(59.2%) and they were identified as Poliplax spinulosa, Laelaps nutalli, Xenopsylla cheopis,Nosopsyllus fasciatus, Pulex irritanse,and Ixodes nymph. Intestinal protozoa that were detected on rats were consisted of Tritrichomunas spp. 45 (9%) and Giardia muris 90(18%).Helminth fauna among the rats throughout the survey revealed the presence of 20 species including the only trematode, Plagiorchis muris. Prevalence rate of helminthes are as follows: Trichosomoides crassicauda 330 (66%), Cysticercus fasciolaris 270(54%), Hymenolepsis nana 214(42.8%), Heterakis spumosa 186(37.2%), S. muris 96 (19.2%), H. diminuta 45 (9%), Syphacia obvelata 12 (2.4%), Moniliformis moniliformis 12(2.4%), Aspicularis tetrapetra 8 (1.6%), Capillaria annulosa 6(1.2%), Trichuris muris 5 (1%), Nippostronglyus brasiliensis 4 (0.8%), Rictularia spp 3(0.6%), Gongylonema spp 3(0.6%), Strongyloides rati 3 (0.6%), Mastophorus muris 2 (0.4%), Anoplocephalid sp 2 (0.4%) and one occurrence of C. hepatica. Based on available data this is the first report of Plagiorchis muris, Capillaria annulosa, and Mastophorus muris in the country. In conclusion, control measures and rat monitoring should be considered in public health and veterinary sectors specifically in the areas with rat overpopulation.

CS42 - Drug Resistance and Production

Thursday, August, 13, 2009

CS42.1

Emerging Ivermectin Resistance in Scabies Mites from Northern Australia

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Ivermectin is used widely in veterinary practice for the treatment of sarcoptic mange, and is also used increasingly to treat human *Sarcoptes scabiei* infestations. Previous reports of ivermectin resistance in *S. scabiei* collected from northern Australia indicated that prospective monitoring was required to detect the further emergence of ivermectin resistance in this endemic region. Bioassays of ivermectin sensitivity are routinely performed on live *S. scabiei* mites collected from crusted scabies patients admitted to hospital. We recently completed a longitudinal analysis of this data and found that median survival times to ivermectin in vitro have doubled over the ten-year period investigated. Sequential in vitro sensitivity data collected from a single patient over a course of ivermectin treatment confirms that selection for ivermectin tolerant mites can occur rapidly and persist once established. Quantitative RT-PCR analysis of mites collected from patients before and after ivermectin treatment shows that a P-glycoprotein and multiple glutathione S-transferase genes are up-regulated after ivermectin exposure, suggesting that these molecules may play important roles in the development of ivermectin resistance in *S. scabiei*. Our findings support concerns regarding the sustainability of scabies control programs using ivermectin and highlight the possibility of ivermectin resistance occurring in other mange host species.

CS42.2

Interactions of Sarcoptes Scabiei Delta-Class GSTs with Various Acaricides

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The mite Sarcoptes scabiei causes the disease sarcoptic mange or scabies, which affects both animals and humans worldwide. The infection is immunopathological and arises because of the mite and the tunnels it digs in the skin of its host. The ectoparasite not only causes suffering in the host but also financial losses in e.g. pig herds. Descriptions of resistance to acaricides among S. scabiei are more and more common, but very little is known about the underlying mechanisms. Glutathione transferases (GSTs) are a family of multifunctional enzymes with fundamental roles in the cellular detoxication. These enzymes have been linked to resistance to various insecticides and suggested to play an important role in detoxifying permethrin in resistant S. scabiei mites. Here we report the crystal structure of one S. scabiei delta-class GST. The 3D-structure was then used for homology studies on two other delta-class GSTs identified from S. scabiei. We also investigated potential interactions between these enzymes and three acaricides; ivermectin, lindane and permethrin, by docking analysis. With the aid of HPLC and mass spectrometry analysis we have used an in vitro system to study if the various acaricides were metabolised directly by the delta-class GSTs. In conclusion, by a combination of various modelling tools and biochemical studies we have shown that it is possible to start unravel the complex issue of acaricide resistance in S. scabiei.

CS42.3

Efficacy of Monepantel Against a Haemonchus contortus Isolate That is Highly Resistant to a Number of Commercially Available Anthelmintics

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Studies were conducted to assess the efficacy of monepantel (an Amino-Acetonitrile Derivative) against fourth stage larvae and adults of a Haemonchus contortus isolate that has known resistance (based on reductions in worm count) to many of the commercially available anthelmintics at recommended doses; fenbendazole (0% efficacy), levamisole (0%), ivermectin (0%), moxidectin (25%), closantel (57%), oxfendazole/levamisole combination (4%) and an oxfendazole/levamisole/abamectin combination (6%).

Study 1 was conducted in Switzerland where lambs were treated orally with ZOLVIX® at 2.5 mg/kg (monepantel, 25 g/L) when experimental infections were at the fourth larval stage. Efficacy, based on reduction in worm counts was 99.7% when compared to the untreated control group.

Study 2 was completed in Australia. In this study, lambs were treated orally with ZOLVIX at 2.5 mg/kg after experimental infections had developed to the adult stage. Efficacy was again calculated using worm burdens and was >99.9%.

These studies demonstrate that monepantel is very effective against multi-resistant H. contortus and will be an important tool in the management of anthelmintic resistance.

ZOLVIX and monepantel are not registered or available for sale in Canada.

CS42.4

Comparative Efficacies of Five Long Acting Anthelmintic Products Against Nematodes in Sheep with Confirmed Anthelmintic Resistance to Three Single Actives

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An initial FECRT carried out on a commercial sheep property confirmed albendazole, levamisole and ivermectin resistance, with efficacies of 81, 79 and 85.0 %.respectively. With no effective single action anthelmintic available the use of persistently administered anthelmintics was investigated

92 ewe hoggets were randomly selected for a FECRT, comparing five long acting anthelmintic products and run on pasture for 100 days. Hoggets were randomly allocated to one of six groups, of which received one of the following anthelmintic treatments, Cydectin LA Injection for Sheep, Ivomec Maximiser capsules, Extender 100 capsules, Bionic capsules and Extender Max capsules plus a undrenched control. All animals were faecal sampled on day 0, 10, 20, 30, 50 and 101 post treatment and weighed on days 0, 30, 50 and 101. Larval coprocultures were carried out on all groups at every sampling.

On day 30 all control animals were drenched due to high FEC levels and animal health concerns. Mean FEC of the control group rose to 1780 epg by day 30, indicating a significant parasite challenge was experienced by all groups. By day 50 the liveweight of the control animals was reduced by 35% (3kg) compared to other treated groups. Four treatments (products) displayed efficacies ranging between 99.3 and 100% on days 10, 20, 30, 50 and 101 however with a maximum efficacy of 82% the extender 100 group failed to achieve effective control. There where no significant differences in liveweight found between anthelmintic treatments. Larval coprocultures revealed differences between species due to treatment products.

CS42.5

Evaluation of a Larval Migration Inhibition Assay in Equine Cyathostomin Nematodes

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Presently there are no in vitro diagnostic assays for anthelmintic resistance validated for use in equine parasites. The egg hatch assay and the larval development assay, which have proven quite useful with ruminant parasites, have so far demonstrated limited usefulness with the cyathostomins and have not been properly validated. In this study we used two different isolates of cyathostomin nematodes with no known prior exposure to avermectin/milbemycin drugs in a larval migration inhibition assay (LMIA). Third-stage larvae were evaluated for their ability to migrate while varying assay parameters in order to optimize the consistency of the dose-response for 3 different avermectin drugs (ivermectin, eprinomectin and doramectin). Factors favoring migration included: using exsheathed larvae, DMSO concentrations <0.5%, a saline media, and a 2-hour migration time. Eprinomectin and doramectin had smaller 95% CI for EC50 than ivermectin, suggesting that these drugs yield more consistent results than ivermectin. However, mean EC50 of the two AM-naive isolates were significantly different. These data demonstrate that the LMIA exhibits several features indicating it has potential as a diagnostic assay for avermectin/milbemycin resistance in cyathostomes. However, the fact that two different AM-naïve worm isolates had significantly different mean EC50 values suggest that many more isolates need to be tested. Further optimization and testing with known avermectin-sensitive and -resistant isolates are required before it can be determined if this assay will be a viable op-

tion for detecting and measuring avermectin resistance in cyathostomin nematodes of horses.

CS42.6

Mammary Gland Development Is Affected by Anthelmyntic Treatment in Dairy Heifer Calves. (Hormonal Control of Milk Production in Bovines. Effect of Verminous nematodiasis During the Cow's Development and Reproductive Stages. Part 2: Effect of Parasitism During Development –ANPCYT- PICT04 21-20294)

Mejía, Miguel E.[2]; Perri, Adrián F.[1]; Licoff, Nicolás[2]; Lazaro, Luciana[2]; Formía, Néstor[3]; Becú-Villalobos, Damasia[1]; Lacau-Mengido, Isabel M.[1]

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Nematode gastroenteritis is a severe impairment for beef and dairy calves' growth in grazing systems. As we previously demonstrated that IGF-1 is diminished in parasitized dairy heifers, and this hormone has been involved in prepubertal mammary growth, we wished to determine if parasite infection could also affect mammary gland development. Forty new born female Holstein calves were randomly assigned to treated (systematic treatments with ivermectin, fenbendazole or levamisole to minimize parasite burden) or untreated group. At 22, 32, 42 and 70 weeks of age, blood samples were taken from all animals for IGF-1 determination by RIA, and six heifers per group were randomly assigned to mammary biopsying. Biopsy samples were taken using a Tru-Core® Biopsy Needle, Medical Device Technologies, Inc.. Histological development of the mammary parenchyma was evaluated and compared between groups. Mammary parenchyma was embedded in fat pad, conforming ductal developing structures of epithelial cells. As the animal grew up more organized ductal structures were observed. Heifers in the treated group had higher ratio of epithelial cells/total area at 22 weeks of age. IGF-1 increased with age and was augmented in the treated group, as expected. We conclude that effective nematode control, to minimize parasite burden, during development could increase early ductal mammogenesis in association with increased IGF-1 levels. Molecular studies will be performed in further studies.

CS43 - Physiology, Pharmacology and Control

Thursday, August, 13, 2009

CS43.1

RNA interference as a Tool to Probe Gene Function in Cestodes?

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RNA interference (RNAi) is an accepted method of investigating gene function via post-transcriptional gene suppression and has become an established tool in the identification/validation of novel drug targets. Cestodes of livestock pose an ever increasing burden on the agricultural sector worldwide and resistance to anthelmintic treatments is now emerging, e.g. to benzimidazoles in the cyclophyllidean cestode Moniezia expansa. This demonstrates the need for novel mode-of-action chemotherapeutics/vaccines to treat tapeworm infections of livestock. Although RNAi protocols have been published for some free living planarians, one monogenean and three trematode species (Schistosoma mansoni, Schistosoma japonicum and Fasciola hepatica), they have not been documented in cestodes. This study aims to develop RNAi protocols in M. expansa. Here we report attempts to silence both neuronally expressed neuropeptide F (Mx-npf) and the more widely expressed actin in M. expansa adults. Although silencing was not seen for Mx-npf through RT-PCR analyses, consistent knock down was seen for Mx-actin transcripts (71±4%) ($P<0.002$) following soaking in Mx-actin-dsRNA. The impact of Mx-actin silencing on worm phenotype was established using muscle tension recordings in which only 20±12% ($P<0.009$) of worm preparations (compared to control) showed a normal response to the addition of praziquantel. A significant decrease in Mx-actin levels was recorded by both reduced actin-immunostaining in the cestode tegument and supporting musculature ($P<0.04$) and suppression of native actin protein levels in worm extracts probed with anti-actin-antiserum quantified by western blot (55±13% $P<0.015$). The data indicate that cestodes do possess a functional RNAi pathway, although neuronal genes may be refractory to silencing.

CS43.2

Enzymes of Glutathione Biosynthetic Pathway in Rodent Malaria Parasite, Plasmodium Berghei Induce Humoral Immune Response

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The tripeptide glutathione (gamma-L-glutamyl-cystinyl glycine; GSH) in malaria parasite is a key modulator of the intracellular reducing environment that provides protection against reactive oxygen species (ROS), maintains protein thiols in reduced state and degrades non-polymerised ferriprotoporphyrin-IX. The level of GSH in malaria parasite is regulated through reduction of oxidized glutathione to GSH by glutathione reductase (GR), GSH synthesis by gamma-glutamyl cysteine synthetase (r-GCS) and glutathione synthetase (GS) and by glutathione-S-transferases (GSTs). These enzymes were studied in rodent malaria parasite, *Plasmodium berghei* . Cell-free *P. berghei* contained 0.617±0.08 u/mg, 0.027±0.008 u/mg, 0.711±0.001 u/mg and 0.443±0.001 u/mg of GR, GST, r-GCS and GS activity respectively. Subcellular fractionation of total parasite homogenate demonstrated that GR, r-GCS and GS were mainly confined to the cytosolic part of the parasite.

GR, r-GCS and GS were purified through ammonium sulphate and column chromatography. SDS-PAGE revealed GR and GS as 25kDa and 70kDa proteins respectively while rGCS is of two subunits of 66kDa and 57kDa. All the purified enzymes induced humoral immune response in mice determined by immunosorbent and immunofluorescence assays, however, only GS exhibited significant *in vivo* protection of the immunized animals. Effect of antimalarials and kinetic properties of these enzymes and other results will be presented.

CS43.3

Identification of Abomasal Nematode Parasites of Sheep Using Lectin Binding

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Lectin staining has been proposed as a means of characterising nematode parasites particularly for eggs and L3, which can be difficult to identify morphologically. Nineteen biotinylated lectins were screened for specific binding to glycoprotein groups on the surface of eggs, L3s (sheathed and exsheathed) and adult stages of *Haemonchus contortus* (H.c.) and *Teladorsagia circumcincta* (T.c.). Phosphate buffered saline was an adequate medium for most lectins, although some required added Ca^{2+} and/or Mn^{2+} for optimum binding. Bound lectin was visualised with streptavidin using a fluorescent microscope. All stages of the two species could be differentiated on their lectin staining pattern, although lectin binding to L3 was less intense. Adults showed the strongest staining; thirteen lectins bound to H.c. and twelve to T.c. adults, with species differentiated by binding of Concavalin A (Con A) and *Griffonia simplicifolia I* Agglutinin (BGS I) to H.c. and *Ulex europaeus Agglutinin* (UEA) to T.c. In agreement with previous studies, the eggs could be distinguished by Peanut Agglutinin (PNA) binding only to H.c. There was little binding to the surface of exsheathed L3, but species could be differentiated by binding to the cuticular openings in the head and/or tail region. Seven lectins bound to T.c. and six to the surface of sheathed L3 H.c. Of these a few lectins bound only to the surface of one species. Wheat Germ Agglutinin (WGA) revealed the surface striations on H.c., whereas others bound less intensely. Identification methods using combinations of lectins are being developed for nematode parasites.

CS43.4

Developing Quality Generic Anthelmintics

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The presence of generic parasiticides in the market generates controversy. Initially generic anthelmintics entered the regions of the world where patent laws were lax and the regulatory hurdles were easy to overcome. They were seen as being of doubtful quality. However, in the EU and the USA, patent laws have to be complied with and the quality of data required for a marketing authorisation is the same as for a novel product. Furthermore, with trade in meat becoming global, regulators in the regions where standards were lower are now demanding high quality data that complies with internationally accepted guidelines such as VICH. The development of a generic anthelmintic starts with sourcing quality active substances from manufacturers that comply with EU /USA Good Manufacturing Practices (GMP). Since the exact formulation of the pioneer product is rarely known in detail, this has to be de-formulated so that the company can start to develop a generic version. It can take 10-12 months to develop a simple tablet formulation with one active. Blood bioequivalence has to be demonstrated with the pioneer and these studies must be compliant with Good Laboratory Practice (GLP). Only if the active has low bioavailability can a controlled test with the dose limiting parasite be done comparing the pioneer and the generic. A residue study is an additional requirement in the USA for food producing animals. At present the minimum dossier review times taken by the Regulatory Authorities in the EU and USA are 210 and 700 days respectively.

CS43.5

In vitro and in vivo Anthelmintic Activity of Trianthema portulacastrum L. and Musa paradisiaca L. against Gastrointestinal nematodes of Sheep (Ovis aries)

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Anthelmintic activity of Trianthema (T.) portulacastrum L. (Aizoaceae) whole plant and Musa (M.) paradisiaca L. (Musaceae) leaves against prevalent gastrointestinal worms of sheep was evaluated that may justify their traditional use in veterinary clinical medicine.

In vitro anthelmintic activity of the crude aqueous methanolic extract (CAME) of both the plants was determined using mature female Haemonchus (H.) contortus and their eggs in adult motility assay (AMA) and egg hatch test (EHT), respectively. In vivo anthelmintic activity of crude powder (CP) and CAME in increasing doses (1.0–8.0 g kg-1) was determined in sheep naturally infected with mixed species of nematodes using fecal egg count reduction test (FECRT).

CAME of T. portulacastrum and M. paradisiaca showed a strong in vitro anthelmintic activity and pronounced inhibitory effects on H. contortus egg hatching as observed through AMA and EHT, respectively. Both plants exhibited dose and time dependent anthelmintic effects on live worm as well as egg hatching. M. paradisiaca (LC50 = 2.13 µg mL-1) was found to be more potent than T. portulacastrum (LC50 = 2.41 µg mL-1) in EHT. However, in vivo, maximum reduction in eggs per gram (EPG) of faeces was recorded as 85.6% and 80.7% with CAME of T. portulacastrum and M. paradisiaca at 8.0 g kg-1 on 15th day post-treatment, respectively as compared to that of Levamisole (7.5 mg kg-1) that caused 97.0% reduction in EPG.

The data showed that both plants possess strong anthelmintic activity in vitro and in vivo, thus, justifying their use in the traditional medicine system of Pakistan.

CS43.6

Activity of Vibrio Cholerae Biofilm Supernatants Against Giardia duodenalis

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Introduction: Many bacteria form biofilms as a physical barrier to resist predation by heterotrophic protozoa and to promote environmental persistence. However, recent research demonstrates chemical factors inhibitory to protozoa are also produced by some biofilms. In this study, the activity of biofilm derived chemical factors against the parasitic protozoa Giardia duodenalis was examined.

Methods: The anti-giardial activity of two strains of Vibrio cholerae biofilm supernatants was investigated. These strains were a wild type strain (El-Tor) and a hapR mutant, which is a quorum sensing regulator deficient strain. Biofilms were grown for 3 days, and the activity of their supernatants against G. duodenalis trophozoites was examined using a resazurin reduction assay. Doubling dilutions of supernatant ranging from 10% - 1.25% final concentration were incubated with G. duodenalis for 48 hours. Metronidazole was used as a positive control in the assay and lyophilised biofilm culture media was used as a negative control. Incubations were carried out in quadruplicate and the number of viable G. duodenalis trophozoites quantified by measuring absorbance.

Results and Conclusion: Chemical factors inhibitory to G. duodenalis were not produced by wild type V. cholera biofilms. In contrast, supernatants from hapR mutant biofilms significantly decreased G. duodenalis viability. Based on these results, chemical factors inhibitory to G. duodenalis are produced by V. cholera biofilms. However, production of these chemical factors appears to be repressed by quorum sensing regulation carried out by the biofilms. Characterization of these chemical factors may lead to the development of new chemotherapies for parasitic protozoa.

CS44 - Strategic Control

Thursday, August, 13, 2009

CS44.1

Efficacy of Imidacloprid 10 % + Moxidectin 2.5 % (Advantage Multi®, Advocate®) Topical Solution Against *Crenosoma vulpis* Infection in Artificially Infected Dogs

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Crenosoma vulpis is a metastrongylid lungworm of wild and domestic canids and is a cause of chronic respiratory disease in dogs in parts of North America and Europe. The purpose of this study was to determine the efficacy of imidacloprid 10 % + moxidectin 2.5 % (Advantage Multi®, Advocate®) Topical Solution against *C. vulpis* infection in artificially infected dogs. Beagles (9 M, 9 F) were each given 100 infective third-stage larvae of *C. vulpis* in gelatin capsules. Fecal samples were monitored for first-stage larvae from 3-8 weeks post-infection (PI). Sixteen dogs (8 M, 8 F) with the highest fecal larval counts were stratified by gender and larval counts and randomly assigned to one of two treatment groups. Group 1 was untreated; Group 2 was given a single topical treatment of Advocate® (10 mg/kg imidacloprid/2.5 mg/kg moxidectin) at 4 weeks PI. Group 2 fecal larval shedding ceased within 7 days of treatment and then remained negative. Mean larval shedding in Group 1 dogs ranged from 7.9-39.2 larvae per gram. Dogs were euthanized at 8 weeks PI and the lungs were removed and examined for the presence of adult

worms by lung flush. The mean (geometric) number for adult *C. vulpis* recovered in untreated dogs was 70.0 (range 58 to 87) compared with 0.0 in animals treated with Advocate®. The resulting efficacy against *C. vulpis* was 100 %. The number of *C. vulpis* was significantly lower for treated dogs than the burden shown in the untreated group (p = 0.003).

CS44.2

Efficacy and Safety of Imidacloprid 10%/Moxidectin 1% and Emodepside 2.1%/Praziquantel 8.6% Spot-On Formulations in the Treatment of Feline Aelurostrongylosis

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The efficacy and safety of two antiparasitic spot-on formulations containing imidacloprid 10%/moxidectin 1% (Advocate®, Bayer Animal Health, Germany) and emodepside 2.1%/praziquantel 8.6% (Profender®, Bayer Animal Health, Germany), each administered once, in the treatment of natural feline infection with the strongylid lungworm Aelurostrongylus abstrusus have been evaluated. The efficacy of both products was tested in comparison to a control oral formulation containing fenbendazole 18.75% (Panacur® Oral Paste, Intervet, UK) administered over three consecutive days. Efficacy assessment was based on larvae per gram of faeces (lpg) counts measured on Days 28±2 following treatment and compared to pre-treatment counts on Days -6 to -2. Thirty-six cats treated either with Advocate® (n=12), Profender® (n=12) or with Panacur® (n=12) were included in the trial. Mean lpg at Days -6/-2 and 28±2 were respectively 47.5 and 0 for Advocate®, 63.8 and 1.3 for Profender® and 33.8 and 1.3 for Panacur®. The cure approach in each treatment groups was calculated on the basis of a reduction of lpg counts of at least 90%. On Days 28±2, 100% reduction from pre-treatment baseline was reached by Advocate®, 99.38% by Profender® and 99.29% by Panacur®. No treated cats showed adverse events. This trial demonstrated that all three evaluated products are safe and effective in the treatment of aelurostrongylosis in cats. Nonetheless, both Advocate® and Profender® spot-on formulations present major advantages compared to the oral paste, represented by the possibility of a single dose and the easy-to-apply dermal administration.

CS44.3

Selective Anthelmintic Treatment Scheme for Goats in Tropical Mexico: Two Year Field Validation

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Introduction: Selective anthelmintic (AH) treatment may reduce the number of animals treated per year to maintain a "refugia" of susceptible worms. A selective treatment scheme was validated using two levels of nematode eggs per gram of faeces (500 vs. 750 EPG).

Methodology: A goat herd of 170 adult goats was monitored on two consecutive years (2005 and 2006). Every month goats were checked for FAMACHA® and body condition score (BCS). Combinations of those scores were used to select animals for faecal sampling. Thresholds for AH treatment were 500 EPG (year 2005) and 750 EPG (year 2006). Data from both years were compared using 2x2 tables and the odds ratios were determined.

Results: A total of 2263 and 2009 events were recorded (2005 and 2006 respectively). In 2005, 13% of the events were treated and 8% during 2006. More animals were treated when 500 EPG was used as threshold compared to 750 EPG (288 vs. 169 respectively, OR = 1.59, 95%CI = 1.29-1.95, P<0.001). On year 2005, 60 animals of the 170 in the herd, were not treated and 90 on year 2006. No animal died due to GIN infection during the two years. No indication of reduced productivity of dams was recorded. When 750 EPG was used as threshold for treatment there was a greater risk of recording animals on FAMACHA@ 5 (16 vs. 29; OR = 2.06, 95%CI = 1.07 – 3.97, P<0.01).

Conclusion: Selective treatment combining FAMACHA® and faecal egg counts was feasible, but work burden was considerable.

CS44.4

Transmission of Intestinal Parasites of Alpacas in the Mid-Atlantic Region, U.S.A.

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Alpacas have recently become popular in the U.S. Because breeding animals are very valuable, most owners prefer intensive parasite control programs, which may result in unnecessary anthelmintic treatments. This study examined parasite transmission in alpacas during the year under typical

management conditions in a region where *Haemonchus contortus* is economically important in small ruminants and also infects alpacas. Sixteen mature male alpacas were divided into 2 groups. Each group grazed a separate 1 hectare pasture previously used for small ruminants. One group received ivermectin (0.4 mg/kg) at 6-week intervals (standard program for control of *Parelaphostrongylus tenuis*). In the second group an alpaca was dewormed if its fecal strongylid egg count exceeded 200 eggs/g (epg). Manure piles were removed regularly. Body weight, packed cell volume, FAMACHA score and fecal egg counts were determined every 2 weeks. Fecal samples were evaluated by Modified Wisconsin technique. Mean strongylid epg did not exceed 52 in both groups during the study. Treatment threshold was exceeded in one sample. *Haemonchus* and *Trichostrongylus* were predominant genera during the grazing season. No signs of parasitic disease were observed and FAMACHA scores averaged 2. Impact of nematodes on production parameters could not be accurately evaluated because macrolide resistance was present. Shearing in the spring appeared to be associated with an increase in epg, indicating that deworming at this time may be of value. These results suggest that in some situations adult alpacas may not require the same intensive control for H. contortus used in sheep and goats.

CS44.5

Evaluation of the FAMACHA© System in South American Camelids

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Introduction: The FAMACHA© system was developed in South Africa as a means to apply a selective anthelmintic treatment regimen to sheep infected with Haemonchus contortus. With FAMACHA©, the ocular mucous membranes of sheep are examined and compared to a laminated card depicting 5 illustrations of ocular membrane colors ranging from a score of 1 (red, nonanemic), to a score of 5 (white, severely anemic). The FAMACHA card was validated in the United States (US) in 2004 for use in small ruminants, based on highly significant correlations between packed cell volumes (PCV), FAMACHA© eye scores, and fecal egg counts (FEC). Since then, the FAMACHA© system has been widely accepted and used by the small ruminant industry in the US. Recent identification of multiple anthelmintic-resistant H. contortus on camelid farms in the southeastern US has prompted interest in the applicability of the FAMACHA© system.

Methods: FAMACHA© eye scores, PCVs and FECs were measured in 671 camelids on 21 southeastern US llama and alpaca farms with documented Haemonchus contortus.

Results: Animals with FAMACHA© scores of 1 to 5 had mean PCV of 31%, 28%, 27%, 22% and 16%, respectively. Animals with FAMACHA© scores of 1 to 5 had mean FEC (EPG) of 139, 284, 567, 1238, and 4,047, respectively.

Discussion: Preliminary results indicate that FAMACHA© scores demonstrate discriminatory value in camelids. A full statistical analysis is underway, which will enable us to make further inferences on the accuracy and usefulness of FAMACHA© in South American camelids.

CS44.6

In vitro Evaluation of Anthelmintic Activity of Artimisia indica and Artimisia roxburghiana Against Gastrointestinal Nematodes of Sheep

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Assessment on the survival of Haemonchus contortus was made to evaluate the anthelmintic activity of A. indica and A. roxburghiana plants, as alternative sources of anthelmintics. In present study was carried out to screen the ovicidal, larvicidal and wormicidal activity of methanolic extracts of A. indica and A. roxburghiana of indigenous plants species. Results revealed that both Artemisia plants have anthelmintic efficacy against all parasitic stages as compared to control group. However, A. indica showed a higher anthelmintic activity than A. roxburghiana. Methanolic extracts of both A. indica and A. roxburghiana exhibited the following ovicidal (85 + 21.2; 80 + 28.3), larvicidal (18 + 2.8; 17 + 4.2) and wormicidal activity (8.5 + 2.1; 8 + 2.8), respectively at a concentration of 50 mg/mL and found insignificant ($P > 0.05$) to Albendazole. It is suggested that more in-depth studies are required to characterize the active ingredient responsible for anthelminitic activity in both these plants. It is also suggested that these results are needed to be replicate at in vivo stage in order to authenticate their efficacy against gastrointestinal nematodes of small ruminants in Pakistan.

CS45 - Diagnosis

Thursday, August, 13, 2009

CS45.1

Detection and Differentiation of Coccidian oocysts by Real-Time PCR with Melting Curve Analysis

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Rapid and reliable detection and identification of coccidian oocysts are essential for various animal and public health purposes. Traditional morphological techniques can identify large and unique oocysts, but are often subjective and require parasitological expertise. The objective of this study was to develop a real-time PCR (qPCR) assay with melting curve analysis (MCA) to detect, differentiate and identify DNA from coccidia species of zoonotic and food safety concern. A universal coccidia primer cocktail was designed and employed to amplify DNA from *Cryptosporidium parvum, Toxoplasma gondii, Cyclospora cayetanensis*, and several species of *Eimeria, Sarcocystis*, and *Isospora* using qPCR with SYBR green detection. MCA was performed following amplification and melting temperatures (Tm) were determined for each species based on multiple replicates. A standard curve was constructed from diluted *T. gondii* oocyst DNA to estimate assay sensitivity. The qPCR assay consistently detected DNA from as few as 10 oocysts. Tm data analysis showed that *C. cayetanensis, C. parvum, Cryptosporidium muris, T. gondii, Eimeria necatrix, Eimeria acervulina, Isospora suis, Sarcocystis cruzi*, and *Sarcocystis muris* can each be identified by unique melting curves and differentiated based on Tm ($P < 0.05$). DNA of coccidian oocysts in food or clinical diagnostic samples can be sensitively detected, reliably differentiated, and identified using qPCR with MCA. This assay may also be used to detect other life-cycle stages of coccidia in tissues, fluids and other matrices. MCA studies on multiple isolates of each species will further validate the assay and support its application as a routine diagnostic screening tool.

CS45.2

Evaluation of Species-Specific Oligoprobes for Identification of Taeniid Cestodes of Canids

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Taeniid cestodes cause industrial losses through the condemnation of meat and offal. Some cause severe illness in humans, such as cystic and alveolar hydatid diseases. Taeniid infections in definitive hosts cannot be diagnosed specifically by faecal egg detection, due to the morphological similarities of the eggs among taeniid species (*Echinococcus* spp. and *Taenia* spp.). In some situations, the canids can harbour several species of these genuses simultaneously. It is necessary to develop a test which can differentiate taeniid eggs presented in an infected animal to a species level. Nowadays, molecular techniques represent promise in achieving an accurate diagnosis. Reverse line blot hybridization, a PCR based technique, has been shown to be a rapid, very sensitive and specific technique which species-specific oligoprobes can be used to screen a large number of samples for known groups of pathogens. A mitochondrial gene, NADH dehydrogenase subunit 1 (ND1) was chosen as target. Common primers for cestode ND1 sequence were designed. The sequences obtained using direct sequencing method, were compared with the available sequences registered in GeneBank using BLAST search to find homology. The obtained sequences and the registered ones were aligned and were used to design species-specific probes for common canine taeniid cestodes in the regions where the greatest inter-species differences but no intra-species polymorphisms were observed. The specificity and sensitivity of probe candidates are being evaluated by dot blot assay using samples of *Taenia crassiceps, T. hydatigena, T. multiceps, T. ovis, T. pisiformis, T. taeniaeformis, Echinococcus granulosus, E. multilocularis and E. vogeli*.

CS45.3

Sarcocystis Species in Cervids – Molecular Data are Necessary for Correct Species Identification!

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Introduction: Most *Sarcocystis* species are assumed to be intermediate host specific and species identification has traditionally been based on sarcocyst morphology in the intermediate host. Several *Sarocystis* species are commonly found in different cervid hosts, but the species descriptions have previously not been supported by molecular data. Our aim was to identify and characterise, by both morphological and molecular methods, *Sarcocystis* species in different cervids, examine their evolutionary relationships, and determine whether some *Sarcocystis* species are shared by these hosts.

Materials and Methods: Samples were obtained from Norwegian red deer, roe deer, reindeer and moose. Sarcocysts were excised from skeletal and cardiac muscle and examined by light microscopy, scanning electron microscopy, and sequence analysis (including phylogeny) of the 18S rRNA gene and the associated ITS-1 region.

Results and Conclusion: We found:

Reindeer: Six morphologically and genetically distinct *Sarcocystis* species; *S. grueneri, S. hardangeri, S. rangi, S. rangiferi, S. tarandi,* and *S. tarandivulpes*. Roe deer: Two morphologically and genetically distinct species; *S. gracilis* and *S. oviformis*. Moose: Five species; three of these (*S. alces, S. scandinavica* and *S. ovalis*) were characterised both morphologically and genetically, and two species were discovered by molecular methods only. Red deer: Five morphologically and genetically distinct species, three of which were also found in reindeer, and two in moose.

Sarcocystis species in cervids seem to have evolved with their definitive hosts and to be adapted to one intermediate host species, but some species might occasionally cause light infections in other cervids. Sarcocysts of a given species may vary in morphology; however, sarcocysts of several species might also be very similar and can only be distinguished genetically. We therefore conclude that molecular methods are necessary for correct identification of *Sarcocystis* species in cervids.

CS45.4

Detection of *Taenia saginata* cysticerci in Experimentally Infected Cattle by Histology and PCR

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Taenia saginata cysticerci occur rarely, sporadically, or commonly in beef, depending on the country of origin. Reliable detection of cysticerci in samples collected at carcass inspection is necessary to prevent human infection. Histological examination of tissue sections is the most common method for cysticerci identification, but has limited sensitivity on degenerating specimens and requires significant expertise. A sensitive and non-subjective PCR assay may be more suitable for detection. Currently, molecular assays are not used in regulatory diagnostic protocols to detect bovine cysticerci in meat samples, although primers have been developed to amplify *Taenia saginata*. The performance of a PCR assay for the detection of bovine cysticerci was assessed using tissue samples from experimentally infected cattle. A standard histological method identified degenerated cysticerci in 61 samples obtained from experimentally infected cattle. Two sets of previously published primers were used in PCR assays on adjacent sections of the histologically positive samples containing cysticerci. Specific primers amplified 328 bp of the LSU rRNA or 521 bp from the *cox1* gene. Combined, the PCR assays identified *T. saginata* DNA in 50 of the 61 samples, consisting of heart (6 of 8), skeletal muscles (43 of 51), and liver (1 of 2). This demonstrates that although the PCR assay did not detect all positive samples, it can amplify DNA from different tissue types, and could be a valuable supplementary diagnostic tool. Development, evaluation, and optimization of other primers should be done before implementing a PCR assay for routine diagnosis of bovine cysticercosis.

CS45.5

Diagnostic Methods for Encephalitozoonosis in Pet Rabbits

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Encephalitozoon cuniculi is common in rabbits; however, diagnosis in correlation with clinical signs still remains a major challenge. Different diagnostic methods were compared for the determination of *E. cuniculi* in naturally infected animals. Group I (n=33) showed symptoms suggestive of encephalitozoonosis (central nervous and/or renal diseases) and group II (n=38) served as control. All animals were tested by IIFAT, histological examination including special spore staining and PCR. Infections with *E. cuniculi* could be determined in 78.8 % of the rabbits of group I and in 57.9 % of group II by histological examination combined with spore staining. In group I 69.7 % and in group II 50.0 % showed a seroconversion. As conventional PCR was only sensitive for eye lenses in cases of phacoclastic uveitis (n=10; 100 %), nested PCR was performed for organs and body fluids. In group I 63.6 % and in group II 42.1 % of the animals were positive in organ samples. Nested PCR of urine was positive in 29.7 % (n=37). All 25 samples of cerebrospinal fluid tested negative in nested PCR. Histological examination combined with special staining was the most sensitive method for post mortem diagnosis. Nested PCR was a good post mortem method to investigate organs of seropositive animals with histological lesions in the brain and kidneys. However, it was unsuitable for rabbits which were seronegative or showed interstitial nephritis without encephalitis, although spores could be detected. In living animals conventional PCR of eyes with phacoclastic is an excellent diagnostic method, while nested PCR of body fluids was unreliable, probably due to the sporadic presence of spores.

CS45.6

Parasites of the African Painted Dog (*Lycaon pictus*) in Wild and Captive Populations: Potential Conservation Impacts.

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Due to the endangered status of African Painted Dogs *(Lycaon pictus)* it is important to understand what parasitic diseases they are exposed to and to what effect these are

having on the rapidly declining wild populations. Conversely zoo collections of these animals are under different pressures due to their captive lifestyle such as stress, nutrition, inbreeding and intensive housing.

Faecal samples were collected from captive populations housed at Perth Zoo, Monarto and Adelaide Zoos and DeWildt Wildlife Trust in South Africa. Wild populations have been sampled from Zambia and Namibia with further sampling to be undertaken Zimbabwe and South Africa. Samples have been analysed via microscopy and parasites observed identified to genus. *Giardia* cysts and *Spirometra* sp. were detected in captive populations while parasite eggs of Taeniidae, Ancylostomatidae and Sarcocystis were detected in the wild populations. Molecular characterisation was then conducted in order to characterise those parasites found. Of particular interest is the zoonotic potential of the *Giardia* sp. detected in captive animals and the determination of *Echinococcus* sp. from the Taeniid ova found. Further sampling will add statistical rigour in order to quantify faunal structure.

CS46 - Coccidia

Thursday, August, 13, 2009

CS46.1

The Epidemiological Survey of Coccidia in Wild Crane Flocks in Japan by Fecal DNA Based Identification of Crane Species and Individual Bird

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Introduction: In Japan, there are mainly 3 crane species, red-crowned (*Grus japonensis*), hooded (*G. monacha*) and white-naped (*G. vipio*) cranes. About 1,000 red-crowned cranes inhabit Hokkaido, a northern island of Japan all year round. About 12,000 hooded and white-naped cranes migrate from Siberia to Izumi in Kyushu, the southern island of Japan in winter. Coccidiosis is a major parasitic disease of cranes because it is lethal to young birds. In this study, the infection status was investigated in each crane flock in Japan by fecal DNA analysis.

Methods: Fecal DNAs were extracted from feces collected in feeding stations in Hokkaido and Izumi in winter, and were used as PCR templates. A part of mitochondrial 16S rRNA gene of the crane was amplified and sequenced in order to distinguish crane species. Individual of red-crowned crane was recognized by fragment analysis of fecal DNA using 7 genetic and 2 sex-linked markers. Crane species and individual identification techniques enabled accurate surveys of anonymous fecal samples. To detect coccidia excreted in crane feces, PCR-based capillary electrophoresis approach was applied. ITS-2 region was amplified by nested PCR with 2 sets of primers specific for the genus *Eimeria* and subjected to capillary electrophoresis in an ABI 3130xl Genetic Analyzer.

Results: About 90% of hooded and white-naped cranes in Izumi excreted coccidia, while about only 5% of red-crowned cranes in Hokkaido were positive.

CS46.2

Eimeria praecox: Pathological Effects and Variability in Virulence of Field Strains

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Introduction: Eimeria praecox, an apicomplexan parasite infecting chickens, has traditionally been considered a mildly pathogenic species compared to say, E. acervulina, a common cause of coccidiosis.

Methods: Using body weight gain (BWG) as the main criterion, the virulences of recent field strains of E. praecox from Wales and the USA were compared with English laboratory strains (Houghton) of E. praecox and E. acervulina.

The pathogenesis in chickens of E. praecox compared to E. acervulina, using intestinal lesions, mucosal integrity, and BWG as criteria was also investigated.

Results: A recent field strain of E. praecox from Wales was more virulent than E. acervulina, which was more virulent than E. praecox from USA and E. praecox (Houghton laboratory strain).

When pathogenesis was investigated, both species caused statistically significant reductions in BWG at the lowest inocula tested (500,000 sporulated oocysts per bird of E. praecox and 250,000 of E. acervulina). E. praecox from Wales, like E. acervulina, sometimes caused actual body weight loss. E. acervulina caused gross, variably shaped, pathognomonic lesions, but E. praecox of all 3 strains caused micro-lesions visible in fresh tissue only with a dissecting microscope. Both E. acervulina and E. praecox caused villous erosion and atrophy. No mortalities occurred in birds receiving up to one million sporulated oocysts each of either species.

Conclusion: Considering the potentially high virulence of E. praecox now demonstrated and its known world-wide distribution, the inclusion of this species in live oocyst vaccines against chicken coccidiosis is fully justified.

CS46.3

Identifying Humane Endpoints in Turkeys Artificially Infected with Coccidiosis

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Coccidiosis remains one of the major concerns in turkey production. Clinical disease associated with coccidiosis primarily affects turkeys between the ages of 3-10 weeks and is characterised by enteritis, watery or mucoid diarrhoea and anorexia contributing to overall poor flock performance. Studies of coccidiostats in artificially infected turkey poults are essential to determine the efficacy of novel products and relicensing of existing products. These studies are regulated in the UK by the Animals (Scientific Procedures) Act 1986, whilst guidance is provided by the WAAVP and the EMEA for study conduct and infection rates. Currently under WAAVP guidelines, deaths are considered acceptable in the infected but untreated control group as a confirmation of disease presence. This conflicts with obligations under the European Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purpose (Council of Europe, 1986) and subsequent EU legislation and the Animals (Scientific Procedures) Act 1986, where there is an obligation to minimise harm and suffering. This study set out to identify humane end points before death for infected poults. Preliminary data on clinical signs which preceded death were characterised and then used to identify birds that had coccidiosis. These birds were then euthanased and the presence of coccidiosis confirmed. The aim of this preliminary project was to begin to identify, define and then apply clear humane endpoints. The study demonstrated that valid efficacy data can be achieved whilst minimising the suffering to experimental birds by applying humane endpoints.

CS47 - Canine Parasites

Thursday, August, 13, 2009

CS47.1

Prophylactic Use of Imidacloprid/Moxidectin Spot-On Solution in Dogs Experimentally Infected with *Angiostrongylus vasorum*

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A controlled, randomized, blinded dose-confirmation study was conducted to evaluate the prophylactic efficacy and safety of imidacloprid 10 mg/kg / moxidectin 2.5 mg/kg body weight spot-on solution in dogs experimentally infected with 200 L3 of *Angiostrongylus vasorum*. Twenty-four adult dogs were randomly allocated to three study groups of 8 dogs each. Animals in group 1 were treated 4 days post infection (dpi), those in group 2 at day 32 pi, and the dogs in group 3 were left untreated. All dogs were euthanized and necropsied 56-59 dpi to determine the worm burdens by reverse lung perfusion. In the control group, excretion of L1 of *A. vasorum* started 47 dpi in three dogs and all dogs excreted L1 at least on one sample day before euthanasia (0.1-32.5 larvae per gram of faeces). A mean of 99 (SD 42.8) adult parasites were recovered in the post-mortem examinations. In contrast, no L1 at all were found in the faeces of dogs in groups 1 and 2, nor were there any adult parasites detected at necropsy. Respiratory symptoms were observed in dogs in groups 2 and 3. Pathological findings in the lungs correlated with the treatment groups: in the animals in group 1, no or minimal lesions were found, while in those in group 2, dispersed patterns of pale pink, slightly raised and consolidated foci were present in all lung lobes. In contrast, the lungs of the dogs from group 3 were severely affected: large confluent areas were hardened, raised and discoloured, with frequent haemorrhagic patches. Pneumonia, thrombi and parasites were histologically confirmed. The lung lymph nodes were regularly enlarged. Hence, imidacloprid/moxidectin spot-on effectively eliminated L4 stages and immature adult *A. vasorum* in experimentally infected dogs and prevented patent infections. The earlier an infected dog was treated, the less severe pathological lesions in the lungs were observed.

CS47.2

Clinical and Laboratory Findings in Dogs Experimentally Infected with *Angiostrongylus vasorum* and First Results of New Diagnostic Serological ELISAs Using Monoclonal and Polyclonal Antibodies

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Experimental infections with *Angiostrongylus vasorum* third-stage larvae (L3) originating from experimentally infected *Biomphalaria glabrata* snails were induced in 3 separate experiments involving 22 dogs (Beagles) in total. In trial 1,

three dogs each were inoculated with 50 or 500 L3, (groups A and B), and eight dogs in each of trials 2 and 3 (groups C and D) with 200 L3. Increased respiration rates and respiratory sounds were observed starting from day 42 post infection (dpi). Typical observations, which became more distinct with increasing time after infection, were panting, abdominally accentuated and deepened respiration with intensified inspiratory and/or expiratory sounds. Faeces containing blood and mucus were occasionally observed during the study. Loss of appetite with weight reduction was common. Radiology performed on dogs in groups A and B showed prominent alterations of the lung parenchyma on 56 dpi which had progressed on 90 dpi. These dogs developed neutrophilic leucocytosis with left shift particularly from 49 dpi on, as well as occasional mild anaemia, thrombocytopenia, basophilia, eosinophilia and monocytosis. Coagulation parameters (PT, PTT, TT) were not altered at any time. First-stage larvae (L1) were detected in faecal samples by the Baermann technique from 47-55 dpi in all dogs. Patency lasted until the end of the study (90 dpi for groups A and B, 56-59 for group C and 76-78 for group D). During patency, there were intermittent days with negative faecal examinations. In a total of 10 dogs, reverse lung perfusion was applied after euthanasia. Ten adult worms were recovered from one dog in group A, 170 from one dog in group B. A mean of 99 (SD: 42.8) adult parasites were found in the dogs of group C. In order to enhance the sensitivity of *A. vasorum* diagnosis, serological tests using monoclonal and polyclonal antibodies were developed for the detection of circulating antigens.

CS47.3

Evaluation of a Combination of Doxycycline and Ivermectin in the Treatment of Naturally-Acquired Dirofilaria immitis Infection in Dogs

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Dirofilaria immitis is a filarial nematode that causes canine heartworm disease (HWD) in many countries of the world and also harbours the bacterial endosymbiont Wolbachia. There is only one drug registered for adulticidal therapy against canine heartworm, melarsomine, but treatment is often followed by severe pulmonary thrombosis. Long-term monthly administration of the macrocyclic lactone (ML) ivermectin has been shown to be macrofilaricidal against D. immitis. It has been reported that monthly administration of prophylactic doses of ivermectin resulted in complete elimination of adult worms in experimentally-infected dogs in 36 months. More recently, the adulticide activity of monthly ivermectin was evaluated in naturally-infected dogs from an endemic area. After 24 months, approximately 65% of infected dogs were negative for the presence of adult parasites. Several treated dogs, however, showed worsening of clinical and radiological signs associated with infection. Recent studies have shown that elimination of the bacterial endosymbiont Wolbachia through antibiotic treatment of the filarial-infected host is macrofilaricidal against both human and animal filarial worms. We have recently reported that a combination of long-term ivermectin and doxycycline treatment had significant micro-and macro-filaricidal effects against D. immitis when compared to either drug administered alone to experimentally-infected dogs. The present study evaluated the effect of a combination of doxycycline orally. (Ronoxan®, Merial Italy) at 10mg/kg/day for 30 days and with 0.6ug/kg of ivermectin orally(Cardiotek®, Merial Italy) once every fifteen days for 6 months on the parasitological, cardiac and pulmonary parameters of dogs with naturally-acquired HWD. Dogs were controlled once a month and microfilarial counts, circulating antigen levels, radiographic appearance of lungs and echocardiographic patterns were evaluated. All dogs showed dramatic decline in circulating microfilariae and 100 % of the animals were negative at 60 days from the beginning of treatment, while 60% of dogs were antigen negative at the end of the study. Lung patterns worsened in 50% of dogs at 30-60 days, likely indicating an inflammatory reaction to the death of adult worms and at the same times, cardiovascular patterns (right cardiac chamber enlargement; acceleration time (AT) of pulmonary artery, tricuspid regurgitation) appeared to worsen. However, all dogs were vastly improved by the end of the study. Results would suggest that a a combination of doxycycline and ivermectin could be a valid alternative for adulticide therapy in dogs with heartworm disease.

CS47.4

Development of an in vitro bioassay to detect the presence of anthelmintic resistance in Dirofilaria immitis.

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Heartworm disease is a significant threat to canine health. Over the past few decades, the prevalence of heartworm infection in pet dogs has been greatly reduced by monthly prophylaxis using anthelmintics of the avermectin/milbemycin class. However, recent reports of heartworm infections in dogs receiving documented monthly prophylaxis are cause for concern. Two possible explanations for this observation are failure of owner compliance in ensuring proper administration of prophylaxis, and the development of anthelmintic resistance. However, it is currently not possible to distinguish these two scenarios because compliance is not possible to verify, and there are no validated assays for detecting resistance in D. immitis. In order to address this problem, we

developed a larval migration inhibition bioassay (LMIA) for D. immitis, using a 96-well plate format. Dirofilaria immitis L3 obtained from mosquitoes fed on microfilaremic dog blood were incubated for 3 h in the presence of increasing concentrations of ivermectin. Following incubation, L3 were transferred to wells containing a 20-micron mesh filter, and numbers of larvae migrating through the mesh were measured after 12 h. Preliminary results indicate that the LMIA produces a sigmoidal dose response with D. immitis L3. We are currently in the process of further optimizing this assay and testing the dose response characteristics of a variety of avermectin/milbemycin drugs. Our goal is to validate this assay so it can be used to screen D. immitis L3 produced from microfilaremic blood samples obtained from dogs for which a failure of anthelmintic prophylaxis has been reported.

CS47.5

Summary of the Efficacy Data of Emodepside Plus Praziquantel Flavoured Tablets (Profender® Tablets for Dogs) Against Mature and Immature Nematode Infection in Dogs

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Modern anthelmintics for dogs are required to be effective against patent as well as immature nematode infections for the prevention of zoonoses and parasitic disease of the host.

The presentation gives an overview of the data on the efficacy of a novel flavoured tablet formulation of emodepside plus praziquantel against mature and immature nematode infections in dogs. Twenty one placebo controlled GCP dose confirmation studies were conducted under laboratory conditions in Germany, Ireland, South Africa and the US that included experimental and natural nematode infections. The tablets were used at the minimum recommended dose of 1 mg emodepside and 5 mg praziquantel per kg body weight. The following efficacies were observed: > 99 % against immature adult and adult stages of *Trichuris vulpis*, > 95 % against immature adult and adult stages of *Uncinaria stenocephala* and *Ancylostoma caninum*, > 95% against L4, immature adult and adult stages of *Toxascaris leonina*, and > 92 % against L3, L4, immature adult and adult stages of *Toxocara canis*. A non-interference study demonstrated that addition of praziquantel did not diminish the efficacy of emodepside. Furthermore, a multicenter field study conducted in 354 dogs in France, Germany, Portugal and Slovakia confirmed the results of the laboratory studies. The absence of significant adverse events in all studies indicated that treatment of dogs with emodepside plus praziquantel tablets was safe as well as highly effective. Thus this novel anthelmintic drug provides complete treatment of the relevant stages of gastrointestinal nematodes in the dog.

CS48 - Trypanosomes

Thursday, August, 13, 2009

CS48.1

Characterisation of Trypanosoma vivax Isolates from Ugandan Cattle and Goats using Microsatellite Markers

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Microsatellite DNA polymorphisms can be utilised to assess the intraspecific genetic diversity and hence are useful for characterisation of species and strains of trypanosomes. Here, we present four new microsatellite markers specific for T. vivax. The GeneDB partial shotgun 5x coverage sequence of T. vivax available as of 1st August 2005 was used and targeted the genomic sequence of T. vivax that has no cross amplification with other livestock-infective trypanosomes. Only di-; tri-; tetra;- and pentanucleotide microsatellites not less than five units were selected. Although pentanucleotide repeats on screening appeared to have the desired variability, they gave poorer PCR products compared to di-, tri- and tetranucleotide repeats. Mononucleotide repeats presented difficulty in detecting visible bands on agarose gels from their amplification, they were omitted from this study. Clear length polymorphism was obtained with (GTA)16 while (CACT)15 gave size and length variability. Clear constant size bands were obtained from (TTA)24 microsatellite, approximately 150 base pairs long and (CA)26, approximately 180-200 base pairs. These findings suggest that different subtypes of T. vivax exist in Uganda; the polymorphic forms derived from microsatellite band size differences may suggest this parasite exhibits virulence differences as has been shown in T. congolense subtypes.

CS48.2

Epidemiology of Trypanosomiasis on the Jos Plateau

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The Jos plateau in North-Central Nigeria covers an area of 8000km2 at an average altitude of 1,280m. It is historically

free of tsetse flies and trypanosomiasis but is surrounded on all sides by infested lowlands that contain sleeping sickness foci and are endemic for animal trypanosomiasis. The lack of disease and abundant pasture attracted many pastoral cattle herders and the plateau is now an area of intense animal production which plays a significant part in the national cattle industry.

Within the past 20 years trypanosomiasis has appeared in the area with consequent reduction in the numbers and economic potential of cattle in the area. Despite these losses, the epidemiology of the disease in this area is poorly understood.

A longitudinal cluster survey was carried out in 2008 to determine the prevalence of trypanosomiasis in cattle using molecular methods. The results of molecular analysis and the effects of risk factors such as altitude, land use patterns and seasonal migrations will be discussed.

CS48.3

Evaluation of Famacha Cards as a Penside Diagnostic Test for African Animal Trypanosomiasis

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African animal trypanosomiasis is one of the most important livestock diseases in Sub-Saharan Africa, causing 3 – 7 million cattle deaths a year and millions of dollars in lost income. This situation is compounded by the lack of accurate diagnosis in the resource poor, rural areas where the disease causes the most damage. Thus, poor diagnosis often leads to non treatment of affected animals or unnecessary treatment of unaffected animals – wasting resources and promoting drug resistance. There is great need for a simple, cheap, reliable penside diagnostic test to combat AAT where it matters most.

Anaemia is one of the most consistent signs of the disease and is often used as a reliable indicator of infection. However, methods for penside evaluation of anaemia are also limited. To tackle the problem of unnecessary treatments for Haemonchus contortus in South Africa, the FAMACHA© card was developed for assessment of anaemia in sheep by comparing ocular mucous membranes to a colour scale. If this method could be successfully applied to trypanosomiasis, it would help to improve diagnosis and reduce the number of unnecessary treatments.

In this study, anaemia in cattle was assessed using both FAMACHA and Hemocue 201 haemoglobinometer and results compared to assess the sensitivity. Famacha results are also compared with molecular diagnosis of trypanosomiasis to assess the specificity of FAMACHA© as a diagnostic tool for this disease.

CS48.4

A Real Time PCR Assay to Quantify and Estimate Mixed Infection Load of Glossina Species with Trypanosoma congolense savannah and Trypanosoma brucei brucei

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Field studies have reported the presence of mixed trypanosome infections within tsetse flies. In the field, these infections may arise from feeding on hosts who are carriers of a mixed infection or by sequential feeding on several differently infected animals. In the laboratory, acquisition of these parasites can be achieved through sequential and/or simultaneous feeding. To date, the level of parasites within these established mixed populations within the midgut of the tsetse fly has not been investigated. In a series of experiments we used quantitative PCR (qPCR) to look at the populations of Trypanosoma brucei brucei and T. congolense. savannah present in the midguts of Glossina morsitans moristans (15 days post infection). Differing infective doses (one-5 x104 parasites/ blood meal) were used to initiate infection (both simultaneously and sequentially) in both single and mixed infections.

The outcome of these experiments will be discussed, with particular attention to the possibility of competition between these two species of trypanosome The efficiency of this experimental approach in terms of sensitivity and specificity were also evaluated and demonstrate that qPCR is an effective method of quantifying trypanosome load within the insect vector and may be applicable to similar studies in the vertebrate host

CS48.5

Low Host Specificity in Trypanosomes Associated with Native Australian Wildlife

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The non-pathogenic trypanosomes are often cited as having had a long evolutionary history with their vertebrate hosts. Host specificity has developed in some species, which are mow unable to infect or become established in alternative hosts. Here we report on an investigation into the diversity of trypanosomes associated with native wildlife in Western Australia based on divergence of the 18S rRNA gene, and show that the degree of host specificity appears to be low, with multiple genotypes present within some host species, and single genotypes appearing within multiple divergent host species. The evolutionary processes responsible for the

apparent gregariousness of Australian trypanosomes are not known, and further work is required to generate a more complete picture, but it appears that a significant degree of ecological host switching has occurred, and that closely related genotypes have evolved to infect multiple hosts within geographically distinct areas.

CS48.6

Market Cattle Movements and Risk of Spread of T. b. rhodesiense to Northern Uganda

Selby, Richard Jamaes[1]; Amongi Acup, Christine[2]; Agwai, Benard[2]; Von Wissman, Beatrix[1]; Picozzi, Kim[1]; Waiswa, Charles[2]; Kabasa, John David[2]; Eisler, Mark[1]; Welburn, Sue[1]

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The link between the spread of acute Human African Trypanosomiasis (caused by the zoonotic parasite Trypanosoma brucei rhodesiense) and cattle movement gave rise to the strengthening of existing Ugandan governmental policy, that cattle traded at market must be treated with trypanocidal drugs prior to movement. As the purchaser is responsible for the cost of treatment it is not unexpected that a significant proportion of animals may be moved without treatment.

With the national policy being complex to enforce at informal and formal markets there has to date been no study to quantify the numbers of cattle moving from districts known to have cases of zoonotic sleeping sickness (T.b. rhodesiense) in either man or animals to areas believed to be free from infection. Within Uganda civil unrest has resulted in the decimation of the cattle population within the northern regions, Since December 2006, government and rebel forces have been engaged in ongoing peace talks. Increasing political stability has resulted in displaced peoples returning to their homelands to farm. Since regions of Uganda endemic for T. b. rhodesiense were largely unaffected by the recent conflict it follows that cattle from these regions are in demand to supply the demand for cattle restocking in the North.

This study assesses the prevalence of T. b. rhodesiense within the cattle population at ten of the largest cattle markets engaged in trading cattle northwards and the Northwards traffic of cattle from these markets over the previous two years and examines the potential for T. b. rhodesiense to spread into previously unaffected districts of Uganda.

CS49 - Drug Receptors and Resistance

Thursday, August, 13, 2009

CS49.1

The Response of Teladorsagia circumcincta to Ivermectin Exposure in Vitro - a Transcriptomic Analysis

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Anthelmintic resistance has a genetic basis - resistant worms produce resistant offspring. Recent research has focused on the molecular mechanisms underlying resistance with a view to identifying genetic markers for rapid detection. Historically, research has focused on the identification of single nucleotide polymorphisms (SNPs) or other mutations in the drug's target gene(s). However, with one or two exceptions and only with the benzimidazole anthelmintics, this has not been a fruitful exercise. Attention has focused more recently on the role of gene expression in the resistance phenotype. Candidate resistance genes include xenobiotic drug efflux pumps such as the p-glycoproteins and drug handling systems such as the cytochrome P450s. These have been shown, in some cases, to display altered expression between susceptible and resistant isolates, which may be either constitutive or inducible by exposure to anthelmintic. In the present study, we have adopted a more global approach to gene expression analysis. We have exposed a triple-resistant isolate of Teladorsagia circumcincta to ivermectin in vitro and examined its transcriptomic response using Roche454 sequence analysis. A total of 98,686 sequences averaging 250bp were obtained; 43,344 from the untreated control and 55,341 from the ivermectin exposed sample. Initial assembly clustered the raw data into 1,659 and 2,049 contigs, respectively, the largest being 3,735bp in length. Bioinformatic analysis is underway to rank contig abundance, provide BLAST identities, subtract "noise" and compare the digital expression levels of the respective contigs. It is hoped this exercise will provide an unbiased analysis of the response of T.circumcincta to drug exposure. Furthermore, it should provide further insight into the molecular mechanisms underlying drug resistance and help identify some potential new leads for its detection.

CS49.2

The Cytochrome P450 Family in the Parasitic Nematode*Haemonchus contortus*

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Infection by parasitic nematodes is one of the most serious health problems of grazing livestock worldwide and resistance to the anthelmintics necessary for their control is becoming widespread.

Cytochrome P450s (CYPs) are a family of drug-metabolising enzymes, present in many organisms; a CYP is responsible for multi-drug resistance in *Drosophila melanogaster* and metabolises anthelmintics in humans. The role of CYPs in drug metabolism by nematodes is unclear. The aim of this project is to characterise the CYP gene family in *Haemonchus contortus* with a view to investigating roles in drug resistance.

We have annotated supercontigs containing 95 fragments of partial gene sequence and are using these as tags to assay gene expression by real-time PCR. 68 out of the 95 CYP tags were found to be constitutively expressed in L3 larvae or adults. Expression levels of the CYP tags have been compared between life cycle stages, sexes, tissues, and after anthelmintic and other drug-exposures, using both susceptible and resistant isolates.

We are also applying new massively parallel sequencing technologies to assay the *Haemonchus contortus* transcriptome at the whole genome level, in order to confirm our CYP annotations, assemble full length genes, and compare CYP expression. It will also provide a more global approach to investigate drug-associated changes in gene expression in the parasite.

CS49.3

Structural Analysis of ACR-23 and Potential AAD Binding

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Aminoacetonitrile derivatives (AADs) are novel anthelmintic drugs with potent activity against veterinary and human parasitic nematodes resistant to all currently available anthelmintics. AADs are thought to act on ligand-gated ion channels in the nematode specific DEG-3 subfamily of nicotinic acetylcholine receptors (nAChR). Resistance in Caenorhabditis elegans is associated with mutations in acr-23 but not the closely related acr-20 gene. Details of AAD binding are currently unknown. Our objective was to use homology modelling to identify structural differences between acr-20 and acr-23 that might elucidate AAD-protein interaction.

Homology models of the ligand-binding domains of acr-20 and acr-23 were constructed with MODELLER in Discovery Studio 2.0 using the mouse nAChR template (PDB:2QC1) and PROMALS3D multiple sequence alignment of the DEG-3 subfamily. Models with the lowest DOPE scores were further refined. Subunits were superimposed onto the pentameric scaffold of AChBP to model the fivefold radial symmetry of a homopentamer. Cavities were identified using the Binding Site tool in Discovery Studio. The largest cavity found on acr-23 was in close proximity to three substitutions that lead to AAD resistance. This cavity is present in ACR-20, but greatly reduced in volume. These results suggest that AADs may act by binding directly to ACR-23 and that identification of similar binding pockets on parasitic ion-channels could predict sensitivity to AADs.

CS49.4

Acetylcholine Receptor Subunit Genes from Ancylostoma Caninum: Altered Expression Patterns Associated with Pyrantel Resistance

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The molecular mechanism of resistance to nicotinic agonist anthelmintics such as pyrantel and levamisole in nematodes of medical and veterinary significance is poorly understood. The identification of pyrantel-resistant isolates of the canine hookworm, Ancylostoma caninum, provides an opportunity to explore, at a molecular level, the mechanism of cholinergic resistance in this species. We have cloned three A. caninum nicotinic acetylcholine receptor subunit genes orthologous to components of the pyrantel-sensitive nicotinic acetylcholine receptor in C. elegans, and have also obtained partial sequence for a further three subunit genes thought not to be constituents of the pyrantel-sensitive receptor. We have compared sequences and relative transcription levels for these genes in two A. caninum isolates showing high-or low-level resistance to pyrantel. While no polymorphisms of likely significance between the two isolates were observed, quantitative analysis of gene transcription levels revealed significantly lower expression of the three putative pyrantel receptor subunits (AAR-38, -63 and -29) in the highly pyrantel resistant isolate compared to the isolate showing low-level resistance. In contrast, expression of the three subunits thought not to constitute the pyrantel receptor (AAR-19, -15 and -8) were either not significantly different between the two isolates, or slightly higher in the highly-resistant isolate. This data suggests that reduced expression of the genes coding for nicotinic acetylcholine receptor subunits that form the pyrantel –sensitive receptors may be a component of the pyrantel resistance mechanism in A. caninum.

CS49.5

Study of the Beta-Tubulin Gen and The Resistance Against Macrocyclic Lactones in *Teladorsagia circumcincta*

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Up to date some markers were associated with resistance of nematodes to ivermectin (IV) in sheep. The relationship between SNPs in the beta-tubulin gen and the resistance against macrocyclic lactones (ML) have been described in different strains of Haemonchus contortus. In the present study, the possible SNPs at the codons 200, 198 and 167 in the beta-tubulin gen of field strains of Teladorsagia circumcincta were analyzed. A faecal egg count reduction test (FECRT) was conducted in 5 flocks to know the prevalence of resistance against IV. The results of the FERCT suggested that one flock was classified as resistant (90%), another one susceptible (98%) and the remaining were borderlines between resistance and susceptibility (93-94%). Third-stage larvae (L3) were recovered from pool cultures before and after the treatments. From each strain an average of 75 L3 were collected for two molecular tests. L3 of T. circumcincta were identified using specie-specific with ITS-2 primers. The PCR results revealed that between 48-79% of the L3 were T. circumcincta in pre-treatment isolates and between 3-85% in post-treatment isolates. Afterwards, the pyrosequencing assay was carried out in T. circumcincta larvae to detect the mutations in the beta-tubulin gene; however, in none L3 were tested the mutation in any position. The possible relationship between certain species of the field strains with the resistance phenomenon and/or the insufficient number of tested L3 could be factors related with the absence of mutations. Because of these results, more studies should be conducted in strains with higher levels of resistance.

CS49.6

LEVAMISOLE Resistance in Gastrointestinal Nematodes Investigated at the Molecular Level

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Levamisole is a broad-spectrum anthelmintic drug widely used to control parasitic nematodes in livestock. However, the high efficacy of levamisole against the gastrointestinal nematodes in sheep and goats has been compromised by the development of resistance in field populations. Levamisole acts as an agonist of the nicotinic acetylcholine receptor (nAChR) that is an important determinant of signal transmission at the neuromuscular junction. In the free-living nematode *Caenorhabditis elegans*, the levamisole-sensitive nAChR is composed of five multi-transmembrane spanning subunits encoded by *unc-29*, *lev-1*, *unc-63*, *unc-38* and *lev-8* genes and mutants lacking one of those genes are resistant to levamisole. Based on C. elegans molecular data, we have identified and sequenced *unc-29*, *lev-1*, *unc-63* and *unc-38* orthologs isolated from the trichostrongylid nematodes *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* that are causing major economic losses to sheep industry throughout the world. Phylogenetic relationship analyses of the nAChR subunit family indicated high evolutionary conservation among nematode species. To investigate molecular mechanisms involved in levamisole resistance, gene sequences and mRNA transcription levels were compared between levamisole resistant and susceptible *H. contortus*. Interestingly, expression of alternatively spliced mRNAs was specifically detected in resistant isolates and their functional relevance in levamisole resistance is in progress.

CS49.7

Genetic Polymorphisms in the Ancillary Proteins of Acetylcholine Receptors from *Haemonchus contortus* Associated with Levamisole Resistance

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Introduction: Gastrointestinal nematodes constitute a veterinary problem of great importance due to growing prevalence of anthelmintic resistance. Though more scarce than for other anthelmintic classes, emergence of resistance to levamisole in *Haemonchus contortus* compromises sustainable worm control in small ruminant flocks. Levamisole is a cholinergic agonist which activates the excitatory nicotinic acetylcholine receptors (nAChRs) present at the neuromuscular junctions of nematodes, resulting in spastic paralysis and death of worms at high concentrations. In the free-living nematode *Caenorhabditis elegans*, genetic screens for resistance to levamisole identified genes encoding five subunits (UNC-29, UNC-38, UNC-63, LEV-1, LEV-8) contributing to the heteromeric levamisole-sensitive nAChR and three ancillary proteins (RIC-3, UNC-50, UNC-74) required for the assembly and trafficking of the receptor. Here we describe the identification and characterization of the orthologs of *ric-3*, *unc-50*, and *unc-74* genes in the parasitic nematode *H. contortus*.

Methods: To investigate molecular mechanisms involved in *H. contortus* levamisole resistance, we performed semi-quantitative RT-PCR analysis to compare mRNA expression levels of *Hc-ric-3*, *Hc-unc-50*, and *Hc-unc-74* in three levamisole-resistant versus susceptible isolates. We also cloned and sequenced full length cDNAs to search for polymorphisms of interest.

Results: Sequence alignments showed that *Hc-ric-3*, *Hc-unc-50*, and *Hc-unc-74* genes are evolutionarily conserved. Changes in transcript levels as well as sequence polymorphisms in these ancillary factor genes were found to be associated with levamisole resistance.

Conclusion: These results represent a significant step towards reconstitution and functional investigations of the levamisole-sensitive nAChR receptor from *H. contortus*.

CS49.8

Investigation of Anthelmintic Metabolism by Nematodes Using the Model Organism *Caenorhabditis elegans*

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Resistance to anthelmintics used to treat parasitic nematodes of veterinary importance represents a serious welfare and economic problem for the livestock production industry. Research into the mechanisms by which parasites develop resistance is necessary to prolong the life of the available drugs and to minimise development of resistance to new classes. Metabolism of anthelmintic compounds by parasites is a possible mechanism of resistance that has received little research, despite there being precedence in the case of insecticide resistance. Due to the more advanced molecular tools available and comparative ease of manipulation, we have used the model nematode *Caenorhabditis elegans* to investigate this further. By using HPLC-MS techniques we have definitively shown that *C. elegans* is able to metabolise at least one class of anthelmintic compound. In addition, whole genome microarrays and real-time quantitative PCR has identified clusters of genes that are potentially involved in xenobiotic metabolism, which are significantly upregulated in the presence of anthelmintic. These include members of the cytochrome P450 family, glutathione-s-transferases and UDP-glucuronsyltransferases. Characterisation of the expression patterns of several of these genes of interest has been undertaken and their ability to confer anthelmintic resistance following overexpression is currently being investigated.

CS50 - Immunology

Thursday, August, 13, 2009

CS50.1

Porcine coccidiosis – Investigations on the Cellular Immune Response Against *Isospora Suis*

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Isospora suis is the causative agent of neonatal porcine coccidiosis, a frequent disease in suckling piglets. This infection leads to a massive damage of the epithelium in the jejunum and ileum, and subsequently to a reduced uptake of nutrients by the mucosal surface. Thus, the weight gain of piglets can be severely reduced and secondary infections may increase mortality. Despite its economic and veterinary importance, interactions between the parasite and the immune system of the host are still poorly understood.

To address these interactions in secondary infections, piglets were infected with *I. suis* on the third day of life and re-infected after six months. Thereafter lymphocytes were isolated from blood (PBMC), spleen and mesenteric lymph nodes (MLN) and analysed for their antigen-specific reactivity in vitro in IFN- ELISPOTs and proliferation assays.

Isospora-specific production of IFN- was detected in PBMC and splenocytes, proliferation only in lymphocytes derived from MLN. After MACS-depletion of distinct T-lymphocyte subpopulations $CD4^+$ T-helper cells and TCR- T cells but not cytotoxic T lymphocytes (CTL) were identified as antigen-specific IFN- producers among PBMC and splenocytes. In contrast, antigen-specific $CD8^+$ CTL seemed to represent the proliferating T-cell subset in the MLN.

Hence, an antigen-specific reaction of T cells could be demonstrated after secondary infections of pigs with *I. suis*.

CS50.2

Mucus and the Mucosal Response to Parasites: Lessons from Proteomics

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The mucus layer which lines the gastrointestinal tract protects the mucosal epithelia from such threats as pathogens

and pH extremes, but is finely tuned to permit the absorption of nutrients. Gastrointestinal helminth infection profoundly alters the nature of the mucus layer, becoming generally thicker and more viscous; a response which is believed to aid parasite expulsion. The gel forming properties of mucins in maintaining the mucus barrier are well known, but in general, the protein composition of mucus and the interactions of component proteins, are not well understood.

Our shotgun proteomic studies of sheep abomasal mucus are in accordance with the small number of similar assays of mucus from other anatomical locations, in that it contains a mixture of epithelial / mucus cell secreted proteins, plasma derived proteins and other cellular derived proteins (such as structural, cytoplasmic and nuclear derived proteins). If we are to assume that this mixture of proteins represents the natural state of mucus, then in order to explain how parasite infection and the Th2 response affects mucus, we must first understand how these disparate mucus components combine and interact to produce the normal mucus layer.

For example, nuclear histones are consistently found in mucus, presumably derived mainly through degradation of sloughed epithelial cells. Histones are potently cytolytic and their proteolytic fragments are antimicrobial. Proteomic and immunohistochemical evidence will be presented, which begins to define the interactions between histones and mucus components. The relevance of these interactions in the response to parasite infection will be discussed.

CS50.3

Abomasal Foxp3+ T Cell Responses Following Primary and Secondary Infection with Teladorsagia circumcincta

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Teladorsagia circumcincta is the major cause of ovine parasitic gastroenteritis in temperate regions, including the UK. Sheep that have been previously exposed to infection develop a degree of immunity to the parasite. The exact mechanisms by which this immunity develops are not known, although local mucosal immune responses appear to be critical.

By analogy with helminth infections in other species T. circumcincta may induce suppressive regulatory T (Treg) cells, and the potential balance between effector and regulatory T cell populations in the abomasum may be a critical factor in determining the outcome of infection. Foxp3 is a key transcription factor required for generation and maintenance of Treg in rodents and humans, and is the most widely used marker for Treg in these species. In this study we cloned and expressed ovine Foxp3 for the purpose of developing assays to identify Foxp3+ T cells in sheep. Using an anti-human Foxp3 antibody that was shown to cross-react with ovine Foxp3, we have quantified abomasal Foxp3+ T cell in uninfected-worm free sheep, and at days 5 and 10 following challenge of naïve or immune sheep. Challenge of naïve sheep resulted in a significant increase in abomasal Foxp3+ T cells numbers 10 days post-challenge compared to uninfected controls ($P < 0.01$). In contrast, challenge of immune sheep did not result in any significant increase in abomasal Foxp3+ T cells. These results suggest that Treg responses to T. circumcincta infection may be different in naïve and immune sheep.

CS50.4

Effects of Ostertagia ostertagi on the Abomasal Mucus Barrier in Cattle

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Infections with gastrointestinal nematodes typically induce a Th2-type immune response that leads to rapid worm expulsion. At the time of expulsion, goblet cell hyperplasia and upregulation of mucins and/or altered mucin glycosylation have been observed and the rapidity of worm expulsion seems to be directly correlated with these phenomenons. In contrast, protective immunity against the cattle parasite Ostertagia ostertagi develops slowly and is incomplete, even after months of exposure. It is unclear how this abomasal parasite deviates or escapes from the immune system, but it is possible that the parasite achieves this by actively modifying the mucus layer. To investigate this hypothesis we have analysed both the quantitative and qualitative changes in the mucus layer before and after a primary infection. Semi-quantitative and quantitative reverse transcriptase (RT)-PCRs were used to analyse the transcriptional regulation of 10 different abomasal mucins, 3 trefoil factor family peptides, a glycosyl-transferase involved in mucin production and intelectin 2, a molecule which can alter the character of mucus. The outcome of this analysis indicated that an O. ostertagi infection causes significant alterations in the nature of the mucus layer. The major effects were seen on day 24 post-challenge infection with a 120-fold up-regulation of intelectin 2 ($p<0.05$) and a 7-fold downregulation of MUC5 ($p<0.05$). This MUC5 gene is the major secreted mucin component of the abomasal mucus layer. The transcriptional downregulation of this gene could severely impede the protective role of the mucus barrier.

CS50.5

Differential Cellular Responses Between Two Local Canarian Island Sheep Breeds Are Associated with a Differential Ability to Resist *Haemonchus contortus* Parasite Infection

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One of the main restrictions on increased global sheep productivity is the susceptibility of these animals to gastrointestinal (gi) nematode infections. The rising incidence of resistance to chemical drenches makes the development of new ways to reduce the impact of parasite infection on productivity essential. The identification and selection of local breeds resistant to gi infection could help in breeding programmes and identification of novel resistance pathways for vaccine development. Recently, we have shown that a local sheep breed to the Canary Islands, the Canaria Hair Breed (CHB) sheep is resistant to infection with the gi nematode, *Haemonchus contortus*. Adult worm growth, establishment and female fecundity were all impaired (Gonzalez et al. 2008, Vet. Parasitol. 153, pg. 374). Interestingly, the data suggests that the protective mechanism in CHB sheep is directed primarily against adult parasites, suggesting a novel resistant mechanism in this sheep breed. Here, we present detailed comparative studies of local and systemic immune cellular changes in CHB and CS sheep infected with *H. contortus* over a period of 28 days. Results presented here demonstrate that both breeds of sheep develop different immune responses and select cellular responses are correlated with the development of greater resistance of CHB sheep to adult *H. contortus* infection.

CS50.6

Local Cytokine Expression During *Dictyocaulus viviparus* Infection in Cattle

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The cattle lungworm, *Dictyocaulus viviparus*, is a parasite of great importance when raising cattle on pasture. It causes parasitic bronchitis that typically affects animals during their first grazing season. The project aimed to study local cytokine mRNA expression in bronchoalveolar lavage fluid (BALF) cells from experimentally infected calves, using quantitative real-time RT-PCR. The kind of cytokines elicited at primary encounter with an infectious agent determines the nature of ensuing specific immune responses and thereby determines if protective immunity is achieved. Three groups of calves were included in the study. Group 1 was infected days 1 and 70, group 2 was infected day 70 only and group 3 remained uninfected. Calves were inoculated orally with 500 *D. viviparus* third-stage larvae each. BALF samples were collected one week before infection and at weekly intervals for four weeks after infection. PCRs for bovine cytokines IL-2, IL-4, IL-5 and IL-10 were established as well as for a T-cell specific (CD3) and general (GAPDH, -actin and cyclophilin A) house-keeping genes. Expression of all four cytokines was detectable and IL 10 showed the highest expression. Preliminary results, calculated by relating cytokine expression levels to the general house-keeping genes GAPDH and -actin, showed that most calves had an increased IL-4 mRNA expression after primary infection but not after re-infection. The level of expression of IL-2, IL-5 and IL-10 varied during the experiment and seemed unrelated to the parasite infection. These results suggest a role for locally induced IL-4 in the instigation of immunity to *D. viviparus* in naïve animals.

CS50.7

Humoral and Cellular Immune Response and Transplacental Transmission in Cows Experimentally Infected with Neospora caninum NC-6 Argentina Strain

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Neospora caninum infection is a major cause of abortion in cattle. The objective of this study was to evaluate the humoral and cellular immune response in cattle during an experimental infection with N. caninum NC-6 Argentina strain and the likelihood of transplacental transmission. Pregnant cows (65 days of gestation) seropositive (SP) and seronegative (SN) to N. caninum were inoculated intravenously with 1 x 108 tachyzoites of NC-6 Argentina strain or PBS as negative control and slaughtered 40 days post-inoculation. Sera were analyzed for N. caninum antibodies by indirect fluorescent antibody test and antibody titer geometric means were analyzed by ANOVA. Blood samples were stimulated in vitro to evaluate gamma interferon (IFN) production. Tissues from dams and fetuses were analyzed by PCR for N. caninum DNA. Inoculated animals significantly increased N. caninum antibody titers and IFN production respect to controls. One

SP cow aborted a SP fetus, and the remaining fetuses were viable. All viable fetuses had histopathologic lesions. The PCR was positive in all fetuses from SN cows and in 2/3 fetuses from SP cows. Neospora caninum DNA characterization is currently underway. No N. caninum DNA was detected in cow brains. This is the first report of experimental infection of cows with a N. caninum isolate from Argentina, and its consequent humoral and cellular immune response and transplacental transmission of the parasite.

CS50.8

Response of CD4 and CD25 Positive T-Cells During Infection in Sheep Selected for Either Resistance or Resilience to Gastrointestinal Parasites

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The immune response in sheep to gastrointestinal parasites incurs a considerable nutritional penalty. The induction of mucosal tolerance to gastrointestinal parasites in sheep may provide a mechanism through which this nutritional penalty can be reduced. Mucosal tolerance is regulated by the production of $CD4^+$ and $CD25^+$ regulatory T-cells (T-reg). However, little information is available on the T-reg cell responses in sheep. This study examined the development of $CD4^+$ and $CD25^+$ cell populations during the course of infection in lines of animals selected for either resistance or resilience to gastrointestinal parasites. Twenty animals were allocated to one of four treatments (n=5) in a 2x2 design with factors being either breed (resistant (RT) or resilient (RL)) or infection (infected with *Trichostrongylus colubriformis* (I) or not infected (N)). Measurement of $CD4^+$ and $CD25^+$ cell proportions in white blood cells (WBC) using flow cytometry was performed every two weeks for 16 weeks. Proportions of WBC's that were $CD4^+$ were consistently greater in RL animals ($P=0.008$) and were not influenced by infection ($P=0.56$). Proportions of $CD4^+$ WBC's that were $CD25^+$ were consistently 30% greater in RTN than RLN animals ($P<0.001$). Compared to their uninfected controls, infection increased the proportion of $CD4^+$ cells that were also $CD25^+$ from day 34 of infection by 32% and 5% in RTI and RLI, respectively, ($P=0.021$). The results indicate differences in the T-cell populations between lines of animals that have been selected for either resistance or resilience to gastrointestinal nematodes which may have implications for the induction of mucosal tolerance.

CS51 - Epidemiology

Thursday, August, 13, 2009

CS51.1

Examinations in Endoparasite Prevalences in Dogs and Cats in Animal Shelters in Lower-Saxony Germany and Investigations of Anthelmintic Resistance

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In 2006 and 2007 a total of 445 dogs and 837 cats from 26 animal shelters in the state of Lower Saxony in Germany were examined on endoparasite occurrence after fecal sample and flotation examination. For 341 dogs and 584 cats additionally an Idexx SNAP® Giardia Test was used for detection of Giardia-coproantigen.

In general, 9.4 % of dogs and 33.6 % of cats showed endoparasites. For dogs, the following species could be detected: Toxocara canis in 4.0 %, Isospora spp. in 2.5 %, Giardia spp., Trichuris vulpis and hookworms in 0.9 % each, Capillaria spp. in 0.4 %, Toxascaris leonina and Hammondia-like oocysts in 0.2 % each. For cats, stages were found coproscopically with following frequencies: Toxocara cati in 27.1 %, Isospora spp. in 7.5 %, Capillaria spp. in 5.0 %, taeniid cestodes in 2.0 %, hookworms in 1.1 %, Giardia sp. in 0.7 %, Aelurostrongylus abstrusus in 1.0 % and Toxoplasma-like oocysts in 0.1 %. Cats and dogs of one year or lower showed significantly more frequently endoparasites in the faeces than older animals. Toxocara cati and Isospora spp. were significantly more frequent in younger cats and dogs than in older animals. The Giardia-coproantigen test revealed 11.4 % of dog and 6.8 % of cat samples positive.

The Egg Count Reduction Test was performed with fenbendazole, pyrantel and milbemycinoxime. However, the low case numbers limit the interpretation. In general, no lack of efficacy was observed for the 3 compounds tested

CS51.2

Distribution and Characterization of Heterobilharzia americana Infections in the Dog in Texas

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The trematode, *Heterobilharzia americana*, has been reported in a wide range of wildlife definitive hosts across the southeastern United States. Limited data are available on the prevalence of this schistosome parasite in canine hosts. Reviewing the parasitologic and biopsy/necropsy histopathology records from the Texas Veterinary Medical Diagnostic Laboratory and from the Texas A&M University College of Veterinary Medicine from 2003 - 2007, a surprising number of 127 canine cases were identified. Using a fecal sedimentation diagnostic test, 120 dogs were positive for the distinctive *H. americana* eggs. These results probably underestimate the true canine infection rate, since this fecal method is rarely used on a routine basis on dog samples. Based on microscopic examination of post mortem or biopsy materials, 14 clinical cases were identified, including 7 cases with both fecal and histopathological positive results. The clinical histories of these dogs ranged from asymptomatic to acute, unexpected deaths. The geospatial locations of these cases were analyzed by county using ARCMap software. Twenty-five of 254 Texas counties had at least one infected dog. There was distinct clustering of cases along the Gulf coast and in association with the major urban populations in the state. These data indicate that canine infection with *H. americana* is not a rare occurrence, nor is it geographically limited to the Gulf coast region within the state. The information derived from this study may be applicable to adjacent geographic regions across the southern USA.

CS51.3

A Survey on *Dictyocaulus Viviparus* Antibodies in Bulk Milk of Dairy Herds in Northern Germany

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Dictyocaulosis, caused by the bovine lungworm *Dictyocaulus viviparus*, is a parasitic disease of cattle occurring worldwide in temperate areas

The parasite is predominantly found in calves and heifers during their first grazing season and exposure induces a protective immunity. To maintain this immunity, which lasts for 6-12 month, 'booster' infections are necessary from year to year. Therefore, even cows can suffer from lungworms either if they become infected for the first time on pasture as adults of if they have lost immunity due to the absence or scarcity of infective lungworm larvae during previous grazing seasons.

The aim of this study was to determine the prevalence of dictyocaulosis in dairy herds in the north-western part of Germany and to evaluate epidemiological factors that may influence the seroprevalance of the disease in this area.

Nearly 900 bulk milk samples were collected from different farms in January, September, and November 2006 and 2008. These samples were tested for antibodies against *D. viviparus* by a milk-ELISA based on recombinant major sperm protein as antigen.

In January 2006 and 2008, 19.8 % and 12.8 % of dairy farms, respectively, were positive for *D. viviparus* antibodies. The bulk milk samples collected in September and November 2006 revealed 37.2 % and 42.1 % positive dairy herds in contrast to only 6.9 % and 6.6 % in the same months in 2008.

CS51.4

Geographic Information System Surveillance of Fasciolosis in Cattle During 2003 to 2008 in Southern Brazil

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Fasciolosis is an endemic disease in some parts of the world, reducing productivity, fertility, use of cattle by-products in slaughterhouses (livers), as well as increasing the costs of treatment and, in severe cases, death of the animals. The disease affects ruminants but there is a growing concern of zoonotic Fasciolosis. Currently there is a shortage of information about the epidemiology of this disease in Brazil. Geoprocessing information may help to demonstrate and monitor the epidemiological situation of endemic areas in southern part of Brazil, a region that has more than 25 million heads of cattle. The objective of this work was to correlate data from slaughterhouses on the detection of Fasciola hepatica in the liver of cattle with climate data in the Southern States (Paraná, PR; Santa Catarian, SC; and Rio Grande do Sul, RS) of Brazil. The data was organized by city and month for six years (2003-2008) to construct an epidemiological GIS map of the disease. The data were obtained from the Federal Inspection System. RS presented the highest average of infected livers (18.7%) followed by SC (10.1%) and PR (0.7%). It is concluded that the animals in the regions of Campanha (RS) and the coast of SC have the highest incidence of the disease (above 20%) and the appropriate climate to favor the F. hepatica life cycle. This will serve to develop a new set of strategies to implement a proper parasite control.

CS51.5

North American Pathogenic Giant Liver Fluke (Fascioloides magna) Is Spreading in Central Europe

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Fascioloides magna was introduced into Europe with imported game animals (Natural Park La Mandria near Turin, Italy) in the second half of the 19th century. Since then *F. magna* has been spreading in Europe and recently several enzootic areas with prevalence up to 95 % have been established in Czech Republic, Slovakia, Austria, Hungary and Croatia. The common definitive hosts of *F. magna* in Europe are cervids; red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and roe deer (*Capreolus capreolus*), but domestic ruminants, such as cattle (dead-end hosts), sheep and goats (aberrant hosts), can also be infected when pastures are shared with cervids. For definitive and dead-end hosts fascioloidosis is accompanied by significant pathological changes of liver and other organs or tissues, leading to decrease of fitness (e.g. body weight). For the aberrant hosts the infection is usually fatal. Therefore a reliable diagnostic method especially for domestic ruminants is required.

We employed the enzyme-linked immunosorbent assay (ELISA) and immunoblot to determine serum antibody response of goats experimentally infected with *F. magna* and related species Fasciola hepatica as a comparative model. The cross-reaction of serum antibodies against antigens from *F. magna* and *F. hepatica* excretory-secretory products (ESP) was recorded by ELISA, whereas two dimensional immunoblot analysis revealed species-specific proteins in ESP with no cross-reaction. These proteins (possibly covering cysteine peptidase - cathepsin L) could be used as potential immunodiagnostic markers.

CS51.6

Epidemiology of Warble Fly in Wild Deer in Scotland and England

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This study was designed to investigate the epidemiology of warble fly infestation in wild deer in Scotland and England in collaboration with the Forestry Commission, a commercial game dealer, Dr. Doug Colwell (Lethbridge, Canada) and Professor Domenico Otranto (Bari, Italy). The carcasses (n=569) and hides (n=2651) of deer culled between March 2005 and September 2008 were examined for warble larvae and scar tissue, respectively. Larvae were recovered from Scottish deer only and identified on morphological and molecular grounds as *Hypoderma diana*; no *H. bovis* or *lineatum* were identified. The prevalence of infection based on the recovery of warble larvae during the spring was 10.4% in red deer (n=557; mean intensity (± SEM) 6.7 (± 1.0), range 1-36) and 25% in roe deer (n=12; mean intensity (± SEM) 1.7 (± 0.3), range 1-2). The prevalence of infection based on the identification of scars in hides examined during the spring and autumn was 26.9% in red deer (n=1374; mean intensity (± SEM) 10.4 (± 0.5), range 1-191); the hides of other deer species (n=844; roe, sika, Pere David's, fallow and muntjac) from Scotland were unaffected. New and old warble scars seen in the spring suggested that scars associated with the emergence of the previous year's larvae remained visible for ≥1 year. Infected deer were distributed largely in northern and western Scotland. Hides from 433 deer (roe, fallow, muntjac, red, Chinese water deer, sika) in England showed no evidence of infection. Funding was provided by Merial Animal Health and the Royal Veterinary College.

CS51.7

Mange in Alpacas, Llamas and Goats in the UK: Incidence and Risk

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A retrospective postal questionnaire was used to obtain information about the prevalence of mange and its association with husbandry-related risk factors, in alpaca, llama and goat herds in the UK. In total 1797 questionnaires were sent out to members of the British Alpaca Society, the British Llama Society and the British Goat Society, giving response rates of 40.4%, 29.3% and 22.8% from the three groups, respectively. Between January and December 2007, mange was reported in 52.2% (151 of 292), 14% (9 of 66) and 21% (41 of 194) alpaca, llama and goat herds, respectively. However, only 37-51% of the farmers had their diagnosis of mange confirmed by a veterinarian or animal health laboratory. In herds where the causal agent was confirmed: psoroptic, sarcoptic, chorioptic and mixed infections were all reported, with chorioptic mange reported most frequently. Risk analysis showed that the prevalence of mange in alpacas was significantly associated with herd size and the country from which the animals were imported. Alpaca farmers who had larger herds were more likely to report mange and farmers who imported their animals from Peru were 1.5 times more likely to report mange than farmers who imported animals from elsewhere or who did not import. There was no significant confounding between these two risk factors. The results show that mange continues to be a major problem for camelids and goats in the UK, and suggests that inadequate control or quarantine is a major factor in allowing infested alpacas to be imported.

CS51.8

Clinical Paragonimiasis in Southeastern Nigeria: Social Structures and Demographic Epistemology

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Paragonimiasis is a neglected but emerging zoonotic parasitic infection in Nigeria. Structured questionnaire was administered in six selected communities in eastern Nigeria, Bende, Ohafia, Choba, Emohua, Oyigbo and Odukpani where crab-consumption have been observed to be significant, as a prelude to a large-scale epidemiological study. In all, 68.8% of those interviewed eat crabs, mostly from indigenous rural people from coastal villages and villages from river basins. There was a possible relationship between crab consumption and mother's educational level, income level, awareness of epidemiological implications of eating crabs; but there was no association between consumption of crabs and sex, age, religious beliefs, nor occupation although fishermen and farmers presented important clusters of eaters. Mother's educational level was perhaps the central factor in Paragonimiasis epidemiology in the region. Crab consumption increased with age. Sudanautes africanus infectivity rate was 16.4%. Those that consume crabs reported of various symptoms of Paragonimiasis such as cough (77.6%), haemoptysis (14.2%), chest pain (43.3%), epilepsy (3.7%) and headache (71.2%). These symptoms were discussed in relation to demographic and social structures.

CS52 - Strategic Control

Thursday, August, 13, 2009

CS52.1

Correlation of Famacha© Anaemia Scoring with *Haemonchus contortus* Infections in Swiss Goat Flocks

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Introduction: FAMACHA© is a useful system developed for clinical evaluation of anaemia in ruminants, competent of detecting infections with blood-sucking *Haemonchus sp* in sub-Saharan Africa. The present study aimed to evaluate the accuracy of FAMACHA© for goat flocks in Switzerland. Previous studies verified the correlation of FAMACHA©-categories and the packed cell volume (PCV). Additionally, we investigated the correlation of FAMACHA©-scores and faecal egg counts (FEC).

Methods: The system determines the degree of anaemia by scoring the colour of the eye mucosa from category 1 (red = non-anaemic) to 5 (white = highly-anaemic), based on the FAMACHA©-colour-chart. Goats from six farms in Central Switzerland were scored for anaemia at four-week intervals, from May to October 2008. Simultaneously, PCV and FEC were individually ascertained. FEC, PCV and FAMACHA©-scores were statistically compared to evaluate the efficacy of FAMACHA© in detecting Haemonchus contortus infections.

Results: The FAMACHA©-scoring and PCV correlated significantly in all months of the study. The sensitivity of FAMACHA© in detecting anaemic goats was 86%, using the anaemia criteria cut-offs FAMACHA©-categories ≥3 and PCV <24%. The sensitivity of the method for detecting goats which needed a treatment was >76%, with regard to FEC of *Haemonchus contortus* (treatment cut-offs: FAMACHA ≥3 and FEC >300 epg or >600 epg). In addition, the use of FAMACHA categories ≥3, as a treatment indicator, revealed that 64% of the animals were recommended for treatment. These results indicate the suitability of FAMACHA© as a tool for a targeted selective anthelmintic treatment of goat flocks in Switzerland.

CS52.2

Production Consequences of Weight-Based Targeted Selective Treatment of Nematodes in Sheep

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Targeted selective treatment (TST), in which a proportion of the flock is left untreated to preserve anthelmintic-susceptible genotypes in refugia, are increasingly advocated. This approach relies on efficient indicators, applicable on commercial farms, to identify individuals that can be left untreated without fear of disease or production loss. Electronic identification and automated weighing technology enables use of short-term changes in weight gain as a workable indicator for TST, but its uptake will be highly dependent on the likely production penalty from leaving the fastest growing animals untreated. On a commercial flock in south-west UK, the weight gain of lambs of various breeds was tracked every one to four weeks in summer in 2007 (n = 508) and 2008 (n = 144), and a variable proportion of the fastest growing individuals that also appeared to be in good condition with little breech soiling was left untreated during whole-flock dosing in June, July and August. Between 10 and 25 % of lambs were selected for non-treatment on at least one occasion (c.f. 2-3 treatments of other lambs). Subsequent weight gain of untreated animals was not reduced relative to their peers over the whole grazing season. Faecal egg counts from

untreated individuals at the time of treatment did not differ significantly from those of the rest of the flock, showing that untreated animals contributed effectively to refugia. Results suggest that weight-gain based TST can be applied without production loss on intensive sheep farms in temperate areas. This work was funded by EU FP-6 STREP FOOD-CT-2005-022851-PARASOL.

CS52.3

Antioxidants as drug targets for the control of Haemonchus contortus

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Parasitic nematodes require antioxidant enzymes to combat the reactive oxygen species produced not only by their own metabolism but also by the host defence. This suggests the antioxidant enzymes may provide effective targets for parasite control. We have characterised the thioredoxin system of the economically important parasitic nematode, Haemonchus contortus. This consists of peroxiredoxins, which detoxify hydrogen peroxide, and thioredoxin and thioredoxin reductase which regenerate the active peroxiredoxin. Thioredoxin reductase is an evolutionarily diverse enzyme with two homologues identified in H. contortus, one of which contains a selenocysteine residue similar to that found in higher eukaryotes, while the other enzyme is significantly different. Two homologues of the peroxiredoxins have also been identified in H. contortus, both with high and specific activity in in vitro assays. The cytoplasmic peroxiredoxin is also highly antigenic and is secreted by the parasite, making it accessible to the host's immune system. Recent evidence suggests that parasite peroxiredoxins are involved in host-parasite interactions where they may regulate the host's immune system. Using the closely related model nematode Caenorhabditis elegans, we have investigated the role of these enzymes in nematode survival and response to drug treatment. The importance of these enzymes, their diversity and their accessibility to the host immune system, makes them prime candidates for drug and/or vaccine targets. Supported by a scholarship from Meat and Livestock Australia.

CS52.4

Performance of lambs in a Scottish field trial that were subjected to either a targeted treatment or targeted selective treatment anthelmintic regime

Greer, Andrew Walter[1, 2]; Kenyon, Fiona[2]; Bartley, David J.[2]; Burgess, Charlotte G.[2]; Donnan, Alison A.[2]; McBean, David[2]; Bartley, Yvonne[2]; Jackson, Frank[2]

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The performance of grazing lambs that were subjected to either a targeted treatment (TT) or targeted selective treatment (TST) anthelmintic regime was evaluated. TT animals received three whole-flock treatments administered at strategic times throughout the grazing season. TST animals were administered anthelmintic on an individual basis according to need as determined by a decision support model which predicted animal performance. Treatment regimes were compared to both a neo-suppressive treatment (NST) in which a whole-flock treatment was administered every four weeks and a metaphylactic/therapeutic treatment (MT) which consisted of a whole flock treatment administered when clinical signs of parasitism were evident. The results for two consecutive grazing seasons are presented. The mean number of treatments per individual during 2007 was 3.0, 2.6, 5.0 and 2.0 and during 2008 was 3.0, 1.9, 5.0 and 2.0 for TT, TST, NST and MT groups, respectively. Mean liveweight gain over the two years was similar for TT, TST and NST, being 146 s.e. 2.4, 147 s.e. 3.2 and 148 s.e. 3.5 g.d-1, but was reduced in the MT animals, being 126 s.e. 2.8 g.d-1 ($P<0.001$). Both TT and TST treatment regimes appear to offer a means of reducing anthelmintic usage with a minimal cost to animal performance, providing sufficient parasitological knowledge is available to determine a suitable TT regime. Furthermore, variation in the number of treatments required for individuals in the TST regime suggested this approach may provide information that will aid the selection of animals that are less reliant on anthelmintic intervention.

CS52.5

Serum Pepsinogen Levels to Monitor Gastrointestinal Nematode Infections in Cattle Revisited

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In response to the increasing concerns on the development of anthelmintic resistance, targeted and selective anthelmintic treatment methods are promoted. Serum pepsinogen levels are traditionally considered as a suitable parameter to

evaluate levels of infection with gastrointestinal nematodes in first-season grazing (FSG) calves, but has not become widely established tool in veterinary diagnostic labs. The objectives of this study were to revisit serum pepsinogen concentration as diagnostic parameter. First, we investigated the reproducibility of the pepsinogen assay between different labs. Next, a field survey was performed including 669 farms. Based on the observed infection levels, the variations in pepsinogen levels between animals and herds and optimal sample size were calculated. On a subset of herds, advice was given to the farmer and veterinarian based on pepsinogen levels of FSG calves at housing on the applied control strategy in the next generation of FSG calves. We observed a poor reproducibility of the assay between different labs. Of 82 herds that were followed-up, 32 herds could be adviced to reduce chemoprophylaxis in the next generation of FSG calves. This resulted in a significant decrease in number of anthelmintic treatments and slightly increased infection levels in the next generation of calves. We conclude that the serum pepsinogen level is a suitable parameter to monitor GI nematode infections in FSG calves supporting target anthelmintic treatments to the most infected herds, but more efforts are needed to standardize the assay among veterinary labs.

CS52.6

Targeted Selective Treatments Maintain Susceptibility to Ivermectin Treatment in Lambs in a Scottish Field Trial

Kenyon, Fiona[1]; Greer, Andrew W.[1, 2]; Bartley, David J.[1]; Burgess, Charlotte G.[1]; Donnan, Alison A.[1]; McBean, David W.[1]; Bartley, Yvonne[1]; Jackson, Frank[1]

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A replicated field study has been conducted in lambs over 3 years to compare the effects of different ivermectin treatment strategies on the development of anthelmintic resistance in gastrointestinal parasites. A targeted selective treatment (TST) regime; where individual animals were selected for treatment on the basis of need, was evaluated against targeted treatments (TT); whole group treatments at strategically appropriate times, neo-suppressive treatments (NST); whole group treatment every 4 weeks and metaphylactic/therapeutic treatments (MT); whole group treatments given upon the appearance of clinical signs of disease in some individuals. Early and late season worm burdens were obtained from tracer lambs each year. Results show that the NST group consistently had the lowest mean worm burdens (mean 11,720) followed by the TST (mean 23,479), TT (mean 24,841) and MT (mean 26,950). Faecal samples were collected every two weeks for faecal egg count (FEC) analysis and faecal egg count reduction tests (FECRT) were conducted after every anthelmintic treatment. For every treatment given to an animal in the TST group, 2.08, 1.25 and 0.96 treatments were given to the NST, TT and MT groups respectively. FECRT results reveal that the mean efficacy was maintained in the TST, TT and MT groups, with efficacies of 95%, 94.7% and 99.2% respectively, whereas mean efficacy reduced each year in the NST group to a low of 79% in 2008. Implementation of either TST or TT approaches can help to slow the rate of development of anthelmintic resistance and provide options for the sustainable use of anthelmintics.

CS52.7

Random Anthelmintic Treatments in Sheep: Targeted Selective Treatment for the Dummies

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Targeted selective anthelmintic treatments are an alternative for sustainable use of available drugs against gastrointestinal strongyles. We treated monthly 20% of the flock at random-based on previous modelling investigations. The lambs were grazed on pasture from April to October. The paddocks were contaminated with Haemonchus contortus, Teladorsagia circumcincta, Trichostrongylus colubriformis and Trichostrongylus axei that were partly resistant to benzimidazoles (approximately 50% efficacy based on faecal egg reduction test). Ivermectin was used since the gastrointestinal nematodes were susceptible to this drug. The random targeted selective treatment (RTST) on lambs grazed two paddocks was compared to a monthly treatment (MT)on lambs grazed on two paddocks. The parasitic infection in november (e.g. at the end of the grazing season) was higher in RTST than in MT, based either on EPG (520 RTST vs 210 monthly treated) or worm counts at necropsy (total worms 2700 vs 1500, significant at $p<0.05$). There was a significant decrease in T.colubriformis and increase of Te. circumcincta in monthly treated lambs. Efficacy of benzimidazole evaluated on FECR test was 80% and 40% on worm counts) in RTST and significantly lower in MT. This indicates a positive effect on the efficacy of the benzimidazoles possibly due to modification of helminth fauna. The weight of lambs at the end of grazing season were 39 in RTST vs 38.5 kg in MT, and the carcasses weighted 16.6 kg RTST vs 17.6 in MT, the differences being nonsignificant. Thus random treatment of lambs appears to be equivalent to monthly "suppressive" treatments, at least in medium intensity infection.

CS52.8

A Participatory Approach for Small-Scale Caribbean Farmers in Goat Health Research and Extension

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Studies in KwaZulu-Natal Province, South Africa indicated that small-scale farmers considered diarrhoea, gastro-intestinal helminth infection and poor reproductive performance to be major problems in their goats. The situation was exacerbated by a lack of information on goat health and management. In order to facilitate acquisition of the skills and knowledge necessary to improve goat management and address the major problem of Haemonchus spp. infection, a collaborative project was developed with the farmers. The methodology involved participation of the farmers in on-farm experimentation and in the development of a "Goatkeepers' Animal Health Care Manual". The process nurtured "champions" in the local farming community who acted as important role models and advisors for other farmers. Several areas for future collaboration were identified, including assisting the farmers to develop their analytical and record-keeping skills. The efficacy of the project was enhanced by an interdisciplinary approach involving staff from universities, research Institutes and extension practitioners collaborating with the farming community. This approach is applicable to other similar small-scale farming systems, including those in the Caribbean. There is a paucity of knowledge of the epidemiology of livestock helminths in the Caribbean. However pilot studies on Grenada, Montserrat, Nevis and Dominica indicated that Haemonchus is the predominant nematode genus in sheep, goats and cattle. An overview of the participatory process is thus presented as a stimulus for discussion of approaches to optimise goat health research and extension in the Caribbean.

CS53 - Arctic Symposium

Thursday, August, 13, 2009

CS53.1

Parasitic Zoonoses in the Arctic

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Zoonoses, or diseases passed between vertebrate animals and humans, can pose risk to human health and are varied in nature. The epidemiology of zoonoses is in large part determined by the relationships between animals and humans within their shared environment. As climates change throughout the world, they are expected to impact the epidemiology of parasitic diseases via changes in the biotic and abiotic elements of ecosystems. Arctic ecosystems are likely to be particularly vulnerable to these impacts of climate change, and may also serve as sentinels for other parts of the world. Now more than ever, quality research in Arctic parasitology is essential to gain vital baseline data, understand and investigate changes in the epidemiology of parasitic diseases, and to control negative impacts on human and animal health.

In this session, we bring together researchers representing a wide array of disciplines to showcase several research programs in arctic parasitology and to discuss avenues to improve future research endeavours. This session will provide the opportunity to demonstrate the importance of an ecosystem approach to investigating disease in Arctic populations, how to avoid common biases in Arctic wildlife research, and how molecular epidemiological tools may be used to improve our understanding of Arctic parasitology.

CS54 - Giardia and Cryptosporidium Symposium

Thursday, August, 13, 2009

CS54.1

Giardia and Cryptosporidium Symposium

Olson, Merle E.[1]; Buret, Andre[2]; Ralston, Brenda[3]; O'Handley, Ryan[4]; Dixon, Brent[5]; Thompson, R.C. Andrew[4]

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Leading Giardia and Cryptosporidium researchers will review the current state of knowledge of Giardia and Cryptosporidium infection in domestic and wild animals. They will focus on the most recent discoveries from their laboratories as well as others. The topics that will be covered will include 1) How Giardia and Cryptosporidium Cause Clinical Signs; 2) Impact of Giardiasis and Cryptosporidiosis on performance in Food animals; 3) Treatment and prevention of Giardiasis and Cryptosporidium in Animals; 4) Identification of Giardia and Cryptosporidium in animals and the environment 5) Zoonotic Significance of Giardia and Cryptosporidium.

CS55 - Non-pharma Control

Thursday, August, 13, 2009

CS55.1

Evaluation of Plantain (Plantango lanceolata) as Suitable Peri-Parturient Feed for Multiple Bearing Ewes

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Current NZ pasture systems (perennial ryegrass/white clover) appear to be sub optimal in supply of metabolizable protein (MP) for lactating ewes and an increase in MP supply to lactating ewe is beneficial to ewe performance. The performance of ewes and their lambs grazing either plantain or perennial ryegrass was evaluated.

120 twin bearing ewes were randomly allocated to one of two pasture treatments, with 3 replicates and run for two years (2x3x2). Animals were set stocked on Perennial ryegrass or Plantain, from pre-lambing until weaning. Stocking rates were reviewed weekly to maintain pasture covers in year one whereas in year 2 all treatments were set stocked. All ewes were infected with 10,000 (year 1) and 30,000 (year 2) Teladorsadagia circumcincta larvae 7 days before parturition. FEC and liveweights of ewes and lambs were monitored weekly.

Increased levels of parasitism seen in year two, related to an increase in larval challenge given, resulting in 3x higher ewe FEC. Large differences seen in mean ewe liveweights between treatments where significant ($p<0.001$) across years. In both years Ewe FEC for Plantain treatment were significantly lower than ewes grazing Perennial ryegrass ($p=0.025$). Lamb growth rates of 302 and 362 g/d for Perennial and Plantain treatments respectively, were significantly different ($p<0.05$). FEC of Perennial lambs were lower than Plantain lambs ($P<0.05$) in year one but not in year 2. Differences in ewe and lamb performance could not be solely attributed to intake differences. Implications of the resultant pasture contamination and improved lamb growth will be discussed.

CS55.2

Kinetics of Capture and Infection of Infective Larvae of Trichostrongylides and Free-Living Nematodes panagrellus by Duddingtonia flagrans

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Duddingtonia flagrans, a nematophagous fungus, has been investigated during the last years as an agent for biological control of gastrointestinal nematodes of production animals. However, little is known concerning the kinetics of D. flagrans capture and infection on parasites. In this study these parameters were evaluated in the infective larvae (L3) of Trichostrongylides of sheep and free-living nematodes Panagrellus. The fungus was inoculated in plates containing an agar medium and the nematodes for 07 days. The interaction was monitored for 1, 3, 4, 5, 10, 15, 20 and 25h and the images were obtained using an optical microscopy. After 1h of L3 or Panagrellus sp. inoculation it was possible to observed the presence of traps and the capture of the nematodes. The nematodes were alive in the 1st and 3rd h while with 4 h, the infection and death of the Panagrellus occurred. Only with 15h of interaction the fungus invaded L3 and the region of penetration showed cellular modification with amorphous appearance and the presence of hyphae. In this time, the hyphae had filled the whole body of Panagrellus. The complete occupation of the body of L3 was with 20h of interaction. L3 was completely destroyed after 25h leaving only its cuticle. There were no specific sites in the cuticle of nematodes for hyphae adhesion and penetration. The process of capture, infection, death and degradation of nematodes by the fungus D. flagrans is completed in 25h in vitro.

CS55.3

Differences in the Response of the Motility and Exsheathment Process of *Haemonchus contortus* Larvae Exposed to Tropical Tannin Rich Sources

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Introduction: Tannin rich plants (TRP) are being tested for anthelmintic effects with different *in vitro* techniques. Differences in sensibility between techniques are unknown. The present trial compared the sensibility of the larval motility and exsheathment assays on *Haemonchus contortus* larvae exposed to tropical tannin rich sources.

Methods: Water/Acetonic extracts of *Acacia gaumeri*, *Brosimum alicastrum*, *Havardia albicans* and *Leucaena leucocephala* were used. Content of total phenols (TP), total tannins (TT) and condensed tannins (CT) were measured for each extract. Larval response to extracts was compared between the Larval Migration Inhibition (LMI) and the larval exsheathment assays with *H. contortus* L3 larvae. Increasing concentrations of lyophilized extracts were used (75, 150, 300, 600, 1200 µg/ml PBS). A General Lineal Model (GLM) test was used to determine the dose-effect in the LMI test or the difference in the percentage of exsheathed larvae between control and treated groups.

Results: The LMI test showed a dose-dependent anthelmintic effect for *H. albicans* ($P<0.001$) and *A. gaumeri* ($P<0.05$), the plants with the highest levels of TP, TT and CT, but not for *L. leucocephala* and *B. alicastrum*. In contrast, the exsheathment process was affected ($P<0.001$) by all doses of *H. albicans* and *A. gaumeri* extracts and a dose-dependent process was found for *B. alicastrum* and *L. leucocephala*.

Conclusions: Results showed that tropical TRP extracts are more potent inhibitors of the exsheathment process than the motility of *H. contortus* L3. This information underlines the need for standardization of the methodological procedures to evaluate anthelmintic properties of plants.

CS55.4

In vivo Anti-Coccidial Efficacy of Sainfoin Against Eimeria spp in Lambs

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The prospect of exploiting the antiparasitic effects of plants that contain plant secondary metabolites in order to provide an alternative to chemical treatments in grazing livestock has stimulated research in this area. Sainfoin (Onobrychis viciifolia) is a legume that contains a variety of such compounds as phenolic glucosides, flavonols, flavonol glycosides and condensed tannins. Several studies have investigated the effect of sainfoin against nematodes but information on coccidia remains scarce. The aim of this study was to screen the in vivo anticoccidial effect of sainfoin when fed as hay to naturally infected lambs. The trial took place on a farm where the presence of Eimeria among lambs was previously confirmed. Three groups of 12 lambs each were included assigned to either: group A (receiving sainfoin hay); group B (receiving lucerne hay and no further treatment) and group C (receiving lucerne hay and treated twice during the trial (week 1 and 5) with diclazuril at the recommended dose rate). The trial started at weaning of the animals and lasted for 2 months. During this period, at weekly intervals, faecal consistency scores and oocyst excretion (opg) were recorded. The results showed reductions in the mean opg number in group A compared to group B (i.e – 50.2%, - 47.7% and -60.3% 6, 7 and 8 weeks post weaning respectively). In contrast, oocyst excretion in group C increased significantly after both treatments being much higher at the end of the trial compared to group B (66.1%) possibly indicating that treatment obstructed host resistance appearance.

CS55.5

Anthelmintic Activity of Some Plants with Particular Reference to Their Condensed Tannin Content

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Anthelmintic activity of 32 plants was evaluated in vitro [adult motility assay (AMA), egg hatch test (EHT) and larval development assay (LDA)] and/or in vivo [fecal egg count reduction test (FECRT) in sheep]. The anthelmintic activity was exhibited by Butea monosperma, Calotropis procera, Camellia sinensis and Azadirachta indica in AMA, EHT and LDA, by Morus alba, Areca catechu, Eucalyptus camaldulensis and Artemisia brevifolia in EHT and LDA, by Zingiber officinale in LDA and AMA, by Melia azedarach and Fumaria parviflora in EHT and by Syzgium cumini, Mallotus philippinensis, Caesalpinia bonduc, Eremostachys vicaryi, Vernonia anthelmintica in AMA. The best in vivo anthelmintic activity against gastrointestinal nematodes of sheep based on reduction in fecal egg counts (EPG) was exhibited by crude methanol extracts of Cassia fistula [93.3%; day 15 post treatment (PT)] followed by Butea monospema (91.7%), Syzgium cuminii (88.5%), Terminalia arjuna (87.3%), Zyziphus nummularia (87%), Acacia nilotica (80.1%), Albizia lebbeck (76.7%)

and Fumaria parviflora (54%) @ 3 g kg-1 body weight. Plants having higher condensed tannin (CT) content [>6 to 10% of dry matter (DM)] as assayed by Butanol-HCl method resulted in higher (87.0 to 91.7%) reduction in EPG compared with 54 to 80.1% reduction with plants having lower levels of CT content (>3 to 6% of DM). It was concluded that (i) ovicidal and/or larvicidal effects of plants indicate presence of a variety of chemicals having anthelmintic potential, and (ii) CT content of the plants have an important role in their anthelmintic activity both in vitro and in vivo.

CS55.6

Mode of Action of Phytohaemagglutinin lectin on the Control of Gastrointestinal Parasites in Sheep

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Lectins are well known to have potent immunostimulatory properties. Additionally we have shown an inhibitory effect on larval feeding of ovine gastrointestinal parasite (GIP) in vitro. However, the use of plant lectins in the control GIP in vivo and their effect on the immune response of the host has not been evaluated. This study examined the ability of the plant lectin phytohaemagglutinin (PHA) to control GIP in sheep. Twenty-four lambs were allocated to one of four groups (n=6) in a 2x2 factorial design with treatments being either infection (-P: no infection vs. +P: mixed infection with Teladorsagia circumcincta and Trichostrongylus colubriformis) or PHA administration (-L: no PHA vs. +L: oral dose of 80 mg PHA/animal/d), for 6 weeks. Compared with their non-dosed counterparts, PHA significantly reduced the faecal egg counts (egg/g) between days 25 and 36 post infection (P=0.033) and had a tendency to reduce the ability of larvae to penetrate abomasal tissue, as evaluated by an in vitro larval establishment test (P=0.063). PHA did not affect cell populations of the intestine. In abomasal tissue of parasitised animals, PHA induced an increase in the number of PAS-positive cells (P=0.034) and had a tendency (P=0.060) to reduce T helper cells. No changes in mucosal mast cells were observed in any of the animals. These results indicate that PHA may have a transient effect on adult fecundity and/or local mucosal immune responses during GIP infection, although further studies are required to define both the direct and indirect effects of PHA in vivo.

CS55.7

In vitro and in vivo Anthelmintic Activity of Some Browse Mediterranean Plants Against Parasitic Nematodes

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Anthelmintic resistance in gastrointestinal nematodes imposes to find alternative solutions to chemical treatments. Among those, tannin-rich plants with anthelmintic properties represents an innovative option. Most current data have been acquired on legume forages. Information on browse plants remains few. Our objectives were i) to screen the in vitro effects on Haemonchus contortus of 7 plant extracts (carob, pear tree, kermes oak, Pistachia, sainfoin, chest nut and olive) collected from the Mediterranean area; ii) to verify the role of tannins in those effects; iii) to verify in vivo these anthelmintic effects.

The in vitro screening was measured by the Larval Migration Inhibition Assay. Statistical analyses indicated significant differences in LMIA for all extracts except olive. After adding an inhibitor of tannins to extracts, the LMIA values were restored to control values for all active plants, except pistachia and carob, confirming main role tannins.

In the in vivo experiment, 48 lambs composed 6 experimental groups, depending on the diet given prior infection (D-14): pistachia (G1), oak (G2), carob (G3), sainfoin (G4). Two control groups received lucerne (G5 and 6). On D0, G1 to G5 were infected with H. contortus and Trichostrongylus colubriformis, G6 remaining uninfected. Parasitological and pathophysiological measurements were performed weekly post infection (PI). After slaughtering (D45 PI), worm counts and fecundity were assessed. The consumption of the plants was associated with significant decreases of egg excretion, overall related with a decreased worm fecundity. Significant reductions of T. colubriformis populations were also observed with carob and sainfoin.

CS55.8

Effects of Artemisinin and Artemisia Annua Products on Experimental Haemonchus contortus Infection in Gerbils (Meriones unguiculatus)

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Haemonchus contortus is a blood-sucking abomasal parasite responsible for major losses to small ruminant producers worldwide. Resistance of this nematode to commercial anthelmintics has produced a demand for alternative control methods. Artemisia annua is an herb used as an ancient Chinese remedy for various ailments including malaria and helminth infections. Artemisinin, a compound isolated from A. annua, has been shown experimentally to have activity against schistosomes. Artemisinin and A. annua aqueous extract (AE) and ethanolic extract (EE) were evaluated against H. contortus in a gerbil model. In all experiments, gerbils were infected with 600 third-stage larvae. In one experiment, gerbils were treated orally with 400 mg/kg artemisinin once or 200 mg/kg artemisinin daily for 5 days. In a second experiment, gerbils were treated daily for 5 days with 600 mg/kg AE or EE. On day 9 post-infection, gerbils were killed, their stomachs removed, and the worms counted. Artemisinin once or daily for 5 days did not show anthelmintic activity (-25% and -35% parasite reduction, respectively) compared to untreated control groups. Artemisia annua AE and EE caused 2% and 25% parasite reduction, respectively, compared to an untreated control group. Differences were not statistically significant in either experiment. Artemisinin and A. annua extracts did not significantly affect H. contortus in gerbils at the given dosages. Further studies are being conducted to assess the effects of an essential oil of A. annua and an increased dosage of A. annua ethanolic extract.

CS56 - Epidemiology

Thursday, August, 13, 2009

CS56.1

Toxoplasma gondii Infection in Greek Dairy Small Ruminant Flocks Detected by ELISA in Blood Samples

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Infection with Toxoplasma gondii is one of the most common parasitic infections in humans and other warm-blooded animals. Except of its zoonotic importance, T. gondii infection in sheep and goats can cause several economically important reproductive problems such as impaired reproduction performance and abortions. The objective of this study was to explore the presence and the extent of T. gondii infection in dairy small ruminants in Greece by conducting a seroepidemiological survey. For this, blood samples were collected from 1,511 sheep and 544 goats coming from 69 different private farms (35 of sheep, 11 of goats and 23 mixed) situated in two different regions of Northern Greece. Moreover, in order to identify putative risk factors, information on history of abortions and management practices was collected, using a farmers' questionnaire. An in-house ELISA based on affinity purified p30 (Tg SAG1) from T. gondii tachyzoites was applied. Seropositive animals were found in 98,5% of the flocks (100% of the sheep and mixed flocks and 91 % of the goat flocks) while the overall prevalence was 47% for sheep and 28% for goats. The mountainous area reveals a lower seroprevalence (41 %) as compared to the coastal area (67%). In conclusion, Toxoplasma gondii infection is extremely widespread among dairy sheep and goats in Greece. Those results strongly indicate a high level of exposure of sheep and goats to this parasite fact that has to be further explored, in order to obtain information on its contribution to livestock abortion problems and its zoonotic prospective.

CS56.2

Preliminary Research into the Lifecycle of Haemogregarina fitzsimonsi Dias, 1953 and its Possible Use as a Bio-indicator for Host Population Health

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Research regarding apicomplexan haematozoa in southern African tortoises is almost nonexistent, with the exception of that in the early 1900's and Dias' (1953) research in Mozambique. South Africa has the world's highest biodiversity of tortoises and therefore may harbour an equivalent apicomplexan biodiversity. Apicomplexans recorded by Dias (1953), Haemogregarina fitzsimonsi, Haemogregarina parvula and Haemoproteus balazuci, have recently been recorded infecting 6/14 tortoise species in South Africa. Lifecycles regarding the two haemogregarines require elucidation for correct taxonomic placement. Siddall (1995), placed all species of chelonian haemogregarines into the genus Haemogregarina (sensu stricto), the parasite being strictly transmitted by a leech vector. This may be incorrect for terrestrial chelonians, as was found with the Palaearctic Testudo graeca and T. marginata infected with Hemolivia mauritanica (Sergent and Sergent, 1904) transmitted by the tick vector, Hyalomma aegyptium. Giemsa's-stained thin blood smears and tick species collected from Chersina angulata and Stigmochelys pardalis, infected with the most prevalent apicomplexan haematozoan, H. fitzsimonsi, were screened. Ticks fixed in 10% neutral buffered formalin from infected individuals were studied using standard histological techniques as well as transmission electron microscopy. If sporogony occurs within the gut epithelial cells of the tick

combined with the erythrocytic merogony already found in the tortoises, it may be possible to determine if H. fitzsimonsi and possibly H. parvula belong to the genus Hemolivia. Additionally, prevalence and intensity of apicomplexans will be recorded along with tortoise body condition to determine if these parasites may be used as bio-indicators of wild tortoise population health.

CS56.3

***Toxocara vitulorum*: First Field Case in the Netherlands**

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In September 2008, we were warned by a practitioner that she had found a lot of *Ascaris*-like eggs in the faeces of a suckling calf which showed clinical signs such as diarrhoea and weakness. A sample of the faeces was sent to the GD (Animal Health Service). We found an EPG of 25,000 and it was confirmed that we were dealing with a *Toxocara vitulorum* infection. More calves of the herd were sampled and some of them were also positive. After housing in November, all calves were treated with doramectin. In faecal samples taken after treatment, no eggs were found. Because calves can become infected by drinking milk from their dam, the family lines of the positive calves were analyzed. It was clear that the infected calves belonged to two different family lines. The oldest cow on the farm that could be regarded as carrying the infection was born in January 1998. This was a cow of the Piemontese breed. In the nineties, this breed was introduced on the farm after import from France. It is likely that one or more of the imported animals carried the *T. vitulorum* infection. It is also likely that the infection had been present on the farm since that time, but without serious clinical signs in the calves and thus without faecal examinations. It is not clear why no clinical signs had been observed in the past. To prevent the further spread of *T. vitulorum*, it was decided to slaughter the whole herd before turn out in 2009 and to prevent grazing of susceptible stock this year on the pasture which was contaminated in 2008.

CS56.4

Fenbendazole Treatment Results in an Increased Productivity in Calves Experimentally Infected with Giardia duodenalis

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A total of 28 Holstein-Friesian calves were experimentally infected with 100,000 Giardia duodenalis cysts. Eleven days later, animals were allocated in two groups of 14 animals each, based on pre-treatment cyst counts. Animals either received an oral treatment with fenbendazole at 15 mg/kg/day during 3 consecutive days, or a placebo (water). From Day 3 (D3) after treatment, cyst excretion was determined three times a week during 4 consecutive weeks, using a commercial immunofluorescence assay. The faecal consistency and general health were recorded on a daily basis. The weight was recorded prior to treatment and weekly thereafter. Data were compared between treatment groups using the Mann Whitney-U test. During the experimental period, there was a high (93-100%) reduction in cyst excretion in the treated animals compared to the control animals, resulting in a significant reduction of the cumulative cyst excretion on D28 (98%, $P < 0.001$). The faecal consistency was significantly better ($P < 0.002$) in the treated group compared to the control group, although none of the animals displayed overt diarrhea. Prior to treatment the weight did not differ between both groups. At the end of the 4 week experimental period however, the animals in the fenbendazole treated group gained on average 2.84 kg (102 g/day) more than the control animals ($P < 0.031$). In this study, for the first time a decreased productivity in Giardia duodenalis infected calves is demonstrated. Furthermore, the results of the present study indicate the benefit of a treatment with fenbendazole on weight gain and faecal consistency.

CS57 - Genetics / Other

Thursday, August, 13, 2009

CS57.1

Morphological and Genetic Diversity of Trichuris spp. Recovered from Humans and Pigs

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The nematodes, Trichuris suis and Trichuris trichiura are believed to be two separate but closely related species. The aim of our study was to examine the morphological and genetic diversity of Trichuris spp. recovered from pigs and humans. Sympatric worm material isolated from 10 humans and 5 pigs in Uganda supplemented with T. suis from Tanzania, Denmark and USA and T. trichiura from England, was obtained. Based on morphology, worms from the two hosts could only

be discriminated by the length of the male spicule (t-test, $p<0.001$). The second internal transcribed spacer (ITS-2) in the r-DNA was amplified by PCR and cloned. Between 1 and 6 clones from 20 worm were sequenced, which resulted in 49 human-derived and 45 pig-derived sequences that could be allocated into as many as 56 different haplotypes. A very large intra-individual variation was found within the human-derived sequences (0.2 – 45.0%) compared to the pig derived sequences (0.2 – 1.4%). This was due to the fact that the human-derived worms consisted of two main ITS-2 sequence types; one of them being unique (69% of the human-derived sequences, consensus sequence 481 nucleotides long) and the other being identical to the sequence type found in pig-derived worms (31% of the human-derived worms, consensus sequence 531 nucleotides long). The results indicated that the nematodes found in pigs belong to a genetically distinct species (T. suis) whereas the nematodes in humans showed considerable genetic variability either related to ancestral polymorphism or more recent cross-breeding between T. trichiura and T. suis.

CS57.2

Genomic Variability Among Schistosoma japonicum Isolates from China Revealed by ISSR Markers

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In the present study, inter-simple sequence repeats (ISSR) markers were used to examine the genetic diversity and population structure of Schistosoma japonicum isolates from different endemic provinces in mainland China, using S. japonicum from Japan and S. mansoni for comparison. Of the 30 primers screened, 4 produced highly reproducible ISSR fragments. Using these primers, 107 discernible DNA fragments were generated with 105 (98.13%) being polymorphic, indicating considerable genetic variation among the examined S. japonicum isolates. The percentage of polymorphic bands among S. japonicum isolates from mainland China and Japan was 82.24%, 43.93% among mountainous type isolates and 64.49% among lake/marshland type isolates from mainland China. The average gene diversity (HE) was estimated to be 0.2291 within mainland China, 0.2446 among all the S. japonicum isolates, and 0.2730 between S. japonicum and S. mansoni. Significantly higher level of genetic differentiation among S. japonicum populations in mainland China was detected based on Nei's genetic diversity analysis (17.40%), Shannon's index analysis (19.41%), and Bayesian method (8.45%). Phylogenetic analysis revealed that all of the S. japonicum samples were grouped into two clades, the first contained isolates from mainland China, and the other one contained samples from Japan. Within the cluster of isolates from mainland China, isolates from mountainous Sichuan and Yunnan provinces grouped together, whereas isolates from lake/marshland regions (Anhui, Jiangsu and Hubei provinces) clustered together. The results of present study demonstrated that the ISSR markers are useful for studying genetic diversity and population structure of S. japonicum isolates from mainland China.

CS57.3

Atypical Toxoplasma gondii Genotypes in Western Australian Wildlife Species

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Tissue samples from 20 species of native Western Australian mammals, including Kangaroos, Woylies, Quolls, Phascogales, Numbats and Bandicoots were screened for *Toxoplasma gondii* using multi-locus nested-PCR at the B1, SAG2, and SAG3 genes. Seventeen of these species (85%) were found to be infected with *T. gondii*, at an overall prevalence of 90% (127 of a total of 141 individuals). Highly variable strain types were revealed. A total of 8 genotypes were identified based on the nucleotide sites that are commonly used for typing strains. At the B1 locus, 31 single nucleotide polymorphisms among isolates from Kangaroos were identified that comprised 4 distinct alleles including 9 specimens with a Type I allele; 8 with a Type II/III allele; 22 with a novel allele designated Atypical 1; and 6 with another new allele referred to as Atypical 2. Detailed analysis among the kangaroo samples indicated that different organs from the same individual Kangaroos often possessed different *T. gondii* genotypes, and different organs were associated with different parasite burdens. The highly variable strain types identified may directly reflect the unique eco-systems in Western Australia, and the variation of susceptibility among marsupials may be highly dependent on the genotype of *T. gondii* infecting.

CS57.4

The Biodiversity and Systematics of Marine Fish Parasitic Isopods of the Family Cymothoidea from Southern Africa

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Parasitic isopods are rarely studied in South Africa and thus little information is available on the biodiversity of these parasites regarding their occurrence, distribution and hosts. This data is necessary to understand the effects these

parasites will have on the South African fish populations and the aquatic environment as a whole. These isopods infect a number of different species in the marine and estuarine environments and have been recorded to have negative impacts on the fish industry when encountered in large infestations. This is due to the isopods causing skin damage, lesions, and anaemia and may even lead to the death of the host in extreme cases. Over the last five years, a significant amount of cymothoid isopods were collected and analysed from numerous sampling trips along the South African coast. Preliminary results already show some specimens which do not conform to those known in South Africa and may be a species not previously recorded in this region or even a new species. Since these cymothoid isopods are rarely studied there is also much confusion in their identification and thus all this information will be looked at in detail and revised to provide an extensive and accurate account of the cymothoid family. This work will be published and available for avid fishermen who observe these ectoparasites on their fish hosts.

CS58 - Diagnosis and Control

Thursday, August, 13, 2009

CS58.1

Identification of Vaccine Candidates Against Poultry Red Mite, Dermanyssus gallinae

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Dermanyssus gallinae, the poultry red mite (PRM) is a blood-feeding ectoparasite that infests many bird species. We are working towards identifying PRM antigens that could be used to generate protective immune responses.

Whole PRM extract was fractionated into PBS soluble, membrane associated, integral membrane and insoluble protein fractions. These protein fractions were injected into Lohmann Brown hens and IgY antibodies, isolated from egg yolk six weeks post primary injection, were fed to PRM in an in vitro feeding assay. Antibodies, raised against the PBS soluble proteins, were found to significantly increase the mortality of the PRM when compared to antibodies generated in the control group (QuilA adjuvant only), $p = 0.017$. The PBS soluble proteins were sub-fractionated and injected into hens for subsequent testing in the in vitro feeding assay. In addition, other proteins, including known allergens, have been characterised in the PRM, such as tropomyosin, paramyosin and histamine release factor. Recombinant forms of these proteins have been made and antibodies have been raised in rabbits and hens for use in immunolocalisation and in vitro feeding assays.

Thus, these in vitro studies provide an important and relatively rapid method to identify possible vaccine candidates to control the PRM, and are an important first step before in vivo field trials.

CS58.2

Cathepsin Proteases: their Potential as Vaccines for the Control of Dermanyssus gallinae Infestations in Commercial Poultry Houses

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The legislated withdrawal of acaricides and the development of acaricide resistance have hampered efforts to control Dermanyssus gallinae in commercial poultry units. Previous studies demonstrated the feasibility of vaccination with D. gallinae proteins as a valid mite control strategy (Bartley et al., 2009 & Wright et al., 2009).

In order to preferentially identify genes encoding antigens that are upregulated during/after feeding we constructed a suppressive subtractive hybridisation cDNA library. The cDNA prepared from starved mites was subtracted from cDNA prepared from fed mites; the remaining gene population representing those upregulated in feeding were extensively sequenced. Analysis of the resulting gene database revealed a variety of putative digestive enzymes and proteins associated with oviposition.

We identified an aspartyl protease (Dg-CatD) that exhibited 49% identity to the longepsin protein; a lysosomal cathepsin D-like protease found in the midgut and salivary glands of the Haemaphysalis longicornis tick with a putative role in haemoglobin proteolysis. Also identified was a cysteine protease (Dg-CatL), which exhibited 35% identity to cathepsin L-like proteins from a variety of parasitic invertebrates with several putative functions described e.g. haemoglobin digestion, vitellogenin protolysis and host-parasite interactions. Both proteins were predicted to possess N-terminal signal peptides associated with lysosomal location (Dg-CatD) and secretion into the extracellular milieu (Dg-CatL).

Recombinant forms of Dg-CatD and L were expressed, purified and characterised. Antibodies specific for Dg-CatD and L were generated in hens and the effect of anti-Dg-CatD/L antibody-enriched blood meal on mite survival assessed. The potential of mite Cathepsins as vaccine candidates will be discussed.

CS58.3

Development of Innovative Method Based on Fungal Entomopathogens for Control of the Economically-Important African Ticks

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Ticks & Tick-borne diseases are considered the greatest animal disease problem in Africa. The conventional method of tick control using chemical acaricides is fraught with several problems e.g. environmental pollution, chemical residues in meat, milk products and in wool, development of tick resistance and the exorbitant costs. Alternative innovative and cost-effective methods of tick control are therefore needed. One promising method is biological control using the entomopathogenic fungi, Beauveria bassiana and Metarhizium anisopliae.

Beauveria bassiana and M. anisopliae induced high mortalities in various developmental stages of Rhipicephalus appendiculatus, Amblyomma variegatum and Rhipicephalus evertsi evertsi.

Both fungi, when applied on R. appendiculatus feeding on cattle in the field induced higher mortality than when applied to off-host ticks in the laboratory. Engorged R. appendiculatus from cattle sprayed with B. bassiana and M. anisopliae and maintained either in the field (vegetation) or in the laboratory produced very similar mortality results. Rhipicephalus appendiculatus sprayed with conidia of B. bassiana and M. anisopliae when feeding on cattle and thereafter maintained in the field exhibited significantly higher mortality than ticks infected off-host and then maintained in the field

When adult R. appendiculatus and A. variegatum were exposed to water and oil formulations of B. bassiana and M. anisopliae and maintained in the field, the oil formulations of both fungi induced significantly higher mortalities than the corresponding water formulations. Infection of engorged female R. appendiculatus by M. anisopliae and B. bassiana induced significant reductions in fecundity and egg hatchability .

Rhipicephalus appendiculatus tick populations on cattle in paddocks sprayed with M. anisopliae and B. bassiana were significantly lower than on cattle in control paddocks sprayed with water. Metarhizium anisopliae also induced high mortality in larvae, nymphs and adults of the red legged tick, Rhipicephalus evertsi evertsi . The mortality increased with conidial concentration with oil performing better than water formulation.

In conclusion, B. bassiana and M. anisopliae have potential for biological control of African ticks,including A. variegatum which has spread beyond the African borders to the Caribeans and threatening to invade the USA and Canada.

CS58.4

Is *Dirofilaria Repens* Spreading in Germany?

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Dirofilaria repens is a zoonotic nematode causing human and canine subcutaneous dirofilariasis. The first autochthonous case of canine dirofilariasis in Germany was registered in Middle Upper Rhine valley in July 2004. To obtain data about further cases of infection and to identify a potential endemic area 44 hunting dogs of 36 owners living in the Middle Upper Rhine region were examined for presence of microfilariae in June 2007. Furthermore a kennel of 29 sled dogs living near to Berlin (distanced 500 km northeast of Middle Upper Rhine) was examined in October of the same year, due to clinical suspicion of cutaneous dirofilariasis of one animal. While 30 of 44 hunting dogs had no travel history, the sled dogs left North Germany only in late autumn and winter months for sled dog races. To identify the pathogens, modified Knott-test was performed and the positive results were confirmed by PCR.

D. repens microfilariae were detected in blood samples of 3 (6,8%) dogs from Middle Upper Rhine without travel history, indicating infection at the local site. Concerning the sled dogs, five animals were found to be positive for *D. repens* microfilariae, being presumably also infected in Germany based on the seasonal travel pattern. Positive sled dogs were treated by doxycycline (approx. 5 mg/kg) and ivermectine (50 µg/kg) for 6 weeks. No microfilariae could be detected on 4th and 19th week after treatment.

The present study suggests the occurrence of at least one endemic area for *D. repens* in Germany.

CS58.5

The Molecular Identification of and the Histological Changes Induced by Anisakis Larvae (Nematoda: anisakidae) in Atlantic Salmon (Salmo salar L)

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The impetus for this study was anecdotal reports from anglers during 2006 and 2007 of heavy parasitic infections in large numbers of wild Atlantic salmon Salmo salar L. The molecular identification of Anisakis simplex sensu stricto larvae found during the winter of 2007/2008 in spent adult

ranched Atlantic salmon, which had been reared to the smolt stage at the freshwater hatchery of the Marine Institute before being released to the sea, will be described. Identification of the larvae from the adult salmon was accomplished by amplification, RFLP and sequencing of the ITS region of rDNA. In addition larvae found in five post-smolts which had just begun their marine migration and had been captured in May, 2008 in the northeast Atlantic during a research cruise of the Marine Institute's research vessel Celtic Explorer were also identified as Anisakis simplex sensu stricto. In addition to using the ITS region identification of the larvae was further confirmed by amplification and sequencing of the mitochondrial Cox 2 gene.

Histological changes induced by these parasites will also be detailed. The majority of larvae were found loosely coiled and attached to the serosal surface of the pylorus caeca and liver. Histological examination revealed that in addition to those parasites attached to the mesentery in the peritoneal cavity, larvae were also present in the substance of the liver and intestinal wall. The majority of larvae were encapsulated in a thin fibrous capsule with a minimal inflammatory cellular reaction. The inflammatory changes in the liver were also mild but the reaction in the intestinal wall was more severe. There was displacement of the circular musculature, disruption of the stratum compactum with extensive eosinophilic granular cell and a limited macrophage and mononuclear cell infiltration. Many of the eosinophilic cells were undergoing degranulation.

This is the first description of Anisakis simplex infection in ranched salmon. It is also demonstrates for the first time that salmon smolts can become parasitised with these larvae very early in the marine migratory phase of their life cycle.

CS59 - Companion Animal Symposium

Thursday, August, 13, 2009

CS59.1

An Update on Selected Zoonotic Diseases of Companion Animals

Ballweber, Lora[4]; Bowman, Dwight[1]; Blagburn, Byron[3]; Little, Susan[2]

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The presentations included in this symposium are review summaries of a recent roundtable involving parasitologists and infectious diseases experts from CAPC, CDC, AAVP and various veterinary colleges.

These presentations will focus on new infrmation, misconceptions and unanswered questions worthy of consideration.

Susan Little: Tick transmitted diseases in the US. This presentation will discuss recent incidence studies. Growing ranges of distribution of both tick species and vector borne diseases are a very effective surveilance tool and an indication of disease range and incidence in humans

Lora Ballweber: Toxoplasmosis is a significant zoonotic disease often attributed to exposure to cats, The reality is that there are more significant risks of infection that often go ignored. The expected outcome of this presentation is a greater understanding of incidence and source of infection.

Dwight Bowman: Giardiasia and Cryptosporidium are protozoan infections that commonly impact both people and pets. There has long been concern that animals were a source of infection for humans. Recent evidence demonstrates that this is not the case. The expectd outcome of this presentation is an increased awareness of disease source in both people and animals.

Bryron Blagburn: Ascariasis and Heartworm Disease as zoonotic disease.

Both Roundworms and Heartworms are common and widely distributed in the US. Roundworms have potential devistating results in zoonotic infections and human heaartworm disease presents a diagnostic challenge in human medicine of real significance. The expected outcome of this presentation is increased awareness of the incidence of these diseases and their implications to human health.

Companion Animals increasingly live in close contact with people. The benefits of pet ownership and the human animal bond have been well studied and documented. Significant numbers of companion animals are parasitized with organisms that have great relevance to human health and in particular in immunocompromised individuals.

Increasingly the health of umans is tied to the health of animals both as disease sentinals and as sources of infection. The benefits of pet ownership to society can not be overstated but the ptentials for zoonotic parasite transmission must not be understated.

CS60 - Babesiosis / Anaplasmosis

Thursday, August, 13, 2009

CS60.1

Erythrocyte Surface Protrusions are not Required for Accumulation of Babesia canis-Infected Erythrocytes in vivo

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Accumulation of infected erythrocytes in the microcirculation has been described in malaria and babesiosis. Accumulation in the brain is linked to cerebral malaria and cerebral babesiosis, respectively. The processes that lead to these accumulations are not completely resolved. Erythrocytes infected with Plasmodium falciparum express proteins on their surface that are clustered into knob-like structures, which mediate specific interactions with receptors on vascular endothelial cells resulting in the intravascular accumulation of the infected erythrocytes. More recently, similar structures have also been described on erythrocytes infected with Babesia bovis. In dogs that are experimentally infected with Babesia canis parasites, we have also observed accumulations of infected erythrocytes in capillaries of different organs including brain, lung, kidney, intestine, muscle and heart. Interestingly, infected erythrocytes obtained directly from these animals were devoid of any knob-like protrusions; hence there is no apparent straightforward correlation between parasite accumulation and the presence of knobs on infected erythrocytes. Here, we present data that support an additional mechanism for parasite accumulation. Bovine and canine babesiosis (due to B. bovis and B. canis respectively) both trigger inflammatory responses that are associated with a phase of hypotension that may lead to stasis of the blood circulation in small capillaries. Local proliferation not only increases the number of infected erythrocytes in small capillaries but also liberates erythrocyte material that triggers coagulation, leading to the formation of a fibrin plug. Further parasite proliferation may lead to capillaries that are packed with infected erythrocytes. This may impact on supportive treatment of animals with babesiosis and humans with P. falciparum malaria.

CS60.2

Molecular Differentiation of *Anaplasma phagocytophilum* Strains Involved in Canine Infections in Germany

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Anaplasma phagocytophilum causes Canine Granulocytic Anaplasmosis (CGA) in dogs. It is transmitted in central Europe by *Ixodes ricinus*, a tick with an exophilic questing behaviour feeding on a wide variety of vertebrate hosts. The reclassification of the *Ehrlichia phagocytophila*-genogroup [*E. phagocytophila*, *E. equi* and the Human Granulocytic Ehrlichiosis (HGE) agent] in 2001 suggested the new species *A. phagocytophilum*. However, there is a great heterogeneity within this species concerning geographic origin, vector and host species, and pathogenicity.

Dogs with *A. phagocytophilum* infection (n=45), as detected by real-time PCR, were included in this study on genetic diversity of canine *A. phagocytophilum* infections in Germany. Amplification of the partial *16S rRNA* gene was successful in all samples, while amplification of the full *msp2* gene was obtained for 17 samples. Products were sequenced and analyzed with bioinformatic tools.

Altogether seven *16S rRNA* and five *msp2* gene types were found differing in up to eight nucleotide positions. Double infections were found on the basis of both *16S rRNA* (n=2) and *msp2* gene (n=1).

The *16S rRNA* gene-type found in 22 dogs was identical to one identified in cattle in Switzerland, in dogs with CGA from Sweden and from questing *I. ricinus* ticks in a city park in Germany. The *msp2* gene types differed greatly from sequences derived form bovine, ovine or human hosts, available in the GenBank.

These results indicate that different strains of *A. phagocytophilum* may be involved in CGA in Germany. The implications of this finding, especially concerning pathogenicity, need further investigations.

CS60.3

Development of a Loop-Mediated Isothermal Amplification Method for Rapid Diagnosis of Babesia microti Infection

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Babesia microti is a tick-transmitted, intraerythrocytic protozoan parasite and is usually seen in small wild rodents. Recently, increased recognition of the prevalence of human babesiosis, because of rising a concern about the potential risk for the transmission by blood transfusion, has provided a motivation to develop an easy and definitive diagnostic method for the infection of the causative agent, B. microti. In the present study, in order to develop a simple and rapid molecular detecting method of Babesia microti gene, we used a loop-mediated isothermal amplification (LAMP) technique, combining with a practicable method for collecting DNA from blood; heat-treated blood samples were directly used for the LAMP assay without DNA purification. We designed a set of four LAMP primers targeting for small subunit ribosomal DNA gene of B. microti, and analyzed the LAMP products in electrophoresis. Standard PCR assay was used as the control method for the diagnostic detection. The designed LAMP primers positively amplified the LAMP amplicons of typical ladder patterns from the B. microti DNA, but not from various negative control samples, including normal murine and human DNAs, and also other protozoan species. The sensitivity of the LAMP assay was 100 times higher than that of classical PCR method. Availability of the quantitative Real-Time LAMP assay was also confirmed in mice model for the clinical diagnosis of B. microti infection. These findings indicate that the LAMP assay is a new convenient method for rapid molecular diagnosis of B. microti infection.

CS60.4

Quantitative Real Time PCR for evaluation of Borrelia burgdorferi s.l. and Anaplasma phagocytophilum in ticks in the region of Hannover, Northern Germany

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This study aimed to monitor the tick population density in recreational areas in the city of Hannover, Germany, and to investigate the prevalence of Borrelia burgdorferi s.l. as well as Anaplasma phagocytophilum in these ticks as an example of local epidemiological investigations in public Greens in Central Europe. Real-time PCR was applied, validated and used.

8802 ticks were collected between March and October 2005 in 12 places of five defined areas of the city of Hannover with flagging. The collecting sites were characterized by the following parameters: easy access, different types of vegetation and use by the public for leisure. The occurrence of different B. burgdorferi genospecies, B. valaisiana and spielmanii was studied using two different PCR detection methods. The seasonal activity showed a peak around June and July, and the minimal activity was around September and October. Subsequently, genus-specific ITS2-PCR as qPCR was performed. 915 positive ITS2-PCR results were differentiated using a species-specific rpoB-PCR for the identification of five Borrelia species. 3962 ticks were evaluated by qPCR: 23.09% of these ticks were positive. Among different places, the highest prevalence was found with 36.01% and the lowest with less than 6%.

A. phagocytophilum was detected in 3.15% of the ticks examined. A real time PCR detecting the major surface protein (msp) 2 gene was used for this investigation. Cumulation in adult ticks could be described (4.1% compared to 2.3% in nymphs), mixed infection with Borrelia spp. were observed in 0.85% of the ticks tested.

CS60.5

Detection of Babesia DNA in Cattle Fever Ticks Using a Reverse Line Blot

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The cattle fever ticks (CFT) *Rhipicephalus (Boophilus) microplus* and *R. annulatus* are vectors of bovine babesiosis, a deadly protozoan parasite endemic in Mexico. These ticks, along with the parasites *Babesia bovis* and *B. bigemina*, were eradicated from the continental United States in 1943 with the exception of a permanent quarantine zone along the border between Texas and Mexico. The detection of CFT on cattle and deer outside of the permanent quarantine zone has led to the recent expansion of this zone with additional temporary quarantine areas. The spread of these ticks and their potential to vector *Babesia* spp. underscore the need to develop accurate and rapid diagnostic tests for use not only in cattle but also for detection of *Babesia* spp. infected ticks. The Reverse Line Blot (RLB) has advantages over other detection assays in that multiple *Babesia* species can be detected and identified simultaneously from a single DNA sample. A RLB based on nucleotide probes specific to the 18s ribosomal RNA sequences of several *Babesia* spp. is being developed and tested to determine their overall prevalence in CFT eggs, larvae, and adult females. The data obtained from the RLB quanitfies the exposure of cattle and deer to these pathogens and is critical to evaluate the risk of babesiosis infection.

CS61 - Parasite Extinction Symposium

Thursday, August, 13, 2009

CS61.1

Should We Worry About Parasite Extinction?

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Despite the fact that most species on the planet adopt a parasitic life style, parasitism is still widely viewed as something we should try to eliminate. Parasite conservation therefore seems a bizarre aim to most. In fact, parasites can play a fundamental role in host population regulation, competition and community structure, and are a prime selective force for animal evolution. The general aims of conservation ecology have moved from preserving individual species or even their habitats to protecting ecosystem function, of which parasites form an important part. Even in applied veterinary medicine, preservation of some parasite challenge is an important part of sustainable control where this relies on acquired immunity. By aiming for parasite elimination, we furthermore encourage parasites to evolve survival strategies that might increase the risk of disease.

This symposium will explore observed and potential effects of parasite extinction on natural populations and processes, and on interactions with domestic hosts. Changes in climate can strongly influence parasite transmission, and potentially the population viability of both hosts and parasites. Consequences for parasites depend on their capacity to adapt, leading to changes in epidemiology, including at the wildlife-livestock boundary. In domestic animals, therapy has placed strong selective pressures on parasites, which have adapted to the threat of extinction most obviously through drug resistance but also by other changes in biology. These should be considered when designing and deploying tools for control. Discussion will explore what action, if any, could be taken to conserve parasites as part of natural ecosystems, and whether control strategies in domestic animals should routinely seek to conserve a part of the parasite population.

Poster Presentations

Aquaculture - Sea Lice

Tuesday, August, 11, 2009

PO1.1

New Classification Criteria of Argulus spp from Statistical and Taxonomic studies of Argulidae in Guangdong-China

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The genus Argulus as a fish ectoparasite has worldwide distribution. Pathogenic effect of this parasite on its host is unrestricted on skin as it causes a local inflammation and manifests itself as skin lesions which could become secondarily infected by bacteria, and is also known to be a vector of certain viruses, as well as a vector of nematode larvae of the family Skrjabillanidae. The main objectives of our study were to record the existing species of Argulus in Guangdong and to present a statistical result by using formulae proposed by Margolis (1982),and to derive new classification criteria by using scanning electron microscopy(SEM). In order to perform an extensive survey in Guangdong water bodies, 27 sites (rivers, lakes, fish farms, reservoirs, etc) were investigated for Argulus. Fishes as major host of Argulus were sampled by angling; trap net, long-lining and Gill-netting, plankton net also was used to collect the parasites in free swimming status, parasites were detached from its hosts and individually saved into a test tube containing 70% ethanol. Identification of Argulus was based on light microscopy and SEM. Twelve species of fishes were collected and detected with a significant variety of 15 species of Argulus. High percentage of presence was recorded among different species of Argulus, prevalence percentage was between 20%-35%, abundance was between 0.23-1.14 and mean density was between 1.19-4.07, and different levels of infection were also detected according to the specific fish species. Intensity was greatly influenced by season and its peak as 35 parasites on individual fish was noticed in late summer of 2008. General differences among species were recognized using light microscopy based on international morphological classification criteria, while SEM was able to elucidate more strict details on the level of each appendage of studied parasite and its content. These might be considered as a new classification criteria on Argulus spp, such as specific number of setae of cephalic appendages, sucking disks rods number and its sclerites structure and number, second maxillae segments number, structure and its scales shape, pre-oral spine, overlapping segments of second antenna, thorax segments scales shape, flagella position on swimming legs, scales scattering on swimming legs. This work was supported by grant from National Natural Science Foundation of China (grant no. 30671577)

Babesiosis / Anaplasmoris

Tuesday, August, 11, 2009

PO1.2

Molecular detection of Babesia rossi and Hepatozoon sp. in African Wild Dogs (Lycaon pictus) in South Africa

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1. University of Pretoria, Pretoria, South Africa; 2. Utrecht Centre for Tick-borne Diseases, Utrecht University, Utrecht, Netherlands

Blood specimens from wild dogs (n=301) were obtained from De Wildt Cheetah and Wildlife Centre (Pretoria) and five game reserves (4 in the North-West Province and 1 in Limpopo Province), South Africa. Specimens were screened for Babesia, Theileria, Hepatozoon and Ehrlichia / Anaplasma species using PCR and Reverse Line Blot (RLB) assays. Positive results were obtained in 18 (6%) wild dogs. Sixteen specimens were found positive for B. rossi and two dogs were Hepatozoon sp. positive. It appears that these tick-borne pathogens are not widely distributed in wild dog populations.

PO1.3

Epidemiological Study on Some Blood Parasites of Cattle in Gharbia District, Egypt

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This study was carried to clarify the epidemiology of the blood parasites among some farm animals in Gharbia district, Egypt. The work was done in different seasons in the period from September 2001 to August 2003.

Examination of 2632 of cattle (1000 native and 1632 of mixed breeds) and 1220 buffaloes by stained blood films, IFA & ELISA and the results revealed that the highest incidence rate of Babesia bigemina was in mixed cattle being 0.92 %, and it was followed by native cattle breed (0.3%), while in buffaloes, the incidence was zero%. The results also revealed that, the highest incidence of Babesia bigemina among cattle in Gharbia district was in Autumn season being 1%, while during Spring and Winter the incidence was very low being 0.14% in both seasons.

In native breed cattle, the prevalence of positive animals in IFA test to Babesia bigemina infection in spring was 20%, in summer was 4.4%, in winter was 5% and the lowest incidence was in autumn being 2.5%.

In mixed breed cattle, the prevalence of positive animals in indirect fluorescence antibody (IFA) test to Babesia bigemina infection in spring was 27.5%, in summer was 4.4%, in winter was 7.5% and the lowest incidence was in autumn being 0%.

In buffaloes, the highest incidence of animals positive in IFA technique to Babesia bigemina antibodies was in summer 8.8%, then during spring being 5%, while in both winter and autumn the incidence was 0% and 2.5% respectively.

Concerning the animal age, utilizing blood film examination the incidence of Babesia bigemina was in cattle over 6 years, where the lowest incidence being 0.43%, while the mid aged cattle between 3-6 years old were found having the highest incidence that was 0.92% and the incidence of Babesia bigemina was 0.72% in cattle under 3 years old.

Babesia bovis (using stained blood film) was of rare occurrence, that only found in mixed breed cattle was (0.12%) and only during autumn (0.21%), in native breeds using IFA test the infection rate of Theileria annulata schizonts parasites in buffaloes was higher than cattle.

Also the results detected the tick infestation of the examined animals and Boophilus annulatus and Hyalomma anatollicum were observed.

PO1.4

Identification of Genetic Variants of *Anaplasma phagocytophilum* in Goat Flocks in Central Switzerland

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Anaplasma phagocytophilum, transmitted by hard ticks of the genus *Ixodes*, causes tick-borne fever (TBF) of ruminants in Europe. Clinical manifestations in cattle and sheep are frequent, particularly after first exposure to tick-infested pastures. Little is known about the impact of *A. phagocytophilum* in goats. However, there have been isolated reports on outbreaks of TBF in domestic and free-living feral goats.

Six goat flocks (72 animals) in Switzerland were investigated for *A. phagocytophilum* DNA from May to October 2008. The goats were kept on pastures where exemplary tick flagging revealed the presence of *I. ricinus*. DNA extracts from EDTA blood-samples were used for real-time PCR targeting the *msp2* gene of *A. phagocytophilum*. Positive samples were tested with nested PCR for amplification of the partial *16S rRNA* gene for sequencing.

Four samples were positive over the study period. Sequencing revealed three different sequence variants. Two of them have been previously reported in ticks in Sweden and Germany, in roe deer in the Czech Republic and in humans in Canada. *16S rRNA* sequences obtained from cattle with TBF from the same region in Switzerland differed in up to 3 nucleotide positions.

Although no clinical signs which may be attributed to *A. phagocytophilum* infection were observed, the results show that *A. phagocytophilum* infection occurs in goats in Switzerland. This may contribute to the maintenance of the tick-rodent-ruminant cycle in nature. Further studies on this bacterial agent, from both goats and ticks from this region, will be necessary to clarify the impact of this finding.

PO1.5

A Pilot Study of Tick Control and Seroprevalence of Anaplasma phagocytophilum in Selected Danish Sheep Herds

Enemark, Heidi Larsen[1, 5]; Agerholm, Joergen Steen[2]; Stamphoej, Inga[3]; Dahl, Jan[4, 6]; Rasmussen, Mette[5]; Nielsen, Bent[6]

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Introduction: *Anaplasma phagocytophilum* may cause clinical disease in several species. In sheep the infection may cause considerable productivity losses. High prevalence of *A. phagocytophilum* has been found in roe deer and ticks in Denmark but well-documented clinical disease in sheep has not been observed.

Aims: Study 1) determination of seroprevalence at herd level 2) significance of anaplasmosis on mortality 3) effect of tick control on mortality and seroprevalence.

Methods: 169 lambs from 3 herds (with 200, 60 and 300 ewes respectively) previously diagnosed with anaplasmosis based on antibodies. On each farm either males or females were treated with Bayticol® pour-on Vet 4 times with monthly intervals from turn out on tick infested areas. Serum samples were collected 3 times with monthly intervals around 4 weeks following turn out. Serotiters were determined using IFAT (cut-off: 1:40). Ticks were counted at each herd visit, and all dead lambs (N=3) were necropsied.

Results: No signs of clinical anaplasmosis were observed. Necropsied lambs died of other causes.

Seroprevalences were high in all herds: 76%, 60% and 51%; 85%, 100% and 100% in herd 1 and 2 respectively; and 100%

at all visits in herd 3. No significant differences in seroprevalence were seen between treated/controls. Number of ticks was reduced approximately 50% (P<0.05 in herd 3) in two herds. Due to lack of data this was not calculated for herd 2. Significantly more Spelsau than cross breeds seroconverted.

Conclusions: Bayticol® pour-on Vet reduced the number of ticks significantly; however seroprevalence was very high in all herds. The serological method could not distinguish between maternal/acquired antibodies which complicated evaluation of results. Breed related differences concerning sensitivity to anaplasmosis appear to exist. Anaplasmosis is prevalent among Danish sheep and clinical significance deserves further research.

Control

Tuesday, August, 11, 2009

PO1.6

Efficacy of a Combination Product Containing Pyrantel, Febantel and Praziquantel (Drontal® Plus Flavour, Bayer) Against Experimental Infection with *Ancylostoma ceylanicum* in Dogs

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Ancylostoma ceylanicum is a prevalent hookworm species in dogs in Asian countries. A recent survey also confirmed the prevalence of this parasite in Australia. This study was performed to test the efficacy of a combination product containing pyrantel, febantel and praziquantel (Drontal® Plus Flavour, Bayer) against *Ancylostoma ceylanicum* infection in dogs. Twelve dogs were subcutaneously injected with 300 L3 of *A. ceylanicum*. Standard floatation technique was used from day 7 post infection onwards daily to determine the start of egg shedding. From day 14 post infection onwards, after confirmation of egg shedding in all dogs, individual eggs per gram (epg) counts were performed daily using McMaster technique. The dogs were randomly allocated into control and treatment group based on epg counts. At day 20 post infection each dog in the treatment group received a combination tablet containing pyrantel, febantel and praziquantel orally at the manufacturers recommended dose, whereas the control group remained untreated. Epg counts were performed daily for 14 days post treatment. The treatment rapidly reduced egg shedding within 3 days post treatment compared to the control group. No eggs were found in the treated dogs from day 4 post treatment onwards whereas the epg counts remained high (4469 + 2064) in the untreated control group. This trial demonstrated that a combination tablet containing pyrantel, febantel and praziquantel given at the manufacturers recommended dose is effective against infection with *A. ceylanicum* in dogs.

PO1.7

Field Evaluation of the Palatability of a New Oral ivermectin Formulation in Horses

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Introduction: Horses are most frequently dewormed using oral pastes which may be difficult to administer for certain owners. Three field studies were conducted to evaluate the palatability of a new oral ivermectin formulation, a soft chew, in horses.

Methods: Two studies in the US and one in Germany were conducted in a total of 206 horses. One hundred thirty-one adults and 75 foals of various breeds were enrolled and ranged in ages of 6 weeks to 31 years. They were offered a new oral ivermectin formulation (Vectin® chewable tablets, Intervet-SP AH) at the dose of 0.2 mg/kg BW in the same conditions in all sites. The soft chews were first presented to the animals in the hand or by placing them in their empty feed bucket. Voluntary acceptance (palatability) by the horses was defined as consumption of all chews offered within 20 minutes. The palatability rate was calculated as the number of horses consuming all chews divided by the total number of horses offered chews.

Results: Combining the results of all three studies, a total of 153 out of 206 horses consumed the whole dose representing an overall palatability rate of 74.3%. The rate was higher in foals (80%, 60/75) than in adults (71%, 93/131). Palatability was the highest in ponies, warmblood and quarter horses and lower in thoroughbreds.

Conclusion: The new oral ivermectin formulation was readily consumed by the large majority of the horses.

Diagnosis / PCR

Tuesday, August, 11, 2009

PO1.8

Molecular Characterization of *Dicrocoelium dendriticum* and *D. chinensis* Obtained from Japanese Serow (*Capricornis crispus*) and Sika Deer (*Cervus nippon*) in Iwate, Japan

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Dicrocoelium dendriticum and *D. chinensis* have been found in Japanese serow (*Capricornis crispus*) and sika deer (*Cervus Nippon*) in Iwate, Japan. This study was designated to phylogenically characterize the 2 *Dicrocoelium* species and to compare them with the other species and geographical isolates of *Dicrocoelium*. We used 42 flukes of *Dicrocoelium* obtained from 4 Japanese serow and 9 sika deer in 12 areas of Iwate Prefecture (40° 27′ N – 38° 44′ N, 142° 04′E – 140° 39′E), Japan. The flukes were identified by morphological features. Genomic DNA was extracted from each flukes. The flagments of nuclear ribosomal ITS2 were amplified by PCR and sequenced directly. Fifteen and 27 flukes were identified as *D. dendriticum* and *D. chinensis*, respectively. *D. dendriticum* was detected from serow in the western areas, while *D. chinensis* was from serow and sika deer in the eastern areas. These findings suggest that the distribution of the two species relates to geographical factors rather than host species. The ITS2 fragments of *D. dendriticum* and *D. chinensis* were 239bp and 238bp respectively, and 48.1% and 46.6% respectively, in GC contents. The ITS2 fragments of *D. dendriticum* showed the similarity of 99.6-100% in each other and of 98.8-99.2% with the Italian isolate of the species (DQ379986). Intraspecific variation of ITS2 among specimens of *D. chinensis* was not detected except for a fluke having a heterogeneous nucleotide site. The ITS2 similarity between the present isolates and Austrian Isolates (EF547132) of *D. orientalis* that is a synonym of *D. chinensis* was 98.7-99.6%. The ITS2 fragments of *D. dendriticum* and *D. chinensis* had the similarity of 94.6-96.4% in each other and of 86.2-87.0% with that (EF102026) of *D. hospes*. The differences among the ITS2 sequences seemed to be useful criteria for the discrimination between *Dicrocoelium* species.

PO1.9

Production and Characterization of Monoclonal Antibodies Against Taenia Saginata Metacestode Antigens

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Cysticercosis is a major cause of economic loss in bovine production because of meat condemnation to control teniasis-cysticercosis complex. Chemotherapy can be used to control infection in cattle and diagnostic tests to monitor treatment should detect live parasites or their products. Monoclonal antibodies against somatic (TAEB) and cyst fluid (TAEF) Taenia saginata metacestode are being produced by fusion procedures using spleen cells from immunized BALB/c mice. Five fusions were performed for each antigen preparation and the supernatant from these cell cultures were screened by indirect ELISA assay. Ten hybrids were selected and cloned. Cloned cell lines were grown in tissue culture to collect the monoclonal antibodies, resulting in 18 IgG1 and 26 IgM reactive clones for TAEB and 9 IgG1 and 9 IgM for TAEF. These clones were selected for ascitic fluid production by injection in BALB/c mice and further harvesting. Western blotting is being performed to evaluate the antigenic component recognition. In the near future, these monoclonal antibodies will be used in a capture ELISA for bovine cysticercosis diagnosis.

PO1.10

Is it Possible to Detect Taenia saginata DNA in Blood Samples of Naturally Infected Calves?

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Bovine cysticercosis is a public health problem as well as the cause of serious damage in the agro-cattle industry, resulting in the condemnation or treatment of carcasses detected as positive. In Brazil, bovine cysticercosis has routinely been diagnosed by post-mortem inspection, although several researchers have developed ante-mortem diagnostic methods with variable specificity and sensitivity. We evaluated the amplification of circulating T. saginata DNA by polymerase chain reaction (PCR) as a diagnostic tool to detect bovine cysticercosis. Initially, we evaluated five primers (which have been previously described) and established which experimentally contaminated blood fraction (cell, serum or plasma) resulted in DNA fragment amplification by PCR. The best

detection limit was observed in the serum fractions (pellet and supernatant) with the use of the SCAR K primers. However, blood samples of naturally infected animals considered positive for the presence of viable cyst forms under Brazilian federal meat inspection abattoirs didn't result in the desired DNA fragment amplification by PCR. New studies are being performed to evaluate the influence of parasitic load in the detection of circulating DNA from T. saginata.

PO1.11

Pattern of Recognition of *Besnoitia besnoiti* Tachyzoite and Bradyzoite Antigens by Naturally Infected Cattle

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Bovine besnoitiosis is caused by the protozoan parasite *Besnoitia besnoiti*. Many recent cases have been described in different European countries, which may be indicative of the expansion of the disease in the future. Many infected animals remain asymptomatic, so that serological tests are essential tools for diagnosis. The objective of the present work was to identify *B. besnoiti* tachyzoite and bradyzoite immunodominant antigens (IDAs). IDAs were recognized by SDS-PAGE under reducing conditions and western blot analysis. Positive sera from symptomatic (n=18) and asymptomatic (n=18) cattle came from *B. besnoiti* endemic infected herds and were positive by IFAT, whereas negative sera (4) came from besnoitiosis free herds and were also negative by IFAT. Up to 28 tachyzoite antigens in the range of 8.5 kDa to 190.8 kDa were recognised. Based on the frequency of recognition 6 IDAs (14.2, 33, 37.1, 39.6, 46.3 and 190.8 kDa) were identified. The 37.1 kDa antigen was recognised by 100% of sera, usually as an intense band. On the other hand, 30 bradyzoite antigens in the range of 8.5 kDa to 187.9 kDa were detected. Seven bradyzoite IDAs (8.5, 15.1, 16.8, 19.0, 34.7, 38.6 and 124.4 kDa) were identified and two of them (15.1 and 16.8 kDa) were considered as the most immunogenic ones. Moreover animals with clinical signs recognised a significant higher number of bradyzoite antigens. Additionally significant cross reactions with other closely related apicomplexan parasites were not detected. This is the first description of *B. besnoiti* bradyzoite antigens. In addition the identification of tachyzoite and bradyzoite IDAs may be useful for development of vaccines, as well as diagnostic tools in order to differentiate acute and chronic infection. Further proteomic studies are needed in order to identify stage specific proteins.

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PO1.12

Analysis of the Correlation Between EPG Counts and the Number of Female Gastrointestinal Nematodes in a Bovine Population

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Infections in ruminants by gastrointestinal nematodes cause extreme economic losses due to both mortality and morbidity. This is often characterized by diminished productivity in these animals. Diagnosis of infections caused by gastrointestinal nematodes (in vivo) is principally based on the variable counts of eggs per gram of feces (EPG). The present study aimed to evaluate the correlation between EPG counts (roundworm) and parasitism intensity by females of different nematode genera seen in bovine populations. Forty naturally infected bovine bulls and cows aged eight to twelve months were euthanized for analysis. EPG counts were conducted in all bovine on the day of necropsy. Our results revealed the presence of six helminth genera, with the following Pearson's correlation coefficients (r): Haemonchus, 0.36; Cooperia, 0.40; Trichostrongylus, -0.23; Ostertagia, -0.07; Oesophagostomum, -0.09; and Bunostomum, -0.16. The total number of female helminthes and mean EPG counts presented an r-value of approximately 0.47. This result suggests that a weak positive correlation exists between EPG counts and the mean number of female nematodes present in necropsied bovine. This study, the first in the literature, confirms that the coprological exam (EPG) should be interpreted using extreme caution.

PO1.13

Serodetection of *Ehrlichia canis* Infection in Dogs in Punjab, India

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Ehrlichia canis parasitize circulating monocytes and causes a serious and potentially fatal disease in dogs. Although in Punjab the first case of canine monocytic ehrlichiosis was diagnosed in 1992, the epidemiological seroprevalence for *E. canis* in the State was not conducted since then. The object-

ive of the present study was to use Dot-ELISA Immunocomb® assay to detect antibodies against *E. canis* in blood samples of 60 pet dogs suspected to be naturally infected with *E. canis* in the Small Animal Clinics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab (India). The privately owned dogs were examined physically for ticks and peripheral blood smears stained with Wright's-Giemsa were examined microscopically. Antibodies reactive with *E. canis* were detected in plasma in 48 samples out of 60 by immuno comb® Dot-ELISA. Out of the 48 positive samples, 19 samples (39.58%) were low positive (1:20-1:40), 15 samples (31.25 %) medium positive (1:80-1:640) and 14 samples (29.16%) high positive titre (>1280), respectively. Titre equivalent 1:80 or more was considered as positive. On microscopic examination, only two dogs with serological evidence of ehrlichiosis revealed typical *E. canis* morulae. Inadequate number of platelets, anaemia and leucopenia was observed in seropositive dogs. Results suggest that *E. canis* circulates in dogs in India in low non-detectable numbers and is transmitted by *Rhipicephalus sanguineus*. Further studies are needed to know whether serologically positive animals should be treated or not regardless the presence or absence of morulae in monocytes when blood is examined microscopically.

Drug Resistance

Tuesday, August, 11, 2009

PO1.14

Enzyme-Linked Immunoassay Using T Gondii Gra7 Antigen for Antibody Detection in Pigs

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Pigs infected with Toxoplasma gondii are considered an important source of infection for humans in several countries. The aim of this study was to use an ELISA test with T. gondii rGra 7 antigen for antibody detection in slaughter pigs from indoor and outdoor farms. A total of 474 sera previously tested by inmunoflurescence and western blot were analyzed. A recombinant T. gondii protein (rGra7) cloned in a prokaryotic expression vector was used as antigen in an indirect enzyme-linked immunoassay (ELISA). Serum samples diluted 1:25 were tested in duplicate. Rabbit anti- pig IgG horseradish peroxidase conjugate and ABTS were used. Optical densities were measured at 405nm and cut off was previously determined using ROC analysis. The ELISA showed 89.2% sensitivity and 93.7% specificity. Antibodies were detected in 189/238 (79.4%) pigs from outdoor farms and in 127/236 (53%) pigs from indoor farms. Protein rGra7 can be used as ELISA antigen for antibody detection to T. gondii in pigs. Further studies evaluating this technique for T. gondii antibody detection in different stages of infection and animal categories related to safe meat processing are currently being conducted.

PO1.15

Anthelmintic Resistance of Nematodes in Communally Grazed Sheep

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A survey was conducted on the occurrence of anthelmintic resistance of nematodes in communally grazed sheep and goats in a semi-arid area of Zeerust, South Africa, from January to March 2008. In the herds belonging to 8 smallholder sheep farmers and 10 smallholder goat farmers, the efficacies of albendazole, ivermectin and closantel were tested by faecal egg count reduction tests where 80% efficacy was considered the cut off for anthelmintic resistance. The results of the faecal egg count reduction tests showed more than 80% efficacy with all the drugs used in most cases, but there were notable exceptions. In one case, closantel, albendazole and ivermectin displayed efficacies of 74%, 70% and 79% respectively while on another farm albendazole showed an efficacy of 76% in sheep. In goats resistance was shown on one farm with an efficacy of 79% to albendazole. The occurrence of anthelmintic resistance in this farming sector is of concern and steps should be taken to prevent its further spread and development to avoid a situation developing as on numerous commercial sheep farms in South Africa where resistance is very common.

PO1.16

Anthelmintic Resistant Nematodes in English Cattle

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We described the first macrocyclic lactone resistant bovine nematodes (Cooperia sp) in the northern hemisphere ten years ago (Stafford and Coles. Vet.Rec. 144, 659). To determine how far resistance had spread 1300 farmers were contacted by letter and phone and offered free tests for resistance. Farmers were asked to collect 10 faecal samples from the ground at the time of treatment using an anthelmintic of their choice and post them for egg counting. If sufficient eggs were present a further ten samples were

collected and posted 14 days later. Initial counts were made using the McMaster technique and a second egg count using the FLOTAC method sensitive to one e.p.g. The response was very disappointing with only 35 agreeing to take part. Of the 18 who had sufficient eggs at the first count and sent second samples 17 had used a macrocyclic lactone. The large majority were given as pour-ons. On 15/17 farms efficacy was below 98% whilst on three farms egg counts remained the same or increased following treatment. On most farms where faecal larval cultures were successful Cooperia sp were present but on four farms the dominant species after treatment was Ostertagia. Since Ostertagia is the most serious of the gastro-intestinal nematodes of cattle this gives cause for concern and will be re-investigated in 2009. Supported by EBLEX. Part of the PARASOL programme.

PO1.17

Efficacy of Four Fasciolicides Against a Natural Acquired *Fasciola hepatica* Infection in a Hill Sheep Research Farm, Ireland

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The main method of control of *Fasciola hepatica* infection in Ireland is through the use of fasciolicides. A randomised trial was conducted to evaluate the current efficacy of triclabendazole, closantel, oxyclozanide or nitroxynil against *F. hepatica* in naturally infected sheep on the Teagasc Hill Sheep Research Farm, located in Leenane, Co.Galway. One hundred and forty Scottish Blackface and crossbred ewes were weighed and randomly allotted into 4 groups of 35 animals each. In accordance with manufacturer's recommendations Groups 1, 3 and 4 were treated with triclabendazole(10 mg/kg), closantel (10 mg/kg) and oxyclozanide (15mg/kg) *per os* and Group 2 nitroxynil administered subcutaneously (10 mg/kg). Individual faecal samples were taken per rectum on day of treatment (Day 0) and on days 7, 14, 21 and 56 post treatment. The number of *F. hepatica* eggs per gram of faeces was determined using the sedimentation technique and the efficacy of each anthelmintic was calculated in terms of the percentage reduction in egg count at each time point.

The results in the study showed a 100% reduction in egg counts by day 14 post treatment in Groups 2-4. However, the results for Group 1 indicated lower efficacy levels, with reductions of 51%, 57%, 70% and 67% by day 7, 14, 21 and 56 days post treatment, respectively. These results are highly indicative of triclabendazole resistant *F. hepatica* in sheep on this farm. Furthermore the results indicate 21 days may be an appropriate time interval post treatment to determine faecal egg count reduction.

PO1.18

Ivermectin Resistance - a Role for Gene Expression?

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Teladorsagia circumcincta is the most important gastrointestinal nematode parasite of sheep in the UK; it is also the principal drug resistant genus. There are increasing reports of multidrug resistance, including resistance to macrocyclic lactone anthelmintics, most notably ivermectin. Resistance is thought to come about either by genetic mutations in the drug's binding site and/or changes in gene expression of the parasite's drug handling mechanisms. Candidate resistance genes include drug efflux pumps such as the P-glycoproteins (Pgps) and drug handling systems such as the cytochrome P450s (P450s). Previous inhibitor studies with *T. circumcincta* have implicated Pgps in the ivermectin resistant phenotype. We have identified eleven novel Pgps and three P450s gene sequences from *T. circumcincta*. Real-time PCR analysis reveals that some of these exhibit an altered gene expression pattern between ivermectin susceptible and resistant parasite isolates. Single nucleotide polymorphisms (SNPs) have also been identified within some of these genes. These genetic changes require further validation but may provide a lead in the search for molecular markers for ivermectin resistance.

PO1.19

An Appraisal of the Helminth Control Practices and Anthelmintic Resistance in Gastrointestinal Nematodes of Sheep in Punjab-Pakistan

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Introduction: In the backdrop of high prevalence of helminths, this study was aimed at appraisal of helminth control practices and screening of nematode populations of sheep for anthelmintic resistance (AR).

Methods: For appraisal of helminth control practices, retrospective data (10 years) from four farm records were analyzed and relevant information was also collected from the farmers/qualified staff. Nematode populations of sheep were screened for AR using fecal egg count reduction test (FECRT).

Results: In the last 10 years, benzimidazoles were most frequently (n=57/113) used anthelmintics followed by levamisole (n=38/113). The frequency of underdose was higher

(n=23/38) for levamisole compared with benzimidazoles (n=12/57). The frequency of using anthelmintics ranged from 3.3 to 4.0 dewormings per year with an interval of 70 to 113.8 days on different farms. Majority (n=116/200) of the respondents were found to use modern anthelmintics. A reasonable minority, however, also used both modern and botanical anthelmintics (n=42/200) or only botanicals (n=28/200). FECRT indicated development of resistance in H. contortus, Trichostrongylus colubriformis and Ostertagia species, and a tendency for the development of resistance in Cooperia curticei against benzimidazoles. Haemonchus contortus was the only nematode resistant to levamisole. There was no evidence of development of resistance in nematodes against ivermectin.

Conclusion: There is a strong need to educate the farmers and qualified staff about the judicious selection and application of anthelmintics, and other standard worm control/management practices. Rotation and correct use of anthelmintics may help in the management of benizimidazole resistance and delaying the development of resistance against levamisole.

PO1.20

Putative Involvement of the Nematode -Aminobutyric Acid (GABA) Receptor in the Mode of Action of the Anthelmintic Emodepsid

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Identification of new anthelmintic substances with different modes of action is of major importance namely due to the course of emerging anthelmintic resistance in parasitic nematodes.

Emodepside belongs to a new class of anthelmintic drugs, the cyclooctadepsipeptides. Its efficacy against anthelmintic resistant nematode populations has been demonstrated. However, its mode of action is not completely understood yet. Different putative receptors for Emodepside have been discovered during the last years. The calcium-activated potassium channel SLO-1 and the G-protein coupled receptor lathrophilin-1 have been suggested to play important roles in the mode of action of Emodepside in nematodes. The aim of this study is to investigate a possible involvement of a further putative Emodepside receptor, the -Aminobutyric acid (GABA) receptor.

The motility of wild-type C. elegans and unc-49 GABA receptor knock-out mutants exposed to different concentrations of Emodepside was investigated. The wild-type C. elegans showed a significantly reduced number of body bends compared to the GABA receptor knock-out mutants at increasing Emodepside concentrations. Furthermore, the effect of the common anthelmintic Piperazin and the neurotransmitter GABA on wild-type C. elegans and GABA receptor knock-out mutants was analysed in this study. Although Piperazin is supposed to have GABA agonistic effects, the motility inhibition by Piperazin was the same for wild-type C. elegans and GABA receptor knock-out mutants. The effect of GABA was somewhat, but not significantly lower on the GABA knock-out mutants.

The observed findings substantiate an involvement of the nematode GABA receptor in the Emodepside mode of action. The Toxocara canis GABA receptor coding sequence was obtained and will be used for subsequent rescue experiments.

PO1.21

Control of Gastro-Intestinal Nematodes in Sheep with the New Amino-Acetonitrile Derivative, ZOLVIX® (Monepantel)

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ZOLVIX® (25 g/L monepantel) is the first product from the recently discovered Amino-Acetonitrile Derivative (AAD) class of anthelmintics to be developed for use in sheep.

Dose determination studies were conducted in Australia and Switzerland to identify the appropriate therapeutic dose of monepantel when formulated for the oral treatment of sheep to control adult and fourth stage larvae (L4) of gastrointestinal (GI) nematodes. The observed efficacies were strongly suggestive of a suitable minimum dose of 2.5 mg/kg liveweight.

In a second step, the high activity of monepantel at the indicated dose was confirmed against adult and larval stages of GI-nematodes in naturally and experimentally infected sheep in Australia, Switzerland and New Zealand.

Most of the identified worm species could be controlled completely or close to 100%. Least susceptible species were identified as Oesophagostomum venulosum, Cooperia curticei (L4 stage) and Nematodirus spathiger (L4 stage). In contrast, Haemonchus contortus were completely eliminated at 1.25 mg/kg. Noteworthy is the fact that, ZOLVIX was able to control nematode isolates that were resistant to the most important anthelmintic classes, i.e. the benzimidazoles, levamisoles and/or macrocyclic lactones. It can be concluded that the AADs will be a valuable tool in the hands of veterinarians and sheep farmers to control GI-nematode infections, especially in regions with widespread anthelmintic resistance.

ZOLVIX and monepantel are not registered or available for sale in Canada.

PO1.22

Anthelmintic Resistance of *Haemonchus contortus* in Small Ruminant Flocks in Southern Germany and Switzerland

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Frequent use of anthelmintic treatment against gastrointestinal nematodes (GIN) led to a selection of resistant GIN strains and consequently to the spreading of anthelmintic resistance (AR). In the context of an epidemiological study in Southern Germany and Switzerland, four farms with clinically reported AR were investigated. Therefore, faecal samples were analysed by Faecal Egg Count Reduction Tests (FECRT) following WAAVP recommendations. Accordingly, resistance was considered present if the Faecal Egg Count Reduction (FECR) was less than 95% and the lower 95% confidence limit for the reduction was less than 90%. The investigation was performed in two sheep flocks (Suffolk and Dorper) and in two goat flocks (16 boer, 21 dairy-goats). The sheep flocks were divided into three treatment groups of ten sheep each, which were sampled on treatment day and ten days later. Mean FECR was 70.8% and 55.3% in albendazole-groups (3.8 mg/kg BW), 52.4% in the fenbendazole-group (5 mg/kg BW), 47.3% in the oxfendazole-group (5 mg/kg BW), and 100% and 44.3% in moxidectin-groups (0.2 mg/kg BW), respectively. All goats were treated with eprinomectin (PourOn-formulation, 1 mg/kg BW). Mean FECR was 28.2% and 27.5% on day 13 after treatment. Coprocultures of all nine individual treatment-groups revealed that the predominant species after treatment was *Haemonchus contortus*.

The study confirmed the existence of resistant *H. contortus*-strains against eprinomectin in goats and against albendazole, oxfendazole, fenbendazole and moxidectin in sheep in Southern Germany and Switzerland. Therefore it is important to establish new treatment schemes to prevent the further spreading of resistance.

PO1.23

A Comparative Study on the Effect of Triclabendazole Sulphoxide on Egg Production and Hatch Rate in Different Liver Fluke Isolates

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Triclabendazole (TCBZ) is the main drug used to treat fascioliasis in both humans and livestock. Resistance to TCBZ was first recorded in Australia in 1995 and has since been observed in various parts of Europe. Fasciola hepatica, like many other helminth parasites, has a high reproductive turnover. Once the adult fluke is established in the bile ducts of the definitive host, it can produce around 25,000 eggs per day. Due to the increase in resistance to anthelmintic drugs in veterinary medicine, a number of standardised methods are used to detect resistance in the field. The in vivo faecal egg count reduction test (FECRT) and the in vitro egg hatch assay (EHA) for benzimidazoles are commonly used to detect resistance in nematode species. However, the effect of anthelmintic treatment on egg output, larval development and egg hatch rate is currently unknown for the liver fluke. This study aims to investigate the egg production, egg development and hatch rate of different isolates in response to treatment with triclabendazole sulphoxide (TCBZ.SO) in vitro. A significant decrease in egg production from susceptible TCBZ.SO-treated flukes and disruption to reproductive structures was observed. In contrast, resistant TCBZ.SO-treated flukes showed minimal disruption to reproductive structures and egg production. The data suggest that TCBZ.SO does have an effect on egg production in vitro. An egg hatch assay for detection of resistance is under development.

Epidemiology

Tuesday, August, 11, 2009

PO1.25

Natural Infections of *Angiostrongylus vasorum* and *Crenosoma vulpis* in Dogs in Germany

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Angiostrongylus vasorum is a highly pathogenic metastrongylid nematode that infects the right side of the heart and pulmonary arteries whereas adult *Crenosoma vulpis* resides in the distal aspects of the bronchial tree of dogs and wild canids. Both parasites have been found in many countries in Europe and appear to be endemic in distinct areas in France, Denmark, and Great Britain. However, until know only few data are available on the prevalence of these parasites in Germany. In order to assess the occurrence of these nematode parasites in Germany fecal samples of 773 dogs with clinical signs of respiratory diseases were collected from September 2007 to February 2009. The Baermann funnel technique was used to examine the samples for presence of lungworm larvae. *A. vasorum* and *C. vulpis* were found in 56 (7.2%) and 47 (6.1%) fecal samples, respectively. 30 *A. vasorum* and 11 *C. vulpis* positive dogs were located in Baden-Wuerttemberg, 12 and 14 in North Rhine-Westphalia, 3 and 4 in Bavaria, 1 and 6 in Rhineland-Palatinate, 7 and 4 in Saarland, 1 and 2 in Saxony, respectively. In Brandenburg only 2 dogs with *A. vasorum* and in Hesse, Lower Saxony and Thuringia 4, 1 and 1

dog with *C. vulpis*, respectively were detected. These surprisingly high prevalence rates indicate that both parasites are endemic in Germany. Due to the high number of lungworm-positive dogs veterinarians should consider infections with *A. vasorum* and *C. vulpis* as a differential diagnosis in dogs with symptoms of respiratory diseases in Germany.

PO1.26

Seroprevalence of Toxoplasma gondii Infection in Small Ruminants from Romania

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Toxoplasmosis is a widespread zoonosis caused by the intracellular protozoan parasite Toxoplasma gondii. Limited epidemiological informations are available about the seroprevalence of Toxoplasma gondii in small ruminants in Romania, and a high incidence of infection would have implications for public health. Aims: The objective of this study was to assess the seroprevalence of antibodies to T. gondii in small ruminants from centre and north-west of Roamania (Transylvania, Maramures, Banat and Crisana).

Methods: Serum samples from 1570 sheep (1453 ewes and 117 lambs) and 431 goats (401 adult goats and 30 kids) were tested for antibodies (IgG) to T. gondii by a commercial ELISA kit (Chekit Toxotest, IDEXX Laboratories, Switzerland), at a sera dilution of 1:400.

Results: Antibodies to T. gondii were found in 64.34%(935/1453) ewes, 25.23%(27/107) 6-months-one-year old lambs, 50%(5/10) 1-month old. The lowest prevalence in sheep were noticed in Transylvania and Crisana regions with 61.1%(454/743) and 61%(133/218) respectively, positive serum samples. The highest prevalence was revealed in Banat and Maramures regions with 75.30%(125/166) and 68.4%(223/326) respectively, positive serum samples. The differences between the regions were significantly (p<0,0001). In adult goats the prevalence of anti-T. gondii antibodies was 59.35%(238/401); 4.73%(19/401) of samples were doubtful. In kids only one serum sample was positive (1/30). The highest seroprevalence was registered in goats from Maramures region 83.33%(70/84). In Crisana region the seroprevalence was 67.27%(111/165), and 7.27%(12/165) of samples were doubtful. The lowest prevalence was in Transylvania region, being 37.5%(57/152); the number of doubtful samples was 7/152(4.6%). The differences between the three regions were significantly (p<0,0001).

Conclusions: Because of high prevalence of anti-T. gondii antibodies in small ruminants there is an important risk of human contamination in certain regions of Romania by consumption of row milk and meet.

PO1.27

Alaria alata: a Trematode Identified in Wild Boars Muscles in France

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The trematode Alaria alata was identified in wild boar meat during meat inspection for Trichinella spp detection in Eastern part of France. The larval stage recovered in wild boars was mesocercariae, which is in this paratenic host a larva migrans. Adult larvae were found in the small intestine of foxes hunted in the same area as wild boars. From 2003 to 2008, positive animals were identified in North East of France, but more recently a focus also emerged in the centre of the country. Since few publications described Alaria spp as zoonotic pathogen, wild boar carcasses were destroyed or sent for well-cooked preparations with a significant markdown value. Experiments on freezing resistance were performed to propose a method to cure the meat. After 5 days at -18°C, one alive larva was found. In order to identify the tissue location of A. alata, a mouse was infected per os with 6 larvae. Unfortunately, none of the 6 larvae were identified after 10 days of infection. The non-tissue specific location and the size of this trematode prompted us to propose an adapted protocol for Alaria diagnosis in meat. Moreover, molecular markers are under development to differentiate A. alata isolates and identify the source of wild boar's contamination in wildlife. The lacks of knowledge on this parasite, its life cycle and hosts, but also the zoonotic feature render difficult its control during meat inspection and the prevention regarding human health.

PO1.28

Prevalence of *Tritrichomonas foetus* and *Campylobacter fetus* Infections in a Spanish Endangered Beef Cattle Breed (Asturiana De La Montaña)

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Bovine trichomonosis and bovine campylobacteriosis are two venereal diseases which are considered important infectious causes of reproductive failure in extensively managed beef cattle and where natural mating is a common practice. The purpose of this study was to investigate the prevalence and risk factors associated with *Tritrichomonas foetus* and *Campylobacter fetus* infections in beef bulls from a local endangered beef cattle breed, Asturiana de la Montaña. This breed is used from ancient times in mountain pastoral systems in sustainable conditions. Preputial smegma samples were collected from 103 bulls (16.4% of the total bull population), from 65 herds (11% of the total). Detection of the pathogens was performed by microscopic examination, culture and PCR. Epidemiological data were collected by means of a questionnaire. The presence of *C. fetus* was not detected in these samples. The percentage of *T. foetus* infected bulls was 31.06 % with a herd prevalence of 41.53 %. The average age was 61.5 months for positive animals and 52.7 for controls. No risk factor could be associated with *T. foetus* infection. Regarding reproductive data, an increase of heat repeat was the only variable associated with *T. foetus*. Furthermore, higher percentages of cows not in calf (21.5 vs. 16.9) and longer calving intervals (479 days vs. 424) were found in positive herds. In summary, the results reported here highlight the importance of the disease in this breed and will be useful for future risk assessment and/or management plans for the prevention and control of bovine trichomonosis.

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PO1.29

Prevalence of *Sarcocystis* spp in Argentinean Cattle

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Sarcocystis infections affect cattle worldwide and are frequently asymptomatic. Bovines are intermediate hosts for *S. cruzi*, *S. hirsuta* and *S. hominis*, which have canids, felids and primates as respective definitive hosts. The objective of this study was to determine prevalence of different *Sarcocystis* spp in cattle from Argentina. Blood, heart and loin samples (n=300) were collected at slaughter from 142 cows, 73 steers, 72 heifers and 13 bulls from 8 provinces in Argentina. Ten grams of muscle were homogenized in a meat grinder with PBS, centrifuged, filtered and observed in a stereomicroscope. Sarcocysts were classified as thin wall (*S. cruzi*) or thick wall cysts (*S. hirsuta* or *S. hominis*), and a subset was further characterized by transmission electron microscopy (TEM). Antibody detection was performed by immunofluorescence antibody test (IFAT) using *S. cruzi* bradyzoites as antigen and serum samples diluted at 1/25, 1/200 and 1/800. Ninety nine point three percent of heart samples had *S. cruzi* and no thick wall sarcocysts; 72% of loin samples had *S. cruzi* sarcocysts, 27% had thick wall sarcocysts, and 25.6% had both kinds of sarcocyst. Thick wall sarcocysts were detected in samples from 7 provinces. Thick wall sarcocysts were identified as *S. hominis* and *S. hirsuta* by TEM. IFAT titer distribution was as follows: 23.7% of animals had a titer of 25, 55% had a titer of 200, 21% had a titer of 800 and 0.3% were negative. This is the first report of *S. hominis* and *S. hirsuta* in Argentina.

PO1.30

Development of Microsatellite Marker Panels for Trichostronglid Nematodes of Ruminants

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For population genetic studies microsatellite markers are considered useful if they possess two important attributes. The first is a significant level of polymorphism to discriminate between different isolates. The second is the ability to consistently amplify from a range of possibly very genetically divergent isolates. Previous attempts to isolate specific microsatellites for Haemonchus contortus and Teladorsagia circumcincta have encountered a high level of null-genotypes (non-amplification) making the isolation of useful markers extremely laborious.

We have developed a more efficient screening process which we have used to filter through significantly more potential microsatellites, identified by Tandem Repeat Finder (Benson, 1999), in order to isolate the few 'robust' and useful markers for population genetic purposes. From the screening of 80 potential microsatellites we have identified a panel of 8 Haemonchus contortus specific microsatellites. We present here the screening procedure adopted for the two important parasitic nematode species, H. contortus and Teladorsagia circumcincta and the use of the resulting panels of microsatellites for the genetic characterization of a range of genetically divergent isolates.

PO1.31

Increased Parasitic Burden by Haemonchus in Bovine in Brazil

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Studies concerning parasitic prevalence and intensity by helminthes species in bovine conducted during the 1990's in Brazil revealed a significant predominance of Cooperia in relation to Haemonchus, maintaining approximately 76% and 14% of the helminth load, respectively. With a similar objective, 76 bovine naturally infected by nematodes, including both bulls and cows aged eight to twelve months, were necropsied in the State of Minas Gerais. Sixteen helminth species were detected, presenting the following prevalences and mean infection intensities: Haemonchus placei (100.00%; 3895.5); Haemonchus similis (28.95%; 159.58); Cooperia punctata (100.00%; 5594,95); Cooperia spatulata (32.89%; 137.83); Cooperia pectinata (34.21%; 1010.54); Trichostrongylus axei (69.74%; 239.22); Trichostrongylus colubriformis (10.53%; 10.79); Trichostrongylus longyspicularis (2.63%; 0.53); Ostertagia ostertagi (2.63%; 3.12); Ostertagia lyrata (2.63%; 1.47); Ostertagia trifurcata (1.32%; 0.26); Oesophagostomum radiatum (94.74%; 470.89); Trichuris discolor (47.37%; 32.51); Strongyloides papillosus (1.32%; 0.07); Capillaria bovis (9.21%; 0.93) and Bunostomum phlebotomum (2.63%; 0.26). The results obtained verified that the two most frequently detected helminth genera were Cooperia (58.34%) and Haemonchus (35.08%), showing a very different correlation from previously published studies. Considering that Haemonchus is significantly more pathogenic to bovine than Cooperia, the increase in its parasitic intensity (from 14% to 35%) observed in this study suggests that the methods of strategic and chemotherapeutic control used to date must be reviewed. Moreover, the significant increase in parasitism by Haemonchus and the consequent decrease by Cooperia, determined by the 76 necropsies conducted in this work, reveal a growing morbidity of parasitism by nematodes in bovine located in Brazil.

PO1.32

The Influence of Queen Elisabeth National Park on the Prevalence of Selected Hemoparasites of Cattle in Uganda

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Introduction: The location of cattle farms around Queen Elisabeth National Park (QENP) frequently exposes farmed cattle to interaction with wildlife. The purpose of this study was to investigate whether the proximity of farms to QENP increased the prevalence of hemoparasites among farmed cows

Methods: Cross-sectional studies were conducted during the summer periods of 2004, 2005, and 2006, in cattle farms surrounding QENP. Blood samples were obtained from 131 cows belonging to 13 farms. Blood smears stained with Diff-quik stain were examined microscopically for evidence of hemoparasites. Coordinates of Global Positioning System (GPS) of these farms were taken. The distance between the farm and QENP was recorded as a dichotomous variable (< 5 miles or > 5 miles). Data were analysed using Excel Data Plus at a significant level of =0.05

Results: The prevalence was 51% (95%CI 49, 62) for Theileria parva; 15% (95%CI 9, 21) for Anaplasma marginale and 8% (95%CI 4,15) for Babesia bigemina. Cattle farms located within 5 miles of QENP had a statistically significant higher prevalence of Theileria parva (2 = 8.3, df = 1, p= 0.004) and Anaplsama marginale (2 = 6.8, df = 1, p= 0.009) than farms located more than five miles from QENP. Farm location had no significant influence on the prevalence of Babesia bigemina (2 = 3.3, df = 1, p= 0.07).

Conclusion: These findings accentuate the role played by wildlife in the distribution of the vectors responsible for spreading Theileria parva and Anaplsama marginale among cow populations raised in the QENP area.

PO1.33

Reindeer Nematodes Infect Sheep

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The increasing number of sheep (*Ovis aries*) in the semi-domesticated reindeer (*Rangifer tarandus tarandus*) herding area in northern Finland leads to winter-fed reindeer sequentially sharing corrals with sheep. This causes potential for cross-infection of gastrointestinal nematodes between these two ruminant species. To elucidate this potential, this study was designed on a reindeer-and-sheep farm. In September-November, slaughter animal abomasa and proximal small intestines were collected and examined for gastrointestinal nematodes. Twelve reindeer and 8 sheep had shared a corral. Twelve reindeer had no known contact with sheep and 12 sheep from another farm had no apparent contact with other ruminants. Both reindeer groups shared free ranging areas with wild moose (*Alces alces*). Also two moose were examined. The parasites were collected, counted and identified. The following species were found in reindeer: *Ostertagia gruehneri, Ostertagia arctica, Spiculopteragia dagestanica,*

Nematodirus tarandi, Nematodirella longissimespiculata and *Bunostomum trigonocephalum*. Sheep were found infected with *Teladorsagia circumcincta, Teladorsagia trifurcata, Ostertagia gruehneri, Ostertagia arctica, Nematodirus filicollis* and *Nematodirus spathiger. Spiculopteragia dagestanica* and *Ostertagia gruehneri* were identified in moose. *Ostertagia gruehneri*, which is considered a reindeer parasite, was only found in the group of sheep that had shared a corral with reindeer. These sheep were not found to be infected with other abomasal nematodes. The reindeer that had shared a corral with sheep were not infected with nematodes usually having sheep as their primary host. However, as the group of sheep did not harbour them, either, it is obvious that there was no sheep nematode infection pressure to reindeer. On other locations, reindeer have been found to harbour sheep parasites.

Food Borne Parasites

Tuesday, August, 11, 2009

PO1.34

Toxoplasma gondii in Ireland

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Toxoplasma gondii is a protozoan parasite which can be transmitted to humans by consumption of undercooked meat and whose prevalence can be decreased by good farm management including rodent control. The Health Protection Surveillance Centre in Ireland has reported 173 cases of human toxoplasmosis in last 4 years but very little is known about the prevalence in animals.

Serum and meat samples from sheep, pigs, chickens and deer were collected from Irish abattoirs in 2007. Age, sex and origin of the samples were recorded. In addition rodent (*Apodemus sylvaticus*, *Sorex minutus*) tissue samples were also collected. Sera were tested for *T. gondii* antibodies by a commercial semi-quantitative latex agglutination test and meat samples by nested PCR targeting the multicopy 18S-5.8S rDNA internal transcribed spacer (ITS1) region of *T. gondii*.

Antibodies to *T. gondii* were found in 35.5 % (103/292) of sheep; 4.7% (15/317) of pig; 1% (3/301) of chicken and 6.6% (23/348) of deer samples (titre ≥ 1:64). Age-related differences were found, with a significantly (P>0.05) higher prevalence in adult sheep and pigs. There was no significant difference in seroprevalence between males and females and different farm locations. Out of 177 meat samples only 1 pig sample was found to be positive in contrast with 18% (20/110) of rodent samples.

This study shows that a significant proportion of Irish food animals examined have been exposed to *T. gondii* and therefore could represent a public health risk for persons that handle or consume raw or undercooked meat.

PO1.35

Wild Boar Surveillance for *Toxoplasma gondii* Infection in the U.S.

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Toxoplasma gondii is an important zoonotic parasite worldwide. Human disease results from congenital infection, accidental ingestion of oocysts in the environment, or from ingestion of tissue cysts in raw or undercooked meat. Among domesticated food animals, chickens, sheep, goats and pigs are known to be infected at varying rates, depending on husbandry. Infection rates in wildlife vary as well, but are generally higher than in domesticated species. In the United States, wild boar are hunted for meat and often used to make cured products which would not involve cooking or freezing to inactivate Toxoplasma tissue cysts. However, nothing is known of the prevalence of Toxoplasma in wild boar in the U.S. In an effort to determine the risk of human exposure to Toxoplasma from wild boar, we undertook a serological study using samples collected during an ongoing national survey conducted by the U.S. Department of Agriculture. Approximately 2000 samples per year were tested beginning in 2007 to the present. Samples were tested using a commercial kit (SafePath Toxoplasma Immunoassay Kit) according to the manufacturer's instructions. The location of positive animals was determined and spatially plotted using the longitude and latitude coordinates recorded for each collected sample. Results of this study indicate that 16% of wild boars nationwide are infected with Toxoplasma, while infection rates in individual states range from 0-30%. Consumption of meat from wild boar therefore poses a significant risk for infection with Toxoplasma.

PO1.36

Neem Oil Organic Neem® (*Azadirachta indica*) to Combat *Rhipicephalus microplus* Tick

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Rhipicephalus microplus tick is present throughout Brazil, and is responsible for great economic losses to the cattle industry. The consumer market is demanding a new position

in the sector of food production to reduce the generation of chemical waste, and the use of alternative medicines can be a good strategy for this purpose. The Neem oil is extracted from the plant Azadirachta indica originally from India that has several medical indications. The objective of this research was to determine the effectiveness of a commercial Neem oil Organic Neem® against cattle tick by in vitro (Lab. Parasitic Diseases, UFPR) and in vivo (Parana Reference Centre for Agroecology, CPRA) assays. In vitro tests used engorged females, which were weighed, immersed for 5 min in 12 concentrations ranging from 0.16% to 10.24% and a control group in triplicates and kept in incubator (80% RHA, 27 °C) for 14 days. Eggs were then incubated for another 26 days under the same conditions for the visual assessment of hatching. In vivo test was conducted spraying 12 adult Jersey cows. Six animals were treated with A. indica at 3% concentration as indicated by the manufacturer. Engorged ticks were counted 9 times after treatment, and daily during the first week. The results from both studies revealed no significant differences in between treatments. The regression analysis showed no correlation between the increase of the concentration of Neem and its effects as ectoparasiticide.

PO1.37

Improving Smallholder Pig Farmers' Knowledge on *Taenia solium* Has the Potential for Reducing Porcine and Human Cysticercosis Incidences in Endemic Areas of Tanzania

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Introduction: *Taenia solium* causes cysticercosis in pigs and humans, leading to great economic losses and human suffering due to condemnation of infected pigs and disabilities in neurocysticercotic patients. Health education of rural smallholder pig farmers and their livestock management advisors in northern Tanzania led to an important reduction of porcine cysticercosis incidence in sentinel pigs, an indication of the potential for reducing human cysticercosis incidence. The health educational package developed with community involvement in northern Tanzania was adapted and evaluated in the southern region.

Methods: In 2008, 700 smallholder rural pig farmers and 14 livestock extension workers in Chunya district were trained on *T. solium* transmission, impact, prevention and control. A five item questionnaire was self-administered to 117 (17%) of the smallholder pig farmers immediately before and after the training to measure their knowledge on *T. solium* and attitude towards consumption of infected pork. Data were analysed using the McNemar test for paired proportions.

Results: Responses on all knowledge questions improved significantly ($P < 0.001$). Following the training, significantly more people (72.6%) informed they would not consume infected pork as compared to before the training (15.4%) ($P<0.0001$), an increased negative attitude towards consumption of infected pork.

Conclusion: These findings indicate the usefulness of health education in the control of *T. solium* infections in endemic areas. Further studies are needed to determine optimal frequency, schedule, and coverage of health education intervention for national or regional control of the parasite. Combined interventions are recommended for ultimate elimination of *T. solium* in Tanzania.

PO1.38

A Method to Detect *Toxoplasma gondii* DNA in Fresh Pork Sausage

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A DNA extraction protocol followed by a nested PCR to detect *Toxoplasma gondii* from samples of fresh sausage has been set to use for screening samples taken in local butcher shops. The PCR is targeting the rRNA locus with the first primers amplifying the intergenic region between 18S and 25S rRNA gene. The nested primers amplify specifically the internal transcribed spacer 1 (ITS1) region. By this method from 2 grams of fresh sausage spiked with a serial dilution of *T. gondii* DNA a positive signal could be detected up to 5 pg of DNA in the sample. Toxoplasmosis can be spread by ingestion of contaminated food and water. In a European multicentre analysis, fresh sausage consumption was reported as one of the major cause for toxoplasmosis transmission in Italy. Infections are quite often asymptomatic but they can be particularly severe when the primary infection occurs during pregnancy (congenital toxoplasmosis) and in cases of immuno-depression. Seroprevalence from 20 to 80% is observed worldwide in both humans and animals depending on age, life style, hygienic conditions. In a previous serological survey in Sicilian sheep flocks a peak up to 65% of positive results have been reported but an ongoing serological screening on swine reveals, so far, positive results to a maximum of 19%. Since the tasting of fresh raw pork sausage is a quite spread habit among Sicilian consumers, even low seroprevalence can represent a high risk factor for the transmission of toxoplasmosis.

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Immunology / Vaccines

Tuesday, August, 11, 2009

PO1.39

Immunization of Chickens with ISCOMs Assembled Recombinant SO7 Antigen and Purified Saponins Gg-6 and Ah-6 Induced Protection Against Eimeria tenella Infection

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Introduction: Avian coccidiosis, caused by various species of the genus Eimeria, is estimated to cost the world-wide poultry industry several billion dollars annually. At present many studies on control of poultry coccidiosis have centered on elicitation of protective immune response to parasite infection by development of various kinds of parasite vaccines. In the current study immunostimulation complexes (ISCOMs) containing recombinant SO7 antigen and purified saponins Gg-6 or Ah-6, were evaluated in their ability to stimulate humoral immunity and to protect chickens against a challenge infection with Eimeria tenella parasite.

Methods: ISCOMs assembled SO7 recombinant antigen (a highly conserved 24 kDa protein associated with the parasite's refractile body) and saponins Gg-6 and Ah-6 isolated from native plants Glycyrrhiza glabra and Aesculus hippocastanum by HPLC fractionation, were prepared. ISCOMs incorporated irrelevant antigens rather than Eimeria antigens were also used for immunization experiments.

Results and Discussion: 1-day old chickens were immunized intranasally with various ISCOMs preparations. Antibody production, serum carotenoid and nitrate-nitrite levels, oocyst production and weight gain were measured after challenge of birds with 50,000 Eimieria tenella oocysts per bird. It was shown that a single immunization with ISCOMs in dose of 6ug per chicken stimulated high levels of specific humoral immune responses, prevented increases in serum nitrate-nitrite levels, reduced oocyst output and prevented reduction in weight gain. Activity of immune responses and protection against challenge was much higher when SO7 antigen was assembled with Gg-6 or Ah-6 saponins into ISCOMs in comparison with SO7 antigen alone or ISCOMs incorporated irrelevant than coccidian antigens. In general, the results of study indicate that ISCOMs containing SO7 antigen and saponins Gg-6 and Ah-6 are suitable for preparation of a highly immunogenic coccidia vaccine protected chickens against Eimeria tenella infection. Research supported USDA ARS-ISTC grant #K-525p.

PO1.40

Early Local Cellular Response and Free Radical Mediated Cytotoxicity in Rats Immunised with a Recombinant by Juvenile Fasciola hepatica cathepsin B3 (CB3) and Infected with Fluke Metacercariae

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In the present experiment we investigated the ability of a recombinant CB3 to induce a protective immune response. We examined T cells, NK cells and free radicals production in the peritoneal fluid of rats at an early stage of infection with Fasciola hepatica.

Male Spraque-Dawley rats were vaccinated, subcutaneously, three times with CB3 and montanide. The control groups were injected only with the adjuvant. All groups were infected with 30 fluke metacercariae and euthanized 15 hours post infection (pi), 4 days pi and 4 weeks pi, respectively. The peritoneal fluid of experimental animals was analyzed by flow cytometry to estimate cell phenotypes and intracellular free radicals production. At 4 weeks pi, flukes were recovered to estimate a protectivity .

A reduction in the fluke burden was found in vaccinated rats.

In immunised rats there was a distinct increase in the cellular response. NK cells dominated in the peritoneal fluid of vaccinated rats, as early as 15 h pi. These groups generated very high levels of inducible NO and superoxide compared to infected controls.

Our results suggest that immunization stimulates free radical production by rat peritoneal cells upon contact with NEJ F. hepatica parasites and that this defence mechanism could be associated with resistance to F. hepatica infection.

PO1.41

The Humoral Response of Sheep Vaccinated with a Recombinant Cathepsin L (CL1) and Phosphoglycerate Kinase (PGK) of Fasciola hepatica and Challenged with Fluke Metacercariae.

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An efficient vaccine against Fasciola hepatica is intensely sought for. The main focus is on recombinant fluke antigens. The aim of this study was to evaluate the humoral response of sheep vaccinated with two recombinant liver fluke vaccine candidates.

Male merinos sheep were immunized twice with 100µg of either CL1 (received from University College Dublin) or PGK emulsified in Quil A. The non-vaccinated controls received

the adjuvant only. A month after the last immunization, all animals were challenged with 100 fluke metacercariae. Antibody titers against the vaccine antigens were determined by ELISA every 2 weeks.

There was a strong IgG response in all vaccinated animals starting from the 2 week after the first immunization. The second dose of the antigen resulted in another boost of antibody titers. There was a slight decline in the IgG response thereafter. The control group exhibited no significant antibody response during this period. Infection boosted the humoral response against CL1, but not PGK. The IgG titers for vaccinated animals were significantly higher compared to the controls. Non-vaccinated animals showed a lower antibody response to PGK compared to CL1 throughout the entire experiment.

These results indicate that CL1 is more exposed to the host's immune system than PGK, during the course of natural infection. Quil A was proven to be a potent adjuvant for sheep, inducing a strong and rapid humoral response in vaccinated animals.

Research funded by the EC, DELIVER project, contract No. FOOD-CT-2005-023025.

PO1.42

Recombinant Interleukin-4 Enhances Campylobacter jejuni Invasion of Intestinal Pig Epithelial Cells (Ipec-1)

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Campylobacter jejuni, a leading cause of bacterial gastroenteritis, has different host age distributions and disease expression in developing and developed countries. Polymicrobial infections may contribute to this, such as Trichuris, which elicits type 2 cytokines (including IL-4) and downregulates type 1 immunity. In previous studies, gnotobiotic piglets infected with C. jejuni and Trichuris suis had bloody diarrhea and marked gastrointestinal pathology, including bacterial invasion into epithelial cells and macrophages. Neonatal swine given these dual infections had elevated IL-4 and IL-10 responses in feces. In the studies reported here, we hypothesized that IL-4 or IL-10 enhances invasion of intestinal pig epithelial cells (IPEC-1) by C. jejuni. 10-14-day old IPEC-1 cells were pretreated with recombinant IL-4 (rIL-4) or rIL-10 for 5 hours and then challenged with C. jejuni. Cells pretreated with rIL-4 were viable and showed approximately 6 fold increases in C. jejuni (but not Escherichia coli DH5α) internalization compared to cells with no pretreatment. Enhanced C. jejuni invasion was rIL-4 dose dependent and reversed by addition of anti-IL-4 antibody. Preincubation with rIL-10 did not significantly alter C. jejuni internalization. Transepithelial electrical resistance (TEER) was significantly reduced following rIL-4 treatment, but not rIL-10 treatment. After rIL-4 pretreatment and C. jejuni challenge, light microscopy showed vacuolated cells with damaged paracellular junctions. Transmission electron microscopy (TEM) showed multiple internalized bacteria. Most were in the cytoplasm, but some were within or adjacent to vacuoles. We conclude that rIL-4 damages paracellular junctions and alters the physiology of these epithelial cells allowing increased invasion of C. jejuni.

PO1.43

Variation of Antibody Titers to *Toxoplasma gondii*, *Neospora caninum* and *Sarcocystis* spp in Naturally Infected Goats

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Toxoplasma gondii is an important cause of abortion in goats; less frecuently abortion is caused by *Neospora caninum* or *Sarcocystis* spp. The objective was to evaluate antibody titer variation for these parasites during pregnancy. Blood samples from 42 goats were collected at 3 time points: February (pregnancy diagnosis), May (1st kidding season) and September (2nd kidding season), in a farm with previous *T. gondii* abortions and positive serology for *N. caninum*. Sera were tested by immunofluorescence antibody test (IFAT) for detection of antibodies to *T. gondii*, *N. caninum* and *Sarcocystis* spp from 1:25 to end dilution. Abortion occured in 13 goats, and 3 kids died within the fist week of age. For each blood sampling, mean titers for *T. gondii* were 1769.2, 2380.5, 1666.7 and frecuency of detection was 100 % at all sampling points; for *N. caninum* mean titers were 117.3, 141.5, 157.7 and frecuency of detection was 35.8%, 43.9% and 28.6%; mean titers for *Sarcocystis* spp were 105.1, 101.2, 96.2 and frecuency of detection was 97.4%, 95.1% and 97.6%. The high titers and variation in *T. gondii* antibody detection suggests that infection was active during pregnancy and could be the cause of abortions.

PO1.44

***Trichuris suis* Immunomodulates an Autoimmune Disease (Multiple Sclerosis) in Rats**

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Helminth infections are known to have a potent systemic immunomodulatory effect on the host immune response, reducing the impact of autoimmune diseases. The swine whipworm, *Trichuris suis*, thus alleviates symptoms in Crohns Disease and Ulcerative Colitis patients, and several other helminths have successfully been used to improve signs of disease in experimental animal models of e.g. multiple sclerosis and type-1 diabetes. To study the immunomodulatory effect of *T.suis*, we first demonstrated that *T.suis* eggs are able to hatch in a rat and that larvae invade the epithelial cells of the caecum and proximal colon. Thereafter the aim was to investigate whether inoculations with *T.suis* eggs would affect the progress of Experimental Autoimmune Encephalomyelitis (EAE) in rats, a model for multiple sclerosis. An EAE response was induced in 16 Dark Agouti rats and the animals were scored daily to monitor the progress of disease. From the time the first symptoms (score 1) of disease appeared, 8 rats were inoculated with 7.000 *T.suis* eggs 3 times a week, while the other 8 rats were kept as non-infected controls. The treatment with *T.suis* continued until the rats were euthanized, when their clinical score reached 4 or at the latest 14 days after the first symptoms. There was a significant (P=0.0011) improvement of the clinical scores in the group of rats treated with *T.suis* compared to the untreated rats. The results from this preliminary study indicate that EAE rats may be a suitable model for helminth-induced immunomodulation.

PO1.45

Early Interleukin-10 Production in Mice Infected with *Neospora caninum* Tachyzoites

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Here the immune response of BALB/c mice inoculated with 5 10^5 or 5 10^6 *Neospora caninum* tachyzoites (NcT) was studied 24h after the parasitic challenge. At this time after infection a stimulatory effect of NcT on spleen B and T cells was already observed, as assessed by surface CD69 expression. However, no direct stimulatory effect of NcT on these lymphocyte populations was observed as assessed *in vitro* on NcT-challenged purified splenic T or B cells. An apoptotic effect of NcT on these responding cells was observed, more marked on the latter population. In the spleen of the NcT-infected mice, up-regulation of the co-stimulatory molecules CD40, CD80 and CD86 was observed on the surface of conventional ($CD11c^{high}$) dendritic cells (DCs). In agreement with this observation, *in vitro* -differentiated DCs were shown to internalize NcT. At this early time after infection studied here, increased Interleukin-10 (IL-10) serum protein and splenic mRNA levels were detected in the infected mice. Although its cellular source was not identified in vivo, *in vitro* results indicate that macrophages are likely candidates. The early IL-10 production observed in the NcT-challenged mice indicates that this cvtokine may play a role in the successful establishment of *N. caninum* infection in the murine host.

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PO1.46

Immune Response Induced by DNA Vaccine Encoding Microneme Protein 6 (MIC6) of Toxoplasma gondii in Mice

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Infection with the intracellular protozoan parasite Toxoplasma gondii causes serious public health problems and is of great economic importance worldwide. Microneme proteins which are responsible for adhesion and invasion have been implicated as vaccine candidates. In this study, we constructed DNA vaccine expressing microneme protein 6 (MIC6), and evaluated its immune response induced in Kunming mice. The sequence of gene encoding MIC6 was inserted into the eukaryotic expression vector pVAXI. We immunized Kunming mice intramuscularly. After immunization, we evaluated the immune response using lymphoproliferative assay, cytokine, antibody measurements and the survival times of the lethal challenged mice. The results showed that the group immunized with pVAX-MIC6 developed a high level of specific antibody responses against T. gondii lysate antigen (TLA), a strong lymphoproliferative response, and significant levels of IL-2, IL-10 and IFN- production, compared with the other groups immunized with empty plasmid or phosphate-buffered saline, respectively. These results demonstrate that pVAX-MIC6 could induce significant humoral and cellular Th1 immune responses. After lethal challenge, the mice immunized with the pVAX-MIC6 showed an increased survival time (13.3±1.2 days) compared with controls who died within 7 days of challenge. Our data demonstrate that MIC6 triggered a stronger humoral and cellular response against T. gondii, and that the antigen is potential target for the further development of a vaccine.

Leishmaniasis

Tuesday, August, 11, 2009

PO1.47

Canine Visceral Leishmaniasis Diagnosis

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Canine Visceral Leishmaniasis (CVL) is caused in Brazil by a protozoa parasite of the *Leishmania (Leishmania) chagasi* species, which dog is the main domestic host. The purpose of this study was to evaluate the CVL diagnosis by ELISA, indirect fluorescence antibody test (IFAT), histochemical (HE), immunohistochemical (IMHC) and PCR using liver, spleen and lymph node tissues from 34 dogs with different clinical signs. A comparative analysis among tests was also done by Kappa index. Positive dogs were euthanized by the Control Center of Zoonotic Diseases. Serological tests (RIFI and ELISA) and parasite direct microscopic examination (HE and IMHC) detected the highest numbers of positive dogs in polisymptomatic (92.0%) followed by oligosymptomatic (57.0%) and asymptomatic (12.5%) dogs. In addition, 60.0% of dogs were positives, 15.0% were negatives, but 26.5% were classified as suspect because of serological and parasitological inconclusive results. Furthermore, PCR confirmed the positive results and detected DNA in tissues from 100% of negative dogs and 89.0% suspects raising the animal positivism index up to 97.0%. The positivism indexes of ELISA, IMHC, RIFI and HE were 65.0%, 62.0%, 56.0% and 56.0%, respectively. More intense parasite load of intact amastigotes was seen in spleen and lymph node tissues than in liver. By comparative analysis it was observed a low level of concordance between PCR and IFAT, ELISA or IMHC, but good between IFAT and IMHC. PCR was the most sensitive method for a definitive diagnosis when serological and parasitological tests were not able to detect positive dogs for this disease.

Non-Pharma Control

Tuesday, August, 11, 2009

PO1.48

Failure of a Commercial Herbal 'Internal Parasite Control' Product to Control Cyathostomes in Donkeys in the UK

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The objective of this study part was to assess the efficacy of a commercially available herbal product, 'Verm-X' at controlling cyathostome infection in donkeys. This product claims to repel parasites using a blend of herbs. Forty donkeys (2-38 years) were randomly assigned to a placebo or treatment group. Faecal egg counts (FEC) were carried out for all animals immediately prior to administration of treatment or placebo treatments. Donkeys assigned to the treatment group were administered the product according to manufacturers instructions over a 5 day period, the product was administered in a brown bread sandwich with apple sauce. Placebo group donkeys received a brown bread and apple sauce sandwich. According to manufacturers recommendations a further FEC was carried out two weeks post treatment to determine faecal egg count reduction (FECR). The treatment group showed a significant increase in FEC (P=0.009) as did the untreated group. FEC both pre- and post-treatment were not significantly different between donkeys receiving treatment and placebo. Neither group showed a reduction in FEC with the maximum FECR in the treatment group being 42% and in the placebo group 43%. FECR was not statistically different between the two groups (P=0.8).

PO1.49

Acaricidal Activity of *Lippia sidoides*, *Melia azedarach* and *Azadirachta indica* Essential Oils on Cattle Tick *Rhipicephalus (Boophilus) microplus*

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Rhipicephalus (Boophilus) microplus is responsible for considerable losses in cattle production due to disease transmission, blood loss, and the cost of control and treatment of transmitted diseases. Natural products seem to resolve environmental problems caused by synthetic acaricide, and many researchers are trying to find out effective natural products to replace synthetic chemicals. The aim of this study is to evaluate the acaricide activity of *Lippia sidoides*, *Melia*

azedarach and *Azadirachta indica* essential oils against larvae and adults of *Rhipicephalus (B.) microplus*. Essential oil of *L. sidoides* (concentrations 2.5; 5.0; 10.0; 15.0 and 25.0 mg/ml), *M. azedarach* and *A. indica* (concentrations 50.0; 100.0; 150.0; 200.0 and 250.0 mg/ml) were tested separately on larvae using the Larval Packet Test (LPT) and against engorged female using Adult Immersion Test (AIT). The lethal concentrations (LC) were calculated using Probit analysis. *M. azedarach* and *A. indica* essential oils showed high LC90 in LPT (higher than 1291 mg/ml). In AIT these oils showed LC90 of 204 mg/ml (*M. azedarach*) and 181 mg/ml (*A. indica*). This result shows that *M. azedarach* and *A. indica* essential oils do not kill the tick but brake the life cycle inhibiting the hatching. *L. sidoides essential* oil showed LC90 of 31 mg/ml in LPT and AIT. This essential oil showed high efficiency against larvae and engorged female of *R. (B.) microplus*. The results point to good and environmental alternative of control of tick and need of further studies to observe the efficiency of *L. sidoides* oil in tests In Vivo.

PO1.50

Effects of Condensed Tannin from Acacia Mearnsii on Goat Infected Naturally with Gastrointestinal helminthes

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The effect of condensed tannins of Acacia mearnsii on endoparasite in goat was investigated using 24 goat kids 12.2 ± 3.4 kg, 4 months-old of the anglo nubian cross breed. The animals were randomly distributed among three treatments with eight animals each. The treatments used were: GCT (animals receiving 24 g of A. mearnsii containing 16.7% of condensed tannin/animal/week), GD (animals not receiving tannin and was drenched with levamisole and closantel) and GC (animals not receiving tannin nor drenched). The experiment lasted 133 days, with animals kept on an Andropogon gayanus mixed with native pasture. Faeces were collected weekly for the egg per gram count (EPG) and the animals were weighed in interval of 14 days. The animals from GD showed total weight gain of 4.9 kg higher than GC 2.6 kg ($P<0.05$), but similar GCT 3.8 kg. Lower values of EPG were observed in GCT and GT than GC ($P = 0.05$). Animals of GTC one time had mean of EPG higher 500, however animals of GC showed three times mean of EPG higher 500. These results showed antiparasitic effect of condensed tannin from A. mernsii representing an alternative for worm control in goat.

PO1.51

In Vitro Efficacy of Commercial Isolates from Plants on Rhipicephalus (boophilus) microplus

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Commercial isolates from plants have potential use to control R. microplus. This study investigated the action of isolates on engorged females and larvae of R. microplus. The females were immersed for five minutes in the substances and incubated (± 28°C and humidity of 80%) for subsequent analysis of the biological parameters. The larvae were tested by the impregnated paper method, with reading after 24h of incubation. The isolates tested with three repetitions were citral (95% pure, Aldrich®), citronelal (85% pure, Dierberger®), geranyl acetate (97% pure, Fluka®), R-(+)-limonene (98% pure, Fluka®) and terpinolene (85% pure, Fluka®). The control was distilled water with 2% of tween. The substances were evaluated on the females, at the following concentrations: 0.31%, 0.63%, 1.25%, 2.5% and 5%. The mean efficacy of these respective concentrations was, for citral: 14%, 0%, 0%, 16% and 30%; citronelal: 10%, 11%, 0%, 9% and 0%; geranyl acetate: 0%, 12%, 0%, 7% and 0%; limonene: 0%, 0%, 6%, 47% and 11%; and terpinolene: 4%, 0%, 10%, 6% and 28%. The substances were tested on the larvae at concentrations of 0.63%, 1.25%, 2.5%, 5% and 10%. The mean efficiency of these concentrations allowed calculating the LC50 and LC99 by the Probit procedure, which were, respectively 0.4% and 1.2% for citral, 1.1% and 3.3% for citronelal, 0.8% and 25.3% for geranyl acetate, 5.2% and 32.3% for limonene and 3.5% and 9.9% for terpinolene. These results indicate that the association of citral with a synthetic active could increase the efficacy against larvae of R. microplus.

PO1.52

Action of Neem Cake (*Azadirachta indica*) on *Haematobia irritans* in Nelore cattle

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Products made from neem could provide effective natural control of cattle ectoparasites without harming the animals, consumers or the environment. The aim of this study was to evaluate the efficacy of commercial neem cake in controlling H. irritans in beef cattle, using two treatments, each with 20 Nelore cows: the control group (supplied only with mineral

salt) and the treated group (2% neem cake mixed with mineral salt according to the manufacturer's recommendations). To divide the animals, two fly counts were carried out 14 and 7 days before of the experiment, to form two homogenous groups. The animals were kept in paddocks 1 km apart. With the animals contained in a chute, the flies (y) were counted on days 7, 14, 21, 28, 35, 42, 49, 56 and 63. The hind region of each animal was photographed and each fly was counted by marking using the Paint Brush program. After statistical tansformation the data were analyzed by the MXED procedure of the SAS program, considering days as repeated measures. There was a statistically significant difference ($P<0.05$) between the effect of the days. There was no statistical difference ($P>0.05$) between the treatments or the physiological state of the animals, because pregnant and non-pregnant cows were distributed uniformly in the two groups. The quantification via HPLC of the azadiractin A and B levels revealed the presence of 421 mg/kg and 151 mg/kg, respectively. We can conclude that the product did not demonstrate efficacy in controlling flies after 63 days of administration.

PO1.53

Effects of Crossbreed Pregnancies on the Abortion Risk of *Neospora*-Infected Dairy Cows

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The aim of the present study was to further analyze that the use of beef bull semen significantly reduce *Neospora caninum* abortion rate in seropositive artificially inseminated (AI) dairy cows, as shown in previous studies. A total of 1115 pregnancies of *N. caninum* seropositive lactating dairy cows were evaluated. Abortion rates were 15.2% (96/633) and 32.2% (155/482) for cows inseminated with beef breed and with Holstein-Friesian semen, respectively. Abortion rates in response to AI using semen from different breeds of beef bulls were 9.9% (30/304), 19.1% (17/89) 19.9% (38/191) and 22.4% (11/49) for Limousin, Piedmontese, Belgium Blue and Charolais bulls, respectively. The highest abortion rates were observed in the dams AI using Holstein-Friesian semen that had high (≥ 30 units) *N. caninum* titration (36.7% abortion, 94/256). The lowest likelihood of abortion occurred in Limousin inseminated cows with low antibody titres, with an abortion rate similar to that in seronegative animals in the analyzed herds (2.1%, 3/145, and 3.2%, 239/7432, respectively). The present results show that different crossbreed pregnancies carry different risks of abortion in *N. caninum*-infected dairy cows. The use of beef bull semen, especially semen from Limousin bulls, can significantly reduce the abortion risk. The maternal *N. caninum*antibody titre also has a clear effect on this risk, with lower risk of abortion if the inseminated *N. caninum* seropositive dams have low antibody titres. Regardless of antibody titre, the insemination of seropositive cows with Limousin bull semen is highly recommended in herds with a high *N. caninum* seroprevalence.

PO1.54

Effect of Heather Consumption on Incoming Larvae and Established Population of *Trichostrongylus colubriformis* in Experimentally-Infected Cashmere Goats

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The consumption of tannin-containing heather seems to be associated with an apparent greater resilience of goats naturally-infected with gastrointestinal nematodes. The current study was performed in experimentally-infected Cashmere goats and had two objectives: firstly, to investigate the effects of heather consumption on the establishment of incoming *Trichostrongylus colubriformis* infective larvae (experiment 1), and secondly, to examine their effects on adult populations of this intestinal trichostrongyle (experiment 2). In experiment 1, 12 castrated male goats were divided into 2 groups: heather supplemented *vs.* non-supplemented. After 2 weeks of adaptation to the diet, all goats were experimentally-infected with 6,000 L3 of *T. colubriformis*. Three weeks after infection goats were slaughtered, worms were counted, and female worm fecundity and development were determined. Heather consumption was associated with close to a significant (P=0.092) reduction on larvae establishment. No effect on fecundity was observed, but the length of female worms was significantly (P<0.001) higher in supplemented goats. In experiment 2, 15 non-lactating goats were experimentally-infected with 6,000 L3 of *T. colubriformis* and were fed high quality lucerne hay. After 6 weeks, 3 groups were established: control, heather supplemented and heather supplemented + polyethylene glycol. Individual faecal egg output was measured weekly. Goats were slaughtered 5 weeks after heather administration and worms were counted, and female worm fecundity and development were determined. Heather administration was associated with a significant (P<0.001) decrease in egg excretion. Although worm counts and female fecundity were lower in supplemented goats, no significant differences were observed.

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vided by a research grant from the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (RTA2007-00098-C03).

PO1.55

Evaluation of Cassava (*Manihot esculenta*) Leaves for Worm Control in Sheep

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Helminthiasis is one of the causes of mortality and morbidity in sheep and goats in Malaysia. However, due to anthelmintic resistance which is escalating in Malaysia and other countries, other alternatives for worm control is needed. One such method is the use of local herbal products for worm control. Cassava is a tropical plant which originated from South America. Previous studies conducted in Vietnam (Thi mui Nguyen *et. al,* 2005) and Cambodia (Seng *et al.* 2006) in goats have proven anthelmintic effect of cassava leaves. Therefore, this study was conducted to evaluate the anthelmintic effect of Malaysian cassava *Manihot esculenta* leaves on nematode parasites of sheep. Twenty Malin breed sheep were randomly selected and equally divided into (untreated) control group(n = 10) and (treated)fresh cassava leaves fed group (n =10). Faecal egg counts (FEC) using the modified McMaster technique was carried out 3 times per week and the FAMACHA score for assessing clinical anaemia was conducted weekly for 3 months. The results indicated that there was no reduction in FEC but Total Worm Count (TWC) showed a reduction of 40 % in the treated group as compared to the untreated group. These results indicate that feeding cassava leaves has an effect on the TWC. Therefore, the use of cassava leaves is a potential alternative for worm control but further work in terms of dosage and toxicity of feeding it in ruminants should be evaluated.

PO1.56

Investigation of Anthelmintic Activity of *Eucalyptus staigeriana* Essential Oil on Goat Gastrointestinal Nematodes

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Gastrointestinal parasitism is one of limiting factors for the breeding of sheep and goats. The increase of anthelmintic resistance and the impact of conventional anthelmintics on the environment have prompted the search for alternative strategies, such as phytotherapy. The aim of this study was to evaluate the anthelmintic efficacy of *Eucalyptus staigeriana* essential oil (EsEO) and its toxicity. The *in vitro* effects of EsEO were determined through the egg hatching test and the inhibition of larval development of *Haemonchus contortus*. The oil was subjected to acute and subacute toxicity. 500 mg/kg EsEO was administrated orally over five days to evaluate its effects on intestinal nematodes in mice. The fecal egg reduction count test was performed using 30 goats naturally infected with gastrointestinal nematodes, divided into three groups: treated with 500 mg/kg EsEO, treated with ivermectin, and an untreated group. Fecal samples were collected from each animal to determine the count of eggs per gram (epg) at 8, 15 and 22 days after treatment. 1.35 and 5.4 mg/ml EsEO inhibited 99.27% and 99.20% *H. contortus* egg hatching and larval development. In subacute toxicity of EsEO, all parameters were found to be in the normal range, and histopathological analysis of organs did not present alterations. At a concentration of 500 mg/kg, the essential oil was 86% effective against mice nematodes. EsEO efficacy against goat gastrointestinal nematodes was 59% at 15th day after treatment. *E. staigeriana* essential oil showed *in vitro* and *in vivo* anthelmintic activity.

PO1.57

Evaluating the Anthelminitic Efficacy of Eleven Browse Plant Extracts from Kenya Using in-vitro Assays

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Introduction: The search for novel approaches in control of ruminant gastro-intestinal nematode infections has been necessitated by development of anthelmintic resistance worldwide. Condensed tannins (CT) are recognised as having anthelmintic activity in small ruminants.

Methodology: Nine native and two introduced browse species from Kenya varying in CT concentrations from 0-10% were collected, dried, ground and evaluated for their anthelmintic potential using egg hatch (EHA), larval development (LDA) and larval migration inhibition (LMI) assays. Two crude extracts were prepared from the leaves [acetone (70%)/water (30%) (AWE), and water (WE)] and were tested in serial dilutions of 25, 5, 1, 0.2, 0.04 and 0.008 mg/ml in 24-well microtitre plates. Thiabendazole (TBZ) was used as the positive control for the EHA and LDA, while tetramisole was used in the LMI.

Results: The AWE preparations had higher activity for the EHA and LDA tests than the WE. The WE had higher activity in the LMI assay than the AWE. Prosopis juliflora AWE leaf extract had the highest potential to inhibit egg hatch close to TBZ. The same plant also had the highest efficacy (93%) at 0.2mg/ml compared to 35% (Acacia tortilis) and 91% (Thiabendazole) for the LDA. Acacia tortilis WE inhibited 80% of the larvae from migrating LMI against 100% for the tetramisole at 1200µg/ml.

Conclusion: Prosopis juliflora (leaves and pods) and Gliricidia sepium extracts had the best activity and may have future promise in integrated agricultural systems where they can be used as browse and therefore may lower nematode infection rates of ruminants.

PO1.58

Evaluation of the Persistent Efficacy of Copper Oxide Wire Particles Against *Haemonchus contortus* in Sheep

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Introduction: Copper oxide wire particles (COWP) have shown a curative effect against *Haemonchus contortus* with an efficacy of 75% under controlled conditions. Field trials in tropical sheep and goats suggested a possible persistent effect against *H. contortus*. The present trial evaluated the persistent efficacy of COWP against *H. contortus*.

Methods: A controlled artificial infection trial was conducted. Twenty four helminth-free lambs (mean weight of 10.8 kg LW) were randomly allocated to 4 groups of six animals. Three groups were treated with a capsule of COWP (2 g of metallic copper): one on day -21 (group -21), another on day -14 (group -14) and the third on day -7 (group -7) before infection. A further group was kept as untreated control (Group C). Artificial infection with *H. contortus* (3700 L3) was performed on day 0. Sheep were humanly slaughtered on days 23 and 24 post-infection. Worm counts were performed.

Results: Geometric mean (+/-SE) for *H. contortus* in group C (1934+/-142) was higher than in group -7 (1104 +/-224) (P=0.025). The group -14 (1334+/-205) showed a tendency to reduce worm burden compared to the control (P=0.054). The worm burden of the group -21 (1720+/-213) was not different to that of the control group P=0.10). Efficacy values, for the treated groups were 42.9, 31.0 and 26.5 % for the group -7, group -14 and group -21, respectively.

Conclusion: The COWP (2 g of copper per animal) can provide a level of protection against re-infection with *H. contortus* for at least 7 days.

Other

Tuesday, August, 11, 2009

PO1.59

Non-Cerebral Coenurosis in Goats in the UA

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During a survey at an abattoir in Dubai in 2008 300 carcasses of young goats aged between three and six months were infested with metacestodes. Two types of cestode larvae were situated in the liver, in the body cavities, under the skin as well as between the muscles. The liver cysts were determined as Cysticercus tenuicollis while all other cestode larvae were typical coenuri with multiple scolices (between 22 and 421) situated in clusters (between four and 17) at the inner surface of the bladder. The volume of the coenuri varied between 0.5 and 40 ml. The rostellum of 300 – 400 m in diameter carried 26 to 32 hooks arranged in two circles. The average length of the larger and smaller hooks was 160 and 114 m, respectively. The location of these coenuri outside the central nervous system and morphological data of the structure of the coenuri and the measurements of the scolices suggest that these larvae might belong to a different strain of M. multiceps or even to a closely related species.

PO1.60

Varroa Destructor a Re-Emergent Pathogen Related to the Bee Depopulation Syndrome

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Bee parasites are intrinsically adapted to bee biology and most of them are considered enzootic in the specific areas where parasite and host have reached equilibrium along the time. Recent data on Varroa prevalence in Spain is supporting the fact that this mite plays a role as an aetiological factor on the disappearance of bees, acting as a re-emergent parasite. The Bee Depopulation Syndrome (BDS) have not been completely understood and the cause or causes of the syndrome are not yet fully acknowledged, although many authors attribute the problem to infectious agents. The higher prevalence of the asian microsporidium (Nosema ceranae) in the European bee (Apis mellifera) in recent years has been related to bee mortality and Varroa mites as well as some viruses, quite probably transmitted by Varroa, seems

also to be participating in the syndrome somehow. Other proposed causes include environmental change-related stresses, malnutrition and pesticides or management. It has also been suggested that it may be due to a combination of many factors and that no single factor is the cause.

BDS was first detected in Spain around 2003 as well as a high Nosema ceranae prevalence and adverse effects on bee survival. But in recent years an increasing Varroa destructor prevalence has been observed in healthy and depopulated hives. Data are presented to establish relationships between different factors analysed by way of a National epidemiological survey and biannual sampling procedure of randomly selected hives. Data are compared with samples sent to a National Diagnostic Laboratory where the detection of Varroa positive hives had been enhanced two or three times in the last years. National political measures are also analysed and possible repercussions on re-emergence of the mite discussed.

PO1.61

Combined Angiostrongylosis and Crenosomosis in a Dutch Dog

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In 2008 a survey showed that *Angiostrongylus vasorum* infections in dogs are endemic in the Netherlands. This case study emphasizes the fact that angiostrongylosis is an endemic disease in the Dutch dog population and one should be aware of double infections with *Crenosoma vulpis*.

A Bull-Terrier, with no history of traveling abroad to an endemic area, was presented at a clinic showing severe coughing which, despite a therapy with antibiotics, and subsequent heart medication, got worse over time. After two months the dog was referred to the University Clinic. A bronchoalveolar lavage was performed and a fecal sample was analyzed using the Baermann technique. Both methods resulted in the detection of larvae of *Angiostrongylus vasorum* and *Crenosoma vulpis*. An adult male of *Crenosoma vulpis* was present in the lavage fluid.

Slugs (e.g. *Arion* spp.) being the intermediate hosts for these nematodes, and foxes, the probable reservoir of both parasites in the Netherlands, make these double infections explainable and to be expected in other cases. In the mentioned survey no double infections were detected, so these double infections will probably occur in very small numbers. For epidemiological reasons, the use of the Baermann technique is recommended as this can provide the most complete diagnosis of the causative agents of lungworm diseases.

Following the reporting of angiostrongylosis being an endemic disease, the growing alertness amongst veterinary clinicians in the Netherlands has resulted in an increased submission of samples for the detection of *Angiostrongylus vasorum* in Dutch dogs.

PO1.62

Gastrointestinal Parasites of Lamas in the Bolivian Andes

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A cross sectional study was conducted to determine prevalences and intensities of gastrointestinal (GIT) parasites in lamas in the Bolivian Andes. A quantitative and qualitative necro-copro-parasitlogical study was performed on 33 lamas between October and December 2007. At the time of necropsy the lamas were aged 1 to >4 years. They originated from 14 different farms in the most lama dense areas of Bolivia: Oruro, Potosi, La Paz and the highlands above Cochabamba. In total 16 different species of nematodes, one cestode species, one trematode species, and one coccidian genus were detected (prevalences in brackets):

In C3 (third stomach compartment): Camelostrongylus mentulatus (33 %), Haemonchus contortus (15 %), Graphinema aucheniae (12 %), Marshallagia occidentalis (6 %), Ostertagia ostertagi (12 %); in the small intestine: Lamanema chavezi (64 %), Nematodirus spathiger (55 %), Nematodirus lamae (12 %), Nematodirus abnormalis (15 %), Cooperia onchophora (9 %), Cooperia surnabada (3 %), Trichostrongylus colubriformis (6 %), Trichostrongylus vitrinus (3 %), Trichostrongylus probolurus (6 %), Moniezia spp. (3 %); in the large intestine: Trichuris spp. (42 %), Skrjabinema spp. (3 %); in the liver: Fasciola hepatica (12 %); in faeces Eimeria spp. (82 %). Pathological changes in the liver were ascribed to be most probably caused by L. chavezi larva migration. The latter species, considered to be the very most pathogenic of all lama GIT nematode species, was also the species detected at the very highest intensity in the present survey, with a mean burden of 2,121 worms per animal.

PO1.63

Teaching Veterinary Parasitology and Parasitic Diseases at the Veterinary Faculty of UNAM in Mexico

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The Faculty of Veterinary Medicine and Zoothecnics (FMVZ) of the National University Autonomous of Mexico (UNAM) is among other 43 veterinary mexican schools the most representative teaching school from Mexico. In 2006 a new study plan was approved from which Veterinary Parasitology was decided to be taught in the third semester. Since it is considered as a coherent subject, it covers general aspects such as clasification, sinonims, hosts, localization, general morphology, life cycle and diagnosis of protozoan, helminths and arthropods of domestic animals. A total of 80 hours (40h lectures and 40 h practicals), are devoted to this course and after 4 partial examinations 11 groups of 50 students each are obliged to pass the final online departamental examination. At the seventh semester, students must take Parasitic Diseases considered as a pre-clinic subject in which teaching is focused to identify the relevance of these diseases in the organs of domestic animals. During the course 40% theorical and 60% practical examinations are in progress and 6 groups of 30 students each learn skills about integration of clinical and epidemiological data for diagnosis. At present we have developed our own diagnosis manual as well as the production of our own parasitology books, since most of them are foreign and epidemiological aspects not always fit with our local obtained data. We are also searching for the oportunity to re-design current parasitology courses to take advantage of new developments in teaching considering that further continuous improvement of these teaching programmes are neccesary. These teaching developments are supported by Project PAPIME-UNAM PE201006.

Parasite Physiology, Pharmacology, Pharmacokinetics

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PO1.64

Pharmacokinetics of Moxidectin and Triclabendazole in Sheep Following a Single Oral Treatment with Either Moxidectin, Triclabendazole or Both

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The purpose of this study was to establish the pharmacological behaviour of moxidectin and triclabendazole (its metabolites triclabendazole sulfoxide and triclabendazole sulfone) for the novel combination product of moxidectin plus triclabendazole after a single oral administration in sheep and to compare the pharmacokinetic profiles of moxidectin and triclabendazole (its metabolites triclabendazole sulfoxide and triclabendazole sulfone) in sheep when administered combined in the same formulation with those of moxidectin and triclabendazole administered alone.

Thirty sheep allocated to five groups were treated orally with either moxidectin alone in the final formulation, triclabendazole alone in the final formulation, moxidectin+triclabendazole, moxidectin intravenously and triclabendazole intravenously. Blood samples were collected at several intervals up to 40 days post treatment and the plasma assayed. The main pharmacokinetic parameters were evaluated and statistically compared.

The main pharmacokinetic parameters were respectively in the combined formulation AUCtot, 57.739 ± 28.594 ng.d.mL-1, Cmax, 12.427 ± 2.581 ng.mL-1, Tmax 0.806 ± 0.301 days, MRT 4.776 ± 2.552 days for moxidectin, AUCtot 608.210 ± 108.790 µg.h.mL-1, Cmax 10.048 ± 1.091 µg.mL-1, Tmax 21.339 ± 6.535 h, MRT 44.212 ± 6.746 h for triclabendazole sulfoxide and AUCtot 839.120 ± 187.640 µg.h.mL-1, Cmax 9.845 ± 1.416 µg. mL-1, Tmax 47.606 ± 0.059 h, MRT 70.863 ± 8.257 h for AUCtot 839.120 ± 187.640 µg.h.mL-1, Cmax 9.845 ± 1.416 µg. mL-1, Tmax 47.606 ± 0.059 h, MRT 70.863 ± 8.257 h.

The bilateral Student t test conducted on the key parameters showed there were no significant differences in PK parameters between actives as administered alone or combined, demonstrating that there was no interaction between actives.

PO1.65

Preliminary Results of an Ongoing Multicentric Field Trial Concerning Efficacy of Metaphylactic Treatment with Toltrazuril on Weight Gain in Dairy In-Housed Calves at Risk of Coccidiosis

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Metaphylactic treatment with toltrazuril in calves at risk of coccidiosis reduces oocysts shedding and improves fecal score significantly. This has been studied thoroughly (Mundt et al. 2005, Mundt et al. 2007). In the present multicentric, randomized, blinded and placebo-controlled field study, the effect of metaphylactic treatment with toltrazuril (Baycox Bovis, Bayer Animal Health, Germany) on daily weight gain of dairy in-housed calves were studied in two Danish dairy cattle farms with confirmed history of coccidiosis caused by Eimeria zuernii and Eimeria bovis. The treatment group (n=9) received one shot toltrazuril (15 mg/kg) and the control group (n=11) received placebo. Treatment was initiated one week before expected outbreak of coccidiosis after regrouping of calves within the farms. Mean age of calves in

treatment and control groups respectively were 78.2±18.17 and 78.8±16.86 days at the start of study (treatment time), and 135.6±19.16 and 136.2±17.89 at the end of the study (pooled data). Mean daily weight gain through the study period was calculated statistically (Wilcoxon-Man-Whitney-U Test, two sided 95% CI) as 0.9511±0.0954 kg in the treatment group and 0.8120±0.1556 kg in the control group which was significantly different ($p<0.05$). Weekly faecal samples demonstrated that prevalence and OPG of E. zuernii and E. bovis were reduced significantly in the treatment group during the 5 weeks post treatment. The present study showed that metaphylactic treatment with toltrazuril in dairy calves at risk of coccidiosis controls oocyst shedding of pathogenic Eimeria sp. and improves daily weight gain significantly.

PO1.66

A Pathologic Study on Experimental Toxoplasmosis in Broiler Chicken

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In this investigation, the clinical signs and histopathological findings due to experimental toxoplasmosis in birds were studied. In this survey, 48 broiler chicks of 25 days old, were randomly distributed into four groups (A, B, C and D). Groups A, B and C were injected intraperitoneally with 5 105, 1 106 and 1.5 106 tachyzoites of Toxoplasma gondii respectively and group D was kept as a control. Before and after experiment, the sera of the chickens were checked against T. gondii antibody .After infection, the clinical signs of all chickens were recorded every day. Also thin blood smears were prepared to check parasitemia. Half of the chickens in each group were slaughtered 25 days after infection and the rest 35 days after infection. Histopathological lesions were observed in the brain, heart, liver, pancreas, kidney, spleen, skeletal muscles, proventriculus and lungs; though the eye did not show any histopathological lesions. These lesions in group A were more severe than other groups (B and C). Although group A has exposed to low dosage of T. gondii tachyzoites, the results indicate that the histopathological in this group were more than the other groups (B and C). Groups B and C which were exposed to high dosage of T. gondii tachyzoites, showed acute toxoplasmosis with low histopathological lesions. Though any stages of the parasite were not found in the histopathological sections of the skeletal muscles, attention must be paid to the potential importance of chicken meat in public health.

PO1.67

Effects of Curcumin (Diferuloylmethane) on Eimeria Tenella sporozoites In-vitro

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The negative effects of coccidiosis on poultry health and productivity and increasing problems related to drug resistance have stimulated the search for novel and alternative methods of control. The present study evaluates the anticoccidial activity of curcumin, the main constituent of turmeric. Its effects were evaluated on Eimeria tenella sporozoites, including morphological alterations, sporozoite viability and infectivity to Madin Darby Bovine Kidney cells (MDBK). Morphological alterations of the sporozoites were recorded as deformation due to swelling and cell membrane corrugations.

Curcumin at concentrations of 25, 50, 100, 200 and 400 µM showed considerable effects on sporozoite morphology and viability in a dose dependent manner after incubation over 3, 6, 18 and 24 h while lower curcumin concentrations (6.25 and 12.5 µM) were not harmful. In comparison to the untreated control, sporozoite infectivity was reduced at curcumin concentrations of 100 µM and 200 µM in a dose dependant manner by 41.64 and 72.81%, respectively. Negative effects of curcumin on MDBK cells were not seen at these concentrations, however, curcumin at concentrations of 1800, 600, and 400 µM were toxic to MDBK cells and affected their proliferation. In conclusion, curcumin exhibited a marked inhibitory effect in vitro on E.tenella sporozoites inducing morphological changes and reducing their viability and infectivity.

PO1.68

Hybridyzation Experiments Indicate Incomplete Isolating Mechanism Between *Fasciola hepatica* and *F. gigantica*

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*Fasciola hepatica*and *F. gigantica* have been commonly identified as the causative agents of fascioliasis in animals and humans. The two species can be discriminated by the sequences of nuclear rDNA and mitochondrial DNA, as well as morphological characters. Recently, the existence of *Fasciola* forms exhibiting heterogeneous sequences between

the 2 species has been reported in some Asian countries including Japan. The *Fasciola* forms seem to be offspring derived from interspecific hybridyzation between *F. hepatica* and *F. gigantica*, indicating that the 2 Fasciola species are considered to be a single species. The purpose of the study is to experimentally clarify the possibility of interspecific hybridyzation between *F. hepatica* and *F. gigantica*. One adult (Fh#3) of *F. hepatica* and 1adult (Fg#20) of *F. gigantica* obtained from a goat experimentally infected with both of the *Fasciola* species were used as parents. Metacercariae of Fh#3 and Fg#20 were produced from snail hosts and individually administered to 2 goats. Thirty-three and 14 adults derived from Fh#3 and Fg#20 were recovered from the livers, and all of the adults showed the heterogeneous genotype between *F. hepatica* and *F. gigantica* (ITS1-Fh/Fg) in nuclear ribosomal ITS1 sequences, suggesting that they are F1 between the 2 species. The F1 adults seemed to have the ability of spermatogenesis, since many sperms were observed in the seminal vesicles. The F1 showed a wide range of variation (1.8-4.7) in the ratio of body length and width. In order to ascertain their fertility, the intrauterine eggs obtained from the F1 adults were observed in the development. One to nine percents of the eggs hatched, while 40-96% and 4-50% died with no development and ceased developing prior to hatch, respectively, suggesting that hybrid breakdown occurred in F2. Viable metacercariae were produced from the hatched miracidia through the snail hosts, and F2 adults were obtained from infected animals. These findings reveal that the isolation mechanism between *F. hepatica* and *F. gigantica* is incomplete.

PO1.69

The Effect of Desiccation on Survival of the Infective Stage Larvae (L3) of *Teladorsagia circumcincta* at Different Constant Temperatures

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The effect of desiccation on survival of the infective stage larvae (L3) of *T. circumcincta* at constant temperatures was determined. Approximately 100 L3 larvae in 1 ml of water in a 55mm diameter Petri dish were placed at 25° C for 24 hr with the lid removed to allow gradual drying (Dessicated Group). A Control Group was also maintained at the same temperature but with the lid retained to ensure no evaporation occurred. Samples were then placed in an incubator at the following constant temperatures: -4°C, 4°C, 17°C, 25°C and 37°C. At intervals of 1, 4, 8, 12, 16, 24 and 32 days, 3 reps for both groups were rehydrated as necessary with a minimum of 2ml of water for 24 hr at 25°C. Larval viability was determined by motility and larvae were considered dead if they were immobile after prodding with a needle under a dissecting microscope. The mean survival at different temperatures for the Control and Dessicated Groups comparing Day 1 to Day 32 respectively were as follows: -4°C (98.3% to 98.5%; 36.1% to 14.5%), 4°C (97.8% to 98.7%; 89.2% to 36.7%), 17°C (97.6% to 98.9%; 39.8% to 13%), 25°C (100% to 97.5%; 78.2% to 5.4%) and 37°C (99.0% to 0.4%; 16.0% to 0%). At 37°C larval survival in the Desiccated Group had declined to 0.4% by Day 4. The survival rate of the Dessicated Group generally dropped faster with time, especially at higher temperatures. The optimal survival rate for the desiccated larvae over time was at 4°C.

PO1.70

Efficacy of an Abamectin+Levamisole Tablet Against an ML-resistant *Cooperia* in Cattle Using a Faecal Egg Count Reduction Test and Occurrence of a Possibly ML-resistant Isolate of *Ostertagia* in Cattle

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45 naturally infected yearling bulls were restrictively randomised into 3 groups. Group 1 remained as untreated controls, Group 2 were treated with ivermectin by subcutaneous injection (0.2mg/kg) and Group 3 were treated with a tablet formulation of abamectin (0.4mg/kg) + levamisole HCl (8mg/kg). Post treatment faecal samples were collected on Day13 and Day28. Faecal egg counts (FEC) were estimated using a modified McMaster technique where each egg counted represented 50eggs/g pretreatment and 25eggs/g post-treatment. All faecal samples were individually coprocultured and FEC were allocated to genera based on the proportions found. Efficacy was estimated by comparing pretreatment with post-treatment FEC corrected for changes in the control group. Pretreatment FEC for *Ostertagia, Trichostrongylus* and *Cooperia* were 53, 105 and 212 eggs/g respectively. Efficacy at Day 13 for *Ostertagia, Trichostrongylus* and *Cooperia* for Group 2 were 99%, 100% and 51% respectively and for Group 3 (abamectin+levamisole) were all 100%. Efficacy at Day 28 for *Ostertagia, Trichostrongylus* and *Cooperia* for Group 2 were 84%, 99% and 5% respectively. The mean allocated FEC for *Ostertagia* at Day 28 was 9.8eggs/g for Group 2 and 8.7eggs/g for Group 3. *Ostertagia* larvae were identified in the cultures of 9/15 animals in Group 2 at Day28. As the claimed efficacy of injectable ivermectin is for efficacy against *Ostertagia* for "at least 14 days", this reduced persistent activity is indicative of some degree of anthelmintic resistance in this genus. These results also show the abamectin+levamisole tablet, with no expectation of persistent activity, was effective against these ML-resistant *Cooperia* and possibly-ML-resistant *Ostertagia*.

PO1.71

Pharmacokinetic Properties and Efficacy of a Spot-On Combination Containing Emodepside Plus Praziquantel (Profender®, Bayer) in Reptiles

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Treatment of nematodiasis in reptiles can be very challenging, especially in stressed, non cooperative or dangerous species. A combination of emodepside and praziquantel (Profender®, Bayer) for topical application may be a promising alternative treatment option for the veterinarian. A pharmacokinetic study has been performed in different reptile species with different types of skin to test penetration of both active ingredients trough the skin. 11 healthy adult reptiles (3 green iguanas, 1 Argentine black-and-white tegu, 1 ball python, 1 corn snake, 2 Savannah monitor lizards, 1 Hermann's tortoise, 1 spur-thigh tortoise, 1 red-eared slider turtle) were tested. Blood was collected before treatment and at 3 intervals (5, 24, and 48 hours) after treatment with topical emodepside/praziquantel, 2 drops/100 g body weight. Pooled serum from 20 untreated turtles and tortoises was collected to establish baseline data. Results showed that both of the actives penetrate the skin and can be found in the serum at levels similar to those seen in cats. However, reptiles with thicker integument (e.g., terrestrial tortoises, green iguanas, ball pythons, Savannah monitors) showed relatively low concentrations for both praziquantel and emodepside 48 hours after administration. In some cases, emodepside concentrations were extremely low (< 2 mcg/L). In addition dose titration studies were performed to establish an effective dose, depending on the type and thickness of the integument. A dose of 4 drops or 0, 12 ml per 100 g of bodyweight was found to be effective also in thick skinned reptiles.

PO1.72

Persistent Activity of Monepantel Against Gastro-Intestinal Nematodes of Sheep

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Knowledge of the persistent activity of an anthelmintic is essential for integrating a product into a drenching regime. An anthelmintic with persistent activity may protect sheep from re-infection by gastro-intestinal parasites for a measured amount of time. Alternatively, decreasing levels of an anthelmintic may accelerate the selection for resistance to the product.

Previous studies have shown that treatment with monepantel (an Amino-Acetonitrile Derivative) at the recommended dose gives no protection against new infections of some gastro-intestinal nematodes. These studies indicated that there was some level of protection (7–10 days) against re-infection with Haemonchus contortus and that the onset of patency in lambs was delayed in serially infected lambs after treatment with monepantel.

A larger, more comprehensive study was conducted to define the presence and extent of persistent activity against H. contortus, Trichostrongylus spp., Teladorsagia spp. and Nematodirus spp. in lambs. Useful persistent activity is unlikely when ZOLVIX® (25 g/L monepantel) is used in nematode control programmes. Other options to manage contamination of pasture with infective larvae should be used such as pasture spelling, grazing with alternate animal species and/or less susceptible ages and classes of sheep.

ZOLVIX and monepantel are not registered or available for sale in Canada.

PO1.73

Glutathione S-Transferases of *Oesophagostomum dentatum* are Involved in Prostaglandin Production

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In previous studies the glutathione S-transferases (GST) of *O. dentatum*, the porcine nodule worm, were isolated from homogenates and characterised, and GST activities and gene expression were demonstrated in all parasitic stages (L3, L4, females and males). Based on the finding that the putative peptide sequences of both isotypes of Od-GST have considerable similarity not only to other nematode GSTs but also to a synthetic prostaglandin (PG) D_2 synthase, its ability to produce PGD_2 was investigated using a commercial test kit. Both worm homogenate and isolated GST from L3 and L4 showed PGD_2 synthase activity after addition of external PGH_2. Activity of cyclooxygenase, an enzyme responsible for PGH_2 production in mammalian systems, could not be unambiguously demonstrated. Together with the finding that GST activity was only detectable in cytosolic and not in microsomal fractions of worm homogenate it can be postulated that GST is the key enzyme for the production of PGD_2 in *O. dentatum*. As inhibition of the enzyme in in vitro nematode cultures leads to growth retardation it can be speculated that PGD_2 plays an intrinsic role for worm development (similar to other primitive organisms). As a bioactive lipid it might additionally be involved in the manipulation of the host since it is also secreted by larval stages of *O. dentatum*. However, the question remains how PGH_2 is provided as a substrate for PGD_2 synthase under natural conditions.

Targeted / Strategic Control

Tuesday, August, 11, 2009

PO1.74

Field Clinical Study with Cydectin triclamox in Sheep

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Moxidectin is a macrocylic lactone with broad spectrum activity against a variety of internal and external parasites and triclabendazole is a well known flukicide effective against adult and immature stages. Recently, an oral formulation containing 0.1 % moxidectin and 5 % triclabendazole has been developed. The objective of this study was to confirm the efficacy of this combination against nematode and fluke infections in sheep under field conditions in France.

Sixty ewes positive for gastrointestinal nematode eggs and Fasciola hepatica antibodies were selected. The ewes were treated either with moxidectin /triclabendazole orally at the recommended volume or with water at the same dose. EPG counts and coprocultures were done pretreatment and on days 21, 28, 34, 43, 49 and 58. Blood samplings were done pretreatment and on days 28 and 58.

Before treatment all the animals were infected by gastrointestinal nematodes and only a few of them were positive for fluke egg counts. However they were all positive for fluke when based on antibody levels. Coprocultures showed that the animals harbored all the major sheep internal parasites. Geometric mean egg counts were significantly lower in the treated group as compared to the control group at all time points and efficacy remained above 90 % at 58 days. Less than 6 control animals were infected with fluke hence the infection level was considered too low to draw any conclusion. No side effects were observed in any of the treated ewes.

The combination of moxidectin and triclabendazole demonstrated to be safe and effective under field conditions in France.

PO1.75

Effects of Condensed Tannins from a Tropical Legume (Mimosa caesalpiniifolia benth) Against Trichostrongylus colubriformis in Sheep

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Legumes rich in condensed tannins (CT) have shown potential control of endoparasites in ruminants. This represents an important sustainable strategy to combat parasites and improve sanitary conditions in the flock. The objective of this trial was to investigate the effects of CT in Mimosa caesalpiniifolia benth (Sansão do campo) against Trichostrongylus colubriformis. Twenty-four lambs (26.89 ± 2.16 kg LW), Santa Inês breed, housed in individual pens, received Cynodon dactylon hay (ad libitum) and 300 g concentrate/animal/day (40% Sansão do campo, 38% corn, 17.8% soybean meal and 4.2% mineral mixture) during 10 weeks. The infected lambs received a total of 3.000 L3 of the T. colubriformis divided three times a week. The experimental design was factorial (2 x 2) concentrate with or without Polietilenoglycol (PEG) and infected or not-infected animals. The sheep were weighted every 15 days and faeces were colleted weekly to count number eggs per gram in faeces (epg). No significant differences were observed between live weight gain between treatments. At the 10Th week the lambs receiving Sansão do campo showed lower epg (519) than those with PEG (969 epg) ($P<0.05$). The CT in M. caesalpiniifolia benth was active against T. colubriformis infection. Sansão do campo can be used as an alternative to control this infection in lambs.

PO1.76

Long Lasting Elimination of *Dirofilaria repens* Microfilariae in Dogs with Monthly Treatments of Moxidectin 2.5 % /Imidacloprid 10 % (Advocate®, Bayer) Spot On

Fok, Eva[2]; Jacsó, Olga[2]; Szebeni, Zsolt[3]; Győrffy, András[4]; Sükösd, László[5]; Lukács, Zoltán[6]; Schaper, Roland[1]

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Nowadays the infection with *Dirofilaria repens* in dogs occurs not only in south-eastern in Europe. Treatment options would be desirable to reduce further spreading and the risk of zoonotic transmission. Currently, there is no licensed treatment

for *D. repens* infections in dogs. Moxidectin has demonstrated efficacy against microfilaria (mf) and safety in dogs infected with *Dirofilaria immitis* and could be an option against *D. repens*. A multi-centre (Budapest/Pécs) field study with 64 dogs previously confirmed positive for *D. repens* mf was conducted where a spot on (Advocate®, Bayer) was tested in different treatment schedules. Treatments were applied to 44 dogs either monthly over 3 month (5 dogs) or 6 months (22 dogs), alternatively biweekly over 6 months (17 dogs) and 20 dogs remained untreated. Mf counts were performed monthly and for further 6 months following the last treatment. Two weeks after the first treatment 38 from 44 dogs were mf negative. Four weeks after initial treatment one dog still showed low mf count. Following the second treatment, all treated dogs were negative. This status was maintained in all treated dogs during the treatment period and the 6 month observation period following the last treatment. These data demonstrate the successful long lasting elimination of microfilariae from positive dogs. Moreover it maybe supposed that adults of zoonotic *D. repens* have been killed based on the observation that no more microfilariae were seen up to 6 months following the end of the treatment period.

PO1.78

Necrotic Enteritis Due to Simultaneous Infection with Isospora suis and Clostridia in Newborn Piglets and its Prevention by Early Treatment with Toltrazuril

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The objective of the study was to investigate the clinical response of newborn piglets exposed to natural *Clostridium perfringens* type A infection to low-dose experimental infection with *Isospora suis*. 51 piglets from 5 sows from a farm with a history of *C. perfringens* type A (ß2) infection were included. The piglets of three litters (n = 31) were experimentally infected with *I. suis* (1000 sporulated oocysts/pig) within the first 4 hours after birth and were randomly assigned into a treatment group (Baycox®; 20 mg toltrazuril/kg BW orally, 12 hrs. p.i..) or into a sham-dosed group. The piglets of the two remaining litters served as uninfected untreated controls. Clinical behaviour, mortality, faecal consistency, faecal oocyst counts, faecal germ counts, and weight development were evaluated for 14 days. One piglet each per study group and litter was necropsied and intestinal tissue samples were taken for pathohistological investigations and in situ hybridisation on study days 3 and 14.

I. suis-infected but untreated piglets developed severe clinical disease with liquid diarrhea and decreased body weight development. 38.5 % of the piglets of this group died during the study period in total, 30.8 % due to necrotic enteritis. In contrast there was no mortality in the toltrazuril treated group or in the uninfected control group.

The hypothesis was supported that concomitant infections early post natum of *I. suis* and *C. perfringens* type A lead to interactions of the two pathogens, increase the metabolically active *C. perfringens* type A and promote the development of necrotic enteritis (NE).

PO1.79

Efficacy of Toltrazuril (Baycox® 5% Suspension) in Natural Infections with Pathogenic *Eimeria spp.* in Housed Lambs

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1. Bayer Animal Health GmbH, Monheim, Germany; 2. Private Practice, Limoges, France; 3. Bayer Santé Division Santé Animale, Puteaux, France

The effect of single oral treatments with toltrazuril (Baycox® 5% suspension) and diclazuril (Vecoxan® suspension orale, 2.5 mg/ml) on naturally acquired *Eimeria* infections in lambs was investigated on a French sheep farm with a known history of coccidiosis. 75 lambs aged 10-14 days (study day 0 = SD 0) were randomised, allocated to three groups and treated (Groups: TOL: 20 mg toltrazuril/kg BW; DIC: 1 mg diclazuril/kg BW; CTRL: untreated). Clinical (faecal consistency, BW) and parasitological parameters (oocyst excretion, opg) were evaluated until SD 60. Coccidial infections (predominantly *E. ovinoidalis*) occurred in all groups (75-80% of the samples). The prevalence in the CTRL animals increased from SD 3 (4.2%) to SD 31 (86.4%) and remained high (>60%). The DIC group started to excrete oocysts early (SD 7) and increased to values comparable to the control on SD 35 with a maximum of 88% at the end of the study. In the TOL group excretion began on SD 14 and remained low until SD 28 when it reached 28%. Prevalences never reached 40% in this group. The number of excreting animals was significantly reduced only in group TOL. The number of excretion days and the average opg decreased significantly in the TOL and DIC groups, with the TOL group showing significantly fewer excretion days and excretion intensities than the DIC group. Changes in faecal consistency were moderate throughout the study. Daily weight gains were not statistically significant. However they were higher in the TOL group compared to groups DIC and CTRL.

PO1.80

Efficacy of Moxidectin/Triclabendazole Oral Solution Against Mixed Nematode and Fluke Infection in Sheep

Parker, Larry David[1]; Bairden, Ken[2]; Rock, David[3]; Blond, Francoise T.[4]

1. Fort Dodge Animal Health, Southampton, United Kingdom; 2. Consultant (Deceased), Glasgow, United Kingdom; 3. Fort Dodge Animal Health, Princeton, NJ, USA; 4. Fort Dodge Animal Health, Tours, France

Background: Moxidectin is an endectocide belonging to the milbemycin family with broad-spectrum activity against internal and external parasites. Triclabendazole is a benzimidazole with well known activity against liver fluke, Fasciola hepatica. This study evaluated the efficacy of a new combination oral solution containing 0.1% w/v moxidectin and 5% w/v triclabendazole in naturally infected sheep.

Method: Fifty eight grazing lambs were allocated to two equal groups based on faecal nematode egg counts (FEC). One group was treated with the combination solution at a dose rate of 0.2 mg/kg moxidectin and 10 mg/kg triclabendazole. The other group acted as controls and received water. Eight animals in each group with the lowest FEC were necropsied 14 days after treatment for the determination of F. hepatica burdens. The remaining 42 animals (21/group) were grazed together as one group on pasture for 56 days. FEC and group coprocultures were carried out on faecal samples collected 21, 28, 35, 42, 49 and 56 days post-treatment.

Results: At least 7 out of the 8 necropsied control animals were infected with early immature (1 – 4mm), late immature (5 – 10mm) and adult (>10mm) stages of F. hepatica. In contrast, no flukes were found in any of the treated animals. FEC were reduced by >99% over the full 56-day period. The combination product was well tolerated.

Conclusion: Moxidectin/Triclabendazole combination oral solution was >99% effective in reducing FEC in grazing lambs over a 56-day period under field conditions in the UK. It was also 100% effective against a natural infection of F. hepatica.

PO1.81

Evaluation of Persistent Efficacy of Moxidectin/ Triclabendazole Oral Solution Against Nematodes in Sheep

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1. Fort Dodge Animal Health, Southampton, United Kingdom; 2. Consultant (Deceased), Glasgow, United Kingdom; 3. Charles River Laboratories, Carlisle, United Kingdom; 4. Fort Dodge Animal Health, Princeton, NJ, USA; 5. Fort Dodge Animal Health, Tours, France

Background: Moxidectin is an endectocide belonging to the milbemycin family with broad-spectrum activity against internal and external parasites. Triclabendazole is a benzimidazole with well known activity against liver fluke. This study evaluated the persistent efficacy of a new combination oral solution containing 0.1% w/v moxidectin and 5% w/v triclabendazole against experimental nematode infections in sheep.

Method: Thirty-one parasite naïve sheep approximately three months old were allocated to 3 groups of 8 and one group of 7 based on bodyweight. Group 1 was an untreated control group. Groups 2, 3 and 4 received the combination oral solution at a dose rate of 0.2 mg/kg moxidectin and 10 mg/kg triclabendazole. Treatment was administered to Group 2 on Day -42, to Group 3 on Day -35, and to Group 4 on Day -28. All sheep were experimentally infected with Teladorsagia circumcincta (8,000 L3) and Haemonchus contortus (4,000 L3) on Day 0. At necropsy, the numbers of DL4 and adult nematodes in the abomasums were determined.

Results: Moxidectin/triclabendazole oral solution was 67.7% effective in preventing infection with H. contortus when administered 42 days prior to challenge, 98.6% effective when administered 35 days prior to challenge, and 99.9% effective at 28 days prior to challenge. The corresponding figures for T. circumcincta were 74.9% at 42 days, 99.7% at 35 days and 100% at 28 days. The combination product was well tolerated.

Conclusion: Moxidectin/Triclabendazole Oral Solution is >95% effective in preventing infection with H. contortus and T. circumcincta for a period of 35 days after treatment.

PO1.82

Successful Treatment of *Dirofilaria repens* Infections in Dogs with Melarsomine (Immiticide®Merial) Against Adults and a Combination of Moxidectin 2.5 % / Imidacloprid 10% (Advocate®, Bayer) Against Microfilaria

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Dirofilaria repens occurs in Europe predominately in southern and south eastern countries. Transportation of dogs from such endemic regions to areas free of *D. repens* bears the risk of introducing this zoonotic disease to non endemic areas. In this study 507 dogs transported from a Hungarian shelter to a shelter close to Cologne from August 2006 - February 2009 were tested for presence of microfilaria (mf). 60 *D. repens* positive dogs were enrolled in a treatment program consisting of an adulticide treatment with melarsomine (Immiticide® Merial, 2 injections 24 h apart), followed by a microfilaricidal treatment with 3 monthly applications of

moxidectin 2.5% / imidacloprid 10% (Advocate®, Bayer) at the standard dose. 35 dogs completed a surveillance period of 6 month following the treatment program. Macrofilaricidal treatment in dogs was tolerated well with few adverse reactions. Microfilaricidal treatment at monthly intervals did not show adverse reactions. All 35 dogs were screened for the presence of microfilaria, all but one stayed negative. It is known from *D. immitis* treatment, that melarsomine at the recommended dose will clear about 50 % -70 % of the dogs from macrofilaria and is ineffective against L 4 stages and early adult stages. This treatment protocol in conjunction with a follow up treatment of 3 monthly doses of moxidectin / imidacloprid is suitable to eliminate infections with *D. repens* almost completely and could be an important measure to avoid introduction of this zoonotic disease from endemic to non endemic areas.

PO1.83

Experimental Infection with *Ancylostom ceylanicum* in Dogs and Efficacy of a Spot on Combination Containing Imidacloprid 10 % and Moxidectin 2.5 % (Advocate®/ Advantage® Multi, Bayer)

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Ancylostoma ceylanicum is a prevalent hookworm species in dogs in Asia and Australia. Since only few anthelmintics are licensed to treat this parasite, an experimental infection model was developed to allow subsequent testing of anthelmintics. Adult hookworms from necropsied dogs have been collected and identified. Female *Ancylostoma ceylanicum* have been isolated and grinded to collect eggs. The eggs were mixed with sterile dog faeces and cultivated by using Hada Mori technique. Two helminth naïve puppies have been infected with 500 L 3 as donor dogs. After patency eggs were collected and cultivated to L3 stage. Twelve dogs were subcutaneously injected with 300 L3 of *A. ceylanicum*. Once patency was confirmed, individual eggs per gram (EPG) counts were performed daily. At day 20 post infection dogs were allocated into treatment and control group. Each dog from the treatment group received a spot on combination containing 10 % imidacloprid and 2.5 % moxidectin at the manufacturers recommended dose. EPG counts were performed daily for 14 days post treatment. The treatment rapidly reduced egg shedding within 3 days post treatment compared to the control group. No eggs were found in the treated dogs from day 4 post treatment onwards. EPG counts remained high (4469 + 2064) in the untreated control group. The spot on combination containing imidacloprid 10 % and moxidectin 2.5 % (Advocate®/ Advantage®, Bayer) given at the recommended dose is highly effective against infection with *A. ceylanicum* in dogs.

PO1.84

FAMACHA© – Big Success in the U.S.!

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FAMACHA©, the innovative clinical technique for managing and treating *Haemonchus contortus* in sheep and goats was developed by Francois "Faffa" Malan in South Africa. The FAMACHA© system allows animals to be treated selectively, thereby decreasing selection pressure on worm populations for development of anthelmintic resistance. Furthermore, FAMACHA© is made available to farmers only through veterinarians or formal training workshops, thus placing the veterinarian back into the role of health advisor for what is the single most important health issue in small ruminant production systems. FAMACHA© was first introduced into the United States in 2002 by Adriano Vatta (Onderstepoort Veterinary Institute, South Africa) when he trained members of the Southern Consortium for Small Ruminant Parasite Control in the FAMACHA© method. Ray M. Kaplan and James E. Miller then modified the FAMACHA©information guide for use by the American small ruminant farmer, and Dr. Kaplan introduced the system in the US at a workshop in Florida in June of 2003. From 7 workshops in 4 states distributing 188 FAMACHA© eye charts in 2003, FAMACHA in the US has grown to include 461 trainers in 47 states and US territories. Approximately 15,000 FAMACHA© eye charts have been distributed in North America since its introduction in 2003, with growing interest and demand each year. Expectations are that this trend will continue and that the FAMACHA© system will establish itself as standard practice for small ruminant anthelmintic treatment in areas where *H. contortus* is an important pathogen.

African Trypanosomes

Wednesday, August, 12, 2009

PO2.1

Quassinoids Isolated from a Medicinal Plant, *Brucea javanica* and their Antitrypanosomal Activities *in vitro*

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Hemoprotozoan diseases, such as trypanosomiasis and piroplasmosis, have threatened livestock and companion animals worldwide. Medicinal plants can be considered as alternative drugs for treatment of these diseases because currently used drugs are needed to improve their side effects, cost performance and so on. The medicinal plant, *Brucea javanica*, is widely distributed throughout Asia. This plant contains a number of quassinoids which have been shown for antiamoebic and antimalarial activities. Recently, we reported that quassinoids also had antibabesial activities. In this study, we examined *in vitro* antitrypanosomal activities of quassinoids with or without liposome.

The dried powder of *B. javanica* fruits was extracted with 70% aqueous methanol and partitioned using ethyl acetate to give aqueous and organic layers. C-20 types of quassinoids were purified from both layers using chromatography-mass spectrometry and their structures were determined using nuclear magnetic resonance. Liposomal formulations of quassinoids were prepared with dipalmitoylphosphatidylcholine, cholesterol and stearylamine. The IC_{50} values were determined for trypomastigotes of *T. evansi* and MRC-5 cell line.

Free bruceine A, B, C, bruceantinol and brusatol from the organic layer showed strong antitrypanosomal activity with IC_{50} values (2.9-17.8 nM) while the standard drugs of diminazene aceturate showed IC_{50} with 8.8 nM. Bruceine D from the aqueous layer has middle activity. The presence of a diosphenol moiety in ring A and the nature of the C-15 side chain are important for their antitrypanosomal activities. Liposomal bruceine B, C and D showed about twice higher antitrypanosomal activities than each free bruceine compound. However, these liposomal formulations appeared to be less toxic on MRC-5 cells.

PO2.2

The Pathogenicity and Drug Resistance of Trypanosoma vivax in Sudanese Calves and Goats

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A survey was conducted at the Blue Nile area, non Tsetse area in Sudan, to determine the prevalence of Trypanosoma vivax in cattle, using haematocrit centrifugation technique, and wet blood film, thin and thick stained smears. The results showed that the prevalence rate during the dry season varied between 6.5 to 2.2% and 6.35 to 1.43% during rainy season. The Tabanids catches were 25±5.1 fly/trap/day in early dry season. This indicated that cattle suffer due to T. vivax which is mechanically quite prevalent in high rates in the area; this rate is depending on seasonality.

The trypanocidal drug resistance studies of T. vivax stock isolated from non-tsetse area were conducted in 32 goats. Trypanocidal drug tested were Homidium bromide (16 goats). The doses tested for homidium bromide were the recommended treatment dose 0.5 /kg body weight and double that dose. The doses tested for diminazine aceturate were the recommended treatment dose 3.5/kg body weight and double that dose.

The T. vivax isolate tested against both drugs relapsed indicating the development of drug resistance against both drugs at the tested doses in Nubian goats (Sudanese local breed). Both groups of goats treated showed significant reduction in packed cell volume (PCV), Hemoglobin (Hb), white blood cells (WBC) and red blood cells (RBC) counts.

The pathological effect of T. vivax isolated from non tsetse area was conducted by experimental infection of 10 Kenana calves (Sudanese local breed). The infected animals showed obvious pathological effects emaciation, anemia, enlargement of lymph nodes, high temperature, increase in heart and respiratory rates, which were positively correlate with peak of parasiteaemia. The blood showed significant haematological changes. The mean value of PCV dropped to 15%, Hb 8%, WBCs 5001.696 ±162.725, RBCs 3554018 ±172889.0. The gross findings showed petechial and echimotic haemorrhages in all the visceral organs spleen, liver and heart. Lymph nodes were considerably enlarged, also proliferative lymphoid changes and infiltration of lyphatic cells were observed in all tissues. Oedema and pulmonary exudates were observed in the lungs. The heart showed myocardial degeneration. The kidneys showed interstitial glomerulonephritis, and inflammatory changes. The testis showed prominent orchitis, epididymitis, atrophic seminepherous tubules and degenerative changes. The brain showed infiltration of lymphoid cells, histocytes, mononuclear and glial cells and necrotic foci.

Generally wide tissue damage and haematic changes were observed in all tissues of the infected animals. Animals died of congestive heart failure.

This study demonstrated the spread of mechanically transmitted Trypanosoma vivax outside the previously known tsetse belt and was also proved to be drug resistant and highly pathogenic.

PO2.3

An Update of the Bovine Trypanosomosis Situation at the Edge of Hluhluwe-Immfolozi Park, Kwazulu-Natal Province, South Africa

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In South Africa, the distribution of tsetse has undergone considerable changes over the last century. Since the nagana outbreak of the 1990's little information is available on the prevalence of the disease in cattle. The aim of this study was to obtain updated data on and assess the contribution of trypanosomosis to the disease burden of cattle kept at the edge of the Hluhluwe-iMfolozi Park, KwaZulu-Natal Province. A survey was conducted at Mvutshini diptank adjacent to the northern edge of the tsetse infested area.Use was made of a purposeful sampling strategy by restricting sampling to animals that the livestock owner considered to be in poor condition. A total number of 76 adult (12 months of age) cattle (Angoni breed) were sampled. From each animal, jugular blood was collected in vacutainer tubes coated with EDTA. Molecular (PCR-RFLP) and parasitological techniques were used to analyse the samples. Of a total of 76 blood samples collected, 26 (34.2%) were parasitologically positive and 46 (60.5%) were positive on PCR-RFLP. All parasitological positive animals were also positive on PCR-RFLP. Almost all infections were due to Trypanosoma congolense savannah subgroup. The average PCV of all animals sampled was 19.8 ± 4.2%. The average PCV of parasitological positive animals (18.6 ± 3.8%) differed little (P>0.05) from the average PCV of parasitological negative animals (20.5 ± 4.4%). Similarly, the average PCV of animals positive on PCR-RFLP (19.6 ± 4.3%) differed little (P>0.05) from the average PCV of animals negative on PCR-RFLP (20.2 ± 4.3%). A total of 63 animals had a PCV 24%. This result is an indication that trypanosomosis is still a problem in the study area.

Diagnosis / PCR

Wednesday, August, 12, 2009

PO2.4

Scanning Surveillence for Parasites-Is it Worth It?

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The veterinary laboratories agency (VLA), an agency of Defra, consists of a regional network of 16 veterinary diagnostic laboratories and two surveillance centres situated throughout England and Wales.

Information on diagnosis of endemic parasite diseases is collated and this long term data provides a base line against which perceived changes in parasite epidemiology can be compared (see paper by van Dijk et al).

However, a number of parasites have been identified recently that had either not previously been recognised in the UK (*Cryptosporidium andersoni*) or had not been reported in certain hosts for a number of years (*Toxocara vitulorum* and *Psoroptes* spp in cattle) . *Cryptosporidia andersoni*, a protozoan parasite that infects the abomasum of cattle, was identified from a faeces sample submitted from an adult dairy cow with diarrhoea.

Toxocara vitulorum, an ascarid parasite that infects the small intestine of buffalo, bison and cattle and is common in tropical areas, was detected in samples from unthrifty suckler calves. Investigations revealed that infection was widespread on this farm, but the source could not be determined. This parasite is considered a potential, but un-proven, zoonotic agent, and is transmitted to calves in the dams' milk.

Psoroptes sp. infestation was diagnosed as causing skin lesions with scab formation, hair loss and severe pruritis over the shoulders and tail-head in animals in a number of cattle herds. Macrocyclic lactones were not effective.

In all instances the veterinary profession and others were alerted to these findings.

PO2.5

Feline *Tritrichomonas foetus* Infection in Korea

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Feline intestinal *Tritrichomonas foetus* infection was first recognized in the USA in 1999 and has so far been reported from the UK, Norway, Switzerland and Australia, but not from Far East Asian countries. In November 2008, two 6-month-old female littermate Siamese cats raised in a household in Korea were referred from a local veterinary clinic with a history of chronic persistent diarrhea. A direct smear examination of fecal specimens revealed numerous trichomonad trophozoites to which repeated treatment with metronidazole did not respond. The trophozoites were isolated by the fecal culture in InPouch™ TF-Feline medium. PCR testing of the isolate based on the amplification of a conserved portion of the *T. foetus* internal transcribed spacer (ITS) region (ITS1 and ITS2) and the 5.8S rRNA gene, and the molecular sequencing of the PCR amplicons confirmed infection with *T. foetus*. This is the first report of feline tritrichomoniasis in Korea.

PO2.6

Is the Origin of Parthenogenic *fasciola* sp. in China? A Study of DNA Types and Morphology in Chineses *fasciola* Specimens

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Parthenogenic (aspermic) *Fasciola* sp. as well as sexual *F. hepatica* and *F. gigantica* has occurred in Asian countries. The aspermic forms show the 3 distinct genotypes representing ITS1-Fh, ITS1-Fg and ITS1-Fh/Fg in nuclear ribosomal ITS1, which had the sequences identical to those of *F. hepatica*, *F. gigantica* and heterogeneous of the 2 species, respectively. Additionally, the aspermic forms phylogenically belong to either groups of *F. hepatica* or *F. gigantica* in the haplotype of mtDNA, although they have diversity in the haplotype. Parthenogenic *Fasciola* specimens that show the identical genotypes in both of ITS1 and NDI have been recently found in Japan, Korea and Vietnam, suggesting they are derived from the common origin. In order to clarify the origin of pathenogenic *Fasciola* sp., the study was designated to analyze *Fasciola* specimens in China based on the genotype of ITS1 and NDI as well as their spermatogenesis. We used 111 *Fasciola* samples from 10 cities in China (Hohhot, Urumqi, Xinin, Yanji, Fuzhou, Guiyang, Wuhan, Kunming, Lhasa, Guangzhou), whose spermatogenesis was checked by the presence of sperm in the seminal vesicles. The genotypes in ITS1 and NDI of the flukes were determined by the sequencing and analyzed phylogenically. All of the *Fasciola* specimens from Hohhot, Urumqi and Xinin were spermic and showed ITS1-Fh, thus identified as *F. hepatica*, similarly all the specimens from Guangzhou were identified as *F gigantica*. The specimens from Yanji, Fuzhou and Lhasa were all parthenogenic and showed the genotype of ITS1-Fh (22.6%), ITS1-Fg (17.7%) or ITS1-Fh/Fg (59.7%). The specimens from Guiyang, Wuhan and Kunming contained *F. gigantica* and parthenogenic forms. The NDI haplotypes of 6, 12 and 8 were identified in *F. hepatica*, *F. gigantica* and aspermic *Fasciola* sp., respectively. The haplotypes representing Fh-C4 and Fg-C2 were detected in both of aspermic *Fasciola* specimens, and spermic *F. hepatica* and *F. gigantica*, respectively, in China, and further the haplotypes were completely identical to the haplotypes shown in parthenogenic *Fasciola* sp. from Japan, Korea and Vietnam described previously. These findings might suggest that parthenogenic *Fasciola* sp. having the haplotype of Fh-C4 and Fg-C2 has originated in China and been introduced into Japan, Korea and Vietnam with infected animals, probably domestic cattle.

PO2.7

Characterization of Egyptian Theileria annulata Strains using SDS-PAGE and RAPD Techniques

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Three strains of Theileria annulata isolated from different geographical Provinces (in Egypt) were characterized using two different methods. The analysis of extracted piroplasm proteins of T.annulata by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) revealed differences in major and minor polypeptide bands of the isolated strains. In addition, different polymorphic bands of the three strains were observed using the Random Amplified Polymorphic DNA (RAPD) markers generated by polymerase chain reaction (PCR).

PO2.8

Seroprevalence of Antibodies to Tick-Borne Pathogens and Heartworm Antigen in Shelter Dogs from the State of Georgia

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The identification of tick-borne pathogens capable of causing disease in dogs can lead to different approaches for disease prevention, management and treatment. The purpose of this study was to determine the prevalence of antibodies to common tick-borne pathogens *(E. canis (Eca), E. chaffeensis (Ech), E. ewingii (Ee), A. phagocytophilum (Aph)*, and *B. burgdorferi (Bb))* in sera from 203 dogs (≥ 1 year-of-age) housed in shelters throughout the state of Georgia during the summer and fall of 2003. Samples were tested using the multi-analyte SNAP® 4Dx® test and by a microtiter plate ELISA for *Ee* antibody. The number of samples reactive in these assays was: *Ee* 11(5.4%), *Eca* 4(2.0%), *Aph* 1(0.5%), *Bb* 2(1.0%) and heartworm Ag 34(16.7%). All samples were further tested using species-specific microtiter plate assays for antibody to *Eca* and *Ech*. Each of the *Eca* SNAP 4Dx reactive samples was reactive in two species-specific *E. chaffeensis* antibody assays and nonreactive in the species-specific *E. canis* antibody assay indicating that all 4 samples were *E. chaffeensis* reactive. Two additional *E. chaffeensis* samples and one additional *E. canis* reactive sample were found using the species-specific assays. In summary, the species-specific results for the tick-borne pathogens found in this population were: *Eca* 1(0.5%), *Ech* 6(3.0%), *Ee* 11(5.4%), *Ap* 1(0.5%), and *Bb* 2(1.0%). The results of the study confirm that *Ech* and *Ee* were the most common sources of *Ehrlichia* seroreactivity in this population reflecting the widespread occurrence of *Amblyomma americanum*, the confirmed vector for both of these agents.

PO2.9

Development of a Species-Specific Molecular Marker to Identify *Haemonchus contortus*.

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Haemonchus contortus is the main gastrointestinal nematode species affecting small ruminants and also cattle in Brazil. Correct nematode identification would assist epidemiological studies and the development of an effective parasitic control program. A sensitive and rapid method using a polymerase chain reaction based technique with a species-specific molecular marker was developed to identify *H. contortus*. Genomic DNA was isolated from one single specimen of each *H. contortus* and *Haemonchus placei*. A segment spanning 3'-end of the 18S rDNA and the 5'-end of the 26S was amplified by PCR in both species, sequenced by using a Dye Terminator chemistry on MegaBACE 1000 sequencing machine and analyzed with SEQUENCE ANALYSER software. *Haemonchus contortus* and *H. placei* DNA sequences were compared by using BLAST 2 SEQUENCES program and a PCR primer pair was designed using PRIMER3 software program. To perform the analysis, genomic DNA was isolated from cattle and sheep blood samples (hosts) and also from 18 morphologically identified adult parasites: *Bunostomum phlebotomum, Bunostomum trigonocephalum, Cooperia curticei, Cooperia pectinata, Cooperia punctata, Cooperia spatulata, H. contortus, H. placei, Haemonchus simillis, Oesophagostomum columbianum, Oesophagostomum radiatum, Trichostrongylus axei, Trichostrongylus colubriformis, Trichuris discolor, Trichuris globulosa* (Nematoda), *Eurytrema* spp. and *Paramphistomum* spp. (Trematoda), and *Moniezia* spp. (Cestoda). PCR reactions using *H. contortus* DNA sample presented a 1750 base pairs (bp) band. No other analyzed helminthes DNA sample showed amplification bands. Bovine and ovine DNA samples presented 650 and 600 bp amplified bands, respectively.

Drug Resistance

Wednesday, August, 12, 2009

PO2.10

Cloning and Functional Characterization of a Cooperia oncophora P-glycoprotein A

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The P-glycoproteins (Pgp) are members of the ABC transporter family which are involved in the mechanism of various processes leading to resistance in protozoa, helminths and cancer cells. Susceptibility of Cooperia oncophora against macrocyclic lactone anthelmintics such as ivermectin (IVM) was increased by the Ppg inhibitor Verapamil (VPL) in a larval development test (LDT) and a larval migration inhibition test (LMIT). In the LDT, a VPL induced decrease in the EC50 of IVM of 100 fold, in the LMIT of 10 fold was observed. In sheep parasitic nematodes PgpA was shown to be involved in resistance processes against IVM. Cloning and characterization of a 4086 bp full-length CoPgpA cDNA, encoding a protein with 1274 amino acids from the susceptible Weybridge isolate and an in vivo IVM-resistant field isolate of C. oncophora, revealed large heterogeneity within, but no major difference between these isolates. Expression of CoPgpA was analyzed by real-time RT-PCR and compared between different life cycle stages in both isolates. Furthermore CoPgpA was stably expressed as HcRed fusion protein in the human HELA cell line for analysis of substrate specificity. For this purpose a competitive transport inhibition assay using quantification of rhodamine 123 accumulation via confocal laser scanning microscopy and flow cytometry was established. This work delivers molecular tools for future analysis of the role of P-glycoprotein in the development of resistance to anthelmintics in cattle parasitic nematodes.

PO2.11

Multiple-Anthelmintic Resistance on a Llama Farm in the Southeastern United States

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Documentation of anthelmintic resistance on goat and sheep farms in the southeastern United States is substantial; however, little is known about its prevalence on llama farms. A study was conducted on a llama farm in Florida to test for the presence of resistance to fenbendazole, levamisole, ivermectin, and moxidectin using both fecal egg count reduction test (FECRT) and larval development assay (LDA, DrenchRite©). Seventy-two llamas were allocated randomly into six treatment groups (n=12/group): fenbendazole oral(FBZ 20 mg/kg), levamisole oral (LEV 12 mg/kg), ivermectin injectable (IVM 0.3 mg/kg), moxidectin oral (MOX 0.3 mg/kg), moxidectin injectable (MOX 0.3 mg/kg), and untreated control. For the LDA, nematode eggs were isolated from a pooled fecal sample collected at the time of treatment. FEC reductions were 0%, 96%, 0%, 90%, and 58% for FBZ, LEV, IVM, MOX PO, and MOX SC, respectively. Based on WAAVP guidelines, these results indicate resistance to all drugs tested except levamisole. In contrast, the LDA data indicated resistance for BZ and IVM, and sensitivity to LEV and MOX. Based on coprocultures, the most common nematode was *Haemonchus contortus*,

followed by *Nematodirus* spp. and *Trichostrongylus* spp. These findings confirm the presence of multiple-anthelmintic resistance on a llama farm in the southeastern US. Furthermore, the incongruent results for MOX in the LDA and FECRT suggest that the dose applied may not be adequate, and that optimal route of administration requires further investigation. . Further research is required to assess the prevalence of anthelmintic resistance on camelid farms in US.

PO2.12

Anthelmintic Efficacy in Cattle Farms in Belgium

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Anthelmintic resistant nematodes have been reported in different regions around the world. However, in Western-Europe the assessment of the problem relies largely on case reposts and no prevalence data based on wide-scale surveys are available. Therefore, we performed a survey to 1) evaluate the efficacy of anthelmintics on cattle farms in Belgium and 2) identify risk factors for reduced efficacy. Anthelmintic efficacy was evaluated by a faecal egg count reduction test that was slightly modified from the current WAAVP guidelines in order to increase feasibility and participation from farmers and veterinarians. Coprocultures on pooled samples were performed on farms with a reduced efficacy. Of 80 farms studied, 71 had positive faecal egg counts and used macrocyclic lactones. Reduced efficacy (faecal egg count reduction < 95%) was observed on 41 % of these 71 farms. Only Cooperia species were found in significant numbers in the copro-cultures post-treatment. Reduced efficacy was significantly associated with farm type (lower efficacy in beef than dairy herds) and the pre-treatment faecal egg counts (lower efficacy in farms with high faecal egg counts). No significant associations were found for previous treatment history and class of macrocyclic lactones. Six farms were revisited and a faecal egg count reduction test was repeated according to the current WAAVP guidelines. Anthelmintic resistance was confirmed in 1 of 6 farms, full anthelmintic efficacy was observed in 3/6 farms, whereas on the remaining farms too few positive animals were for a reliable interpretation.

PO2.13

Time-Course of Triclabendazole Action Against Adult Fasciola hepatica in the Sheep Host

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Fifteen indoor-reared cross-bred sheep with no pre-exposure to Fasciola hepatica were infected with 200 metacercarial cysts of the triclabendazole-susceptible Cullompton isolate of F. hepatica via oral gavage. At 12 weeks post-infection, sheep were dosed with 10m g/kg triclabendazole. Four sheep per time period were euthanized at 48 h and 72 h and 5 sheep were euthanized at 96 h post-treatment. Two control sheep were euthanized alongside the 96 h triclabendazole-treated sheep. Flukes were recovered from each of the sheeps' liver and gall bladder and processed for electron microscopy, immunocytochemistry, histology and HPLC analysis of drug levels.

Scanning electron microscopy revealed that drug disruption increased in severity with increasing time, culminating at 96h post-treatment, with almost complete elimination from the host. From 48 h in vivo, tegumental swelling, blebbing and spine disruption was widespread. By 72 h, disruption had increased in severity, with many flukes exhibiting loss of tegument in their posterior region. In some severely-affected flukes the sloughing of the tegument affected the whole of the fluke. This was particularly noted in specimens from the gall bladder. At 96 h post-treatment, all flukes showed complete loss of tegument and spines, and some flukes were grossly deformed. Transmission electron microscopical analysis of the ultrastructure of the flukes reflected the morphological disruption observed at the surface. HPLC analysis allowed comparison between the degree of morphological disruption observed in individual flukes with levels of drug present in these same flukes.

PO2.14

In vitro Selection and Species Differentiation of Ivermectin Resistant Cyathostome Larvae

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Studies on ivermectin resistance in cyathostomins are hampered by the diversity of cyathostome species in field samples, of which eggs and L3s can not be differentiated morphologically. Therefore, an in vitro selection system combined with a molecular technique to differentiate the species would be useful. In the Larval Migration Inhibition Assay (LMIA), ivermectin inhibits the migration of exsheated L3s through a sieve in a dose dependent way. Larvae from a population fully homogenous for ivermectin susceptibility and incubated at a given ivermectin concentration, will all have an equal chance on migration. Those that have migrated will have the same chance of migrating through a second sieve as they did through the first one. However, if the population is heterogeneous for ivermectin susceptibility,

the fraction of larvae that migrate through each consecutive sieve will increase because each passage selects the least susceptible L3s. We assayed two populations of L3s cultured from horses that were either never treated with ivermectin or treated with subtherapeutic doses of ivermectin for seven years, with a final dose of 40 g ivermectin per kg bodyweight. The migration in the presence of ivermectin was twice as high in the treated population as in the non-treated one. Furthermore, by using 4 consecutive sieves the fraction of migration in the non-treated and the treated populations increased 25 and 8 fold, respectively. This demonstrates that both populations are heterogeneous for ivermectin susceptibility with a broader window in the non-treated population. This offers the possibility for in vitro selection of ivermectin resistant L3s. Reverse Line Blotting showed that in vitro selection of the non-treated population resulted in an increase of Cylicostephanus calicatus from 8 to 68 %, while in the treated population no clear change in species composition was found after migration

PO2.15

Anthelmintic Resistant Nematodes in Sheep and Goats in Norway

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Introduction: The prevalence of anthelmintic resistant nematodes in sheep and goats in Norway was largely unknown before this study.

Methods: Faecal egg count reduction test (FECRT) and larval culture for third stage larvae identification from pooled faeces were performed in 28 sheep and 12 dairy goat flocks. All the flocks were randomly selected from either inland mountain areas or coastal areas in southern Norway or from northern Norway. In each flock, two groups of twelve animals each were treated with benzimidazoles (BZ) or macrocyclic lactones (ML) and one group of twelve animals was used as untreated controls. In sheep flocks only lambs and in goat flocks only adult animals were included.

Results: BZ resistant nematodes (i.e. FECRT less than 95%) were detected in 3 of the 28 (sheep flocks (FECR of : 7, 78 and 85%). Resistance to macrocyclic lactones were suspected in two of the 28 sheep flocks (FECR of 80 and 93%) but the mean egg counts in the control groups were very low in both farms. All the BZ resistant flocks were located in the same county in the coastal area of south-western Norway. Nematodes resistant to benzimidazoles were detected in only one goat flock (FECR: 92%). Third stage larvae Haemonchus were mainly detected in the BZ post-treatment samples in sheep farms whereas Teladorsagia/Trichostrongylus larvae were found in the BZ resistant goat farm.

Conclusion: This study indicates that anthelmintic resistant nematodes are present in small ruminants in Norway.

PO2.16

The Route of Administration Affects Ivermectin Clinical Efficacy Against Resistant Nematodes in Sheep

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Macrocyclic lactone compounds are administered by the subcutaneous and oral routes to sheep and goats. Optimized drug treatment is necessary to deal with resistant nematodes. The aim of this work was to compare the efficacy of ivermectin (IVM) against resistant nematodes following its subcutaneous and intraruminal administration to lambs. Eighteen (18) lambs naturally infected with IVM resistant gastrointestinal nematodes were used. Animals were allocated into three (3) experimental groups (n=6): untreated control, IVM subcutaneously injected (SC) (IVOMEC® Merial Argentina) (0.2 mg/kg) and IVM intraruminally administered (IR) (ORAMEC® Merial Uruguay) (0.2 mg/kg). Individual fecal samples for egg counts were collected at 0, 9 and 15 days post treatment. Post-mortem examination was done at day 15 post-treatment. Adult nematodes recovered from the digestive tract were count and identified by species. The Kruskal Wallis test was applied. IVM effectiveness percentage by genus was always higher after the IR treatment. Efficacies against abomasal parasites were 52.4 % (SC) and 81.9 % (IR). Treatments showed 95.2 % (SC) and 99.9 (IR) of efficacy against intestinal nematodes. The adult nematode counts of Teladorsagia spp., T. axei. and T. colubriformis were statistically different ($p \leq 0.05$) between groups. H. contortus showed be highly resistant after the IVM treatment by both administration routes. The enhanced IVM concentrations recovered at the sites of gastrointestinal nematodes location account for the higher effectiveness obtained after the IR treatment. This advantageous efficacy pattern against resistant nematodes obtained for the oral/IR route should be considered to optimize IVM efficacy.

PO2.17

Trichostrongyle Intestinal Worms Resistant to Ivermectin Treatments in Dairy Calves of the Buenos Aires Province (Argentina)

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A controlled test efficacy of ivermectin against natural trichostrongyle infections in dairy calves of the Buenos Aires province –Argentina- was carried out.

Twelve Holando Argentino castrated male calves of 6 months old and weighing 150 k in average were used. The animals had been grazing on pastures naturally infected with trichostrongyle worms. According to individual live weight and eggs per gram of faeces (e.p.g.) two comparable groups were conformed, named as Group 1 and Group 2 respectively.

The animals of G1 received a single subcutaneous injection of ivermectin at the dose rate of 0.2 mg/ kilo body weight, whereas the G2 remained as non treated control group.

Forteen days after treatment the animals were necropsied following the guidelines published by WAAVP (Wood et.al., 1995) to determine the number of nematode eggs in faeces and worms counting at the level of abomasum, small and large intestines and lungs of treated and control animals.

The faecal egg count reduction test (FECRT, Coles 1992) showed a reduction of 76.2%. The efficacy of ivermectin was 100% ($P < 0.05$) against Haemonchus placei, Ostertagia ostertagi, Trichostrongylus axei, Oesophagostomum spp. and Dictyocaulus viviparus respectively.

The efficacy against Cooperia oncophora, Cooperia pectinata and Cooperia mcmasteri was 80.4% ($P=0.4712$), to Trichostrongylus colubriformis 79% ($P=0.1445$) and against Nematodirus helvetianus 0% ($P=0.5745$).

The results obtained in this trial not only confirm the resistance to ivermectin of the intestinal worms Cooperia spp. and Trichostongylus spp but also establish the first mention in Argentina of resistance to ivermectin of the intestinal worm Nematodirus.

PO2.18

Evaluation of Four Cattle Anthelmintics Using the Fecal Egg Count (FEC) Reduction Test and Polymerase Chain Reaction (PCR) Test for Egg Identification

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Introduction: Efficacy of four anthelmintics was evaluated. The trial was conducted at the NCDA&CS/NCSU Upper Piedmont Research Station near Reidsville, NC in June and July of 2008. FEC and parasite identification was performed pre- and post-treatment by the Bovine Functional Genomics Laboratory, USDA-ARS, Beltsville MD.

Methods: Angus calves (n=125) were weaned at about 200 days of age and a FEC was determined by a commercial lab. Eight days later, calves were sorted by sex, weight and initial FEC and randomly assigned to form five balanced groups. Groups were randomly assigned to receive no dewormer (control), Ivomec Pour-on® (IVPO), Privermectin® (generic topical ivermectin, GIVPO), Ivomec® injectable (IVINJ), or Safeguard® oral liquid (fenbendazole, FBZ). Fecal samples were taken on treatment day and 14 days later, and shipped on ice to the USDA lab which remained blind to treatment. FEC was determined by the modified Wisconsin test. PCR was performed on composite samples from each group.

Results: Control 24.4% reduction; pre-treatment, Cooperia, Ostertagia, Haemonchus; post-treatment, Cooperia, Haemonchus. IVPO 80% reduction; pre-treatment Cooperia, Ostertagia, Haemonchus; post-treatment Cooperia. GIVPO 67.9% reduction; pre-treatment Cooperia, Ostertagia, Haemonchus; post-treatment Cooperia, Ostertagia. IVINJ 73.1% reduction; pre-treatment Cooperia, Ostertagia, Haemonchus; post-treatment Cooperia, Ostertagia. FBZ 100% reduction; pre-treatment Cooperia, Ostertagia, Haemonchus; post-treatment – no eggs.

Significance: Cooperia may be resistant to ivermectin on this farm and are the main reason for the FEC reductions observed. It is important to further develop and evaluate optimum parasite management strategies. PCR to determine changes in parasite populations may improve interpretation of the FEC reduction test.

PO2.19

Absence of Ivermectin Resistance in a Survey on Dairy Goat Nematodes in France

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Introduction: Macrocyclic lactones are more intensively used in dairy goats since the launch of eprinomectin in 1997. Previous results obtained in 2000-2002 have shown that ivermectin resistance could be suspected in 6 out of 36 surveyed French dairy goat farms but necropsic examinations after experimental infections and ivermectin treatment did not confirm the anthelmintic resistance. The 6 goat farms were all located in the same area (Burgundy, eastern France).

Methods: In order to check more accurately the status of goat nematode populations for ivermectin susceptibility in France, a survey was conducted in 2007 and 2008 during

winter time in 22 randomly selected dairy goat herds located in eastern part of France (16) i.e. Burgundy and Rhône area and western part of France (6) i.e. Midi-Pyrénées, Deux-Sèvres and Indre-et-Loire.

On each farm, 30 adult goats were randomly allocated into 2 groups of 15 animals : an untreated control group and an ivermectin treated group (0.4 mg/kg BW per os). Individual faecal egg counts and pooled larval cultures were done 16-17 days after anthelmintic treatment for control and IVE groups. Faecal egg count reductions (FERC) were calculated for IVE group compared to control one, and, when less than 95 per100, were considered as indicative of anthelmintic resistance.

Results: FECR indicated the absence of ivermectin resistance in nematode populations from all the 22 dairy goat farms. The nematode genera involved in control groups were of Teladorsagia / Trichostrongylus, Haemonchus and Oesophagostomum / Chabertia types.

PO2.20

Anthelmintic Resistance in Virginia Small Ruminants at a Critical Level

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Introduction: Modern broad spectrum anthelmintics represent only three pharmacological classes: benzimidazoles (BZ), levamisole/morantel (LM) and macrocyclic lactones (ML). Once resistance develops to any drug in the class, cross-resistance will rapid be expressed. The development of anthelmintic resistance has complicated farm management to the point of threatening small ruminant production systems.

Methods: By combining detailed producer interviews, fecal egg count reduction tests and larval development assays, a picture of anthelmintic resistance and management on 25 Virginia sheep and goat farms was drawn using 2002-09 data. Effectiveness of anthelmintics [fenbendazole (FBZ), albendazole (ABZ), morantel (MOR), levamisole (LEV), ivermectin (IVE) and moxidectin (MOX)] were estimated as resistant, marginal or effective from the composite information and were quantified as 1.0, 0.5 or 0.0 for comparison.

Results: Several critical trends were noted. BZ resistance was widespread with an estimate of 98% (FBZ) and 85% (ABZ). MOR and LEV resistance were estimated on 75% and 52% of farms, respectively. Resistance to IVE and MOX were estimated at 100% and 69%, respectively. MOX demonstrated a time trend, showing only marginal (36%) resistance in 2002-03 and complete failure (100%) in 2006-09 data. Generally Haemonchus predominated, although both resistant Haemonchus (65%) and Trichostrongylus (25%) were cultured on one farm.

Discussion: From these data, it is estimated that 33-50% of Virginia farms no longer have any effective anthelmintic, FBZ and IVE are resistant on over 95% of farms, 20-25% have one effective anthelmintic (LEV or MOX) and less than 15% have both LEV and MOX still effective. Control strategies relying heavily on anthelmitics will fail, and farmers are confronted with extensive modification of management practices.

Ectoparasites

Wednesday, August, 12, 2009

PO2.21

Fleece Kinetic Disposition of Cypermethryn Applied as Backline Treatment on Angora Goats and its Effect on Damalinea caprae Infestation

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The most common external parasites of Angora goats, the chewing louse Damalinia caprae, is a potentially serious problem which can affect the quality and quantity of mohair fiber produced and may reduce animal weight gains.

Sixty Angora goats, 7 months old and naturally infested with D. caprae were used to study the effectivity and kinetic disposition of cypermethryn 6%. Doses were calculated on an individual body weight basis (3 ml/10 kg bw) and applied to the dorsal mid-line from the base of the neck to the butt of the tail of 30 goats. Treated goats were kept in outdoors pens and maintained separately from untreated infested group. One gram of mohair samples were taken at skin level, from each goat, pooled and identified as from the dorsal mid-line and right mid side ribs. Procedures for pesticide determination were carried out following CSIRO and Akre & Macneil (2006) methods. Each sampling was carried out at treatment (day 0) and on days 18, 34, 42, 73, 122 and 139, and the presence of D. caprae registered. Lice counts were assessed by examination of 11 partings and a complete examination was carried out in order to define eradication. Most of the residual pesticide (78 to 91%) on goats was found until day 42 on the mohair from the backline samples (application area). There was an increase in the concentration of the cypermethryn towards day 18 in both two samples (backline and middle-rib). Concentration after day 42 was very low (0.43 to 0.11 g/gr). D. caprae was eradicated by treatment, no live lice were seen from18 days after treatment to the end of the trial (day 139).

This suggests that the concentration of cypermethryn found at fleece level is sufficient to achieve louse eradication.

PO2.22

Efficacy and Safety of Promeris Duo® for the Treatment of Generalized Demodecosis in Dogs from Italy and Albania

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For a total of 26 dogs presenting at veterinary clinics with generalized demodecosis, skin scrapings and clinical examinations were performed before treatment with ProMeris Duo® spot-on (n = 18) or a licensed control product (containing a combination of moxidectin and imidacloprid, n = 8) and repeated at intervals of approximately 28 days. Post-treatment parasite counts were compared to Day 0 counts and clinical signs of demodecosis were evaluated as Demodex-induced skin lesion score and compared with Day 0 scores. The parasitological cure rate was calculated for each treatment group as the proportion of dogs with no Demodex spp. mites detected on two consecutive monthly examinations.

ProMeris Duo reached an at least 90% reduction from baseline in total number of Demodex spp. mites on Day 56 with a maximum reduction of 98.6% on Day 168, whereas in the control group a 90% reduction was reached from Day 84 onwards with a maximum of 92.1% on Day 168. Parasitological cure (no mites) was achieved in 88.9% of the ProMeris Duo group and 75.0% of the control group.

There was a significant improvement in Demodex-induced skin lesion scores from baseline within the ProMeris Duo group for all study days. Final assessment of clinical cure, improvement and failure showed that 94.4% animals of the ProMeris Duo group were cured and 5.6% improved, compared with 62.5% cured, 12.5% improved and 25.0% failed in the control group.

No treatment-related adverse event occurred.

PO2.23

Novel Control of the Sheep Scab Mite, Psoroptes ovis, Through the Application of Bacteriophage Technology

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Introduction: Sheep Scab is a debilitating disease caused by the non-burrowing ectoparasitic mite, Psoroptes ovis. Currently it is endemic in the UK with 50 million sheep at risk from infection, with an annual cost of over £8 million. Although it has previously been eradicated globally it has been reintroduced to a number of countries.

Current controls include organophosphate dips or chemical Injectables, both of which exhibit a number of problems such as resistance, environmental damage and harmful affects on human health.

My PhD project is investigating a novel way of controlling Psoroptes ovis mites, with the use of bacteriophage therapy.

Methods: Current ongoing work involves identification of bacteria associated with P. ovis using ARISA and sequencing techniques. Once identified, the role of the bacteria will be tested for any beneficial/nutritional associations with the mite. Additionally, bacteriophage sampled from the environment will be screened for their effect on the detected bacteria. Bacteriophage has a number of advantages over other forms of control such as specificity and their effects against multi-drug resistant bacteria.

In this way potential beneficial bacteria associated with P. ovis can be targeted to disrupt the lifecycle and hence produce a novel means of controlling sheep scab disease.

PO2.24

Control of the Poultry Red Mite (Dermanyssus gallinae)

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In our study, were investigate 117 small-scale and 7 large-scale laying hen breeding farms with 53.8 % and 57.1 % prevalence, respectively. The poultry red mite spends the most of its life away from the birds. In the farms with battery-cage system mites are hidden under the conveyor belts of eggs (89.5 %). In the farms with slatted flooring systems they tend to be hidden under the wires rods (81 %), in nest boxes (7 %), on the bottom side of feeding bowles (6 %) in small crack and crevices in the poultry house walls. With respect to possibility of long-term survival of mites in shelters, prevention tends to be rather difficult. Due to the lack of new acaricides, the strategy of red poultry mite control should be based on a rotation of various acaricides and tried to use other possible alternative chemicals as insect growth regulators. The potential of combination using of synthetic pyrethroids and chitin-synthesis inhibitor, Triflumuron, was assessed to control populations of the poultry red mite under practical conditions. More then 50 % of reduction of the nymphs and females were noticed on the 57th day and until to 103rd day the mean reduction was 96.2 % (range 89.7-100 %) after combination treatment with lambdacyhalothrin and triflumuron. With respect to the eradication of red mites, the presented results are promising but, in the monitored period, complete extinction of the population of red mites did not occur. Therefore there is a need to use combined treatment

included an adulticidal and larvicidal compound, to control D. gallinae.

Work supported by the basic research of National Reference Laboratory for Pesticides at the University of Veterinary Medicine in Ko ice and Grant KEGA /4284/06.

PO2.25

Lice Genus and Species Frequency on Pigeons Columba livia from Facilities in Mexico

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An ectoparasite searching was carried out in free living doves which jointly lived with other animals where food was available. The biologic material consisted of 50 pigeons from which lice were isolated by rubbing a piece of cotton with alcohol in the whole body including the wings. The collected material was placed in bottles with 70%, alcohol and later they were mounted between slide and coverslide using Hoyer´s liquid Four lice species were observed : Physconelloides zenaidurae, Columbicola columbae, Hohorstiella lata and Bonomiella columbae. The total of lice collected was of 4688 f rom which the 65,78% corresponded to P. zenaidurae; 30,66% to C. columbae; 3,21% to H. lata; and 0,35% to B. columbae, In relation to the frequency on the host the following results were obtained: 98% P. zenaidurae and C. columbae; 46% for H. lata and 6% to B. columbae In a previous study carried out in Mexico was a frequency of C. columbae, 55% and 25% was recorded for the north and south zone of the Federal District, respectively . It is concluded that the presence of Columbicola columbae was ratified and the presence of four genus and specie of lice in domestic doves . In Mexico, the presence and frequency of P zenaidurae H. lata and B columbae has never been detected . Then it can be said that this is the first time that these lice are observed on Columba livia from Mexico

Epidemiology

Wednesday, August, 12, 2009

PO2.26

Gastrointestinal Helminthes of Iranian Red Foxes (Vulpes vulpes)

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As infected red foxes (vulpes vulpes) to parasites have an important role in distribution of zoonotic parasites which threaten public health in human as well as their gastrointestinal helminthes especially in rural regions cause some of dangerous diseases in human beings, was decided to study their intestinal helminthes in Iran. In this Survey, 22 red foxes (vulpes vulpes) were collected from rural and urban regions in the Western provinces of Iran. After autopsy, their intestine was removed and their content searched for the presence of helminthes. Recovered parasites were fixed and stained and finally identified according to the keys and guideline given by Yamaguti(1961),Anderson(1992)and Khalil et al .(1994). The species which were recovered from red foxes is listed as follows:

Ancylostoma caninum (4.54%), Uncinaria stenocephala (13.64%), Oxynema sp. (9.09%), Toxascaris leonina (31.82%), Toxocara canis (4.54%), Rictularia affinis(54.54%), Strongyloides sp. (4.54%) Physaloptera sp. (4.54%), Taenia hydatigena (9.09%), Mesocestoides lineatus (81.82%), Dipylidium caninum (9.09%), Joyeuxiella pasqualei (27.27%), Diplopylidium nolleri (4.54%) Echinococcus granulosus (4.54%), Macroacanthorhynchus hirudinaceus(22.72%), Macroacanthorhynchus sp. (31.81%).

PO2.27

Prevalence of Anti-Toxoplasma gondii and Anti-Neospora caninum Antibodies in Swine from Northeastern Brazil

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A serologic survey was conducted among 130 swine slaughtered in the public slaughterhouse of Patos city, Paraíba State, Northeastern Brazil, to determine the prevalence of anti-Toxoplasma gondii and anti-Neospora caninum antibodies, and to verify possible associations between sex of the animals and antibody prevalence. The sera were analyzed for anti-T. gondii and anti-N. caninum antibodies by indirect fluorescent antibody tests, considering 1:64 and 1:50 dilutions as cut-off points, respectively. The prevalence of anti-T. gondii antibodies was 36.2% [95% IC = 27.9% – 45.0%] with reciprocal titers ranging from 64 to 2048, and anti-N. caninum antibodies was 3.1% (95% IC = 0.8% – 7.7%) with reciprocal titers ranging from 50 to 6400. Three of the four N. caninum positive samples were also positive for T. gondii antibodies. All Neospora and Toxoplasma IFAT-positive sows were also positive for confirmatory immunoblotting techniques using total and purified N. caninum and T. gondii tachyzoite antigens, i.e. p38 (NcSRS2) and p30 (TgSAG1). There were no associations between sex of animals and

prevalence of anti-T. gondii and anti-N. caninum antibodies. This is the first indication of N. caninum natural infection in pigs from Brazil.

PO2.28

The First Evidence of Neosporosis in Farm Dogs in Eastern Poland

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Neospora caninum is an important cause of abortion in dairy cattle worldwide. Dogs are both intermediate and definitive host of N. caninum and play a crucial role in horizontal transmission of this parasite to other animals, especially to cattle.

The presence of N. caninum in Poland has been confirmed serologically in cattle, bison, deer, foxes and dogs from urban and rural areas in south-western Poland.

The objective of this study was to assess the prevalence of antibodies to N. caninum in dogs living in close contact with dairy cattle exhibiting reproductive problems.

A total of 506 cows from 10 farms with reproductive failure were tested for neosporosis. With the use of a commercial ELISA test (IDEXX Laboratories Inc., Westbrook, ME, USA), antibodies against parasite were detected in 169 sera samples (33.4%), the prevalence varied from 10% to 70.2% in different herds.

Additionally, 29 sera samples were collected from dogs of different ages and both sexes, living on farms or in close contact with cow herds.

Antibodies to N. caninum were found in 10 sera samples taken from dogs (34.48%). Two of these dogs came from farms where neosporosis in aborting cows was confirmed.

To our knowledge, this is the first report of neosporosis in farm dogs where the presence of this parasite was also confirmed in aborting cows.

PO2.29

Screening of Plant Compounds for Anthelmintic Activity Against Ovine Gastro Intestinal Nematodes

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Introduction: The increasing prevalence of anthelmintic resistance has led to research examining phytotherapeutics as a means of controlling gastro-intestinal nematodes.

Preliminary screening for 513 European plant extracts has been conducted as part of an EU Framework Six project (www.replace-eu.com) in an attempt to identify plants with anthelmintic activity.

Methods: A variety of in vitro tests can be used for detecting anthelmintic activity but the larval feeding inhibition test has proved to be the most sensitive where feeding activity can be disrupted through direct effects on the neuromusculature and/or other target sites for extract products. The larval feeding inhibition test (LFIT) has been adapted for use with aqueous plant extracts and was used as a primary screen. Polyethylene glycol (PEG) and polyvinylpyrrolidone (PVPP) were used as inhibitors to identify principal active plant secondary metabolites (PSM).

Results: Of the initial 513 plants samples, 119 were active i.e had an LFI50 estimate <1.25mg/ml. Twenty three of the most active plants were subsequently analysed further on the basis of these primary tests. The egg hatch test (EHT), larval exsheathment test (LET) and adult motility test (AMT) were used as a secondary screen. Five plants exhibiting anthelmintic properties from the primary and secondary screens were selected for tertiary in vivo screening. Worm-burden efficacies for the five plant compounds ranged between 0-42% and 21-37% against Haemonchus contortus and Trichostrongylus colubriformis respectively.

PO2.30

Occurrence of Eucoleus aerophilus (Syn. Capillaria aerophila) in Dogs and Cats from Italy

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The present paper reports the occurrence of the lungworm Eucoleus aerophilus (syn. Capillaria aerophila) in dogs and cats from Italy. Individual faecal samples were randomly collected from 569 dogs and 200 cats either referred to veterinary clinics for a range medical problems or to the Parasitological Unit of the Faculty of Veterinary Medicine of Teramo for routine parasitological examination. The minimum animal numbers to be sampled were calculated with the software Win Episcope 2.0, considering an infinite population, an expected prevalence of 5% (cats) or 3% (dogs), a maximum error desired 3% (cats) and 2% (dogs) and a 95% level of confidence. All 769 samples were subjected to two faecal flotation procedures, i.e. by using a sugar solution with a 1.200 specific gravity (s.g.) and a 1.350 s.g. zinc sulphate solution. Sixteen dogs (2.8%) and 11 cats (5.5%) scored positive for eggs of E. aerophilus when samples were processed with either of the two flotation solutions. Overall 14 dogs and 8 cats infected by E. aerophilus showed respiratory symptoms, mainly represented by general respiratory distress, dry

cough, wheezing and sneezing. The results of this study indicate that E. aerophilus is not uncommon and that canine and feline capillariosis is of clinical importance. Given the impact that E. aerophilus infections may have for animal health and welfare and its zoonotic potential, it is strongly advisable to routinely include this parasitosis in the differential diagnosis of (cardio)-respiratory diseases of dogs and cats in Italy as elsewhere.

Equine Par

Wednesday, August, 12, 2009

PO2.31

Habronematosis Due to Habronema muscae in a Stable in the United Arab Emirates

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Habronematosis is a nematode infection in equines caused by Habronema muscae, Habronema microstoma and Draschia megastoma. Nematode larvae found in lung tissue histological cuts of a horse from a farm in Al Dhaid (UAE) were determined to belong to the Habronematidae Family. The clinical examination of the other 18 horses present in the farm revealed summer sores (i.e. cutaneous habronemosis) in two stallions. Nematode larvae were found in 147 (=26.2%) out of 561 male but only in 64 (=8.7%) out of 739 female Musca domestica caught at the farm in November and December 2008. All 15 examined Stomoxis calcitrans were negative for nematode larvae. Flies caught in the stable showed a prevalence of 20.3% while flies trapped outside the stable on the territory of the farm had a much lower prevalence of 1.1%. The intensity of infection varied between one and 29 larvae per head. All larvae retrieved at the fly dissection were undertaken to a ribosomal DNA-targeting semi-nested PCR protocol able to discriminate among the three nematode species. The larvae were identified to be H. muscae.

PO2.32

Investigating the Rhoptry Associated Protein-1 (RAP-1) Gene of Babesia caballi

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A competitive-inhibition enzyme-linked immunosorbent assay (cELISA) developed for the detection of equine antibodies specific for Babesia caballi was tested on 107 South African equine field samples. None of these samples tested positive using the cELISA assay, although ten samples tested positive for B. caballi antibodies using the IFAT test. We therefore characterized the B. caballi RAP-1 gene, which codes for the antigen used in the cELISA assay, by designing three sets of primers to amplify the complete RAP-1 gene (~1800bp). We were only able to amplify the 5' end of the gene (615 bp) from ten South African B. caballi tissue-culture samples, and we only obtained sequence data from seven of these. BLASTN analysis revealed that the sequences showed between 79 and 81% identity to published B. caballi RAP-1 sequences. The GenomeWalker Universal kit (Clonetech) was used to amplify the region flanking the 615 bp B. caballi RAP-1 fragment. Amplified products were cloned into the pGEM-T Easy vector and sequenced using the T7 and SP6 primers. The complete B. caballi RAP-1 gene sequence, comprising a single open reading frame of 1489 bp that encodes a protein of 493 amino acids, was obtained from two samples. BLASTP analysis indicated 65% amino acid identity to published RAP-1 protein sequences. The observed amino acid sequence differences might explain why the cELISA assay was not able to detect any of the South African B. caballi isolates. Redesigning the current cELISA assay using a more conserved protein as the target antigen may overcome this problem.

PO2.33

First Report of Equine Intraocular Nematodiasis Caused by *Parelaphostrongylus tenuis*

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Parelaphostrongylus tenuis, the meningeal-worm of the white-tailed deer (*Odocoileus virginianus*), rarely if ever causes overt disease in this host. When third stage larvae in gastropod intermediate hosts are ingested by other ungulates (cervid and non-cervid alike), the resulting larval migration

through the spinal column and the nervous tissue proper can result in serious or fatal neurologic disease. Infections with *P. tenuis* have been previously reported in horses, but had not been substantiated. Further, there is a prior report of *P. tenuis* in the posterior segment of the eye of a 10-month-old eland antelope (*Taurotragus oryx*) housed in Ohio. To our knowledge, this is the first report of equine intraocular nematodiasis caused by *P. tenuis*. Here we report the surgical recovery of a 45 mm long male metastrongyloid nematode from the anterior chamber of the eye of a 4-year-old Hanoverian horse from Wisconsin, USA. The spicules of the recovered specimen measured approximately 190 to 200 µm in length, while the length of the gubernaculum was 63 µm. Although the specimen was mature, these measurements are on the smaller end of the range for *P. tenuis*, as is almost always the case with those recovered from atypical locations. Bursal structure, as well as size and structural characteristics of the spicules and gubernaculum allowed the identification of the worm as *P. tenuis*. This specimen has been deposited in the United States National Parasite Collection (USNPC 101413).

PO2.34

Use of Purified Antigens for the Early Detection of Strongyle Infection in Horses

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The aim of this study was to evaluate the utility of strongyle excretory/secretory antigens purified by means of gel-exclusion chromatography. One group of eight adult autochthonous Pura Raza Galega (PRG) grazing mares received one dose of commercially available ivermectin (1 mg kg-1 bodyweight) pour on. Fecal and blood samples were individually collected during 21 weeks. Feces were analyzed by the coprological flotation technique. The IgG humoral immune response was assessed by an ELISA procedure and excretory/secretory antigens from a mixture of L3 small strongyles (Cyathostominea, Poteriostomum and Gyalocephalus) purified by an FPLC system. The composition of each of the fractions collected was determined by a chip electrophoresis procedure.

The excretion of strongyle-eggs was suppressed at the 3rd week after treatment (wat). Four protein peaks were collected after the chromatography, named P1 to P4. The antibody response against the P1 and P2 did not decrease after the treatment of the mares. A significant reduction at the 3rd wat was noted by using the peaks P3 and P4 until the 7th wat. Strongyle-eggs were observed 10 wat again. The IgG levels against the four antigens rose up from the 8th wat, two weeks prior to the observation of the eggs in the faeces. We concluded that purified antigens can be useful for the early detection of strongyle infection in horses, especially P3 and P4.

This work was partly supported by the Research Projects XUGA PGIDT06RAG26102PR and 07MDS021261PR (Xunta de Galicia, Spain).

PO2.35

Prescription Only Medicines; an Opportunity or a Burden for Practitioners

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In July 2008 anthelmintics for horses have become Prescription Only Medicines (POM) in the Netherlands. This new regulation should strengthen the advisory role of veterinarians concerning parasite control. To examine the effect of this regulatory change on the approach of veterinarians, we need to know about veterinarian's knowledge and practice regarding worms and worming. Additionally, collected data may identify needs for continuing post-academic education for specific aspects of parasite control.

In October 2008 a questionnaire concerning the period prior to July 2008 was sent to 1265 members of two groups of veterinarians (production animal health and equine health). The questionnaire included questions about worming policies and the use of faecal samples to check for parasite infections. The response rate was 17% (214 veterinarians) distributed over the country. Of these, 174 indicated that they practice equine medicine.

Out of 174 veterinarians 59 indicated that they spend 80% or more of their time on horses. Preliminary results indicate that 77% of veterinarians, at least sometimes, examine faecal samples from horses. If faecal samples are examined this usually is done to confirm a clinical diagnosis. Only 25 % of veterinarians indicate that they examine faecal samples in the context of monitoring.

The current questionnaire will be followed by a second one in three years time. Based on outcomes of this first questionnaire refreshment courses will be developed on parasite control including hands on training on parasitological faecal examination.

PO2.36

Comparison of Coprological and Molecular Techniques for the Diagnosis of *Anoplocephala perfoliata* Infection of the Horse

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Anoplocephala perfoliata is the most common tapeworm parasite of horses and is incriminated as a significant cause of clinical disease. The sensitivities of common coprological diagnostic techniques for *A. perfoliata* infection vary considerably. The present work evaluated and compared the reliability of a recently described coprological FLOTAC technique as well as a modified flotation technique and traditional flotation technique with that of a PCR-based assay for diagnosis *A. perfoliata* infection. Of 43 faecal samples collected from horses bred on a single farm, 19 (44%) resulted positive for the presence of *A. perfoliata* eggs using the FLOTAC technique. From the 19 FLOTAC positive samples the 18 samples (42%) by using a modified flotation technique and 7 samples (16%) examined by traditional flotation technique were also positive. All collected samples were also subjected to a PCR protocol specific for regions of *A. perfoliata* ITSs. Four out of the 19 FLOTAC positive samples and six out of the 24 FLOTAC negative samples were found positive by PCR. In this work, the PCR assay actually showed the unreliability for detecting of *A. perfoliata* eggs probably due to smaller sample size and also as a result of an irregular distribution of *A. perfoliata* eggs in the horse faeces. Nonetheless, the FLOTAC technique scored the highest number of positives compared to the other techniques and may have advantages compared to other methods that allows also estimating of the parasite burden. The results of the present work indicate that the FLOTAC technique as well as a modified flotation technique can be utilized as useful methods for the detection of *A. perfoliata* in faecal samples collected from naturally infected horses.

The financial support of the grant project MSM 6215712403 is acknowledged.

PO2.37

Comparison of Two Commercial Anthelmintics Against Strongylids of Naturally Infected Horses

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The aim of the study was to compare the anthelmintic efficacy of two commercial compounds against gastrointestinal nematodes of naturally infected horses.

Methods: Forty two crossbred equines positive to strongylids were used. They were divided in 3 groups of 14 animals each. Group 1 (G1) received a single oral dose of Eqvalan-Gold (Merial®) containing 200 mcg of Ivermectin and 1 mg of Praziquantel/kg b.w. G2 was treated with Ivermectina Gel (Sanfer®) given as a single oral dose containing 200 µg of Ivermectin and 1 mg of Praziquantel/kg b.w. G3 served as a non-treated control. Efficacy was measured as the percentage reduction of strongylid eggs counted on day 0 against the percentage of eggs identified on days 7, 14, 21 and 28 days after the treatment, respectively.

Results: G1 showed a gradual reduction of eggs exerting an efficacy of 89.5%, 97.5%, 77% and 93% for days 7, 14, 21 and 28, respectively. G2 showed an egg reduction of 100%, 97.8%, 100% and 100% for days 7, 14, 21 y 28. G3 always showed high counts of eggs being the maximum of 14,600 EPG. Statistical comparison showed no difference in efficacy among treated groups but certainly yes in the untreated control. The nematodes identified were Strongylus vulgaris, Strongylus edentatus, Strongylus equinus, Cyathostomum spp, as well as some small strongylids. No Dyctiocaulus nor Anoplocephala or Parascaris were observed during the study. It is concluded that both compounds are efficient to remove gastrointestinal nematodes in horses.Study financially supported by SANFER Laboratories, Mexico.

PO2.38

Effect of Temperature on the Different Stages of Development of Eggs and Larvae of Nematodes Ciatostomíneos (Nematoda-cyathostominae)

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The cyathostome nematodes are most abundant in the large intestine of horses. Climatic conditions influence the dynamics of development of eggs and larvae. This study aimed to evaluate the effect of temperature on the different stages of development of eggs and larvae of ythostome at different seasons. In the laboratory of the Helminthological E.P.P. WONeitz of DPA UFRRJ, take stool samples were collected directly from the rectum of a horse naturally infected at the beginning of each season of the year and were kept under refrigeration (10 º C) and freezing (± -4 º C). Eggs were recovered every 15 days, each sample and observed according to the phases: Morula, gastrula stage, eggs, larvae, L1 and L3. The values are presented in percentage (%). Under refrigeration, Morula stage 19 in spring, 54 in summer 13 in the fall and 44 in winter; stage of gastrula stage 4, 11, 2 and 19 for eggs and larvae were the values 1, 2.5, 1.3 and 4.0 for the season respectively. For the first stage larvae of the values were 32, 30 44 and 33 and L3 are 36, 19, 47 and 61 respect-

ively. On a freezing, stage of Morula 53, 50, 32 and 38, stage of gastrula stage 10, 11, 6 and 11; eggs larvae 4, 5, 3 and 1 for L1 32, 30, 37 and 36 and L3 60, 26 40 and 10 respectively. The development of eggs kept under refrigeration and freezing does not kill eggs.

PO2.39

The Frequency of Strongylid Nematodes of Horses from Different Regions of the State of Rio De Janeiro, Brazil and Evaluation of the Cyathostomins L3 Morphology

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Brazil is the third largest breeder of horses in the world (36 million) used in business, sports, games and recreation and strongylyd is the highest number of parasites of the large intestine of horses with high prevalence of cyathostomin causing various diseases. In order to evaluate the frequency and identify the species of nematodes parasitizing the horses from different regions of Rio de Janeiro, this study was prepared. From October 2008 to February 2009, seventy-two horses were evaluated by counting the number of eggs per gram of faeces (OPG) and fecal culture for identification of larvae of the third stage (L3). The classification was based on morphology and arrangement of the intestinal cells. Among cyathostomin there are various types of distribution of cells and is classified into types I, II and III. Of the 22 horses race sleeve of large Marche Seropédica, the EPG ranged from 100 to 4350 with 100% of carriers. The L3 of cyathostomin (Nematoda-Cyathostominae), 50% had 8 cells arranged in 2 initial cells, followed by 1 row of 6 cells (type I), 40% a single row of 8 cells (type II), 6% Poteriostomum with 16 cells arranged in 2 rows of 8 cells (Type III) and the sub-family Strongylinae 4% Strongylus vulgaris (32 cells). In Itaguaí, 9 crossbred animals of Thoroughbred English (EPB), OPG ranged from 50 to 3600, with 78% parasitized. The L3 frequently 60% (Type I), 33% (Type II), 5% Poteriostomum (Type III) and 2% S. vulgaris. In the mountain region of Petropolis, 41 SRD animals, 95.1% were parasitized, OPG ranged from 50 to 4400. The L3 showed 62% (Type I), 30% (Type II), 1% Poteriostomum (Type III) and 7% S. vulgaris. In all three regions was assessed in the proportion of difference between the types cyathostomin, most frequently type III - Poteriostomum in the region of Baixada Fluminense. It calls attention to S. vulgaris due to the condition that question. Several factors can influence the diversity and parasite load. Project entitled "Prevalence of intestinal parasites of horses in different regions of Rio de Janeiro."

PO2.40

Multi-Centric, Controlled, Randomised Field Clinical Trial to Evaluate and Compare the Stress Response Induced in Horses When Administered Endoparasiticides in Tablet or Paste Formulations

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Introduction: Administering equine anthelmintics in paste formulations is integral in the management of equine parasites. Recently a tablet presentation has been developed. The objective of this clinical trial was to assess whether this new presentation reduces the stress when administering an anthelmintic.

Methods: Horses (n=122) were randomly allocated into three homogenous groups:

Group 1: Single oral administration of 1 tablet for 100kg BW (each containing 200 micrograms/kg ivermectin and 1.5 mg/kg praziquantel; Equimax® Tabs, Virbac)

Group 2: Single oral administration of paste A (containing 200 micrograms/kg ivermectin and 1.5 mg/kg praziquantel; Equimax® Gel, Virbac)

Group 3: Single oral administration of paste B (containing 400 micrograms/kg moxidectin and 2.5 mg/kg praziquantel; Equest® Pramox, Fort Dodge).

Stress response was assessed by monitoring heart rates (using Horse Heart Rate Monitors, Polar Equine) before, during and after the administration of the allocated anthelmintic. Additionally six scored behavioural reactions indicative of stress were assessed.

Results: Mean heart rate before treatment was similar in all groups. Heart rates increased during treatment in all groups, this was significantly lower ($p<0.05$) in Group 1 (compared to Groups 2 and 3). Over 60% of Group 1 presented no or only one behavioural reaction. Approximately 60% treated with pastes presented more than two reactions. Flehmen and pinning ear back reactions were significantly less frequent in Group 1 than in the paste groups ($p<0.05$).

Conclusion: Using the parameters monitored, this study demonstrates that the administration of an equine endoparasiticide in tablet formulation induces less stress compared to a paste formulation.

Global Warming / Parasite, Endangered Species

Wednesday, August, 12, 2009

PO2.41

Four New Species in Armenia Fish Helminthofauna

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Objective: The revelation of helminth fauna of fish from carp pond farms of the Ararat Valley and high-mountain Lake Sevan.

Material and Methods: The collections of the helminthes serve as material. 1850 samples of fish have been investigated: 1540 samples of Cyprinydae (Cyprinus carpio, Hypophthalmichthys molitrix, Aristichthys nobilis, Ctenopharyngodon idella) from pond farms of Ararat Valley and 310 samples of Cyprinydae (Carassius Carassius, Varicorhinus capoeta sevangi, Barbus lacerta goktschaicus) and Salmonidae (Coregonus lavaretus sevanicus) from Lake Sevan. Treatment of the helminthes will be carried out by common methods.

Results: Helminthes were found out in the intestine, on gills and lens.

Monogenea Eudiplozoon nipponicum Goto, 1891 was found on gills of the carps.

The larval forms of trematodes Diplostomum rutili Razmashkin, 1969 Diplostomum mergi Dubois, 1932 were found in the lens of the fishes of all species.

Cestoda Caryophyllaeus fimbriceps Annenkova-Chlopina, 1919 was found in the intestines of carp fish.

Conclusion: 4 new species of the helminthes of freshwater fish were registered in helminthofauna of Armenia. These are - monogenea Eudiplozoon nipponicum Goto, 1891, discovered on gills, metacercariae (larval forms) of 2 species of trematodes - Diplostomum rutili Razmashkin, 1969 and D. mergi Dubois, 1932, which parasite in the crystalline lens and cause the parasitic form of cataract and blindness, and cestoda Caryophyllaeus fimbriceps Annenkova-Chlopina, 1919, discovered in the intestines of the carps.

Monogenea Eudiplozoon nipponicum Goto, 1891 is first registered in helminthofauna f Transcaucasia.

PO2.42

Anisakid Nematods in Fish and Marine Mammals of Northern Canada

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Introduction: Anisakid nematodes have been reported in the Subarctic and Arctic regions throughout the world. Some members of this family such as *Anisakis simplex*and *Pseudoterranova decipiens* are considered zoonotic diseases and have been of public health concern worldwide. In Arctic Canada, studies on Anisakidae nematodes in different species of fish and marine mammals are limited. Our goals are to monitor these parasites throughout the Canadian Arctic and sub Arctic in Inuit marine traditional foods; to determine their regional distribution; to compare with other records of the circumpolar North; to identify Anisakid species and marine fish species at risk for human consumption; to train northerners to collect and preserve parasites for further identification.

Method: Marine fish traditionally consumed by Canadian Inuit are collected. The body cavity is opened and the nematodes are manually collected and preserved for microscopic identification and PCR. The flesh is digested using an HCL / pepsin solution and an incubator. Marine mammal stomachs are cut and nematodes are collected manually.

Results: Preliminary results show that adult Anisakidae worms are present in beluga, seals, and larvae are found in cod and sculpins. *P. decipiens* is the dominant species, but *A. simplex*, and, Contracaecum sp. were also present. Literature review records and this project show that Anisakid nematodes are at least distributed from Labrador to Hudson Bay. More samples are needed to determine which fish species will be of concern for human consumption. We trained 3 northerners and 1 Master's student in fish dissection, parasite collection and preservation methods.

PO2.85

Climate Change-Induced Risk of Winter Tick *Dermacentor albipictus* Infestation on Barren-Ground Caribou in the Northwest Territories, Canada: a Conceptual Model

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Climate change is one of the most important environmental issues of our time and is most pronounced at northern latitudes. Climate change impacts the whole ecosystem, altering the life cycles of plants, animals and micro-organisms, and their interactions. We developed a conceptual model to estimate the effects of climate change on the life cycle of the winter tick *Dermacentor albipictus*, and on the potential risk of infestation of barrenground caribou, *Rangifer tarandus groenlandicus*, populations in the Northwest Territories (NT). The barrenground caribou is the most important large mammal species for aboriginal subsistence hunters, and its populations in the NT are currently declining.

Although there is considerable evidence of significant winter tick infections in moose, *Alces alces*, in southern and central NT, to date, there have been no reports of winter ticks on barrenground caribou. The apparent absence in barrenground caribou may be due to their migratory behaviour. They remain above treeline from late spring through to early fall and then move to below the treeline for winter and may lack temporal and spatial synchrony with questing larval ticks during autumn when infestation would take place. We present preliminary conceptual and mathematical models of the winter tick life cycle under a climate change scenario. The possible consequences of climate change on winter tick distribution, the potential risk of tick infestation of barrenground caribou populations in northern Canada, and consequences on host population dynamics, are discussed.

Heartworm

Wednesday, August, 12, 2009

PO2.43

Dirofilaria immitis (Leidy, 1856) Infection in a Dog, First Autochthonous Case in Hungary

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Owing to the climatic changes a growing number of arthropod-borne diseases have been observed during the last decades, which either rarely had occurred in the country before, or only in imported cases. It has been reported that Dirofilaria repens was particularly frequent among Hungarian dogs during the last years. Heartworm infection in dogs occurs widespread in the world, but in the endemic parts of Europe used to be only the Mediterranean region, So far there have only been publications of imported cases in Hungary. Nowadays, however, it happens that heartworms can be found in dogs during dissection, or that serological tests give positive D. immitis results, although in these cases the origin of the dog was unknown, or the animals became infected abroad.

A 4 year-old, male Hungarian Vizsla dog which had never been abroad was admitted with poor general condition, decrease in body weight, haematemesis and jaundice to the Clinic of Faculty in Budapest. The dog was humanely euthanized two days later following owner's consent because of sudden worsening of clinical conditions. Two adult D. immitis were found in the right ventricle partially coiling around the tricuspid valve. PCR on blood was positive for both Dirofilaria species, but only D. repens microfilariae were found by modified Knott's test. This is the first, confirmed report of autochthonous canine heartworm infection in Hungary. The tourism with pets, the repeated travelling of Italian hunters with their dogs to Hungary and climate changing may have increased the spreading of immitis dirofilariosis.

PO2.44

Autochthonous canine and Feline dirofilariosis in Central Italy: Microscopic and Molecular Evidence

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In Italy Dirofilaria repens is distributed nationwide with different prevalence rates according to the geographical regions, while Dirofilaria immitis is mainly present in hyperendemic foci in the North. Nonetheless, D. immitis has recently spread toward central Italy, where the parasite is now considered endemic. In order to enhance the knowledge of the presence of these filarial nematodes in the central area of Italy (Abruzzo region) individual blood samples were collected random in 2008 from 300 autochthonous animals (i.e. 175 dogs and 125 cats). All samples were subjected to the Knott method and to two PCRs specific for the cox1 and 12S mitochondrial genes of canine and feline filariae. Sixteen dogs were microscopically and molecularly positive for D. repens (n.13), for both D. repens and D. immitis (n.2) and for Dipetalonema reconditum (n.1), while 1 more animal was positive for D. repens only at the PCRs (9.7% overall infection rate). Three and one cats were microscopically and molecularly positive for D. repens and D. immitis respectively (3.2% overall infection rate). Even though the infection rate by Dirofilaria spp. seems to still be low in central Italy, mainly for D. immitis, both parasites are

present and likely spreading southward in our Country. It is thus advisable that dirofilariosis is included into the differential diagnosis of canine and feline cardiopulmonary and skin diseases in southern/central Italy, where the prophylaxis against the vectors should also be implemented. Finally, given the zoonotic potential of these filariae, such scenario represents an important risk factor also for humans.

PO2.45

Heartworm Infection, Prevention, and Treatment, a New Educational CD

Murray, Michael Joseph

Merial Limited, Duluth, GA, USA

A new educational CD, "Heartworm infection, prevention, and treatment", gives veterinarians a novel approach to understanding the complex subject of heartworm prevention and treatment. The CD presents an in-depth look at the interactions of the heartworm lifecycle, various treatment protocols using melarsomine dihydrochloride, and administration of ivermectin heartworm preventive medications. What is unique about this CD is the dynamic view it provides of the interaction of heartworm development within a dog and the outcomes of treatment and prevention practices. Veterinarians can choose among multiple treatment protocols using different case scenarios, which are further illustrated with animations that demonstrate why certain outcomes can occur based on the expected efficacy of treatment protocols. This highly visual depiction makes it easier for veterinarians to understand why certain outcomes of treatment and prevention occur. The CD uses a heartworm infection model that is based on the infection potential in the highly endemic Mississippi River Valley in the United States, but the principles the CD illustrates apply anywhere dogs can be infected by heartworms.

PO2.46

Occurrence of *Dirofilaria immitis* and Tick-Borne Infections Caused by *Anaplasma Phagocytophilum, Borrelia burgdorferi* Sensu lato and *Ehrlichia canis* in Domestic Dogs in France: Results of a Countrywide Serologic Survey

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The occurrence of *Dirofilaria immitis* antigen, and antibodies against tick-borne pathogens in French dogs was analysed in a geographically broad serological survey. Samples were submitted routinely to a commercial laboratory for various diagnostic investigations (Group A; n = 919) or a tentative diagnosis of heartworm disease (Group B; n = 131). All samples were tested for *D. immitis* antigen using two different serological rapid-assay test systems. Samples in Group A were also tested for specific antibodies against three tick-borne pathogens (*A. phagocytophilum, Borrelia burgdorferi* sensu lato and *Ehrlichia canis*). Results were plotted in geographical maps. Occurrence of *D. immitis* antigen in Group A (0.22%; 95% CI: 0.03% - 0.78%) was significantly lower ($p < 0.0001$) than in Group B (6.87%; 95% CI: 3.19% - 12.64%). Heartworm infections in both groups were regionally restricted to the areas of Bouches-du-Rhône and Corsica in the South of France. In Group A the calculated seroprevalence was 2.72% (95% CI: 1.77% - 3.99%) for *A. phagocytophilum,* 1.09% (95% CI: 0.52% - 1.99%) for *B. burgdorferi* and 0.33% (95% CI: 0.07% - 0.95%) for *E. canis* with a distribution of the positive cases throughout the country. Furthermore, co-infections of *Anaplasma* with *B. burgdorferi* (0.22%) or *E. canis* (0.11%) were determined. This study represents the first data of *A. phagocytophilum* seroprevalence in the French dog population.

Non-Pharma Control

Wednesday, August, 12, 2009

PO2.47

Scanning Electron Microscopy of *Haemonchus contortus* Adults After Contact with Extracts of Two Tannin Rich Plants: *Lysiloma latisiliquum* and *Onobrychis viciifolia*

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Introduction: *In vivo* and *in vitro* studies showed anthelmintic (AH) effects associated with the consumption of tannin rich (TR) legumes against gastrointestinal nematodes (infective larvae or adults). Tannins are suspected for the AH properties. The mode of action against nematodes remains obscure. Functional and ultra-structural changes have been described for infective larvae of nematodes. The objective was to identify changes provoked on *Haemonchus contortus* adults after *in vitro* contact with extracts of TR plants.

Methods: Acetonic extracts of the leaves of *Lysiloma latisiliquum* and *Onobrychis viciifolia* were used. Adult *H. contortus* were collected from artificially infected goats. Freeze

dried water/acetonic extracts of *O. viciifolia* and *L. latisiliquum* were used (1200 µg/ml PBS). The *H. contortus* adults were maintained *in vitro* for 24 h in 24 multiwell plates in the following experimental groups: Group A, incubated in PBS (control); Group B incubated with *O. viciifolia* extract; Group C incubated with *L. latisiliquum* extract. After incubation, worms were washed with PBS, fixed in Karnovsky's fixative at 4 °C, dehydrated and prepared according to a standard procedure for scanning electron microscopy (SEM) observation.

Results: Both TR extracts originated thickening of both the longitudinal and transversal cuticular ridges by patches. Such changes might reduce motility of worms and/or possible exchanges with the environment through the cuticle. Aggregates of TR extracts were observed around the bucal capsule and the vulva in females.

Conclusions: These findings suggest that the nutrition and the reproduction, in particular the egg excretion, of the parasitic nematodes are negatively affected.

PO2.48

Antischistosomal Properties of Extracts from Medical Plants from Kakamega, on the Parasite, Schistosoma Mansoni in BALB/c Mice

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Schistosomiasis is a major public health problem in tropical and subtropical regions of the world where an estimated 200 million people are infected and close to a billion people are at risk of contracting the disease. Because it is a chronic insidious disease, it becomes a threat to development as the disease disables men and women during their most productive years. Although Praziquantel is a drug of choice for treatment of schistosomiasis, there have been reports of resistance hence a need for an alternative drug. Oxamniquine is the only alternative to praziquantel for S. mansoni infection but has limited supply because it is expensive. Cheaper treatment of schistosomiasis should be made available to poor communities in endemic areas and plants seem to be a cheaper source for drug development. The aim of this study was to investigate antischistosomal properties of extracts from Medicinal plants used in Kakamega in treatment of BALB/c mice infected with Schistosoma mansoni. Parasitological, cercaricidal and pathological assays were carried out to measure the antischistosomal activity of aqueous and methanol extracts. The mice were infected with S. mansoni and then treated with two doses of either 150mg/kg body weight Solanum or papaya (n=60) or 450mg/kg body weight praziquantel (n=15). Concentrations of plant extracts (5ug/ml, 15ug/ml and 30ug/ml) were used with cercariae in vitro cercaricidal assay. Solanum and papaya extracts illustrated a desirable killing effect on the larvae worm of up to 100%. In worm recovery of different treatments, infected control had the highest number of worms (57 ± 1.3) as PZQ had the lowest (25 ± 1.8). The four treatments: papaya methanol, papaya aqueous, Solanum methanol, Solanum aqueous had worm number counts between the two extremes; (35 ± 2.2), (38 ± 1.9), (33 ± 3.4), (32 ± 1.8) respectively. However, the papaya groups had a higher worm counts compared to the Solanum groups. Granulomas observed followed a similar trend as worm recovery in praziquantel and infected non-treated mice. However in a comparison between papaya and Solanum, Solanum treatments showed to have minimal pathological effect of the two. There was a significant statistical difference between the number of worms recovered from praziquantel-treated mice and those from plant extracts ($p<0.05$). However there was no significant difference ($p>0.05$) between the number of worms recovered from infected non-treated mice and those from plant extracts. This suggests that praziquantel is more effective than plant extracts in the management of S. mansoni infections.

PO2.49

Influence of Dietary Protein Supply on Resistance to Experimental Infections with Haemonchus contortus in Ile De France and Santa Ines Ewes Around Parturition and During Lactation

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Background: Periparturient responses to parasitism may differ between sheep breeds and be sensitive to protein nutrition. Here, interactive effects of protein nutrition and breed were assessed on host responses to parasitism during different reproductive stages.

Methods: Ile de France (IF) and Santa Ines (SI) ewes were infected with 1000 Haemonchus contortus L3 three times every week, from six weeks before expected parturition date until cumulative dose had reached 15,000 L3. Ewes were fed diets formulated to iso-energetically supply 0.8 (LP) or 1.3 (HP) times metabolizable protein requirements during late pregnancy and subsequent lactation. After weaning, ewes received coast-cross hay only. The lambs were weighted weekly since of birth until weaning.

Results: Feeding treatment did not significantly affect faecal egg counts (FEC), packed cell volume (PCV), total plasma protein concentration and ewe or lamb body weight. However, FEC increased from around parturition onwards for all ewes and stayed elevated post weaning. SI ewes had lower FEC than IF ewes throughout ($P<0.05$). Conversely, SI ewes showed mean PCV values significantly higher than IF ewes throughout the trial ($P<0.05$). Total plasma protein concentration for IF ewes was on averaged lower than for SI ewes. At

weaning, IF lambs were consistently heavier than SI lambs (P<0.05).

Conclusions: In general, SI breed better supported the artificial infections with H. contortus than IF breed whilst resistance was not affected by the dietary treatments used. This study was funded by FAPESP.

PO2.50

Effects of Protein Supply on Immunological Responses to Haemonchus contortus in Santa Ines and Ile De France Ewes

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Background: Protein supplementation has long been known to improve the resilience and resistance to gastrointestinal nematodes. The effect of Haemonchus contortus infection on immunological responses in ewes fed with different levels of metabolisable protein (MP) was evaluated in Santa Ines (SI) and Ile de France (IF) sheep in different reproductive stages (gestation, lactation and weaning of lambs).

Methods: IF and SI ewes were infected with 1000 Haemonchus contortus L3 three times every week, from six weeks before expected parturition date until cumulative dose had reached 15,000 L3. Diets were formulated to supply 0.8 times (low protein=LP) or 1.3 times (high protein=HP) MP requirements. After weaning, ewes started receiving only coast-cross hay. Blood samples were collected every week to measure eosinoplhis counts and antibodies. IgG and IgA levels were measured in the sera using ELISA method against L3 and adult crude antigens of H. contortus.

Results: Significantly higher eosinophils counts was detected in SI compared to IF (P<0.05). Increased MP supply increased eosinophil numbers only after weaning (P<0.05). No significant differences were observed between breeds for IgG and IgA levels against L3. However, IF had higher IgG levels against adults than SI, whilst SI had higher IgA levels against adult than IF (P<0.05). Compared to LP ewe, HP ewes had higher levels of IgG against L3 and adult during lactation and higher levels of IgA against adults during late pregnancy and lactation.

Conclusions: The high MP diet was associated with increased immune responses to H. contortus mainly during lactation independent of the breed.

This study was funded by FAPESP.

Parasite Genomic/Proteomics

Wednesday, August, 12, 2009

PO2.51

Transcriptome Analysis of Preadult Hypobiotic and Non-Hypobiotic L5 Stages of the Bovine Lungworm *Dictyocaulus viviparus*

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The bovine lungworm *Dictyocaulus viviparus* belongs to one of the most important parasites in cattle farming in temperate areas. It causes high economic losses as a result of parasitic bronchopneumonia and also death in susceptible cattle. The infective L3 stage larvae develop after ingestion by the host via L4 and preadult L5 stage to adult lungworms. This development can be inhibited in the L4 and preadult L5 stage, in case of unfavourable environmental conditions. The occurrence of inhibited or arrested development is also referred to as hypobiosis. Hypobiosis is of significant importance, ensuring the survival of the parasite from year to year. To identify transcripts specific for the uninhibited and inhibited stages Suppression Subtractive Hybridization (SSH) was performed to create subtracted libraries. Afterwards, 2016 clones of each library were spotted on high density arrays and Differential Screening was carried out using subtracted and unsubtracted probes to verify stage-specific transcription. Those clones containing ESTs identified as differentially transcribed were sequenced followed by EST-processing and clustering. Transcripts were then analysed and annotated by gene ontology search, domain/motif search and assigned to corresponding pathways in other organisms. Predicted proteins were also compared with published sequences in the Parasite genome WU-BLAST2 Nematoda database, NCBI database as well as WormBase and the results will be presented.

PO2.52

Two-Dimensional Fluorescence Difference Gel Electrophoretic (2D-DIGE) Analysis of *Besnoitia besnoiti* Tachyzoites and Bradyzoites

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Besnoitia besnoiti infection in cattle is governed by tachyzoites, related to acute infection, and bradyzoites gathered into macroscopic cysts located on cells of subcutaneous

connective tissue (chronic infection). However, the entire life cycle of this parasite still remains unknown as well as the molecular mechanisms implicated on tachyzoite to bradyzoite conversion. In this sense, a different pattern of antigen recognition has been observed between tachyzoite and bradyzoite extracts. Moreover, the most important tachyzoite immunodominant antigens showed different apparent molecular weight compared with those detected in bradyzoite extracts, which lead us to study the differential expression of stage specific antigens. Thus, in order to identify stage-specific proteins, an Ettan 2D-DIGE approach was performed on tachyzoite and bradyzoite extracts followed by mass spectrometry analysis. A total of 130 and 132 spots were differentially expressed in bradyzoites and tachyzoites, respectively (average ratio ± 1.5, $p < 0.05$ in T- test). Furthermore, 21 differentially expressed spots were selected and analysed by MALDI-TOF/MS. The data obtained were used to search for proteins in databases (NCBI and Swiss PROT/TrEMBL) using MASCOT. As result, 5 bradyzoite specific-proteins (ENO1, GAPDH, LDH2, SOD and a putative ATPase) and 6 tachyzoite specific-proteins (Hsp70prec, Hsp70, PDI, ENO2, LDH1 and a putative RNAse) were identified. The present results set the basis for the development of improved diagnostic tools to differentiate between acute and chronic infection and the identification of new proteins as vaccine candidates. Moreover the role of these proteins in tachyzoite-to-bradyzoite conversion should be a subject of further research.

Acknowledgements: We would like to thank veterinarians A. Morales and J.M. Pereda (SAT Coagral, Guadalajara, Spain) for sending skin biopsies. We wish to acknowledge J. M. Marcén and Vanessa Navarro for their excellent technical assistance and the Proteomics Facility UCM-PCM, a member of ProteoRed network, funded by Genoma España. This work has been supported by a research grant from the Santander-Universidad Complutense de Madrid.

PO2.53

Cryptosporidium Species and Subtype Analysis from Calves and Lambs in North-Western Spain

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Molecular techniques are essential to unravel the public health significance of Cryptosporidium isolates from animals. This protozoan is recognized worldwide as one of the most common enteropathogens causing neonatal diarrhoea in ruminants. The main goal of this research was to investigate the occurrence and zoonotic potential of Cryptosporidium isolates from pre-weaned domestic ruminants in a region of Spain. For this purpose, faecal specimens from diarrhoeic calves (26) and lambs (75) younger than 21 days were collected over a six-month period (February-July 2008) from farms in Galicia (north-western Spain). Samples were examined for the presence of Cryptosporidium oocysts and microscopy-positive specimens were selected for molecular examination. Overall, 11 calves (42 %) and 15 (20%) lambs tested positive. Cryptosporidium species were determined by nested PCR of an SSU rRNA gene fragment and RFLP analysis with the endonucleases SspI, VspI and MboII. Cryptosporidium parvum was identified from all the positive cattle isolates and 57% of sheep isolates. The Cryptosporidium cervine genotype was identified by both restriction analysis and sequencing of PCR products from the remaining 43% of sheep isolates. Two different sequences were seen within the Cryptosporidium cervine genotype which differed by one and four nucleotide polymorphisms from the reference isolate EF362480. Sequence analyses of the glycoprotein (GP60) gene revealed that all the C. parvum isolates belonged to the zoonotic subtype IIaA15G2R1. These data indicate that most isolates from diarrhoeic calves and lambs in this geographical area have zoonotic potential. These is the first report of the Cryptosporidium cervine genotype from ruminants in Spain.

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PO2.54

Gene Expression in Third-Stage Larvae of the Ovine Abomasal Nematode Teladorsagia circumcincta in Response to Changing Environmental Cues

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Parasitic gastroenteritis, caused by trichostrongylid nematodes, is the most commonly diagnosed systemic disease of sheep in the UK. The principal causative nematode (worm) is the abomasal parasite Teladorsagia circumcincta. Control, dependent on the use of anthelmintics, is failing due to the rapid emergence of drug resistance in the target nematodes. Vaccination is a feasible alternative but development is hampered by a lack of knowledge of the host-parasite interaction to incoming larvae, a prime effector of immunity in sheep.

We are seeking to define the molecular interactions between the host site of infection (the true stomach or abomasum) and the incoming larvae – how do larvae respond to changing environmental cues? We are using subtractive suppressive hybridisation to compare gene expression in 3rd stage larvae at exsheathment as they encounter naïve or immune abomasal environments. Here, we describe a

preliminary comparison between quiescent sheathed larvae and larvae exsheathed in a high CO2 environment primed for infection.

Semi-quantitive PCR has shown up-regulation of several genes in the exsheathed population compared to the sheathed larvae. These include sequences with significant homology to activation-associated secreted proteins from T. circumcincta and O. ostertagi, which are similar to pathogenesis related ancyclostoma-secreted proteins from hookworms, and also sequences with homology to ESTs previously detected in L4 stage specific cDNA libraries.

PO2.55

The Comparative Analysis of Hsp90 In Nematodes

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Heat shock protein 90 (Hsp90) represents an ubiquitous group of proteins that function both as molecular chaperones and as stress proteins. Although the gene encoding Hsp90 has been characterized, little is known regarding its function in parasitic nematodes. Previous studies on nematode Hsp90s had demonstrated functional differences in Hsp90 from Caenorhabditis elegans and the parasitic species, Brugia pahangi. C. elegans Hsp90 failed to bind to Geldanamycin (GA), a specific inhibitor of Hsp90, in solid phase pull down assays, while B. pahangi Hsp90 binds to GA. In this study, we examined the GA binding of a range of nematode species from different clades with a view to determining whether the C. elegans GA-resistant phenotype is shared with other nematodes. Our results show that the ability of Hsp90 to bind GA is associated with the life-cycle of the nematodes. Species that have a free-living larval stage in the soil do not bind GA, while species that are obligate parasites (Trichinella and the filarial worms), or which are enclosed within a protective egg shell while in the environment (Ascarids), possess an Hsp90 that binds GA. Hsp90 also has been shown to be involved in acquisition of drug resistance in fungi, where the emergence of fluconazole resistance depends on high levels of Hsp90 and is abolished when Hsp90 function was compromised. Given its conservation throughout evolution, it is possible that Hsp90 may be involved in the acquired resistance of nematode to a variety of drugs. Our studies are using RNAi to reduce hsp90 levels in wild type and ivermectin resistant C. elegans to determine whether Hsp90 may play a similar role in nematodes.

PO2.56

The Putative Cyclooctadepsipeptide Receptor Depsiphilin of *Ancylostoma caninum*

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The G-Protein coupled receptor HC110-R of *H. contortus* and its orthologue in *Caenorhabditis elegans*, the latrophilin-like protein 1 (LAT-1) were to shown play a role in the mode of action of the new anthelmintic compound emodepside. *C. elegans lat-1* knockout mutants showed a decreased paralysing effect of emodepside on the pharyngeal muscle. In the present study, the LAT-1 orthologue in the canine hookworm *Ancylostoma caninum* was identified. It was named as depsiphilin, according to previously detected LAT-1 orthologues in the cattle nematodes *Cooperia oncophora* and *Ostertagia ostertagi*. Identities of the amino acid sequences within the mentioned parasitic nematodes were about 81% to 91%, but only 42% to *C. elegans* LAT-1. To obtain more information about regulation of these receptors and to facilitate phylogenetic and evolutionary analyses of parasitic nematode genes, the genomic structure of *A. caninum* depsiphilin was investigated using GenomeWalker™ technology (Clontech). Thus, 25.000 bp of genomic DNA could be amplified. High consistency concerning the position of introns in comparison to *C. elegans* LAT-1 was observed, although *A. caninum* depsiphilin exhibits much more introns than *C. elegans* LAT-1, which yields also in an increased length of the gene. With a view to possible differences in efficacy on different developmental stages, we analysed the transcription level of *A. caninum* depsiphilin in eggs, L1, L3, male and female adult worms using quantitative real-time PCR. We found a higher transcription of depsiphilin in eggs, which conflicts to own studies showing that hatching is not affected in several gastrointestinal nematodes by emodepside.

PO2.57

The Complete Mitochondrial Genomes of Oesophagostomum dentatum and Oesophagostomum quadrispinulatum (Nematoda: Strongylidea)

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The complete mitochondrial (mt) genome sequences were determined for two Oesophagostomum species, O. dentatum and O. quadrispinulatum. Their sizes of the entire mt genome

are 13,752bp for O. dentatum and 13681bp for O. quadrispinulatum, respectively. The mt genomes of these Oesophagostomum species all encode 12 proteins, two ribosomal RNA and 22 transfer RNA genes, but lack the ATP synthetase subunit 8 gene, which is consistent with all other species of Nematode studied to date, with the exception of Trichinella spiralis. All genes are transcribed in the same direction and have a nucleotide composition high in A and T, but low in G and C. The contents of A+T of the complete genomes are 75.79% for O. dentatum and 77.52% for O. quadrispinulatum. The AT bias has had a significant effect on both the codon usage pattern and amino acid composition of proteins. The mt genome structures for two Oesophagostomum species, including genes and non-coding regions are in the same order as for Ancylostoma duodenale, Necator americanus, and similar to Caenorhabditis elegans and Cooperia oncophora, but differ from Ascaris suum and Anisakis simplex in the location of the AT-rich region, whereas there are substantial differences when compared with Onchocerca volvulus, Dirofiliria immitis and Strongyloides stercoralis. Based on genome organization and amino acid sequence identity, O. dentatum and O. quadrispinulatum were more closely related to A. duodenale and N. americanus, than to C. elegans, C. oncophora, A. suum and A. simplex, but distantly related to O. volvulus, Dirofiliria immitis and Strongyloides stercoralis. Determination of the complete mt genome sequences for two nodule worms of pig should provide a foundation for studying the systematics, population genetics and ecology of these and other nematodes of socio-economic importance.

PO2.58

Sequence Difference in Mitochondrial NADH Dehydrogenase Subunit 1 Gene Among Fasciola spp. of Human and Animal Health Significance

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The present study examined sequence differences in the NADH dehydrogenase subunit 1 gene (nad1) among and within Fasciola hepatica, F. gigantica, and the "intermediate Fasciola" from China, Niger, France and USA. The partial nad1 (pnad1) was amplified from individual Fasciola samples, and the amplicons were directly sequenced. MP and NJ trees were constructed using the software Phylip 3.67 version 4.0 and Mage version 4.0, and ML tree was also constructed using Puzzle version 5.2. Sequence homology analysis was performed using the Megalign program of the software DNAStar version 5.0 by comparing with the corresponding sequence of Fasciola spp. available in GenBankTM. The lengths of all the pnad1 sequences was 446 bp. Sequence comparison revealed that variation in pnad1sequences among F. hepatica samples from China, Niger, French, and USA were 0.2-0.9%, the variation in pnad1 sequences among F. gigantica samples from different geographical locations were 1.8-3.4%. The pnad1 sequences of the "intermediate Fasciola" were more similar to that of F. gigantica, and the inter-specific difference ranged between 5.6-8.1%. Sequence differences in the pnad1 sequences between F. hepatica and F. gigantica were 7.0-8.3%. Phylogenetic analysis using pnad1 revealed that the "intermediate Fasciola" was more closely related to F. gigantica than to F. hepatica. It is concluded that pnad1 sequences can be used as genetic marker for the differentiation of Fasciola spp. and for population genetic studies within Fasciola spp..

PO2.59

Genotypic Characterization of Cryptosporidium Isolates from Cattle in a Slaughterhouse in Tabriz City, Northwestern Iran

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Background & Objectives: Cryptosporidium spp is a common intestinal protozoan parasite that infects a wide range of host including humans and livestock throughout the word.

Methods and Methods: In this study fecal samples were collected from 104 adult cattle in a slaughterhouse in Tabriz, Northwestern Iran. Initial identification of Cryptosporidiosis was carried out by Formalin-ether concentration and Kinyoun acid fast staining method.

Results: 11 (10.5%) adult cattle were found to be positive. These positive samples were genotyped with a small-subunit rRNA-based PCR-restriction fragment length polymorphism analysis. Among 11 analyzed isolates two different species of Cryptosporidium were identified; 63.6% (7 cases) of isolates belonged to C.andersoni and 36.4% (4 cases) to the potentially zoonotic species of C.parvum bovine genotype.

Conclusion: The results of present study showed that two species of Cryptosporidium are responsible for cattle Cryptosporidiosis in this region. The relatively high prevalence of C.parvum bovine genotype suggest that there is a potential risk of zoonotic transmission of C.parvum bovine genotype infection between cattle and human, likely by means of contaminated water or food, or through direct contact in the farmers and veterinary staff.

PO2.60

The Discovery of Lipid Binding Protein Families in the Excretory/Secretory Products of *Haemonchus contortus* using a Novel Iterative Proteomic-Bioinformatic Approach

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Two families of small fatty acid binding families of nematodes, the nematode polyprotein allergens/antigens (NPA) and the fatty acid- and retinol-binding protein (FAR), have been studied since the *Ascaris suum* NPA was shown to be a major allergen. As components of the excretory/secretory products (ESP), a number of roles are proposed for these proteins, including a contribution to host-parasite interactions. To date, no members of these protein families have been reported in *H. contortus*, a major parasite of small ruminants.

Adult *H. contortus* ESP were sequentially resolved by size exclusion and anion-exchange chromatography, and fractions containing 10-25 kDa proteins were analysed by reverse phase HPLC with mass spectrometric detection and sequencing. Data analysis using an iterative proteomic and bioinformatic approach was achieved by the development of a customised database of *H. contortus* proteins derived from EST and genomic databases. Peptide sequence coverage of 46-58% of *H. contortus* NPA and 7-47% of *H. contortus* FAR predicted sequences was achieved.

We can predict at least two *hc-npa* and six *hc-far* genes in this species. Unique expression patterns for each of *hc-far-1* to *-4* genes in the L1, L3 and adult stages were observed by semi-quantitative RT-PCR. We also report the gene structure for the *far* genes, and a phylogeny of the NPA and FAR proteins from *H. contortus* with other nematodes. The NPA and FAR families of *H. contortus* are at least as complex as the *C. elegans* families, and this is likely to be true for other parasitic nematodes.

PO2.61

Comparative Analysis of the H. contortus and C. elegans B-Tubulin Gene Family

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The benzimidazoles act by binding to -tubulin and disrupting microtubule formation. Mutations in the H. contortus isotype-1 and isotype-2 -tubulin genes have well established associations with benzimidazole resistance. Identification of additional H. contortus -tubulin genes and comparative analysis with other nematodes should help us understand the relative importance of the individual genes as benzimidazole targets. The Haemonchus contortus genome sequencing project has generated >750 Mb of shotgun sequence data and although currently assembled as short contigs our analysis suggests the coverage is high and that most, if not all genes, are at least partially represented. For example, in excess of 80% of H. contortus EST sequence data is contained within the current genomic sequence. We have identified two additional -tubulin loci within the H. contortus genome making a current set of four H. contortus -tubulin genes, designated as isotype -1, -2, -3 and -4. Our analysis suggests this is the total complement of -tubulin genes in H. contortus which compares to a family of six genes in C. elegans. The previously known Isotype-1 and -2 are the closest homologues to the benzimidazole target ben-1 and may represent a gene duplication since the most recent common ancestor of the two species. Isotype-3 is a clear orthologue of the C. elegans touch receptor-specific -tubulin mec-7 based on phylogenetic analysis, synteny and expression pattern data. The closest homologue of isotype-4 in C. elegans is the embryonically expressed -tubulin tbb-4.Further analysis of the gene family and the implications for benzimidazole mode of action and resistance will be presented.

Parasite Physiology, Pharmacology, Pharmacokinetics

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PO2.62

PERL - An *in vitro* Model for the Percutaneous Migration of *Ancylostoma caninum* Third-stage Larvae

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Besides oral infection, percutaneous invasion of the host is a common mode of infection for third-stage larvae of the canine hookworm *Ancylostoma caninum*. A previously described *in vitro* model for percutaneous migration was modified and optimised for further investigations. For this purpose, Franz cells, originally developed for pharmacodermatology, were adapted to the needs for small volumes and relatively small pieces of skin. These cells were called percutaneous larval migration chambers, shortly PERL chambers. The conditions for optimum migration rates of larvae were determined, and the influences of several anthelmintics, such as ivermectin, levamisole, emodepside, and pyrantel, on the migration ability of the larvae were analysed. Larvae were preincubated at different concentrations of anthelmintic

for 30 min at room temperature, before they were placed onto the surface of the skin fixed within the PERL chamber. Migration took place at 37°C overnight. Each concentration of anthelmintic was tested in three chambers within one experiment, and each experiment was run three times. These results were compared to the results of conventional larval migration inhibition assays (LMIT), using a sieve with 20 µm meshes as barrier. The PERL chamber assay was shown to be a sensitive and reproducible quantitative *in vitro* system for the investigation of anthelmintic efficacy on the migration ability of infective larvae. Therefore, PERL chambers can be used to examine and to quantify the action of migration inhibiting substances. Furthermore, it represents the basis for investigation of the migration behaviour of skin-penetrating larvae and the production of "percutaneously migrated" larvae for subsequent biomolecular experiments.

PO2.63

Treatment and Prevention of Vertical Transmission of *Toxocara cati* in Cats with an Emodepside / Praziquantel Spot-On Formulation

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Lactogenic transmission of larvae to the offspring after acute infection is an important host finding strategy of *Toxocara cati*. This study aimed to determine the efficacy of emodepside 2.14% / praziquantel 8.58% spot-on (Profender®, Bayer Animal Health GmbH) in the prevention and treatment of lactogenic *T. cati* infections. Eight pregnant domestic shorthair cats were orally infected with 2000 *T. cati* eggs daily on 11 consecutive days starting 50 days after presumed conception. Four queens were treated according to manufacturer's recommendations on day 60 post conception and four queens were left untreated. 28 days after birth, the kittens (n=6) of two untreated queens were treated with the smallest pipette. The two other litters (n=8) were left untreated. The efficacy of emodepside was determined by daily faecal egg counts in pooled faecal samples of each litter, from day 35 until day 56 after birth. Litters in the control group became positive for *T. cati* on day 36 and day 50 after birth. The corresponding mothers became positive 50 and 59 days after the first inoculation. Egg shedding was completely prevented in all four treated queens and their litters (n=10) and in the kittens from the two litters group which were treated 4 weeks after birth. The untreated mothers of the treated litters stayed also coproscopically negative throughout the study, which might be explained by an oral uptake of emodepside through grooming. The treatment was well tolerated by pregnant queens as well as by four weeks old kittens.

PO2.64

Modulation of Drug Cellular Efflux Improves Ivermectin Activity Against Resistant Nematodes: Integrated Pharmaco-Parasitological Assessment

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The involvement of P-glycoprotein (P-gp) on IVM disposition kinetics has been demonstrated. P-gp over expression accounts for enhanced drug efflux in IVM resistant nematodes. The aim of this trial was to assess the effects of loperamide (LPM), a P-gp modulating agent, on IVM pharmacokinetics and efficacy against resistant nematodes in lambs. Eighteen (18) Corriedale lambs naturally infected with gastrointestinal nematodes were allocated into three (3) experimental groups. Group A remained as untreated control. Animals in Groups B and C received IVM (subcutaneously, 0.2 mg/kg) either alone or co-administered with LPM (0.2 mg/kg, twice every 12 h). Blood samples were collected between 0 and 14 days post-treatment and IVM plasma concentrations were determined by HPLC (Pharmacokinetic trial). LPM enhanced the IVM plasma availability ($P<0.05$) and prolonged its elimination half-life ($P<0.05$) in co-administered lambs. Faecal individual samples were collected from animals at days -1 and 14 post-treatment to perform the faecal eggs count reduction test (FECRT). Additionally, at day 14 post-treatment, animals were sacrificed and adult gastrointestinal nematode counts were performed (Efficacy trial). As earlier shown in cattle, the FECRT values increased from 79 % to 96 % after LPM coadministration. The efficacy against Trichostrongylus colubriformis increased from 77.9 % (IVM alone) to 96.3 % (IVM+LPM). A similar trend was observed for Nematodirus spp. LPM modulates the P-gp–mediated intestinal and hepatic excretion of IVM in the host, and it may also interact with the P-gp efflux transport over expressed in resistant nematodes, which would account to increase worm exposure to IVM.

PO2.65

Pharmacological Evaluation of a Combined Albendazole, Ivermectin and Levamisole Formulation in Lambs

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The goals of the current trial were: a) to characterize the plasma pharmacokinetics (PK) of albendazole (ABZ), ivermectin (IVM) and levamisole (LVS) administered either alone or co-administered to lambs; b) to compare the efficacy of the same drugs given separately or co-administered to lambs infected with resistant nematodes. Fifty (50) Corriedale lambs naturally infected with multiple resistant gastrointestinal nematodes were involved. a) "PK study": The animals were allocated into four groups (n=10 each) and intraruminally treated either with ABZ (5 mg/kg), IVM (0.2 mg/kg), LVS (8 mg/kg) or with a combined formulation of ABZ+IVM+LVS (RaiderPlus®, Cibeles, Uruguay) at the same dose rates for each active ingredient. Blood samples were collected over 15 days post-treatment and drug plasma concentrations measured by HPLC. b) "Clinical efficacy trial": An untreated control group (n=10) was included. The efficacy estimation was performed by the faecal egg count reduction test (FECRT). Although LVS kinetics was unaffected, significantly lower (65%) ABZ-sulphoxide and higher (66%) IVM plasma availabilities were obtained after treatment with the combined formulation in comparison to those obtained after the treatment with each drug alone. FECRT values were 64% (ABZ), 83% (IVM), 55% (LVS) and 91% (combined formulation). However, no differences (P>0.05) were observed on faecal egg counts among experimental groups. In conclusion, a PK interaction among drugs was observed and the combined formulation did not offer a clinically relevant increase in efficacy against resistant nematodes. Thus, further understanding of potential pharmacological interactions is needed before drug combined formulations are introduced into the pharmaceutical market.

PO2.66

Efficacy of Moxidectin/Triclabendazole Oral Drench Against Mixed Infections of *Fasciola Hepatica* and Gastrointestinal Nematodes in Sheep

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The aim of the study is to evaluate the efficacy of moxidectin/triclabendazole oral drench (Fort Dodge Animal Health) at a dose rate of 0.2 mg moxidectin/kg bodyweight and 10 mg triclabendazole/kg body weight against an experimental infection of Fasciola hepatica and natural infection of gastrointestinal nematodes (GIN) in sheep. 48 ewes, naturally infected by GIN, were allocated into three groups. At the same time, each group was divided into control and treated sheep. All animals were infected the day 0 of the experiment with 200 metacercariae and then were treated as follows: group 1 at week 4 post-infection (pi), group 2 at week 8 pi, and group 3 at week 12 pi. Finally, sheep were slaughtered 4 weeks after each treatment. Faecal samples were recovered on days 0 and 14 post-treatment to carry out larval cultures to identify the nematode species. After the necropsy of each animal, the adult nematodes and the flukes were picked up for counting and identification. The efficacy of the treatment against nematodes and immature/mature stages of F. hepatica were based on the number of parasites recovered compared to control. The results showed efficacies with values of 100%, 97% and 100% against 4-week, 8-week and 12- week old flukes respectively. Regarding the GIN recovered from abomasum and small intestine the efficacy of the treatment was 100% against Teladorsagia circumcincta and 99% against Trichostrongylus spp and Nematodirus spp. On the other hand, larval cultures confirmed the presence of T. circumcincta (50-98%) and Trichostrongylus spp (3-50%).

PO2.67

Antigiardiasic Effect of the Compound Alpha (Benzimidazole Derivative)

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Giardia intestinalis (syn *G. lamblia*, *G. duodenalis*) is a cosmopolitan zoonotic parasitic disease. Produces diarrea, reduction in weight gain which is an economic lost for the owner. The antigiardiasic treatments are based on derivatives from nitroimidazoles, nitrofuranos, benzimidazole derivatives and nitrotiazol; the efficiency goes from 60-90%, but they have side effects and have resistant strains. Is necessary to find news alternatives for the treatment in veterinary medicine. The alpha compound is a benzimidazole derivative with fasciolicide effect, and it can be candidate as giardicide. The aim of this work is to determine the effect antigiardial *in vitro* of the compound alpha. The essays were carried out on the isolate B43-INP (cow/ assemblages E/B) and the strain WB (assemblage AI). The trophozoites (50000) were exposed to different concentrations of compound alpha 1-40µg/mL and were incubated in TYI-S33 for 24 hrs, after trophozoites were washed and re-cultured in fresh medium for 24 hrs. The controls were trophozoites untreated, DMSO-exposed, trophozoites and killed by frezze-thaw. The cellular viability was determinated by the method colorimetric XTT-PMS and by cellular count. The essays were by triplicate with 3 repetitions. By direct cellular count, the 100% of cellular dead was with 15 µg/mL and 30 µg/mL for the isolates B43 and WB respectively. With XTT-PMS the 99% and 99.22% of dead trophozoites for the isolate B43 and for the WB. The isolate of cow B43 was more sensible to compound alpha that the isolate WB.

PO2.68

Maintenance of Adult Teladorsagia Circumcincta in a Short Term Culture

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Research on nematode parasites like *Teladorsagia circumcincta* is hindered by an inability to maintain adult worm cultures beyond a few days. The method presented allows the maintenance of adult *T.circumcincta* for up to 14 days in culture (with high motility for 10 days) and continuation of egg production for up to 48 hours. Adult worms were raised by infecting sheep with L3 *T.circumcincta* and harvesting adults 21 or 28 days post infection. The adults were washed from the abomasum, set in 1% agar and recovered after migration into saline. They were incubated in cell essential medium (CEM), based on MEM supplemented with 10% FBS, 1% non-essential amino acids, 1% glutamax and 1% penicillin-neomycin-streptomycin mix. The worms were co-cultured with HeLa cells in this medium at 37 °C in an atmosphere of 5% O2, 10% CO2, 85% N2 and 65% humidity. The presence of the cell line was critical, as adult worm survival was greatly reduced in CEM alone after nine days (45 ± 5% versus 70 ± 10% with cells) and those worms surviving had reduced motility. The HeLa cell line could not be replaced by an *E.coli* population or CEM previously exposed to the cell line or supplemented with fatty acids or cholesterol. This method can be used for testing potential anthelmintics on adult worms up to 10 days after the worms have been harvested from sheep. It can also be used for cultivating eggs in sterile conditions for further study.

PO2.69

Plasma Pharmacokinetics of a New Palatable Oral Ivermectin Formulation in Comparison with an Oral Paste in Horses

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Introduction: Plasma pharmacokinetics have been shown to be in close relationship with clinical anthelmintic efficacy in horses. This study was conducted to determine the plasma pharmacokinetics of a new oral ivermectin formulation in comparison with an oral paste in horses.

Methods: Two groups of eight unfed adult horses were treated orally with Vectin® chewable tablets (Intervet-SPAH) or Ivomec®P (Merial) at 0.2 mg/kg BW in a crossover design including a 6 week washout period. Blood was taken 18 times (day 0-21). Plasma ivermectin concentrations were measured using a validated HPLC/fluorescence method (LOQ=0.25 ng/mL). The parameters Cmax, Tmax, AUC0-LOQ, MRT and T>1ng/mL were calculated. To investigate bioequivalence, error variances were estimated from an ANOVA (fixed intra-subject effects: treatment, period, fixed inter subject effect: sequence, random effect: animal within sequence).

Results:

Significant treatment effects ($p<0.05$) were observed for AUC0-LOQ and Cmax.

Mean pharmacokinetic parameters (range)

	Vectin®	Ivomec®P	Bioequivalence
Cmax (ng/mL)	81.1 (59.5-107.2)	64.9 (12.9-100.5)	no
Tmax(h)	4.1 (2-6)	5.4 (2-24)	-
AUC0-LOQ (ng/mL.h)	5129 (3220-8559)	4057 (690-6122)	no
MRT (h)	110.3 (90.8-131.4)	110.9 (70.7-138.6)	yes
T>1ng/mL (days)	20.1 (16-21)	19 (7.4-21)	yes

Discussion: The systemic ivermectin exposure was significantly higher in the Vectin® group than the Ivomec® group as indicated by higher AUC and Cmax values. The variability of the plasma profile was higher in the Ivomec®P group. Although no product was spit out visibly, this might reflect an only partial swallowing of the paste in some horses.

Conclusion: The new chewable tablets led to a higher and more consistent systemic exposure than the paste. This might be correlated with a more consistent antiparasitic efficacy and a lower risk of parasite resistance.

PO2.70

Combined Administration of Fenbendazole and Praziquantel Against Toxocara canis and Ancylostoma caninum in Pregnant Dogs and their Puppies

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The aim of the present study was to evaluate the efficacy of a combined administration of fenbendazole and praziquantel on the elimination of T. canis and A. caninum in pregnant dogs and their puppies. Ten pregnant dogs or lactating with their puppies, all positive to Toxocara and Ancylostoma eggs were included in the trial for treatment. Then single oral administration of 100 mg de fenbendazol y 10 mg de praziquantel (0.5 ml/kg) were given during 3 days followed by daily coprological analyses during 6 days to determine the percentage of egg reduction. The data were submited to an ANOVA to determine posible differences between treatments. Results indicated a percentage of egg reduction for T. canis in the mothers of 57.1, 90.6, 91.0, 96.7 and 98.4 and for

A. caninum, of 96.1, 97.2, 98.0, 98.5 and 98.7% for days 2 to 6 respectively. Reduction on the litter infected with T. canis, was 79.0, 97.2, 98.8, 99.5, 99.9, and for A. caninum 91.1, 99.0, 100, 100 y 100% for days 2 to 6, respectively. Comparison in sex between puppies infected with T canis showed no statistical difference in egg reduction. However, female puppies parasitized with A. caninum showed a 99% egg reduction by day 2 after treatment. The combined administration of these drugs exerts high efficacy either in puppies and mothers from the first day of treatment. Study financially supported by laboratorios SALUD ANIMAL S.A. de C.V.

PO2.71

Determination of the Effectiveness of Levamisole in Goats

Amaral, Carlos H.; Leite Filho, Ronaldo V.; Aguiar, Thiago N.; Sprenger, Lew K.; Gonçalves, Ricardo B.; Franciosi, Aline; Dewes, Adriana; Molento, Marcelo Beltrao

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Gastrointestinal parasites cause great damage in sheep and goats. Levamisole (LEV) is an antiparasitic drug that acts as a cholinergic agonist promoting hyperexcitability and spastic paralysis. This drug has been used by goat producers on what is indicated for sheep (4.5mg/kg). The objective of this work was to determine the effectiveness of LEV in goats compared to sheep. Animals were divided in 4 groups of each species (n=7) belonging to Böer goats and crossbred sheep, with similar management. G1: received 4.5mg/kg, G2: 6.75mg/kg, G3: 9.0mg/kg of LEV, and G4: control. The animals were evaluated for nematode faecal egg (EPG), coproculture, FAMACHA, packed cell volume (PCV), and plasma protein (PP). Comparison was made using RESO. The results of the coproculture showed to be similar before and after treatment in goats. There was a higher prevalence of Trichostrongylus sp. followed by Haemonchus sp. In sheep there a was greater prevalence of Haemonchus sp. followed by Trichostrongylus sp., but this result was reversed after treatment. LEV was ineffective for goats in all treatments (29, 0, and 63%). The effectiveness calculated for sheep was 86, 93 and 99%, respectively. However, only the dose of 9.0 mg/kg was considered to be highly effective. The results have shown a correlation between FAMACHA and PCV (1:0.75). LEV inefficiency in goats may be due to the different kinetics compared to sheep. One should be careful when monitoring and recommending LEV for the control of nematodes in goats.

PO2.72

The Efficacy of Ivermectin in Dexamethasone Treated Young Cattle

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An experiment was carried out to study the possible interaction between immunosuppression and efficacy of anthelmintic treatment in young cattle. Two groups (A and B) each of seven calves were experimentally inoculated at the start of the experiment with an equal mixture of 30,000 infective third stage larvae of Cooperia oncophora and Ostertagia ostertagi. Blood parameters and faecal egg counts (FEC) were then monitored from day 0 until day 35. The calves in B were immunosuppressed by intramuscular injections of short and long term acting dexamethasone (Dexadreson® vet. Intervet 0.08 mg/kg and Vorenvet® vet. Boehringer Ingelheim Vetmedica 0.25 mg/kg) at days 22 and 24, respectively. Three days post patency (day 24) groups A and B were injected subcutaneously with ivermectin (Ivomec inj., Merial) at the normal dose rate (0.2 mg/kg). The faecal egg count revealed a significant difference ($p<0.001$) in FEC patterns between groups A and B. Although, both groups still excreted eggs (100-200 epg) 11 days post anthelmintic treatment, there was a significant ($p=0.025$) difference in the reduction of eggs in groups A and B between days 23 and 35, where group A had a higher reduction. After 35 days, 10 animals from both groups were sacrificed, and established gastrointestinal worms were collected and counted. No Ostertagia were found in the abomasums, but low numbers of Cooperia remained in the small intestines. Overall, this experiment showed that the animals were immunosuppressed by dexamethasone and also indicated that there might be a possible interaction between the efficacy of anthelmintic treatment and immunity.

Zoonoses

Wednesday, August, 12, 2009

PO2.73

Toxoplasma gondii: Evidence of Sexual Transmission in Sheep

Lopes, Welber D. Z.; Santos, Thaís R.; Sakamoto, Cláudio A. M.; Silva, Helenara M.; Vicentini, Frederico F.; Costa, Gustavo H.; Oliveira, Gilson P.; Costa, Alvimar J.

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Reproductive rams experimentally infected with T. gondii were distributed into three groups: GI, one ram inoculated with 2.0x105 oocysts; GII, one ram infected with 1.0x106 tachyzoites; and GIII, one ram maintained as an uninfected control. After inoculation of the rams with T. gondii, 12 reproductive non-pregnant ewes, serologically negative for reproductive diseases, particularly toxoplasmosis, were distributed into three groups. Groups were then synchronized and naturally inseminated by infected rams: GIV, five ewes inseminated by the GI ram; GV, five ewes inseminated by the GII ram; and GVI, two ewes inseminated by the control ram. Serum collected from ewes on days -14, -7 and zero (prior to insemination), and on days 1, 3, 5, 7, 11, 14 and weekly until birthing, was screened for the presence of antibodies against T. gondii using indirect immunofluorescence (IFI). Bioassays were conducted on semen samples, ewe tissue samples, and samples of their respective offspring. After insemination, five of the ewes presented specific antibodies against T. gondii. Two were inseminated by the ram inoculated with oocysts (GI) and three by the ram inoculated with tachyzoites (GII). The bioassay made it possible to determine the presence of T. gondii in semen samples of infected rams on the day of insemination and in ewe tissue samples that presented antibodies against T. gondii after insemination. Results from additional studies (PCR and immunohistochemical), should corroborate this proof of a new toxoplasmosis transmission route. This information would certainly contribute to clarifying the high prevalence of this zoonosis in sheep.

PO2.74

Histopathology of the Reproductive System of Male Sheep and Goats Experimentally Infected with Toxoplasma gondii

Lopes, Welber D. Z. [1]; Santos, Thaís R.[1]; Luvizotto, Maria Cecília R.[2]; Santana, Luís Fernando[1]; Sakamoto, Cláudio A. M.[1]; Silva, Helenara M.[1]; Oliveira, Gilson P.[1]; Costa, Alvimar J.[1]

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This study aimed to describe eventual histopathological alterations in the reproductive systems (testicles, epididymides, seminal vesicles and prostate) of small ruminants with toxoplasmatic infection. We used nine rams and seven bucks, distributed and inoculated with T. gondii, as follows: GI, four rams and bucks (2.0 x 105 P strain oocysts); GII, four rams and three bucks (1.0 x 106 RH strain tachyzoites); and GIII, one ram and one buck maintained as controls. Infection with T. gondii was verified using seroconversion analysis (IIF-IgG) in all of the infected animals from post inoculation day (PID) 7 onward. After PID 70, all of the animals were euthanized and tissue samples (testicle, epididymis, seminal vesicle and prostate) were collected and processed for histological analysis. The principal alterations diagnosed included: perifocal mononuclear interstitial inflammatory infiltrate in the prostate; degeneration and metastatic calcification of the testicles; and mononuclear interstitial infiltrate, pyknosis, acidophilia and necrosis of the muscle fibers surrounding the seminal vesicles. The histopathological findings of this work, together with the isolation of T. gondii in fragments of the reproductive systems examined (immunohistochemistry), in addition to the results obtained by other authors in different tissues, suggest that histological alterations diagnosed in the reproductive system of rams and bucks infected with the respective protozoan are strongly suggestive of toxoplasmatic infection.

PO2.75

Preferred Locations for Cysticercus bovis in Bovine Experimentally Infected with Taenia saginata Eggs

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Preferred locations for Cysticercus bovis were evaluated in the striated skeletal musculature and entrails of 25 bovine, each experimentally infected with 2x104 Taenia saginata eggs. Two bovines were maintained as uninfected controls. All of the experimental bovines were euthanized 90 days post infection. Next, the carcasses were deboned and 26 anatomical regions of each bovine were sliced in fragments of approximately 5 mm in thickness. Examination of the 25 bovine samples inoculated with T. saginata eggs led to the recovery of 9,258 C. bovis (cysticercs). Of the positive samples, 75.03% (6,946) were from the skeletal musculature and 24.97% (2,312) were from the entrails. Accentuated parasitism by C. bovis was verified in the shoulder blade (12.55%), heart (11.02%), liver(9.48%), head (8.51%), chuck roll and neck (8.25%), strip loin and full tenderloin (7.26%), knuckle (6.63%) and back ribs (5.54%), which together totaled 69.24% (5,738) of the cysticercs detected. In contrast, low parasitism occurred in the brain, spleen, tail muscle, kidneys, esophagus and diaphragm, totaling only 3.90% of the 9,258 cysticercs detected. Given these results, it can be concluded that specific skeletal musculature regions, such as the shoulder blade, chuck roll and neck, strip loin and full tenderloin, knuckle, back ribs and top round, which are not officially examined in many countries. To date, these regions have not constituted preferred locations of Cysticercus bovis. These regions deserve greater attention from health inspectors, because they contain a greater number of cysticercs compared to the remaining regions of a carcass parasitized by Taenia saginata larvae.

PO2.76

Histopathological and Immunohistochemical Observations in Pregnant Queens Experimentally Infected with Two Major Clonal Lineages of Toxoplasma gondii from Brazil

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Toxoplasma gondii isolates obtained in Brazil are biologically and genetically divergent from the isolates described in Europe and North America. Histopathological (hematoxylin-eosin staining) and immunohistochemical analyses were conducted on tissue samples from cats that were inoculated with the two major brazilian isolates of T. gondii. The cats were orally inoculated with tissue cysts during the middle third of gestation and distributed into three groups, containing four cats per group. Group A was inoculated with BrI type T. gondii, Group B with BrII type, and Group C was maintained as control group. Miscarriage occurred in one queen from Group A, while premature stillbirth was observed in Group B, of one litter. Among Group A queens, endometria with accentuated inflammatory infiltrates and necrotic areas were observed. In one kitten, verification revealed a lung with areas of atelectasia, emphysema, congestion and inflammatory reaction. In this lung and in the heart, macrophages phagocytosing T. gondii were observed and confirmed by immunohistochemistry. Among Group B queens, the presence of inflammatory cells in skeletal musculature and uteri presenting hemorrhage and discrete inflammatory infiltrates were observed. In the kittens, congestion, atelectasia, emphysema and the presence of inflammatory cells were detected in the lungs and in the encephalus. In one litter, hearts with necrotic areas and the presence of T. gondii inside macrophages were observed and verified by immunohistochemistry. Overall, the isolates of BrI and BrII type T. gondii caused similar histopathological alterations in experimentally infected pregnant cats and their offspring.

PO2.77

Incidence of Cryptosporidium, giardia and Microsporidia in Young Dogs in Brazil

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This study aims to investigate the incidence of Cryptosporidium*null result*, Giardia*null result* and microsporidia in fecal specimens obtained from dogs in their first year of life in the city of São Paulo, Brazil. These protozoans were investigated in a prospective study by repeated fecal sampling of dogs between 2 and 12 months of age, privately owned and from different breeds. Individual fecal samples were collected from 200 dogs with approximately 2, 4, 6, and 12 months old. For the detection of Giardia*null result*, Cryptosporidium*null result* and microsporidia it was used, respectively, the floatation technique with lead sulphate, the Kinyoun method and the Gram-Chromotrope staining. Ninety-eight dogs (49%) had positive results to 1 or more of these protozoan parasites. For Cryptosporidium*null result*, prevalences observed at 2, 4, 6, and 8 months were, respectively, 12.5% (25/200), 10% (20/200), 7.5% (15/200) and 7% (14/200); for Giardia*null result*, these prevalences were 32.5% (65/200), 29% (58/200), 25% (50/200) and 20% (40/200), respectively. For microsporidia, respective prevalences were 5% (10/200), 3% (6/200), 2.5% (5/200) and 0.5% (1/200). It was observed that the highest incidences for all protozoans occurred in 2 months old dogs and progressively declined in the subsequent ages. No significant difference in prevalence was observed between males and females. Giardia*null result* was found to be the most common protozoan, followed by Cryptosporidium*null result* and microsporidia.

PO2.78

Molecular Characterization of Indian Isolate of Schistosoma Spindale Collected During an Outbreak of Cercarial Dermatitis

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Background: Schistosoma spindale is an important animal schistosome which is also responsible for causing severe cercarial dermatitis among farmers in India. Recently, a focus of cercarial dermatitis was found in Tinsukia District of Assam. The aim of this study was to confirm the identify of the species involved using DNA sequencing of the internal transcribed spacer two (ITS2). Methodology: Infected snails were collected from the affected paddy fields were people reported dermatitis. The cercariae were harvested from the snails in the laboratory. These cercariae were used to infect Swiss albino mice and adult schistosome flukes were recovered. DNA was extracted from individual worms and ITS2 region was amplified using PCR and subsequently sequenced using ABI sequencer using BigDye V.3.

Results and Conclusion: The morphological analysis of adult flukes was revealed that the species responsible for cercarial dermatitis was S. spindale. Phylogenetic analysis of the ITS2 region revealed that Indian isolate of S. spindale represented a distinct lineage and occupied an intermediate position between S. haematobium group and S. mansoni.

PO2.79

Serological Survey of Rickettsias of the Spotted Fever Group in Horses and Dogs in Almirante tamandaré, Paraná, Brazil

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Brazilian Spotted Fever (BSF) is a lethal rickettsiosis in humans and is endemic in some Brazilian regions. Horses and dogs can participate in the disease's cycle and, when the sorology is positive, they can be used as sentinel animals in epidemiological studies. The first reported case in the state of Paraná occurred in the municipality of São José dos Pinhais in 2005. This present study was conducted in Almirante Tamandaré, PR which has never had a reported case of BSF and is located 26 km from São José dos Pinhais. Serum samples were collected from 71 horses and 20 dogs from 9 properties in the region. Ticks were also collected from these animals. The owners of these animals responded to a questionnaire in order to obtain information about their management. Serum samples were processed by indirect Immunofluorescent-antibody test (IFAT), using the antigens of Rickettsia rickettsii and R. parkeri. Ticks were analyzed by PCR for Rickettsia sp. Ten animals were identified as seropositive, 6 (8,45%) horses and 4 (20%) dogs, with titers varying from 64 to 1024. All the ticks were negative. The data reveals that Almirante Tamandaré is a vulnerable area for BSF. We consider that BSF has been underdiagnosed in many non-endemic regions.

PO2.80

Experimental Infection with Ancylostom ceylanicum in Dogs and Efficacy of a Spot on Combination Containing Imidacloprid 10 % and Moxidectin 2.5 % (Advocate® / Advantage® Multi, Bayer Animal Health)

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Ancylostoma ceylanicum is a prevalent hookworm species in dogs in Asia and Australia. Since only few anthelmintics are licensed to treat this parasite, an experimental infection model was developed to allow subsequent testing of anthelmintics. Adult hookworms from necropsied dogs have been collected and identified. Female Ancylostoma ceylanicum have been isolated and grinded to collect eggs. The eggs were mixed with sterile dog faeces and cultivated by using Hada Mori technique. Two helminth naïve puppies have been infected with 500 L 3 as donor dogs. After patency eggs were collected and cultivated to L3 stage. Twelve dogs were subcutaneously injected with 300 L3 of A. ceylanicum. Once patency was confirmed, individual eggs per gram (EPG) counts were performed daily. At day 20 post infection dogs were allocated into treatment and control group. Each dog from the treatment group received a spot on combination containing 10 % imidacloprid and 2.5 % moxidectin at the manufacturers recommended dose. EPG counts were performed daily for 14 days post treatment. The treatment rapidly reduced egg shedding within 3 days post treatment compared to the control group. No eggs were found in the treated dogs from day 4 post treatment onwards. EPG counts remained high (4469 + 2064) in the untreated control group. The spot on combination containing imidacloprid 10 % and moxidectin 2,5 % (Advocate® / Advantage® Multi, Bayer Animal Health) given at the recommended dose is highly effective against infection with A. ceylanicum in dogs.

PO2.81

Presence of *Toxocara spp.* in Soil of Altata Beach, Navolato, Sinaloa, Mèxico

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Toxocara spp. is a common intestinal parasite of dogs and cats, and also can result in a condition known as visceral and or ocular larval migrans in humans, which is associated with inflammation of body organs and or the central nervous system, Symptoms of which are caused by the movement of the worms through the body, include fever, coughing, asthma, or pneumoniae; toxocariasis most often occurs in children, who often play or eat dirt contaminated with Toxocara spp. eggs disseminated for defecate of dogs and cats in public areas, and are extremely resistant to adverse environmental conditions, capable of surviving in soil for many months. The objective of this work was to determine the presence of Toxocara spp. in the soil of Altata Beach of Navolato, Sinaloa, Mèxico; this were determined for representative sample described by the technique of Thrus eld (2005) was used: n=[t*SD/L]2. Where n=sample size, t=value normal distribution (Student t) for a 95% con dence level (t = 1.96), L=accepted error or precision (5%), and SD=weighted disease prevalence (%). the number of soil sample determined by random samplings was 171, analyzed by the sedimentation techniques. The results indicate that 57(33.3%) samples of soil of Altata beach were positive to Toxocara spp. It is concluded that the contamination with Toxocara spp. represent danger for the pets and public health for the family community, and visitors frequently use these recreation place. It is necessary implement control strategies and education for the prevention of the infections.

PO2.82

Data on Echinococcosis/Hydatidosis in Slaughtered Animals from Different Areas of Romania

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Cystic echinococcosis, caused by the larval stages (hydatid cysts) of E. granulosus is known as one of the most important parasitic infections in livestock in the world. It can establish itself in many different hosts, including humans, and is regarded as one of the most wide-spread zoonoses. Romania has been included in the mezoendemic countries, in the WHO classification. An epidemiological study was conducted during 2008 year, to assess the current status of hydatidosis in cattle, sheep, and swine slaughtered in 13 abatoirs from three different regions of Romania. Hydatid cyst count and characterization were realized based on routine meat inspection of 5983 cattle, 5799 sheep, and 27446 pigs. The incidence of hydatic cysts in cattle varied from 21.16% to 73.05%. In sheep, the incidence of hydatic cysts was comparable with the one of cattle, with range between 17.98% and 74.5%, while in pigs, echinococcocic cysts had an incidence of 0.69% to 5.05%. Regarding affected organs, the highest percentage was registered in the lung (85.7%, in cattle, and 93.75% in sheep), followed by the liver (57.1% in cattle, and 87.5% in sheep). The biggest number of cysts was found also in the lung (78.6% from total number of cysts in cattle, and 49.5% in sheep). In the liver were found 17% from the total count of cysts in cattle, and 47.6% in sheep. The hydatic cysts developed in spleen and kidney represented less than 3% of the total counted cysts. The present study provides baseline data on the current status of the disease in the area and imposes a rigorous parasitological control of the disease.

PO2.83

Prevalence of Toxocara spp. in Dogs of Fields Fishing of Navolato, Sinaloa, Mèxico

Rubio Robles, Mario Cesar; Gaxiola C., Soila M.; Gaxiola Montoya, Joel; Castro del Campo, Nohemi; Estrada S., Guadalupe; Lopez V., Martin

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Toxocara spp. are common intestinal parasite of dogs and cats, continuously acquire infections from the environment, they may develop a serious illness or even die before a prenatally or lactogenically; not only can cause disease in their respective hosts, also can result in a condition known as visceral and or ocular larval migrans in humans and represents a serious health problem, for animals and humans. The objective of this work was to determine the prevalence of Toxocara spp. in dogs of fields fishing of Navolato, Sinaloa, Mèxico. This were determined for a representative sample with both sexes and cradle described by the technique of Thrus eld (1995) was used: n=[t*SD/L]2. Where n = sample size, t = value of the normal distribution (Student t) for a 95% con dence level (t = 1.96), L = accepted error or precision (5%), and SD = weighted disease prevalence (%). On the basis of the technique described, the total number of sample animals determined for random sampling was 134. For each dog feces were collected rectally by digital stimulus into plastic bags and transported under refrigeration at 4°C to the FMVZ-UAS, and processed by the otation technique with sugar solution. The results indicate that of the 134 dogs analyzed 13 (9.7 %) were positive to Toxocara spp. This be an issue of importance in the community because frequently these dogs roam in and around the town and can distribute parasites, and residents as visitors ignore about parasitic diseases that dogs can transmit them.

PO2.84

Ancylostoma spp. in Sand of Altata Beach of Navolato, Sinaloa, Mèxico

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The moist sand of beaches can act as reservoir or vector of zoonotic parasite potentially pathogens,how Ancylostoma spp. that infect the host by eggs and larvae penetrating the skin of bare feet or hands that have contacted with contaminated sand, and produce Larva migrans, also known as creeping eruption or sandworm eruption, characterized by tortuous migratory lesions of the skin, this represent risk for health humans and pets that have contacted with contaminated sand. The objective of this work was to determine the presence of Ancylostoma spp. in moist sand of Altata beach of Navolato, Sinaloa, Mèxico. this were determined for representative sample described by the technique of Thrus eld (2005) was used: n=[t*SD/L]2. Where n=sample size, t=value of the normal distribution (Student t) for a 95% con dence level (t =1.96), L=accepted error or precision (5%), and SD=weighted disease prevalence (%); the total of composite sample of sand determined by random samplings was 162, took surface moist sand scraping of 100 grams of sand for each sample and deposited it in plastic bags; transferred to the laboratory of parasitology of the FMVZ-UAS to be analyzed by the sedimentation technique. The results indicate that 102 (62.9%) samples were positive to Ancylostoma spp.; It is concluded that the contamination with Ancylostoma spp. represent high risk for the pets and public health, yet

residents as visitors ignore about parasitic diseases that dogs can transmit them it is necessary implement control strategies and education for the prevention of the infections.

Animal Production

Thursday, August, 13, 2009

PO3.1

Resistance of Santa Ines and Crossbred Ewes to Naturally Acquired Gastrointestinal Nematode Infections

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This trial was carried out to evaluate comparatively the degree of resistance to naturally acquired gastrointestinal nematode infections in sheep of the following genetic groups: purebred Santa Ines (SI), and SI crossbred with Dorper (SIxDO), Ile de France (SIxIF), Suffolk (SIxSU), and Texel (SIxTE). Fifteen ewes of each group were raised indoors until 12 months of age. At this age, they were moved to pasture and were evaluated for six months. The highest fecal egg count (FEC) mean was recorded in SIxTE group in February (2020 eggs per gram). However, significant difference between group means occurred only between SI (FEC = 80) and SIxIF (FEC = 347) groups in January. All groups showed a progressive reduction in body weight throughout the experiment with a weight loss from 12.0% (SIxTE) to 15.9% (SIxSU). In general, the animals with higher FEC presented the lowest packed cell volumes and blood eosinophils values. Immunoblobulin G (IgG) levels against *Haemonchus contortus* antigens increased in all groups as a result of the exposition to parasites and remained relatively constant until the end of the study, excepting the SIxSU and SIxTE that showed a rise in IgG levels in the last sampling which coincided with reduction in mean FEC. In conclusion, all breeds evaluated showed promising results when crossbred with the Santa Inês hair sheep. Crossbreeding can increase sheep production with maintenance of a satisfactory degree of resistance, especially to *H. contortus* and *Trichostrongylus colubriformis*, the major nematodes detected in the flock.

PO3.2

Natural Evolution of Neospora caninum Infection in a Dairy Herd of Italy

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The increase of abortion rate from 5.9% to 10.1% apparently due to neosporosis (seroprevalence 31% and 31.5% in 2005 and 2006) in a dairy herd of north-eastern Italy, induced the owners and their veterinarian to explore the epidemiology of the infection and to estimate its economic impact before to apply a control plan. The herd is officially free for brucellosis, leucosis and tubercolosis and vaccinated against BoHV-1, PI3, BRSV and Pasteurella. BVDV is under control by serology on 6-month calves. In the 2-years study the herd was managed as usual, animals were sampled 3 times, aborted foetuses collected for diagnosis and data annotated for statistical association. In total, 480 sera from 219 cattle were tested (cELISA, VRMD Inc) and seroprevalence showed a tendency of significant decrease in time (45.7%-31.5%-28%; p=0.05). In all the samplings seroprevalence was higher in raised animals vs purchased animals and at the 3th sampling additional association was found with increasing age and with gravid/lactating cows vs heifers. In the study period, 25 abortions occurred (abortion rates 8.7% and 6.0%) and Neospora only was found with PCR (7/14). Seroprevalence in aborting cows was significantly higher at the 2nd and 3th sampling. The mean calving interval was always higher in seropositive cows with a tendency to be significant at the 1st and 2nd sampling. Neospora infection was mainly maintained vertically (65%), but a considerable amount of transmission (35%) seemed to be horizontal (seropositive 6-months calves from seronegative mothers). Fertility cost was € 129.75 (USD 163.38) per cow.

PO3.3

Hematolgical Parameters of Goats Naturally Infected by Gastrointestinal Nematodes

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Parasite infection is a serious problem for goats and the species that is the most relevant is *Haemonchus contortus,* which provokes anemia in the animals. A complete examination of the animal health is important to characterize the source of

the anemia. In this way, the aim of this study was to evaluate the effect of two grazing intensity on the hematological parameters of 22 Boer Saanen female goats (40 kg of BW, in average) naturally infected with gastrointestinal nematodes. Eggs counts per gram of feces (EPG), coproculture analysis, Famacha score, packed cell volume (PCV) and haemogram in the goats into low (n=11) and high (n=11) grazing intensity treatments were evaluated every 12 days. It was observed a significantly effect of the interaction between treatment and day of evaluation, whereas animals subjected to low grazing intensity presented higher counting. For all the others parameters there were no differences between treatments (P>0.05). The EPG, in average, were 1033.3 and 1554.5 in the animals submitted to high and low grazing intensities, respectively. In the coproculture analysis, *Haemonchus* sp. was predominant (around 60%), followed by *Trichostrongylus* sp. (around 30%). In average, the goats presented Famacha score 1 and 23% of PCV, beyond the minors counting of hemoglobin and eosinophils were 7.93 g/dL e 3.46 L, respectively. These results showed that grazing intensity did not affect parasite infection in goats, however some hematological alteration can be observed, not directly related to parasite infection.

PO3.4

Ruminant Nematodes in Pasture Under Different Grazing Systems with Sheep and Cattle

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The aim of this study was to evaluate the effect of different pasture management systems on the parasitic load (L3 larvae) in sheep. The experimental period was 99 days, in a rotational system (seven days occupation and 21 days rest) to evaluate different management systems: isolated, alternate and simultaneous with cattle and sheep. The area was eight hectares subdivided in eighteen paddocks using Panicum maximum vr Tanzânia grass. Twenty mixed breed cattle, thirty lambs and fifteen ewes were used (sheep were Santa Inês breed). Grass was collected for recovery and identification of L3 each week pre and post grazing of the paddocks. Medium sized correlations were found between number of L3 of the same larva at entry and exit of the animals. In all systems the decreasing number of larvae was Haemonchus spp, Trichostrongylus spp, Strongyloides spp, Cooperia spp, and Oesophagostomum spp, There was an increase in the degree of infection depending on the system used, with the simultaneous system showing the best results with lowest degree of pasture contamination.

PO3.5

An Efficacy Trial of Four Cattle Anthelmintics Based on Gastrointestinal Nematode Recovery

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In June 2008, 122 yearling heifers were obtained from pastures in northern California with a history of anthelmintic resistance and transported to a dry lot facility in southwestern Idaho, USA. Fifty heifers with the highest fecal egg per gram counts were selected for study enrollment. Candidates were equally randomized to treatment with either injectable ivermectin (Ivomec®, Merial, 0.2 mg kg^{-1}BW), injectable moxidectin (Cydectin®, Fort Dodge, 0.2 mg kg^{-1}BW), oral fenbendazole (Safe-Guard®, Intervet, 5.0 mg kg^{-1}BW), oral oxfendazole (Synanthic®, Fort Dodge, 4.5 mg kg^{-1}BW) or saline. At 14 days post-treatment, nematodes were recovered from the abomasum, small intestine, and large intestine. In the control group, 10/10 animals were parasitized with adult and L4 stages of *Ostertagia ostertagi* and 9/10 with adult *Cooperia spp*. Based on geometric mean percent reduction versus saline controls, fenbendazole, oxfendazole, and moxidectin were >90% efficacious against adult and L4 stages of *O. ostertagi* (P<0.05). Ivermectin was 90% efficacious against adult *O. ostertagi* (P<0.05) and 81% against L4 stages. Versus controls, fenbendazole and oxfendazole were >90% efficacious against adult *Cooperia spp.* while moxidectin was 88% efficacious (P<0.05). Ivermectin treatment resulted in no reduction in adult *Cooperia spp*. The study was sponsored by Fort Dodge Animal Health.

PO3.6

Parasitological Survey and Determination of Metabolic Profiles of Farmed Eland (Taurotragus oryx) in the Czech Republic

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Farmed eland (Taurotragus oryx) were kept under husbandry conditions at the farm (CULS Prague) in the Czech Republic and monitored to obtain information of health status and effects of nutrition. Faecal egg count patterns and clinical signs associated with their gastro-intestinal (GI) parasites were monitored at fortnightly intervals during 9 (Giardia spp.) to 15 months (coccidia and helminths). Cysts of protozoa (Giardia spp.) were recovered in 20,7% (n=87) of individual faecal samples and increased during the grazing season from June (30,00%) to September (36,36%) and peaked in July, August (72,73%). Prevalence of oocysts (Eimeria spp.) was distinctly higher 56,69% (n=157) with 100,00% peak in June and higher

prevalence (≥ 80,00%) from April to October. Nematode eggs infection levels (genus Chabertia, Oesophagostomum, Trichostrongylus spp., Ostertagia, Capillaria) were identify in 57,96% (n=157), using flotation techniques. Our findings suggest different nematode infection due to husbandry conditions but to a lesser extent to species or individual susceptibility. Only a few trematode eggs (Dicrocoelium spp.) by sedimentation method and no lung worms larvae by Bearmann and Vajda methods were confirmed. No differences in faecal egg counts could be found between the sexes and different age groups. No clinical signs, such as loss of faecal consistency, could be correlated with faecal egg counts ($P > 0.05$). To evaluate the use of biochemical analysis were made screening of 20 blood serum samples of elands (Taurotragus oryx) after immobilisation and analyzed by IDEXX Laboratoires ((Cymedica Ltd. comp.) for 13 biochemical parameters using VetTest analyzers, 10 haematological parameters by QBC VetAutoread and 8 blood gasses and electrolytes by VetStat (effect of eland's age and sex on all parameters values by General Linear Mixed Model using the SAS System V 9.1.) Were found only effect of age on creatinine, lower in young animals ($F(4,13)= 5.1$, $P < 0.01$; range= 100 – 165 µmol/l, mean= 142 µmol/l). It follows from results obtained, elands were under stress before immobilisation (high levels of creatine kinase, pH, K+ or glukose) and very low pCO_2 levels. Identification of elands and knowledge of the seasonal variation of their helminths can contribute greatly to a well-adjusted species-specific management and helminth control program. Our results will be used as reference values for further studies and project was supported by Gant Agency of the Czech Republic No. 51120/1161/1603 FRV .

PO3.7

Endoparasitosis and Ectoparasitosis at Pig Farm

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Introduction: Preventive parasitological and dermatological examinations about ecto- and endoparasitic presence were taken twice in year, at spring and autumn, on industrial pig farm, which had its own breeding stock, weaners, growers and finishers. The results of those examinations shown causative agents of parasitic infection, type of those agents, intensity of infection and suggestion about therapy, based on those data.

Methods: This investigation were taken at industrial pig farm, called "Reprocentar", near Leskovac, city in Serbia, an period 1995-2000. The samples of faeces and samples of derma scarification, taken from all categories of animals, were examined by standard parasitological methods. Diagnosis were taken during the autopsy of dead animals, or at the slaughterhouse, too. The faeces samples were taken from 30 animals of any particular category.

Results: There were parasitic infections at all pigs categories, according to results from this trial.

There were protozoan infections with Balantidium coli, Eimeria perminuta and Eimeria debliecki, helminthosis caused with Ascaris suum, Strongiloideus ransomi and ectoparasitosis caused by Sarcoptes scabiei, at piglets to 25 kg of body weight.

Balantidium coli, Eimeria (mixed infections),Ascaris suum, ascaridosis and echinococcosis (at the slaughterhouse) were found at finishers. Scabies were found in 5% of animals in this category.

Parasitic infections with Eimeria sp. Balantidium sp. And Ascaris sp. were found at breeding stock.

Conclusion: According to the results of coprological examination and examination of derma scarification, we made conclusion that ascaridosis and scabies were dominate parasitic infections at this pig farm.

PO3.8

Efficacy of Fenbendazole and Eprinomectin in New York Dairy Replacement Heifers as Determined by Fecal Egg Count Reduction

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Introduction: The objective of the study was to test the field efficacy of fenbendazole (FEN, medicated crumble mixed in the daily ration at 5mg/kg of body weight) and eprinomectin (EP, pour-on formulation applied at 500 mcg/kg of body weight) when administered to dairy replacement heifers in New York State as determined by fecal nematode egg count reduction.

Methods: Between December 15, 2008 and January 30, 2009, a convenience sample of eight dairy farms raising replacement heifers on pasture in the summer and fall were screened for nematode eggs per 3 grams [EP3G] using the Wisconsin Modified Sugar Flotation method done independently by two laboratories. Six herds met the inclusion criterion of an average of at least 10 nematode EP3G of feces. Then, 20 yearling heifers in each herd assigned to receive either fenbendazole or eprinomectin were haphazardly selected for sampling per rectum with coded samples being sent to the two laboratories for EP3G determinations in a blinded fashion. Fourteen days after all animals in each group

were treated, the previously sampled animals were again sampled for EP3G determination.

Results: There were 3 farms and 58 animals in the FEN group and 3 farms and 60 animals in the EP group. Average pre-treatment EP3Gs were 22 in the FEN group and 14 in the EP group.

Discussion: Results of the post-treatment EP3Gs have not yet been fully compiled. Supported by Intervet/Schering-Plough Animal Health and performed under IACUC 2008-0188 at Cornell University.

PO3.9

Evaluation of the Association Between Isospora suis Infection and Post-Weaning Performance on Three Southwestern Ontario Swine Farms

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In recent work, oocysts of Isospora suis were detected in the feces of suckling piglets on 35 of 50 (70%) pig farms in southern Ontario. Furthermore, litters of pigs that were positive for oocysts were significantly more likely to exhibit diarrhea than negative litters. In order to determine the impact of I. suis on production, a prospective cohort study was conducted on a convenience sample of 3 swine farms in Ontario, to determine the association of I. suis infection with weight gain in pigs up to 8 weeks of age. Fecal samples were collected from randomly selected piglets in each of 72 litters and examined for oocysts using the Cornell-Wisconsin centrifugal floatation method. Piglet weight was recorded 6 times (at 1, 2, 3, 4, 5 and 8 weeks of age). If one or more piglets in a litter were found shedding I. suis oocysts at 2 or 3 weeks of age the litter was classified as infected. A linear mixed model with random intercepts was used to examine the effect of infection on weight gain. On average, pigs from infected litters were 1.4 kg (95% CI = 1.1–1.8 kg, $P<0.001$) lighter than pigs from non-infected litters at 62 days of age. Thus, I. suis infection during the nursing stage was associated with significantly lower weights of pigs at 62 days of age.

PO3.10

Paramphistomosis in Argentina: Current Situation

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Introduction: Paramhistomids' importance has been increased since the second half of 90's decade in Argentina, due to new findings where it hasn't been detected before. Studies are carrying out in order to find out their significance in animal's health and production.

Methods: For genus and species identification, flukes from different location were processed trough histological methods. Potential intermediate hosts (IH) snails were collected, and laboratory strains of those were subjected to experimental infections. A three year study was performed in a sheep farm, searching for seasonal trends of amphistome infestation, measured by the fecal egg shedding (FES) and its relationship with environment.

Results: samples were classified as Paramphistomum leydeni. By this time, this is the only detected species of this genus. Regarding the IH, natural infection was detected in Lymnaea viatrix. This was confirmed on a laboratory strain of this snail.

An increase of FES has been seen on spring, related to positive soil hydric balance in previous fall, which indicates a higher infection rate from fall to winter.

Conclusions: P.leydeni was reported in Argentina by the first time, and it showed receptive to L.viatrix as IH. However, we can't deny the existence of another species, and also for the IH. Sheep showed marked seasonality in FES, suggesting a yearly trend of infection for template weather and hydric excesses in fall and spring.

We speculate that recent amphistome spreads has been favored by such factors in different country locations, added to the increase of the livestock movement trough provinces.

PO3.11

Eosinophils in the Small Intestine are Negatively Correlated with Faecal Dry Matter in Merino Sheep Challenged with Trichostrongylus colubriformis and Teladorsagia circumcincta

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Introduction: Diarrhoea is a major problem in sheep production as soft faeces adhere to wool, reducing the value of the fleece and pre-disposing animals to flystrike. Diarrhoea is associated with intake of nematode larvae, but sheep that are highly immune to nematodes still suffer from diarrhoea. The purpose of this experiment was to investigate the immune response of sheep that have been selectively bred for resistance to nematode parasites, and whether this immunity could be responsible for diarrhoea. We hypothesised that inflammatory granulocytes in abomasal and small intestinal tissue sections would be negatively correlated with faecal dry

matter (FDM) following an artificial challenge of nematode larvae.

Methods: Forty 20-month-old Merino rams were selected from a flock that has been bred for low worm egg counts for the past 20 years. Rams were housed indoors and challenged daily with 500 T. colubriformis and 500 T. circumcincta infective larvae for six weeks. Faecal samples were collected weekly and FDM determined. After six weeks the rams were euthanased. Tissue samples were taken from the abomasum and duodenum. The average number of eosinophils, mast cells and globule leukocytes per mm2 of tissue were determined.

Results: There was a significant correlation (r -0.42, P<0.001) between eosinophils in the small intestine and average FDM over the course of the experiment. Eosinophils in the abomasum, and mast cells and globule leukocytes in both organs, were not correlated with FDM.

Conclusions: Eosinophils produced in the duodenal mucosa in response to nematode larval challenge are associated with a significant softening of the faeces. Eosinophils are involved in the immune response to parasites, so it is the rejection of larvae that results in diarrhoea and not a large parasite burden. Therefore, breeding sheep for low worm egg counts will not necessarily reduce diarrhoea.

Diagnosis / PCR

Thursday, August, 13, 2009

PO3.12

PCR Detection of *Taenia spp.* in Rodents in Danish Woodlands

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Introduction: Infections with *Taenia spp*. in wildlife develop into hydatid cysts in various tissues of mammal intermediate hosts. The presence of *Taenia spp*. infection in rodents in areas of active public attendance may raise question about risk of spread of similar cestodes e.g. *Echinococcus multilocularis*. Immature Taeniid cysts in rodents are hardly distinguishable morphologically and therefore more reliable and precise diagnostic tools are developed to overcome this problem.

Methods: Rodents were trapped in 8 Danish woodlands. Cysts or white spots in visceral organs were examined morphologically and by PCR amplification of 12S rRNA and *cox1* genes. Phylogenetic trees were produced and compared with previously published trees.

Results: Lesions on internal organs were recognized in 54 rodents. Of these, 32 were identified as *Taenia by PCR, among which only 14 could be diagnosed morphologically. The incidence rate was higher in woodlands more distant to rural areas and in adult rodents trapped in winter and spring. T. mustelae* was exclusively found in Bank voles aggregated in two out of three woodlands while infections with *T. taeniaformi*s were isolated from three rodent species from six woodlands. Sequence variation among *T. mustelae* was low (0-1.2%, n=17). Sequence variation among 13 isolates of *T. taeniaformis* ranged from 0-1.5%, except one isolate which had sequence variations of 19.5% (12S rRNA) and 10.6% (*cox1)*.

Discussion: PCR gave clear identification of Taenia infections in rodents at the species level. Phylogenetic trees produced from 12S rRNA and *cox1 genes gave different topologies, however, it was consistent with previous studies.*

PO3.13

Preliminary Serological Evaluation of Filter Paper-Collected Blood and Serum from Cats and Mice Experimentally Infected with *Toxoplasma gondii*

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The collection and storage of whole blood or serum on a commercially available filter paper (Advantec) for subsequent detection of anti-*Toxoplasma gondii* antibodies in animals was evaluated using a modified agglutination test (MAT). Sera and whole blood samples were collected at pre-and post-inoculation from mice and sera from cats experimentally infected with *T. gondii* oocysts. Aliquots of whole blood and sera were applied to filter paper strips, dried, and stored at room temperature for up to 2 weeks. Paired sera from the same animals were stored at -20C. Samples were eluted from the filter paper and tested by a commercial MAT (Biomerieux). Antibodies were detected in both serum and filter paper-eluted serum samples from four of five infected cats. One feline filter paper serum sample was positive at a 1/160 dilution, but negative at a 1/16,000 dilution. Fresh and filter paper-eluted serum and blood samples from three *T. gondii*-infected mice were also tested. All samples from two mice were positive at all dilutions, and one mouse was positive at 1/160 dilution for filter paper serum and blood samples, but negative for both at the higher dilution of 1/16000. The data set was small, but showed matching antibody positives at low dilutions, regardless of whether samples were whole blood or serum, or whether or not they were previously blotted to filter paper. Filter strips may provide a convenient, simple and stable collection and storage option for whole

blood or serum samples under adverse conditions or in remote areas.

PO3.14

Comparative Evaluation of Enzyme-Linked Immunosorbent Assay and Western Blotting Using Taenia Solium Cystic Fluid Antigen for Serodiagnosis of Neurocysticercosis in Patients Having Active or Calcified Solitary or Multiple Cystic Lesions

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Neurocysticercosis (NCC) caused by Taenia solium metacestodes, is a serious parasitic infection of central nervous system. The brain CT scan findings (n=80) revealed that solitary cystic lesion was dominant (66.3%) over multiple cystic lesions (33.7%) in NCC cases from Assam located in the northeastern region. The sensitivity of ELISA and Western blot analysis using Taenia solium metacestode cystic fluid antigen was 100% in patients with multiple cystic lesions (active & mixed). In patients having solitary active lesions 84.0% tested positive by western blotting in contrast to ELISA which could detect only 48.6% cases. ELISA was not useful for detection of NCC cases having calcified cystic lesions. In case of patients with multiple (calcified) lesions, only 15.4% of cases were found positive with ELISA, whereas 57.1% of cases tested positive in Western Blot. Similarly, patients having calcified solitary cystic lesions only 5.6% tested positive with ELISA and 40% were positive in Western blotting.

PO3.15

Comparison of Two Purification Systems of Recombinant Protein (MSP) Used in Serodiagnosis of Dictyocaulus viviparus Infections in Cattle

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For several years diagnosis of lungworm infection in cattle has been based on various serological methods such as the enzyme-linked immunosorbent assay (ELISA), because of the low sensitivity and time consuming coprological method. It has also been shown that the Major sperm protein (MSP) (also known in D. viviparus as Dv3-14), which is a small and abundant protein occurring only in nematodes, can be used as the capturing antigen in such assays. In the present study, the cDNA coding a recombinant version of MSP was cloned into Escherichia coli BL21(DE3) chemically competent cells. MSP was then expressed in two systems: 1) as a His-tagged protein in pET14b (Novagen) vector, and 2) as a glutathione-S-transferase (GST) fusion protein in pGEX 6P-1 vector (GE Healthcare). Then the recombinant MSP protein was purified by affinity chromatography using HiTrap chelating High Performance columns (GE Healthcare), whereas the fusion protein MSP-GST was purified using affinity chromatography on Glutathione Sepharose High Performance columns (GE Healthcare). Functional tests, both of the pure recombinant MSP protein and with the fusion protein MSP-GST attached, showed that the expressed proteins were recognized by antibodies in sera from infected cattle. The reactivity and stability of both recombinant proteins, which will be used as the capture antigen in an ELISA, will be further analyzed. The long-term goal is to develop an ELISA assay for the routine diagnosis of Dictyocaulus viviparus infection (in cattle and red deer sera).

PO3.16

Development of a Serum Antibody Elisa to Detect Fasciola hepatica Infections in Horses

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Little is known on the prevalence of Fasciola hepatica in horses, or on the association with liver pathology. In ruminants, the liver fluke is associated with a variety of symptoms, ranging from production losses to mortality. One of the main problems to study the prevalence or clinical relevance of F. hepatica in horses is the difficult diagnosis. As most flukes do not reach the mature stage in the horse, faecal examination for excreted eggs is not reliable. A potential alternative might be the detection of serum antibodies against F. hepatica. In the present study, purified F. hepatica excretory-secretory proteins were used to develop a serum antibody detecting Elisa. Serum samples from confirmed positive horses and from neonatal foals were used as positive and negative controls, respectively. Different serum and substrate dilutions were tested, and a serum dilution of 1/400 combined with a substrate dilution of 1/5000 was found to yield a good segregation between positive and negative control samples. Thereafter, serum samples with different gamma-glutamyl-transferase levels were screened using the optimized Elisa, as this parameter is used to indicate bile duct pathology. All samples were run in duplicate and a mean value for optical density (OD) was used to calculate a corrected OD value (ODr), incorporating the OD values for the positive and negative controls. Overall, there was a positive correlation between gamma-glutamyl-transferase levels and ODr values for F. hepatica. A preliminary cut-off was used with the aim of achieving a high specificity, identifying F. hepatica positive horses, with increasing prevalence in the high gamma-glutamyl-transferase samples.

PO3.17

Preservation of Faecal Samples for Nematode Egg Count Determination and Larvae Identification from Horses, Cattle and Sheep

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The preservation of faecal samples for nematode egg count diagnostic may be compromised if longer periods are required from the sampling place/local to the nearest laboratory. The objective of this study was to determine other forms of storage faecal material to proceed with reliable EPG counts compared with refrigeration at 4 C. Fecal samples were collected from the rectal ampoule of horses, cattle and sheep and were separated into five groups: freezer (-18C), refrigerator (4C), room temperature (18C), incubator (28C) and vacuum at room temperature. The analyses were performed at: 0, 12, 24, 48 h and 5, 10, 15, 20 days. Analyses were made in triplicates for EPG test and for larvae identification. For horses the best results were obtained from vacuum and freezer, where the samples were preserved on those conditions for 15 days. There was no statistical differences between the two groups ($P<0.05$). For cattle, the best result obtained was in vacuum until 10 days, with significant difference from the other conditions ($P<0.05$). For sheep the best way for storage was the refrigerator having viable eggs for 20 days. Meantime, the vacuum may also be recommended for 15 day according to statistical data ($P<0.05$). Larvae identification was possible for more than 10 days for all above methods.

PO3.18

Detection of *Neospora caninum in Naturally Infected Urban Rodents from Great São Paulo Area, SP, Brazil*

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Introduction: *Neospora caninum* is a protozoan parasite of animals that until 1988 was misdiagnosed as : *Toxoplasma gondii*. It has been associated as the major cause of abortion in cattle and neurological problems in dogs worldwide. The findings of: *N. caninum* DNA in naturally infected mice and rodents suggest that these animals might be an important source of infection to carnivore hosts.

Methods: In order to determine the occurrence of: *N. caninum* in urban rodents and their role as reservoirs to dogs, DNA was extracted from tissues of 121 rodents (two: *Mus musculus*, seven : *Rattus norvegicu* s and 112 : *Rattus rattu* s) captured in Great São Paulo area (SP, Brazil) from April 2005 to February 2008. Brain samples from all rodents and heart samples from 82 rodents were tested by nested PCR directed at Nc5 target.

Results: All heart samples were negative. Out of the 121 brain samples, 10 turned out to be positive (eight: *Rattus rattu* s and two: *Rattus norvegicus*). These 10 samples were also tested at ITS1 target gene with agreement at 8 samples (six: *Rattus rattus* and two: *Rattus norvegicu* s). Positivity found was 8.3% (10/121) with one or the other target gene and 6.6% (8/121) considering agreement with both target genes.

Conclusions: These results indicates that urban rodents from Great Sao Paulo area could be : *N. caninum* reservoirs to dogs that could feed on their tissues.

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PO3.19

Assemblages of *Giardia* in Ruminants from Some States of the Mexican Altiplano

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Giardiosis is a re-emerging water-borne disease. Prevalence in ruminants varies from 1-100%. The morphological similarities mask genetic variability, thus several techniques have been used to characterize *Giardia*isolates; fragments of the genes encoding glutamate dehydrogenase (GDH) have described seven assemblages: AI, AII and B (zoonotic), C and D (canids), E (ruminants), F (cats), and G (rats). In Mexico there are no reports about the assemblages in ruminants. The aim of this study was to determine the prevalence of *Giardia* and which are the predominant assemblages in calves and sheep from some states of Mexican Altiplano. Fecal samples were collected from 240 sheep and 156 cows, from the Estado de México, Morelos, Hidalgo, Querétaro and Veracruz. Samples were concentrated by flotation, samples with *Giardia* were processed to obtain cysts, and these ones were excysted in vitro and were kept in axenic cultures. DNA was obtained from trophozoites and the amplification of a fragment (432bp) of gdh was carried out, PCR products were restricted by enzymes Nla IV and Rsa I. Prevalence in sheep was 10.4% and in cattle was 5.76%. Five axenic isolates were obtained from each species; additionally DNA from isolates from 3 sheep and 2 goats was tested too. In sheep mix of assemblages E/B was predominant. In cattle all of them had mix of assemblages E/B. In goats the assemblages were mix of E/B and E/B/AI. These results show that ruminants from some regions of México are a source of assemblages zoonotics.

Ectoparasites

Thursday, August, 13, 2009

PO3.20

Bovine Ticks Control Efficiency of Acaricide Samples Collected from a Spray Tank and Oxidative Treatment for Residual Water Decomposition

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The inadequate application of tick baths is responsible for reducing the efficacy of the products. This study investigated the efficacy of samples of a commercial acaricide (15g of cypermethrin, 25g of chlorpyriphos and 1g of citronelal) on engorged Rhipicephalus (Boophilus) microplus females and the efficiency of oxidative treatments on the reduction of the acaricide's active ingredients. Four different amount of acaricide dilution were evaluated, and the wastewater containing the acaricide's residue were submitted to two Fenton's oxidation conditions, without and with mixing in a mixing camera. In other treatment, it was tree hours exposed to sunlight. Distilled water was used as control. Tick females were immersed for five minutes in the solutions, in triplicate, and incubated for subsequent analysis of the biological parameters. The reduction of the active acaricide ingredients were measured by the residual carbon content (RCC) and by GC/MS. The mean efficacy of the acaricide in the treatments varied from 0% (control) up to 72% (addition of the acaricide according to the label insert instructions, 1:800 v/v). The results demonstrate that the water in the tank still had acaricide residues. The presence of Fenton's reagent drastically reduced the RCC, making it a promising waste treatment, with decomposition efficiency of 53% and 79% without and with mixing, respectively. The reduction of the cypermethrin and chlorpyriphos was from 89% up to 98%. Otherwise, these treatments were still lethal to the engorged female ticks probably because of a subproduct reaction.

PO3.21

First Record of Ceratothoa oestroides (Risso, 1826) and Mothocya rosea (Crustacea: isopoda) from Myripristis murdjan, the Pinecone Soldierfish from Red Sea, Egypt

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Cymothoidae are cosmopolitan, ubiquitous and non specific parasite of fish of different biotopes causing a significant economic losses to fisheries either by killing, stunting or damaging their hosts .This study represents the first record of Ceratothoa oestroides and Mothocya rosea from Red Sea, with a new host record (Myripristis murdjan, the Pinecone soldierfish), adding them to the other resident species of the fish–parasitic isopod family Cymothoidae in Egypt. The infestation rate was 81.25% and 18.7% for Ceratothoa oestroides and Mothocya rosea respectively. Ceratothoa oestroides was recovered from the buccal cavity (61.5%) and the branchial cavity (38.5%), while Mothocya rosea was settle in the branchial cavity. No mixed infection was observed in this study. The recorded cymothoid isopods were fully described and illustrated.

PO3.22

Neem (Azadirachta indica): its Potential for Control of African Ticks of Economic Importance

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Ticks & Tick-borne diseases are considered the greatest animal disease problem in Africa. The conventional method of tick control using chemical acaricides is fraught with several problems e.g. environmental pollution, chemical residues in meat, milk products, wool, development of tick resistance and the exorbitant costs. Alternative environmentally-friendly and cost-effective methods of tick control are therefore needed. Derivatives of Neem tree (Azadirachta indica) a tree native to Burma and arid regions of the Indian subcontinent have traditionally been used by farmers in Asia and Africa to control insect pests of household, agricultural, and medical importance. Although Neem derivatives haves been used for centuries for control of arthropod pests, very limited research has been done on its potential for controlling African ticks.

This paper presents results of effects of Neem compounds on three African tick species.

Neem oil smeared on rabbit ears inhibited attachment of larvae, nymphs and adults of Amblyomma variegatum for several days post-application. Fed and unfed larvae, nymphs and adults of A. variegatum exhibited significant mortalities when treated with Neem oil . The mortalities increased with increasing Neem oil concentrations. Neem seed powder mixed with rabbit pellets and then fed to goats at various concentrations significantly reduced attachment of larvae, nymphs and adults of the tick A. variegatum engorging on the goats. It also reduced engorgement weights, fecundity and hatchability of eggs and increased tick mortality, feeding and molting periods. Eggs of the ticks, Rhipicephalus appendiculatus and Boophilus decoloratus treated with Neem oil exhibited significant reductions in hatchability.

Spraying diluted Neem oil on de-ticked Zebu cattle grazing in tick-infested pastures significantly reduced the number of ticks attaching for 4 to 5 days. These results show that Neem products can provide a suitable environmentally-friendly and cost-effective method of managing African ticks.

In conclusion, the effectiveness of Neem compounds against the African tick A. variegatum may be of great economic importance since this tick has spread rapidly beyond the African borders and is now found even in the Caribbeans threatening to invade the USA and Canada.

PO3.23

Fleece Kinetic Disposition of Cypermethrin Applied as Backline Treatment of Sheep at Shearing and its Effect on Melophagus ovinus

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When sheep are treated with pour-on ectoparasiticides, the active agent needs to move throughout the animal, and more importantly towards skin and fleece to be effective. This study examines the disposition on wool of cypermethrin applied as backline treatment. Twenty Merino sheep, 2 years old and naturally infected with M. ovinus were treated at shearing with a backline formulation of cypermethrin 6%. Two grams of wool samples were taken at skin level from each sheep and pooled as from the dorsal mid-line and right mid side ribs. Each sampling was carried out at shearing (day 0) and on days 6, 14, 34, 45, 74 and 101. Procedures for pesticide determination were carried out following CSIRO and Akre & Macneil (2006) methods. Analysis was done by GC-ECD. Ked infestations were assessed on the same days by examination of the right side of the sheep. Most of the residual pesticide on sheep (73 to 94% of the total) was found until day 45 on wool from backline samples only. There was an increase in concentration towards day 21 in both two samples. Concentrations after day 45 were very low (2.85 to 0.17 g/gr). M. ovinus was not eradicated by treatment. A possible explanation is that the concentration found at fleece levels is too low for maximum effectiveness. Additionally, it raises questions of how effectively the target organisms interact with the main mass of pesticide, and finally, whether exposition to sub-lethal doses, could favor the development of resistance.

Epidemiology

Thursday, August, 13, 2009

PO3.24

Current Studies on Neosporosis in European Bison (Bison bonasus bonasus L.) in Poland

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Neospora caninum, an apicomplexan parasite with a worldwide distribution that infects warm-blooded vertebrates, is a causative agent of infertility, fetus mummification and abortion.

The prevalence of antibodies to Neospora caninum was examined in European bison (Bison bonasus bonasus L.) living in enclosure and the wild in Bia owie a National Forest, Poland. Sera of 145 animals collected in 2007-2008, of different ages and sexes, were tested for N. caninum antibodies using an ELISA test. Positive antibody responses were found in 12 bison (prevalence of 8.3 %). Additionally, all positive sera were tested by Western blot to verify the ELISA results and for the first time by two dimensional electrophoresis (2DE) and 2DE-Western blot. The Western blot results confirmed the presence of specific antibodies against N. caninum immunoreactive proteins, in all ELISA positive sera.

Our latest results confirmed previous data (Cabaj et al. 2005) and strongly indicate the presence of N. caninum in the European bison in Poland at least since 1988 to date and suggest that further studies are needed to evaluate the natural occurence of a sylvatic cycle of N. caninum. The negative effect of this infection on the health status and conservation of the European bison is discussed.

This work was supported by grant No. N303 062 32/2263 of the State Committee for Scientific Research, Warsaw, Poland.

Cited literature:

Cabaj W., Moskwa B., Pastusiak K., Gill J. 2005. Antibodies to Neospora caninum in the blood of European bison (Bison bonasus bonasus L.) living in Poland. Veterinary Parasitology, 128, 163-168.

PO3.25

Prevalence of Cryptosporidium in Children Suffering from Gastroenteritis in Ardabil Hospitals

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Background and Objectives: Cryptosporidium is an intracellular – extracytoplasmic parasite that has taken much attention in last 20 years as a clinically important human pathogen.

Cryptosporidial infection can be transmitted from fecally contaminated food or water and

from animal-human or human-human contact. In immunocompromised persons, the illness is much more severe such as debilitation, fatigue, cholera-like diarrhea, severe abdominal cramps, low-grade fever, severe weight loss and Anorexia. Because there was no regional study about cryptosporidiosis in Ardabil, we carried out this survey to determine the prevalence of cryptosporidiosis among the children hospitalized in Ardabil.

Methods: This descriptive and analytical study was carried out on 371 patients in Sabalab and Aliasghar hospitals of Ardabil. A questionnaire was filled for each patient. Stool samples were examined by concentrated formal - ether method and stained with modified Ziehl-Neelson method. The data were analyzed with SPSS (ver 11) using Chisquare test.

Results: We analyzed 371 stool samples from children with diarrhea. Cryptosporidium

oocysts were detected microscopically in 15 samples. Its prevalence was 4.04% in infected patients. 66.7% of the infected ones were at the age of 6 to 24 months, 20% 25-48 months, and 13.3% 49-72 months.

Conclusion: Because cryptosporidiosis was more prevalent at the age of 6-24 months, health education is more necessary for their mothers.

PO3.26

Epidemiological Study of Gastrointestinal Nematodes in Sheep Under Organic and Conventional Production Systems in Canada

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In order to determine the epidemiology of gastrointestinal nematodes on sheep flocks in Canada, a longitudinal study was carried out May 2006–March 2007 on 23 farms in Ontario and 8 farms in Quebec that were purposively selected. Eight were certified organic (CO), 16 were non-certified organic (NCO) and 7 were conventional (C) farms. On each farm, 10 ewes and 10 female lambs were randomly selected. Monthly visits were undertaken during the grazing season, with two visits in winter. At each visit fecal egg counts (FEC) in eggs per gram (epg) were performed on individual animals, and infective larvae were identified from feces and pasture samples. Multiple clinical parameters were also recorded. Summary statistics and a multilevel mixed linear regression analysis using log epg was fit using SAS 9.1. Overall, the mean FECs were 181 and 509 epg for ewes and lambs, respectively, in Ontario, and 351 and 147 epg for ewes and lambs, respectively, in Quebec. The mean egg counts for the different types of farms were 94, 287 and 235 epg for ewes under CO, NCO and C, while for lambs they were 295, 521 and 417 epg, respectively. From coprocultures, the nematode species that predominated was Teladorsagia sp (50%), followed by Haemonchus sp (19%). In the final model, province, month of sampling, temperature, deworming, age, and body condition score ($P<0.05$) explained part of the variation of FEC. There was also a significant interaction between province, age and month of sampling ($P<0.05$), indicating that FECs depended on the level of each of these three variables.

PO3.27

Finding of Litomosoides Carinii (Nematoda filariidae) in Rats (Rattus norvergicus) Caught in Center of DF Mexico

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Litomosiides carinii is a filarid worm with American distribution, which is frequently seen in rodents like Sigmodon, and other species of mice and rats; in Brazil it has been on Sciurus and Neotoma, in Venezuela on Mus, in Argentina on Holochilus and in Costa Rica on Sygmodon hispidus. It stablishes in the toracic cavity, lung and pleura. Thirthy rats (Rattus norvergicus) were captured in a residential unit of Mexico D.F, in August 2008 there were captured and dissected to observed the thoracic cavity which find nematodes, accumulating a total of 30 worms,which were fixed in alcohol to 70%, after dyed with Mayer,s Haemalumbre, dehydrated in alcohol at different grades mounting them in slides and coverslides using synthetic resin, . At the same time humid preparations of blood were prepared and observed in a dark field microscope being determinate as immature stages (microfilarie). From the 20 female worms prepared, the following measurements were obtained the biggest female worm had 4.2 cm in length, the smallest 2.0 cm (X 3.3.cm), the biggest male was 2.0 cm the smallest 1.0 (X 1.4cm) this small filarid worm was previously reported in Mexico by Caballero (1944) on Sigmodon hispidus . Therefore it is concluded that L. carinii is reported in Rattus norvergicus in Mexico

PO3.28

Prevalence of *Eimeria* spp. in Domestic Bovines, Ovines and Caprines on Commercial Farms in South Africa

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Introduction: *Eimeria* spp. are important pathogens affecting domestic bovines, ovines and caprines. Disease is characterised by enteritis, dehydration and/or poor weight gains, causing economic losses of poor production, costs of treatment and/or prophylaxis, and death. Pathogenic species reported are *Eimeria zuernii*, *E. bovis* and *E. aubernensis* in cattle; *E. bakuensis*, *E. ahsata* and *E. ovinoidalis* in sheep; and *E. arloingi*, *E. christenseni*, *E. caprovina* and *E. ninakohlyakimovae* in goats.

Methods: Faecal samples were collected from domestic livestock, 1 – 9 months of age, in 42 districts in South Africa. Samples were refrigerated, transported to independent diagnostic facilities, and enumerated using a Modified McMaster technique.

Results: A total of 77 bovine, 64 ovine, and 22 caprine samples were examined for the presence of oocysts. In bovines *E. zuernii*, *E. bovis*, *E. ellipsoidalis*, *E. cylindrica*, *E. canadensis*, *E. alabamensis*, *E. pellita*, and *E. wyomingensis* were identified. In ovines *E. bakuensis*, *E. ahsata*, *E. ovinoidalis*, *E. crandallis*, *E. parva*, *E. faurei*, *E. intricata*, *E. weybridgensis* and *E. granulosa* were identified. In caprines *E. arloingi*, *E. christenseni*, *E. caprina*, *E. jolchijevi*, *E. alijevi* and *E. hirci* were identified. Twenty nine (37.7%) bovine, 51 (79.7%) ovine and 14 (63.6%) caprine samples were positive for *Eimeria* spp. The prevalence of diarrhoea at the time of sampling in animals with pathogenic *Eimeria* spp. subsequently identified was 90.9% in bovines, 100% in ovines and 100% in caprines.

Conclusion: The presence of pathogenic intestinal *Eimeria* spp. in domestic bovines, ovines and caprines on commercial farms in South Africa was confirmed.

PO3.29

Prevalence in Gastrointestinal Nematode Parasites of Sheep in the Czech Republic

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During 2004 – 2007 the prevalence in gastrointestinal nematode parasites was studied on 14 sheep farms in the Czech Republic. The study was based on the evaluation of the faecal samples collected directly from the rectum of each ewe of the group. In collections the total number of eggs were counted in every animal in 1 gr of its faeces (EPG) with using the McMaster techniques. Prevalence of gastrointestinal nematodes was 25-98 %, however, the infection intensity was typically low to moderate on the most of the farms. None of the farms was negative for nematodes on faecal exam. Parasitological findings on 10 farms were lower than egg number 150/ 1gr of feaces in most of sheep in a group.

Even if the prevalence ranged from 25 to 98 %, infection intensity on majority of farms was low to moderate. Clinical signs were very usually latent.

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Immunology / Vaccines

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PO3.30

Cytokines Mrna Levels in Brazilian Somali Sheep Resistant and Susceptible to *Haemonchus* spp. Infection

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Cytokines are known to play a central hole in immune mechanisms involved in defense against gastrointestinal nematodes infections. The present study used Real-time RT-PCR to quantify cytokines (IL-4, IL-13, TNF-alpha and IFN-gamma) in two groups of Brazilian Somali sheep: one resistant and other susceptible to *Haemonchus* spp. infection. From a Somali sheep herd, 75 young animals were kept together on pasture without anthelmintic treatment for 4 months. Based on mean fecal egg counts, the eight most resistant and the eight most susceptible animals were slaughtered for parasite recovery and collection of abomasum tissue samples. Real-time RT-PCR was performed using the LightCycler PCR and SYBR Green I dye. RPL-0 (ribosomal protein L-0) was used for normalization and the relative quantification of genes was calculated by REST software. Resistant animals had 9 fold less *Haemonchus* spp. than susceptible ($P<0.05$). TNF-alpha and IFN-gamma were both up-regulated in susceptible animals ($P<0.03$). The other two genes analyzed had the same expression pattern in both groups ($P>0.05$). Higher TNF-alpha can be associated with both TH1 and TH2 response. However, higher IFN-gamma and lack of IL-4 and IL-13 levels indicates a TH1 response in susceptible animals. TH1 cytokines appear

to play an important role in parasite susceptibility in Brazilian Somali Sheep leading to chronic infection. Study funded by FUNCAP, CNPq and Embrapa Caprinos e Ovinos.

PO3.31

Implication of Hydatic Antigens on Cytokines and NO Production in Human hydatidosis

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Human echinococcosis is caused by infection with the larval stage of the cestode Echinococcus granulosus. It constitutes a serious public health problem in various parts of the world, particularly in Algeria. It seems that the variability and severity of the clinical expression of this parasitosis are related to the variety of human immunological responses to the several antigens. The study of these antigens with their multiple immunological effects will be useful in designing strategies to develop early immunodiagnosis of hydatid disease. It could also open new perspectives in the understanding of the pathogenesis of this disease. The aim of our work is to study the immunological response associated with human hydatidosis by evaluating the effect of hydatic antigens on cytokines and NO production. We investigated IL-12, IL-8, IFN- and NO production by mononuclear cells of hydatic patients stimulated with antigens of hydatic cyst (cystic fluid, purified fractions, extracts of protoscoleces, laminated layer and germinal layer). Our results show that all hydatic antigens; excepted laminated layer extract; increase cytokines and NO production in vitro. Our findings underline a strong host-parasite interaction. All these findings have important implications in the diagnosis of human hydatidosis. Moreover, this study highlights the role of parasite laminated layer in regulating the host immune responses against Echinococcus granulosus. Inhibition of these mechanisms seems to be important issue to address during the design of anti-hydatic treatment.

PO3.32

Immunological Aspects of Heterophyidae Infection in Laboratory Animals

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Heterophyiasis is an important food-borne parasitic zoonosis in Egypt, among inhabitants living arround brackish-water lakes especially fisher men and it is a common human parasite in Nile Delta

whole blood was collected with heparin or EDTA as anticoagulant to help in the haematological studies such as (Red Blood Cells conut) RBCs, WBCs (White Blood Cells Count), PCV (Packed cell volume) and Hb (Haemoglobin). Only, marked increase in the total leuckocytic count was recorded, while RBCs, PCV and Hb. were decreased in most results obtained . Total protein and globulin decreased while albumin and A/G ratio increased.

Liver enzymes showing marked increase in AST (aspartate aminotransferase) and increase in ALT (alanine aminotransferase) in dogs and rats denoting that, liver has a role in the response to that infection.

Kidney function tests urea and creatinine showed slight increase at 6 (days post infection) d.p.i.

After preparation of different (antigen) Ag from different collected helminthes, the protein content of each was determined.

The sera of infected animals were collected to find antibodies in their blood against the parasite using ELISA (Enzyme Linked Immuno Sorbent Assay) and using crude Heterophyid antigen collected from their intestines after scarification. The worms washed, homogenized and then centrifuged to collect supernatant fluid as antigens. Indicated that (antibody) Ab start to appear at 9 d.p.i. and increase till 21& 28 d.p.i. and the detection of depend on antigen concentration.

PO3.33

Mouse Transcriptomics-Based Deduction of in vivo Enteric Immune Response to Infection by an Obligate Intracellular Parasite Eimeria falciformis

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Objectives: Intracellular protozoan parasites of genus Eimeria are extremely host- and cell-specific pathogens. Different Eimeria species (~1000) infect their distinct hosts, and parasite infection is confined exclusively to the intestinal epithelium. Our aim is to characterize the host factors that might determine the development and specificity of E. falciformis in a mouse model.

Methods: We have established parasite oocyst infection in a murine model in addition to in vitro infection of mouse cell lines with sporozoites. This research involves in vivo gene expression analyses of Eimeria-infected mouse caecum using whole-genome microarray and in vitro parasite culture.

Results: In vivo infection of mouse with E. falciformis leads to the modulation of about 5400 host genes within 5 days of infection. The cluster analysis indicates these genes to be involved in multiple biological processes including cell adhesion, lipid metabolism, transcription, translation, signal transduction and immune response. Our transcriptomics

data also reveal the substantial induction of IFN -dependent immunity upon natural infection with E. falciformis. These data indicate the recruitment of selected immune cells to the site of infection i.e. caecum and their systematic interaction. In accordance to in vivo data, the cell lines lacking their IFN signalling are better compliant to parasite growth, whereas the standard mouse cell lines are permissive to infection but allow minimal parasite development into its advanced stages. Taken together, these results underscore the influence of immune signalling in allowing and/or sustaining the intracellular development of Eimeria. Moreover, we also demonstrate how a transcriptomics-based approach can be employed to deduce the global host immune response to an intestinal parasite infection.

Conclusion: Our data suggest the host immune response as an important determinant of the development of E. falciformis. Currently, we are establishing the interaction of divergent immune signalling to deduce their biological relevance for in vivo replication of Eimeria in context with other similar intracellular pathogens.

PO3.34

Fasciola hepatica Tegumental Antigen Suppresses Dendritic Cell Maturation and Function

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Parasitic worms and their derived molecules have powerful anti-inflammatory properties and are shown to have therapeutic effects on inflammatory diseases. The helminth, *Fasciola hepatica*, has been reported to suppress antigen-specific Th1 responses in concurrent bacterial infections thus demonstrating its anti-inflammatory ability. Here, *F. hepatica* tegumental antigen (FhTeg) was shown to significantly suppress serum levels of IFN- and IL-12p70 in a model of septic shock. Since dendritic cells (DCs) are a good source of IL-12p70 and critical in driving adaptive immunity we investigated the effect of FhTeg on the activation and function of murine DCs. While FhTeg alone did not induce maturation of DCs, it significantly suppressed cytokine production (IL-12p70, IL-6, IL-10, TNF- and nitrite) and cell surface marker expression (CD80, CD86 and CD40) in DCs maturated with a range of TLR and non-TLR ligands. We have shown that FhTeg works independently of the TLR4 pathway since it still functioned in DCs generated from TLR4 mutant and knockout mice. It also impaired DC function by inhibiting their phagocytic capacity and ability to prime T-cells. It does not appear to target the common components (ERK, JNK or p38) of the TLR pathways; however, it suppressed the active p65 subunit of the transcription factor NF- B in mature DCs which could explain the impairment of pro-inflammatory cytokine production. Overall, our results demonstrate the potent anti-inflammatory properties of FhTeg and its therapeutic potential as an anti-inflammatory agent.

PO3.35

Primary and Secondary Responses of Young Lambs to Trichostrongylus colubriformis Experimental Infections: Kinetics and Individual Variations

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Introduction and Objectives: The adaptive immune response was well characterized in adult sheep selected for enhanced resistance to Trichostronglylus colubriformis but data concerning young and random-bred lambs are scarce. The objectives of this study were i) to define the cytokine genes expression by RT-PCR, ii) to measure the mucosal antibody and cellular responses, iii) to measure the effects of this immune response on the nematode populations during a primary and a secondary T. colubriformis experimental infections. Seventy nine 3 month old Romane lambs, divided into three groups (primary infection, secondary infection and control), were used.

Results: A decrease of the faecal egg counts was observed in the secondary infection. Il-4, Il-13, IFN, Il-12, TNF genes expressions were not significantly different between the three groups, however, high Il-5 gene expressions were noted in some (but not all) individuals during the secondary infection. Antibody responses (IgA and IgG), eosinophil, mast cell and globule leucocytes recruitments in the duodenal mucosa were low during the first exposure and increased gradually during the second one. Strong negative correlations were shown between egg excretions and antibody and cellular recruitment in the duodenal mucosa.

Conclusion: A weak immune response was reported during the primary T. colubriformis infection in young lambs. During the second exposition to this parasite, high individual variations were reported.

PO3.36

Local Cytokine Response During the Early Stages of Infestation by Hypoderma lineatum (Diptera:oestridae)

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Local cytokine responses were studied in three groups of cattle (n=4) experimentally infested with H. lineatum first instars (L1) placed on the skin: G-1 undergoing a primary infestation, G-2 undergoing a secondary infestation and G-3 infested for their third consecutive time. Cattle were infested with 25 first instars deposited on the skin. Skin samples were taken at 0, 6, 12, 48, 96 and 144 hours post infestation (h.p.i.). Interleukin 10 (IL-10), IL-4 and interferon gamma (IFN-) production was studied by immunohistochemistry. IL-4+ cells showed a significant increase at 6 h.p.i. in both reinfested groups (G-2 and G-3) when compared with G-1. In all groups the number of IL-4+ cells decreased significantly at 48 h.p.i.. IL-10+ cells increased in G-1 at 6 and 48 h.p.i., whereas in both reinfested groups increased at 12 h.p.i. with a peak at 48 h.p.i. IFN- + cells showed a significant increment at 6 h.p.i. in all groups, followed by a rapid descent at 12 (G-1 and G-2) and 48 h.p.i. (G-3). Penetration of the skin by H. lineatum did not have any significant effect on IFN- serum concentrations and, except for IL-10 there were no correlation between local production and serum concentrations of cytokines. The increase of both Th1 (IFN-) and Th2-type cytokines (IL-4 and IL-10) indicates that bovine T-cell response during the first phases of the infestation by H. lineatum is apparently a Th0 response.

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PO3.37

Cellular Proliferation and Invasion Efficiency of Different *Neospora caninum* Isolates

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Neospora caninum is a cyst-forming parasite that has been recognised worldwide as a cause of abortion in cattle. Biological characterisation of *N. caninum* isolates has shown significant differences in their capacity to induce pathology and vertical transmission in mice. This broad range of *N. caninum* virulence may be related to the isolate behaviour in the host, including the efficiency of dissemination and the ability to reach high parasite burdens in target organs. Such variations could be the result of differences in doubling time (Td) or invasion rate (IR) into host cells, enhancing resistance to the host immune response. We have examined the *in vitro* behaviour of the Nc-Liv and nine Spanish *N. caninum* isolates, analysing their invasion and proliferation rates. The IR was determined using a red/green immunostaining probe and counting the external and internal parasites with a laser scanning citometer. The IR was the number of tachyzoites that completed the internalisation into the MARC-145 cells at 2, 4, and 6 hours after their inoculation onto the cell-monolayer (P.I.). Proliferation rate was performed using a real time PCR to quantify the tachyzoites at 4, 8, 20, 32, 44, 56 and 68 hours P.I. The Td was determined from exponential intracellular growth period for each isolate. Significant differences in IR were detected at 2 and 4 hours P.I. ($P<0.0001$; Kruskal-Wallis test) and the Td varied from 11 to 15 hours among *N. caninum* isolates ($P<0.04$; Kruskal-Wallis test). These results may help us to explain the differences in pathogenicity exhibited by these isolates.

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PO3.38

Evaluation of the Proinflammatory Cytokines TNF-alpha and IFN in Cattle Naturally Exposed to Warble Flies (Hypoderma sp.)

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Sixteen Rubia gallega beef cattle were bled at monthly intervals, through one entire life cycle of Hypoderma sp. (May 2007-May 2008), in order to evaluate the systemic production of the proinflamatory cytokines TNF-alpha and IFN-gamma. Animals were divided into three groups: The first group was integrated by 4 uninfested calves (G1), G2 composed by 7 primoinfested heifers and G3 integrated by 5 reinfested cows. The infestation of G2 and G3 was confirmed by means of indirect and sandwich ELISA before the start of the study. Cytokines were detected by capture ELISA. The number of warbles on the back of G1 and G2 were recorded in order to correlate with cytokine levels.

The evolution of both cytokines was very similar for the three groups of animals. The differences between uninfested and infested groups (G2 and G3), were only significant for the TNF-alpha, coinciding with the beginning of larval migration into the host. The infestation by Hypoderma did not have any significant effect on IFN-gamma serum concentrations, suggesting that peripheral blood immune response did not reflect accurately local responses occurring during the life cycle of Hypoderma.

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Parasite Genomic / Proteomics

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PO3.39

Epizootiology, Phylogeography and Co-Phylogeny of the French Heartworm, Angiostrongylus vasorum, and its Hosts in North America and Europe

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Canine pulmonary angiostrongylosis is a snail-borne disease that causes cardio-respiratory distress in dogs and other canids. The causative agent is Angiostrongylus vasorum (French Heartworm) a nematode in the Metastrongyloidea surperfamily that parasitizes the right ventricle and pulmonary arteries of dogs and wild carnivores. The distribution of A. vasorum is worldwide, but patchy, with hyper-endemic foci surrounded by areas where it is rarely found. Insular Newfoundland represents the only endemic focus of this parasite in North America. When and how A. vasorum was introduced to Newfoundland remains a mystery and the spread of this parasite from Newfoundland to Atlantic Canada is of great concern.

The number of molecular studies on A. vasorum is limited. This study will use high-resolution microsatellite markers to investigate the extent of differentiation between A. vasorum from sampled areas. Various loci within the nuclear DNA and mitochondrial DNA (mtDNA) will be characterized for A. vasorum isolates collected from Newfoundland and other geographical areas for comparing the genetic make up of the parasite worldwide and to illustrate the spread of A. vasorum throughout Europe and Eastern Canada.

Microsatellite markers are also being used to investigate the differentiation between insular Newfoundland red fox populations and those from mainland populations (i.e. Labrador and the Atlantic provinces). In addition, sequence data from a portion of the mtDNA control region and cytochrome b (cyt b) gene will be used to obtain information on the phylogeographical patterns of the red fox across its Newfoundland range. Phylogeography of both red fox populations and A. vasorum infrapopulations will be compared to infer possible co-phylogenetic relationships.

PO3.40

Identity and Phylogeny of Diplostomum Fish Parasites of the Catfish (Clarias gariepinus) in Tanzanian Freshwaters Based on Sequence Divergence on the ITS1–5.8S–ITS2 rDNA Region

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A study was conducted on fish parasites of genera Diplostomum and Tylodelphys, which are among the more economically important fish parasites in Tanzanian fish husbandry. The objective was to delineate and assess phylogenetic affinities of Diplostomid metacercariae co-existing in the brain cavity of the catfish, Clarias gariepinus collected from Ruvu, Msimbazi and Kilombero rivers, Mindu dam and Lake Victoria. A molecular genetic approach was used in which DNA was extracted from three diplostomid species namely Tylodelphys sp 1, Tylodelphys sp 2 and Diplostomum mashonense. A DNA fragment containing complete ITS1–5.8S–ITS2 region was amplified and sequenced. Analysis of sequence results revealed similar parasite species within Tanzania freshwaters despite morphological and geographical variation. Furthermore, Tanzanian Tylodephys bore strong genetic affinity with a European Tylodelphys sp (94% homology), although the clade of the African Tylodelphys was found to have evolved later than the European and North American Diplostomum species (99% bootstrap support). The results are presented as an effort in understanding and management of parasites in Tanzania fish husbandry.

PO3.41

Stage-Specific Expression of Six Calcium-Dependent Protein Kinases in Cryptosporidium parvum in vitro

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Cryptosporidium spp. are apicomplexan pathogens with zoonotical impact causing intestinal or even respiratory infections in a wide range of hosts.

Cryptosporidium parvumm possesses seven calcium-dependent protein kinases (CDPKs) and four calmodulin domain related protein kinases (CRKs). Such kinases have previously been described in plants, algae and other apicomplexa. Based on phylogenetical and structural analysis, six kinases with classical domain structure were chosen to be analysed for stage-specific expression. The sequences annotated as cgd3_920, cgd5_820, cgd4_3330, cgd2_1300, cgd2_1060 and cgd7_1840 were recovered from CryptoDB.org and were detected by means of nested 3prime RACE-PCR.

A specific antibody was generated for cgd3_920 by immunisation of rabbits. Human ileocecal adenocarcinoma cells

(HCT-8) were infected with sporozoites from 4 x 105 freshly excysted oocysts of *C. parvum* in 6 well plates. The detection of the transcripts was carried out on 3 h, 21h, 27 h, 43 h and 51 h post infection. Only cgd4_3330 was detected at all points in time, while the transcripts of CDPKs were found at 21 h, 43 h and 51 h post infection. In the immunoblot the anti cgd3_920 specific antibody showed strong reaction with an antigen at 56 kDa in protein extracts of excysted oocysts, which corresponds to the calculated molecular weight of cgd3_920 of 55.72 kDa, but presented no reaction with antigen extracts of infected HCT-8 cells.

CDPKs coded by cgd3_920, cgd5_820, cgd4_3330, cgd2_1300, cgd2_1060 and cgd7_1840 of *C. parvum* are transcribed in infected HCT-8 cells and CDPK coded by cgd3_920 is translated in sporozoites but not in infected HCT-8 cells at 48 h.

PO3.42

Identification of Genomic Heterogeneity of Giardia duodenalis Isolates from Human and Animal Reservoirs by Using PCR-RFLP of Glutamate Dehydrogenase (gdh) Coding Gene from the Tabriz

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Background and Objective: Human giardiasis, caused by the intestinal flagellate Giardia duodenalis, is considered a zoonotic infection. Although the role of animals in the transmission to humans is still unclear. This study proposal is identification of genomic heterogeneity of Giardia duodenalis isolates from human and animal.

Materials and Methods: A PCR-RFLP genotyping tool used to characterize morphologically identical of Giardia duodenalis isolates from a variety of host species. A 432 bp fragment of the glutamate dehydrogenese gene (gdh) was amplified and sequenced. For discriminating of the strains, restriction enzyme profiles were determined for each of the assemblages with two enzymes Nla IV and Rsa I.

Results: Analysis of the amplified sequences for these samples revealed a matching with GenBank reference sequences. The pattern of RFLP differences in Giardia duodenalis isolates in the Tabriz city discriminated assemblages A-I, A-II, B-III, B-IV of the human isolates, and A-I from cat isolate.

Conclusion: The molecular identification of assemblage A-I of Giardia duodenalis isolate from cat reveals that this genetic subgroup represents greatest public health risk potential zoonotic transmission in the municipality of Tabriz. Also, genetic subgroups identified from human reveals that genetic diversity of this protozoon in Iran is similar to that of Giardia from other parts of the world.

PO3.43

Assessing *Haemonchus contortus* Gene Function Using RNA Interference in Transgenic *Caenorhabditis elegans*.

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RNA interference (RNAi) is a powerful method to assess gene function. It has been used very successfully in the free-living nematode, *Caenorhabditis elegans*. The effectiveness of this tool in parasitic species such as *Haemonchus contortus*, however, remains to be thoroughly investigated. We are using transgenic *C. elegans* expressing *H. contortus* proteins to assess the ability of the parasite protein to replace the function of the endogenous homologue that has been silenced by RNAi. The system described allows the rapid generation of transgenic animals and the easy assessment of protein expression as the transgenes are fused to GFP.

PO3.44

Genomic and Bioinformatic Characterisation of an ADP/ATP Translocase of Haemonchus contortus

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A complete cDNA (Hc-ant-1) encoding an ADP/ATP translocator (Hc-ANT-1) was isolated from parasitic nematode Haemonchus contortus. This cDNA encodes a protein of 297 amino acids containing characteristic motifs [RRRMMM and PX(D,E)XX(K,R)]. Comparison with homologues from a range of invertebrates and vertebrates revealed relative conservation, with amino acid sequence identity highest in and flanking RRRMMM and in the six hydrophobic transmembrane regions. Phylogenetic analyses of selected, full-length amino acid sequence data showed that Hc-ANT-1 was most closely related to Tv-ANT-1 from the related nematode Trichostrongylus vitrinus and ANT-1.1 from Caenorhabditis elegans. The genomic structure of the full-length Hc-ant-1 gene was most similar to that of Tv-ant-1. Analysis of the region (5'-UTR) upstream of Hc-ant-1 predicted a number of promoter elements, such as CAAT, E-box and GATA transcription factor elements. Transcriptional analysis by real-time PCR showed that Hc-ant-1 was transcribed at a 70-fold higher level in female adults of H. contortus compared with males. Using C. elegans as a surrogate system, temporal and spatial transcription profiles of Hc-ant-1::GFP were similar to those of Ce-ant1.1:GFP; GFP expression was detected predominantly in pharynx, body wall and muscle cells, rectum muscle, hypodermal cells and intestinal cells. RNA interference, conducted by feeding C. elegans with dsRNA from Hc-ant-1 cDNA showed no

gene silencing, despite high nucleotide identities between Hc-ant-1 and Ce-ant1.1. These first insights into ANT-1 of H. contortus provide a foundation for exploring its functional role in this socioeconomically important nematode.

PO3.45

The Single Sporozoite of *Cryptosporidium andersoni* Contains Multiple Copies of 18S rRNA Gene Having Different Genotypes

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Cryptosporida are intracellular protozoan parasites that mainly infect the gastrointestinal tract of a wide range of vertebrates including livestock and humans. The oocyst of the parasites, the stage outside of the hosts, contains four of haploid sporozoites. We observed that *Cryptosporidium andersoni* oocysts isolated from cattle in the northern part of Japan showed two genotypes, i.e. type A that is identical to *C. andersoni* reported previously and type B that has one thymine nucleotide insertion at the part of 609-613 in the 18S rRNA gene of type A. Additionally, we detected these two distinct genotypes within single oocyst. Then, we isolated four single oocysts under the microscope to construct the clone libraries of the 18S rRNA gene of each oocyst, and sequenced five clones per oocyst. As a result, each clone library from four single oocysts showed both types A and B. Subsequently, we tried to analyze single sporozoites. We isolated 24 single sporozoites, and we successfully PCR-amplified two of them. We sequenced 24 clones per each single sporozoite. Although one sporozoite showed only type B, the other one showed type A and B. These results indicated that one single sporozoite could contain both type A and type B 18S rRNA gene. Accordingly, we concluded that the sporozoite of *C. andersoni* had multiple copies of 18S rRNA gene that have different genotypes such as type A and B.

PO3.46

Identification of Putative Emodepside Receptors in Ascarids

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The identification of new mode of action of anthelmintic substances is of great interest for example for the potential identification of new drugs against parasitic nematodes. In the beginning of the 1990s the anthelmintic active substance PF1022A was identified and led to the design of a new anthelmintic drug, Emodepside. Its efficacy against anthelmintic resistant nematode populations has been demonstrated. However, its mode of action is not completely understood yet. The calcium-activated potassium channel SLO-1 has been identified as a putative Emodepside receptor. In Caenorhabditis elegans SLO-1 plays an important role in the mode of action of Emodepside. It has already been demonstrated, that Emodepside is effective against ascarids and that SLO-1 is involved in the anthelmintic effects of Emodepside. Thus, in this study the complete slo-1 cDNA sequence information from Toxocara canis, Ascaris suum and Parascaris equorum were described and subjected to bioinformatical and phylogenetic analysis. Coding DNA sequences ranged between 3300 and 3400 bp in size and amino acid sequence alignments comparing SLO-1 of the three species revealed matches of approximately 90%. Different splicing variants were identified.

Amino acid similarities of the ascarids and C. elegans SLO-1 sequences range from 77% to 81% respectively and the similarity to SLO-1 from Homo sapiens is approximately 50%. Altogether the slo-1 gene family reveals a high level of similarity.

PO3.47

Diversity of *Theileria parva* in South Africa as Shown by PCR-RFLP and Sequence Analysis of the Genes Coding for p104 and the Polymorphic Immunodominant Molecule (PIM)

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Restriction fragment length polymorphism (RFLP) profiles and sequence analysis of the variable region of the genes coding for the p104 and polymorphic immunodominant molecule (PIM) proteins were used to characterize *Theileria parva* parasites that occur in South African buffalo, in cattle from farms with suspected theileriosis and in experimentally infected cattle. Buffalo-derived *T. parva* p104 and PIM RFLP profiles were obtained from most *T. parva* samples collected from buffalo and from some of the cattle samples. Relatively homogeneous PIM profiles were observed from samples originating from buffalo from three game parks, suggesting the presence of dominant genetic variants in *T. parva* parasites in these relatively small isolated buffalo populations. p104 and PIM RFLP profiles and gene sequences similar to that of Muguga, a Kenyan *T. parva* stock that causes East Coast Fever (ECF), were obtained from three T. parva positive cattle samples, although these animals were not diagnosed with

classical ECF. Less polymorphic and atypical PIM and p104 profiles were obtained from some buffalo and cattle samples but these were not similar to known cattle-derived profiles. PIM sequences obtained from clones of these included buffalo-derived (88%) and recombinant (12%) sequences. Three previously reported p104 alleles were identified in this study; however new variants were also obtained suggesting extensive genetic diversity in the South African *T. parva* population. This study has shown that PIM and p104 profiles are more complex than previously thought and the differentiation of *T. parva* parasites in South Africa based on these profiles would be very difficult.

PO3.48

Characterization of Multiple Acetylcholinesterases and Relationship to Organophosphate Resistance in the Southern Cattle Tick, *Rhipicephalus (Boophilus) microplus*

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Three cDNAs have been reported to encode acetylcholinesterase (AChE) in *Rhipicephalus microplus* and were expressed in the baculovirus system. Each of the recombinant proteins exhibited different kinetic properties but shared general biochemical characteristics of AChE including substrate preference for acetylthiocholine over butyrylthiocholine and inhibition by eserine or organophosphate. Recombinant forms of BmAChE1 and BmAChE3 containing specific mutations isolated from organophosphate (OP) resistant strains of *R. microplus* exhibited decreased sensitivity to OP inhibition. All three *R. microplus* AChEs were expressed in synganglion as indicated by qRT-PCR. Multiple forms of each of the *BmAChE* transcripts were sequenced from individual ticks suggesting alternative splicing of transcripts or the presence of more than two alleles (gene duplication) for each transcript. Gene copy number was investigated by quantitative real time PCR using genomic DNA. Investigation of the physiological roles of the three AChEs was investigated by RNA interference. Long dsRNA specific for each of the three *R. microplus* AChE cDNAs, *BmAChE1*, *BmAChE2*, and *BmAChE3* was introduced by microinjection of unfed adult females and subsequent gene silencing effects were monitored by qRT-PCR and phenotypic effects.

Trichinella

Thursday, August, 13, 2009

PO3.49

Preliminary Characterization of Excretory-Secretory Protein Extracts of Trichinella spiralis and Trichinella britovi Muscle-Stage Larvae by Two-Dimensional Electrophoresis

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Trichinella spiralis and Trichinella britovi are intracelullar nematode parasites of mammalian skeletal muscles.

Trichinella species exhibiting different epidemiological features occur in Europe and, two of them, T. spiralis and T. britovi, have been identified so far in Poland.

The objective of our study was to describe the differences between excretory-secretory protein extracts of muscle larvae of two encapsulated species T. spiralis and T. britovi by two-dimensional electrophoresis (2DE).

The protein patterns of T. spiralis and T. britovi, differed remarkably in silver stained 2DE gels. The differences were most evident in the low-molecular weight area.

Additionally, antigenic properties of T. spiralis separated proteins were analyzed with two-dimensional Western Blot using sera from experimentally Trichinella spp. infected pigs. Immunoreactive spots were detected using rabbit anti-pig IgG conjugated with horseradish peroxidase. This preliminary study revealed several protein spots which reacted with specific antibodies. The antigenic patterns of crude extracts of muscle L1 larvae differ also in two-dimensional Western Blot.

Futures studies should concentrate on confirming the proteomic differences between the two species: T. spiralis and T. britovi, such antigenic differences could provide a basis for future serological differentiation of Trichinella species. At the moment it is not possible to recognize the species serologically.

PO3.50

Muscle Distribution of Domestic Trichinella Larvae in Natural Infected Pigs

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Several studies have described the muscle distribution of sylvatic and domestic Trichinella larvae in experimental infection in production animals and wildlife. The present study aimed to determine the muscle distribution of domestic Trichinella larvae in natural infected pigs. Forty muscle samples were collected from each of the ten natural infected pigs (6-months old to 2-years old), identified previously by Trichinella ELISA (SafePath Laboratories, USA) in a Trichinella endemic farm. Four hundred individual artificial digestions were performed on samples varying from 5 to 100 grams, with a mean time for digestion of 45 minutes. The larval burden for diaphragm, as the reference muscle, was varying from 0,01 larvae to 6.3 larvae per gram. Independently of the degree of infection, the digastric muscle and levator labii superiore muscle appeared to harbor the highest number of muscle larvae, with a worm burden of 1.2 to 21.8 LPG and 0.9 to 7.4 LPG respectively. Furthermore, among the first five predilection sites we found the tongue apex, the diaphragm and the pectineus muscle. Work founded by the Romanian Education Ministry thru the "PNII" projects, contract PNII-RU-RP-11/01.10.2007.

PO3.51

qPCR for detection of *Trichinella spp*. in potential Australian wildlife hosts

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No indigenous cases of human or animal trichinellosis have ever been recorded on mainland Australia, however the absence of infection does not necessarily constitute freedom due to cultural practices of eating well-cooked pork as well as the independent circulation of *Trichinella* in wildlife. Because there is a lack of extensive epidemiological investigations in animals within mainland Australia, a truly *Trichinella*-free status has not yet been verified. Therefore, wildlife surveys conducted with sensitive and specific diagnostic methods will be valuable in verifying the region's true *Trichinella* status. Field diagnostics for *Trichinella* detection in wildlife currently rely on the artificial digestion (AD) method which is the gold standard for routine inspection of meat for human consumption. However, the level of sensitivity attained by AD may be insufficient to detect low level infections in wildlife, while use of immunobiological methods such as ELISAs appear to improve sensitivity, at the cost of specificity. Molecular methods have been used until now primarily for taxonomy, although recently Guenther *et al*. (2008), developed a quantitative PCR (qPCR) for the detection of *Trichinella* species found in Europe for meat inspection purposes. Our study sought to assess the usefulness of qPCR as a diagnostic tool in wildlife surveys in Australia, using universal primers targeting the mitochondrial rRNA gene *rrnL* to detect all known species of *Trichinella*, as well as species-specific primers designed for the non-encapsulated species *T. pseudospiralis* and *T. papuae*, which are most likely to be transmitted into mainland Australia. We test the sensitivity and efficacy of the mincing protocol and qPCR to detect *Trichinella* larvae, by simulating low level infections in tissues of potential Australian hosts including fox, dog, crocodile and wild boar.

PO3.52

Serological Detection of Anti-Trichinella Antibodies in Wild Red Foxes (Vulpes vulpes) in Norway Using E/S Antigen and -Tyvelose Glycan Antigen in an Indirect ELISA

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Trichinella surveillance in wildlife has relied on the detection of muscle larvae using digestion techniques. Serology has been proposed as more suitable for large-scale epidemiological studies in wildlife. Sera from 328 wild red foxes (Vulpes vulpes) were tested with both excretory/secretory antigen (E/S) and synthetic -tyvelose glycan antigen, in indirect ELISA tests. The red foxes were also examined for muscle larvae, using individual muscle digestion (magnetic stirrer method) on 10g of quadriceps muscle. Western blot (WB) was carried out on all seropositive samples using crude larval antigen. Both -tyvelose and E/S antigen proved suitable for the detection of antibodies to Trichinella spp. in foxes. Both ELISA antigens performed well, although, the E/S antigen was superior to the -tyvelose antigen, with sera that had been stored at -20°C for more than 10 years. Neither antigen detected all of the samples proven seropositive by WB, and in total 24 of the 27 positive WB sera were identified using both antigens. Serology alone, without WB or muscle digestion, led to a two to three-fold higher seroprevalence estimate respectively. In total, 7.3% (24/328) of the wild red fox population had antibodies to Trichinella on ELISA and WB. Antibodies were identified in red foxes from a further two regions in Norway compared to muscle digestion results. The use of E/S antigen in conjunction with the WB was the

method of choice for the screening of wild red fox populations for Trichinella.

PO3.53

Wild Boar Distribution in Areas with *Trichinella* Findings in Red Foxes – a Risk for Game Meat Consumption?

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The combination of the omnivorous life style of wild boar with the frequent consumption of raw or undercooked (and often uninspected) wild boar meat represents a considerable risk for human *Trichinella* infections. Surveys on the endoparasites of foxes in Austria from 1989 to 2003 revealed *Trichinella*-positive animals in 28 of the 121 districts of Austria. Wild boars can be found in 100 districts, and a total of 18 districts are now known for the simultaneous occurrence of *Trichinella*, foxes and wild boars. An evaluation of different time periods (1989-1993 and 1999-2003) showed that due to the larger range of the wild boar population the overlapping areas expanded in recent times, probably also increasing the risk of parasite transmission from foxes to wild boars. This may cause a transition from the purely domestic life cycle of *Trichinella* in Austria to an intermediate cycle, possibly involving human consumers of wild boar meat. Care has to be taken to implement and maintain appropriate control measures for game meat inspection.

PO3.54

Prevalence and Infectivity to Experimental Rodents of *Trichinella* sp. Obtained from Japanese Black Bears in Iwate Prefecture, Japan

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The bear has been known to be a unique wildlife that causes trichinellosis in humans in Japan. The present study was carried out to clarify the prevalence of *Trichinella* nematodes in Japanese black bears (*Ursus thibetanus*) and the infectivity of the nematoda to experimental rodents. We tested 116 muscle samples from Japanese black bears in Iwate Prefecture between1998 and 2006. The samples were digested with the solution containing 1% hydrochloric acid and 0.5% pepsin and observed for the larvae. As the results, *Trichinella* larvae were detected in 2 muscle samples (1.4%). In order to identify the species of the larvae, the DNA fragments of mitochondrial *cox*I gene were amplified by using genomic DNA extracted from the larvae and sequenced directly. The sequences of the fragments showed the similarity of 98.8% with that (AF129487) of *Trichinella* T9 from a black bear. The *Trichinella* larvae were administered to BALB/c mice and Mongolian gerbils in the dose of 300. The animals were sacrificed on the days of 5, 14, 21, 30 and 40 post-infection and examined for the adults in the small intestines and the larvae in muscles. As the results, the recovery rates of the adults were 18.11 ± 7.69% on day 5 and almost 0% after day 10 for BALB/c mice and were 13- 30% on day 5 to 40 for the gerbils. The larvae were first detected on day 30 with the number of 1287 ± 1005 from the mice and were on day 21. These finding show that the isolate of *Trichinella* sp. has high infectivity to Mongolian gerbil.

PO3.55

Wild Animals as Reservoirs of Trichinella spp. in the Patagonic Region

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Trichinella is a widely distributed zoonotic nematode found primarily in carnivorous mammals. Wildlife can serve as a natural reservoir and play an important role in the spread of the parasites.

The aim of the present study was to assess the occurrence of the genotypes of Trichinella in potential wildlife reservoirs from different endemic areas of the patagonic region of Argentina, where human outbreaks are reported each year. Muscle samples were examined by artificial digestion and larvae were identified at the species level by a nested multiplex polymerase chain reaction analysis and sequencing of cytochrome oxidase I gene (COI). From 2005 to 2008, muscle samples were collected from 35 wild boars (Sus scrofa), 13 mountain lions (Puma Concolor) and 37 foxes (Pseudalopex griseus). Larvae of Trichinella were found in 16 wild boars, where 10 isolates were identified as T. spiralis, and in only one mountain lion that COI sequence analysis was similar to a new sylvatic genotype recently reported from same host and region. These results suggest that in the Patagonia wild boards and mountain lions can act as reservoirs for the maintenance and dissemination of the domestic and sylvatic trichinellosis, respectively.

PO3.56

Potential Use of Deglycosilated *Trichinella spiralis* Muscle Larva Excretory-Secretory Products for the Early Diagnosis of Trichinelosis and to Evaluate Albendazole Treatment Efficacy

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We analyzed the whole antibody (Ab) response to native or deglycosilated *T. spiralis* muscle larva (ML) excretory-secretory products (nESP or dESP) by ELISA in rats treated with albendazole (ABZ) in the early or late larva encapsulation phase. ML establishment and infectivity was also studied to determine the ELISA value in the early diagnosis and prognosis of trichinellosis. Seven-rat groups were infected with 2000 *T. spiralis* ML. Rats were not treated (A) or treated with vehicle (B1,B2,B3) or ABZ, 20 mg/Kg/day (C1,C2,C3) for 3 (B1,C1) or 6 (B2,C2,B3,C3) days starting on day 13 (B1,C1, B2,C2) or 45 (B3,C3) post-infection (pi). Serum samples collected before and at different times after the infection. ML recovery was done by digesting artificially the gastrocnemius to determine ML establishment and viability. Infectivity of ML recovered was tested in mice. In ABZ treated rats, viability of ML recovered from groups C1, C2 and C3 was not affected. ML establishment was reduced in 24 % in group C1 and 44 % in group C2 while it was not affected in group C3. In contrast, only 1 % of the ML recovered from groups C1 and C2 and 61 % from group C3 established the infection in the mouse. An early production of Abs to ML-dES was detected by day 10, peaked on day 14 pi and the highest antibody production was on day 61 in groups A1, B1 as compared with the antibody production to nPES which started lately by day 24 and reached its maximum by day 31 pi. The 3-day ABZ treatment showed a transitory decrease of the early Ab response to dESP from days 14 to 19 and a delay in the late antibody appearance to nESP from days 28 pi. Overall, these results suggest that early recognized antigens from ML-dES are candidates for the early diagnosis of trichinellosis while those lately recognized from nESP may be useful to evaluate ABZ treatment efficacy. *Fellow of COFAA, EDD

PO3.57

Gene Responses in Rat Intestine During Early Phase of *Trichinella* Infection

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Early innate defense against *Trichinella* infection takes place in intestinal mucosa. *T. spiralis* induces a higher infection-intensity in rats than *T. nativa*.

We designed an experimental infection to study if this difference is due to distinct host defensive reaction to these two parasite species. Six rats were infected with *T. spiralis*, six with *T. nativa* and six served as controls. The infection dose was 2000 trichinella larvae. The rats were killed at day 5 post inoculation and RNA was isolated from mucosal scrapings of jejunum. Also, three extra rats for each group were used as infection intensity controls, and euthanized after one month to count the larvae per gram (lpg) values, which were 500 lpg for *T. spiralis* and 0.67 lpg for *T. nativa*.

We utilized rat genome microarray chip (GeneChip®, Affymetrix) with 31 000 probes to analyze host intestinal responses. Microarray data shows, that when compared to controls *Trichinella* infections caused over-expression (p-value <0.05, at least two fold changes) of 866 genes, of which 614 were over-expressed both in *T. spiralis* and *T. nativa* infections. Simultaneously 654 genes were down-regulated, of which 386 were shared in infections with both species. Gene ontology clustering showed that *Trichinella* infection activated several significant biological processes in rat's jejunum associated for example to lipid and organic acid metabolism.

PO3.58

Protein profiling for swine with *Trichinella spiralis* iInfection in Thailand

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Trichinellosis is mainly caused by *Trichinella spiralis*. It was first reported in 1962 in Thailand and since then, trichinellosis cases are reported every year. Traditionally, *Trichinella* infection diagnosis can be done by direct method of finding first stage larvae in the muscle. This technique is however considered to be a time consuming method. Another diagnostic method is indirect diagnosis which is the most commonly used technique based on serology. However it has been reported that there occurs cross reaction between crude larvae extract and other helminthiasis infection. The purpose of this study was to observe the immunoblot profiles and to identify specific protein antigen of infective stage protein antigens derived from *T. spiralis*. The samples were divided into 3 groups. Positive control (group 1) was obtained from 5 swine sera with experimentally confirmed *T. spiralis* infection. Negative control (group 2) originated from 5 swine sera which were confirmed microscopically negative for protozoa and gastrointestinal parasites. Group 3 was derived from 14 swine sera which had been parasitologically confirmed positive for other protozoa and parasitic infections. Crude antigens obtained from the infective larvae of *T. spiralis* were

used in SDS-PAGE and immunoblot. The immunoblot profiles of *T. spiralis* infected swine sera in our study revealed at least 13 bands with molecular weight (MW) ranging from 10 to 55 kilodalton (kDa). They were 39, 36, 35, 30, 29, 27, 24, 21, 19, 18, 15, 14, 11 kDa. The MW protein antigen of 18 kDa is likely to be specific for *T. spiralis* infected sera and would be an interesting protein candidate for the specific diagnosis for trichinellosis in swine production.

PO3.59

Trichinella pseudospiralis in the Eastern Slovakia – a Guest or a Resident?

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Until 1995, Trichinella pseudospiralis, infecting both mammals and birds has been detected only sporadically in sylvatic mammals and birds. However, a substantial increase of the occurrence of this pathogen was registered in EU countries, with reports from France, Finland, Sweden, The Netherlands, Spain, Lithuania, Italy, Germany, Hungary, and Bulgaria.

In Central Europe, the first focus of T. pseudospiralis was documented from pig breeding farm in Eastern Slovakia in 2003. The area where the farm was located is an important crossing of Pan-European bird migration routes from Europe and northern Asia. Given that several species of raptorial birds move from their breeding regions in northern Europe temporarily residing in Eastern Slovakia during the wintertime and with respect to the striking genetic similarity between the Slovak isolate and isolates from Sweden and Finland by DNA (cox1 mitochondrial gene) and allozyme approaches (PGM polymorphism) we suggested that the parasite appeared in our territory due to migration of birds of prey from Scandinavian Peninsula.

Since 2005 several records of T. pseudospiralis in fox and wild boar (mixed infection of T. britovi and T. pseudospiralis) from Eastern Slovakia were reported and question arises whether the species has already constituted a local focus in wildlife (more likely explanation), or it was repeatedly re-introduced several times into this territory from northern-eastern regions. Further monitoring program on T. pseudospiralis in respective animal species is needed to verify their importance as reservoir in the area of Eastern Slovakia.

The work was supported by the Slovak Grant Agency VEGA 2/7186/27.

PO3.60

Trichinelasis in Russia: History and Prospects of Methodology and Technical Set-Up Progress for *Trichinela* Invasia Control of Slaughter Animal Meat

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In present work we give short summary for the history of development and methods for *Trichinellasis* invasia diagnostics in meat products from slaughter animals and evolution of the specialized technical equipment for these methods.

Mandatory trichinelloscopy of meat was primary introduced in Saint- Petersburg at 1881. Since then, much efforts of Russian veterinary and sanitary scientists with engineers were focused at increasing reliability and trustworthiness of the expertise. Trichinelloscopy had passed a long way from the simple magnifying glass and monocular microscope towards digital videomicroscopy with coming automatic *Trichinella* larvae search and detection in the material under study, with minimal part of human labor. The changes in samples preparation approach starting from compressor glasses of various construction for individual trichinelloscopy of carcasses to biochemical separating fermentators for group analysis with increased productivity . The abandoning by Chapter 16 of EU Commission Regulation #2075/2005 of compressor trichinnelloscopy that comes into action since 31.12.09 results in the development of sample preparation and will be aimed at design of high - productivity equipment for individual discharge of Trichinella larvae from single samples of slaughter animal products.

PO3.61

Labor Consuming in Magnetic Stirer Method and Gastros Usage: a Comparative Study

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It is temping to consider the reduction of routine and time-consuming analysis of meat for *Trichinella* larvae presence in two method modalities 1. magnetic stirrer method and 2. processing with Gastros 6E machine. (This model is operating accordance with EU 2075/2005 Regulation prescriptions and has 3 chambers, 2 lt. each.) Three members of laboratory stuff with different experience were carrying out 6 tests each, with chronometry. Preparation procedures, such as mixing an artificial gastric juice, meat probe collection and, afterwards, the rinsing of cylinders are equal for both techniques, so they were not taken into consideration. We have also included the time needed for clearing and washing the labware and

equipment after the analysis, for taking a new digestion (or by the end of the day).

Therefore, it can be speculated that average labor operations were reduced from routine 12 min to 5 min in Gastros-6E duty cycle. The most unexperienced laborant demonstrated the bigger reduction.

Vectors

Thursday, August, 13, 2009

PO3.62

The Effect of *Beauveria bassiana* on Different Stages of *Lutzomyia Longipalpis*

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Visceral leishmaniasis is a zoonosis whose primary vector in Brazil is the phlebotomine *Lutzomyia longipalpis*. Currently, efforts to control the vector have not been effective in reducing the incidence of disease. A possible alternative to current strategies is the biological control of the vector using entomopathogenic fungi. This study evaluates the effects of the fungus, *Beauveria bassiana*, in different developmental stages of *Lu. longipalpis*. Five concentrations of the fungus were utilized ranging from $10^{4 \text{ to} 108}$ conidia/ml, accompanied by controls. The unhatched eggs, larvae and dead adults were sown to reisolate the fungus from the infected material. The fungus was subsequently identified by PCR and DNA sequencing. *B. bassiana* reduced in 59% the egg hatching. The mortality of infected larvae was significant. The longevity of infected adults was lower than that of the negative control, but was not higher than that of the positive control, in which death was instantaneous. The effects of fungal infection on the hatching of eggs laid by infected females was also significant and dose-dependent. With respect to fungal behavior post-infection, only germination and sporulation were significantly higher than the control. The identity of the reisolated fungus was confirmed by automated DNA sequencing post-passage in all insect stages. These data show that *B. bassiana* has good pathogenic potential, primarily on *Lu. longipalpis* larvae and adults. Consequently, the use of this fungus in phlebotomine control programs must be considered as a possible means of reducing the use of chemical insecticides, resulting in benefits to humans and the environment.

PO3.63

Synanthropic Flies as Potential Vectors of Pathogenic Agents

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In the present study we investigated the pathogenic burden of 486 wild flies caught at different animal related places in Dormagen (GER). Most of the caught species belong to muscoid flies (e.g. *Musca domestica*, (I: 62%), followed by blow flies and flesh flies. The flies were examined for the pathogenic agents they carried with standard microbiological and parasitological methods. We could detect a large diversity of different bacterial and fungal species, protozoan and even metazoan species on the exoskeleton and in the intestine of the flies. Among them we could prove life threatening bacteria species such as enteropathogenic *Escherichia coli-strains* (EAEC, EHEC, ETEC, EPEC), potential pathogenic fungi (e.g. *Candida albicans, C. tropicalis*), eggs and larvae of animal helminths (e.g. *Ascaris suum*) as well as the hog louse *Haematopinus suis*.

The present study emphasizes the potential of synanthropic flies (especially the house fly *M. domestica*) as a crucial vector of multiple pathogenic agents.

PO3.64

Molecular Detection of Feline Babesia Species in Field-Collected Ticks (Acari: ixodidae) in South Africa

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Ticks are of vast importance due to their ability to transmit an impressive variety of infectious agents. Emerging arthropod-transmitted parasites of wildlife are potential disease threats and the worldwide picture of ixodid tick-transmitted parasitic diseases is an example of this dynamic situation. The present work describes the molecular detection of feline Babesia spp. in field-collected ixodid ticks which could serve as potential vectors for feline babesiosis in cheetahs at various breeding centres in South Africa. The vegetation at two cheetah breeding centres was dragged for ticks on a monthly basis over a 12 month period. Five tick species namely Amblyomma hebraeum, Amblyomma marmoreum, Haemaphysalis elliptica, Rhipicephalus simus and Rhipicephalus zambeziensis were identified taxonomically. Subsequently, DNA was extracted from immature and adult ticks. The V4 variable region of the parasite 18S rRNA gene was amplified and subjected to the Reverse Line Blot (RLB) hybridization assay for the detection and differentiation of Babesia and Theileria spp. Results have

shown that Babesia spp. could only be detected from Haemaphysalis elliptica ticks. Since Babesia species are transmitted by ixodid ticks, the results suggested that Haemaphysalis elliptica might play an important role as a natural vector in the field-transmission of feline Babesia species.

PO3.65

Annual Dynamics of Ixodes Ricinus and Dermacentor Marginatus (Acari: ixodidae) in Some Areas from North-East, and South-East of Romania

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Ixodes ricinus and Dermacentor marginatus (Acari: Ixodidae), three-host ticks, are the most wide-spread ticks in Romania; they were found in different geographical areas with different ecological particularities, showing a large ecological valence. For good knowledge of their distribution areas in our country, and the risk of tick-borne pathogens, a study on annual dynamics of populations of those two species in the North-East, and the South-East areas of Romania, during of 2008 year, was performed. The annual dynamics of tick populations emphasized differences in starting of questing activity, and maximal parasitism on animals, according to the geographical areas and annual evolution of the main local ecological factors (temperature, humidity, vegetation). The questing activity and maximal parasitism for both species were registered earlier in the South-East than in the North-East areas. D. marginatus ticks had the first appearance in late March, with an activity peak in April, in the South-East areas, while the populations from the North-East appeared later, in late April, with a peak in May. I. ricinus ticks started the questing activity in April, in the South-East areas, with a peak in May, while in the North-East areas the populations came out in May and had a peak in June. A less marked increase of activity was also observed in autumn, for both species, in both areas. The highest activity was registered for D. marginatus and I. ricinus ticks in the South-East areas, between September and November, with a peak in October. The autumn peak (in October) of the two species from the North-East areas was diminished, about half of the peak of tick populations from the South-East. Small number of I. ricinus and D. marginatus were collected in all other months, except December, January, and February.

The temporal distribution of tick species might be an important tool for prediction on the seasonality of tick-borne diseases in Romania.

PO3.66

Experiences About Culture of Two Snails Vectors Species for Fasciola hhepatica

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The objective of the present study was to compare the obtained results of the observation of cultures of two snails Lymnaea (Fossaria) humilis and L. (Fossaria) bulimoides originatinated of a farm of the State of Mexico. The method consisted of collecting mud, algae Oscillatoria sp. and snails, where the cultures were prepared. For it purpose porcelain trays were used and Petri´s box of 14 cm in length were come to sterilize the Earth and calcium carbonate was added, the collected snails were placed in four trays and in a total of 20 Petri´s box, once this means were dried was come to add the algae Oscillatoria spp, for their culture, (approx 15 days for the growth of the algae) later 50 Lymnaea humilis snails were placed of 8 days of born (measured 3X2.5mm of length and diameter respectivelly) and 50 L. bulimoides snails in 2ª tray those that measured 3X3; in 5 Petri´s box, 10 snails of L. bulimoides and L. humilis each species were placed approx 8 days of age in each box. It was added food to the snails when they arrived at 15 days of age, administered commercial fish food in the porcelain trays and the Petri´s box. The cultures were observed each third day until completing of 45 up to 90 days until the death of snails happened as much concludes that L. humilis was major survival for that stops L. bulimoides.

Water-borne Protozoa

Thursday, August, 13, 2009

PO3.67

Detection and Identification of Cryptosporidium species in Water Samples from a River in Ardabil City, Northwestern Iran.

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Cryptosporidium is an opportunistic parasite typically associated with large waterborne outbreaks. Surface waters contaminated with human and animal feces serve as main source for epidemic spread of Cryptosporidium parasites. In this study, we used a small-subunit rRNA-based PCR – Restriction Fragment Length Polymorphism (RFLP) technique to determine the prevalence and to characterize human – infective species of Cryptosporidium parasites in water samples collected from a stream in Ardabil city in Iran. Among

30 samples examined, 11 samples showed positive results. Restriction pattern analysis showed C C. andersony as the most common species with 7 cases; followed by C. parvum, bovine genotype, with 3 cases and C. suis with 1 case. The results indicated that PCR – RFLP technique provides an applicable and feasible method for detection and identification of Cryptosporidium oocysts in environmental water samples. The results, furthermore, demonstrated that wildlife is the major source of Cryptosporidium oocysts in surface water resources in the study region.

PO3.68

Assessment of Helminthological Contamination in Drinking Water Sources of Kathmandu Valley Nepal

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Water is very important to all life, including man. The pollution of water is a threat to the survival and existence of life itself. Contaminated water and water borne diseases are responsible for the deaths of 1.8 million people every year. Over 50,000 children die each year in Nepal from lack of access to safe drinking water. Piped supply of safe drinking water in Kathmandu valley is not sufficient. Because of the reason community people are compelled to use alternative sources and to store water for a long period. Hence the e study was conducted in Kathmandu valley with the objective of determining microbilogical contamination of water sources. The study was conducted in winter and rainy season during 2008. Samples were collected from various water sources (wells, stone spouts, NWSC tap, and stored water). Water samples were tested for bacteriological contamination by using H2S method, Coliplate and Coli-strip techniques were used to determine the density of coliform and E.coli. Microscopic examination was also done using centrifugation and floatation method to observe the presence of protozoan and helminthes parasites. In the study total 160 samples of drinking water was collected from the different sources in the two seasons. Out of total samples collected, well water contributed 54 (345) and NWSC tap water contributed 42 (26%), stored water contributed 48 (30%) and stone spout contributed 16 (10%). Source wise distribution of samples showed that well water contributed the highest number of samples. The results revealed that higher water samples were found contaminated in rainy season. Out of total samples 57.5% water samples were found contaminated. Among the four sources stored water showed comparatively higher contamination (73%) and is followed by well water (59.3%), NWSC tap (47.6%) and stone tap (31.3%) respectively. Microscopic test results showed the presence of eggs of Ascaris spp, Ancylostoma spp, Trichuris spp, Taenia solium and some unidentified nematodes. All the parasites were observed in rainy seasons.

PO3.69

Giardia, Cryptosporidium and Microsporidia Infections in Wild Animals from a Deforestation Area in the State of São Paulo, Brazil

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The occurrence of Giardia *null result*, Cryptosporidium*null result* and microsporidia was investigated in 98 faecal specimens from wildlife animals, captured in an area of deforestation for the construction of two water reservoirs, located in the state of São Paulo (Brazil). Samples were obtained from 46 rodents, 21 marsupials, 16 frogs, 9 bats, 3 tamarins and 3 lizards. For the detection of Giardia*null result*, Cryptosporidium *null result* and microsporidia it was used, respectively, the floatation technique with lead sulphate, the Kinyoun method and the Gram-Chromotrope staining. The total number of parasitized animals by one of these protozoans was 17.35% (17/98). Cysts of Giardia*null result* were found in faecal samples from 2 prehensile-tailed porcupines (Coendou villosus*null result*). The three positive animals for Cryptoporidium*null result* were rodents - 1 montane akodont (Akodon montensis*null result*), 1 ebony akodont (Thaptomyces nigrita*null result*) and 1 guainan squirrel (Sciurus aestuans*null result*). Microporidia spores were seen in the stools of 12 animals - 6 small rodents, including 3 montane akodonts, 1 prehensile-tailed porcupine and 2 pigmy rice rats (Oligoryzomys sp.*null result*); 3 marsupials, including 1 gray slender mouse opossum (Marmosops incanus*null result*) and 2 big eared opossums (Didelphis aurita*null result*); 3 hairy-legged vampire bats (Diphylla ecaudata*null result*). This is the first description of microsporidiosis in wildlife animals in Brazil. The present study emphasizes the importance of the animals, particularly small mammals, as potential sources of protozoan infection to other animal populations, including man, in areas of deforestation.

PO3.70

Survey of Cryptosporidium spp, Giardia spp. and Microsporidia Infections in Capibaras and Opossums in an Ecological Park in São Paulo, Brazil

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Cryptosporidium*null result*, Giardia*null result* and microsporidia are ubiquitous enteric protozoan parasites that infect a wide range of vertebrates hosts. Fecal samples from 35 capibaras (Hydrochaeris hydrochaeris*null result*) and 21

opossums (Didelphis aurita*null result*) living in an ecological park located at the municipality of São Paulo, Brazil, were examined for Cryptosporidium*null result* oocysts, Giardia*null result* cysts and microsporidia spores. For the detection of Giardia*null result*, Cryptosporidium*null result* and microsporidia it was used, respectively, the floatation technique with lead sulphate, the Kinyoun method and the Gram-Chromotrope staining. DNA extracted from feces or rectal swabs was amplified by polymerase chain reaction using parasite-specific small subunits ribosomal RNA gene primers. Cryptosporidium parvum*null result* was found in 4 capibaras (11.4%) and 1 oppossum (4.7%). Giardia intestinalis*null result* was detected in 2 capibaras (5.71%) and Encephalitozoon cuniculi*null result* was found in 3 oppossums (14.3%). The results obtained in the present study demonstrate that, despite the prevalences of these parasites were found to be low, capibaras and opossums may contribute as infective sources within the environment. It is important to emphasize that water is a major conduit for these protozoans and contaminated water is an important source of human infection either by direct consumption or by the use of contaminated water in food processing or preparation.

PO3.71

Identification of a New Species of Cyclospora sp. from Ducks in China

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Cyclospora organisms are intestinal pathogens of vertebrates, which have been found in human cercopitheci colobi papionis and cattle in the world. Cyclospora-like oocysts in the feces of ducks from China were discovered at first, these oocysts were nearly to exactly round with the 7.5-11 m diameters and light green globular things. Two pairs of primers were designed based on 18S rDNA of Eimeriidae, and Nested PCR was used to amplify partial conservative fragments of 18S rRNA gene, and a 294bp fragment was amplified, cloned and sequenced. NCBI Blast analysis online indicated that the partial 18S rDNA is most closely related to the Cyclospora sp., which identities is 98%. In order to certify if the cyclospora sp. is a new species, a new pairs of primers are designed and used to amplify the variant region ITS-1. Sequence analysis indicated that the ITS-1 sequence is species characteristic. So the duck-associated Cyclospora-like organisms may be a new species of Cyclospora sp.. This work is supported by grants from National Natural Science Foundation of China (grant no. 30371082, 30671577) and Natural Science Foundation of Guangdong Province (grant no. 32286).

PO3.72

Synergistic effect of Pyrantel Embonate and Febantel in Elimination of Giardia in a Gerbil Model

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Febantel and a combination of Febantel and Pyrantel embonate are commercially available antiparasitic preparations that have shown to b e effective in the treatment of Giardia infections in companion animals. The objectives of the study were to determine the optimal dose of Febantel, Pyrantel embonate, and a combination of Febantel/Pyrantel embonate required to effectively treat Giardia in a Gerbil model and to determine if there is a synergistic effect with the two drugs.SPF Gerbils were infected by oral inoculation with 105 Giardia duodenalis trophozoites (day 0). On Days 5 to 7 animals (n = 6) were treated once daily via oral gavage with 1) Febantel (160, 80, 40, 20 or 10 mg/kg); 2) Pyrantel embonate (160, 80, 40, 20 or 10 mg/kg), 3) Febantel and Pyrantel embonate (160, 80, 40, 20, or 10 mg/kg); 4) Metronidazole (200 mg/kg); or 5) placebo . Gerbils were euthanized on day 8 (24 hours after last treatment) and duodenal trophozoites were enumerated on a haemocytometer to obtain a concentration of trophozoites/ cm of gut. Febantel alone, effectively eliminated Giardia trophozoites at 160 and 80 mg/kg. Pyrantel embonate did not eliminate Giardia from the animals but significantly reduced parasite counts at all dosages. Febantel combined with Pyrantel embonate effectively eliminated Giardia trophozoites at 160, 80 and 40 mg/kg. Metronidazole did not totally eliminate Giardia trophozoiles from the gut. All placebo treated animals were heavily infected with Giardia trophozoites. Febantel is more effective in elimination of Giardia infections when combined with Pyrantel embonate compared to the agents used alone.

PO3.73

Prevalence and Diagnosis of Giardia Infection in Dogs and Cats Using a Fecal Antigen Test and Fecal Smear

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Giardiasis is a common gastrointestinal infection in dogs and cats but it remains a diagnostic challenge due to intermittent shedding and the small size of the cysts. The new fecal antigen test (SNAP® fecal ELISA Giardia test) was evaluated in Canadian veterinary clinics to determine the prevalence of Giardia in dogs (134 clinics) and cats (94 clinics) with gastrointestinal clinical signs. The fecal antigen test was compared to a fecal smear which was the method used by many veterinary clinics. A total of 1871 dogs and 389 cats were enrolled in the study. The presence of fecal antigens were observed

in 241 (13.0%) and 16 (4.3%) symptomatic dogs and cats respectively. Loose or watery diarrhea with increased frequency was the predominant clinical signs of dogs and cats with diarrhea. When a Bayesian evaluation was performed using the Giardia SNAP® test as a reference the sensitivity the fecal smear was only 31.8% for dogs and 26.7% for cats. The positive predictive value for a fecal smear was only 52.1% in dogs and 28.6% in cats. The SNAP® fecal ELISA Giardia test appears to be a valuable tool in the diagnosis of giardiasis in dogs and cats.

PO3.74

The First Discovery of Cyclospora sp. from Canine Feces in China

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Cyclospora organisms are intestinal parasites causing protracted diarrhea in human and animals. Ingestion of several sporulated oocysts results in acquisition of cyclosporiasis. In the present work, stool samples from dogs were collected and examined by saturated sucrose floatation and modified acid-fast staining. Then sporulation experiment and fluorescence microscope examination were conducted. Cyclospora-like oocysts were discovered firstly in dogs from China. The oocysts were spherical and 7.5-10 m in diameter, with well-defined refractive spheres in each unsporulated oocysts, appearing as morula. After modified acid- fast staining, the organisms appeared faint-pink to red in color, while some oocysts not staining and appearing as "ghost". After sporulation experiment the oocysts produced two sporocysts, each containing two sporozoites. All oocysts displayed a brilliant blue fluorescent outer ring when viewed with fluorescence microscope. To further identify the Cyclospora-like organisms, two pairs of specific primers were designed based on partial 18S rDNA of Cyclospora sp. and used to nested PCR. These primers selectively amplified a 294-bp DNA fragment of the 18S rRNA gene. The amplicons were purified and cloned into PMD19-T vector. The insert was successfully sequenced, followed by analysis using online Blast software, and the phylogenetic tree was constructed using MEGA software. The result revealed the identity between the dog-associated Cyclospora-like organisms and C. cayetanensis was 98, and the Cyclospora-like organisms belong to the group of Cyclospora in the phylogenetic tree. This work was supported by grants from National Natural Science Foundation of China (grant no. 30671577, 30371082).

Zoonoses

Thursday, August, 13, 2009

PO3.75

Seroprevalence and Risk Factors Associated to *Toxoplasma gondii* Infection in Pig Farms from Spain

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A total of 1,782 pig sera from 62 farms sampled from 2007 to 2009 were tested for antibodies against *Toxoplasma gondii* using the modified agglutination test (MAT). Sera titres ≥1:25 were considered positive. The farms were randomly selected from eight different regions of Spain, which account for over 80% of the Spanish pig production. Antibodies to *T. gondii* were found in 328 samples (18.4%; 95%IC: 16.6-20.2). Wide variations within farm seroprevalences (0 to 93%) were observed. There were also statistically significant differences ($P<0.05$) between regions and among age groups. Catalonia, Comunitat Valenciana, Castilla-León and Castilla-La Mancha showed mean seroprevalences > 20%, while the lowest prevalences were observed in Murcia and Aragón (4.4% and 8.1%, respectively). Sows showed statistically significant higher prevalence of infection (23.0%) compared to both, 15 and 20 weeks old piglets (16.1% and 11.6%, respectively). There were not statistically significant differences between farrow-to-finish and piglet production farms. A further cross-sectional study was performed to estimate the associated risk factors to *T. gondii* infection in Catalonia (North-eastern Spain), the highest pig-density area in Spain, by linear regression analysis. The risk factors significantly associated to *T. gondii* seroprevalence in this region were the presence of cats, percentage of mortality at weaning and the presence of outdoor facilities in the farms. The seroprevalence observed in the present study indicates widespread exposure to *T. gondii* among domestic pigs in Spain, which might have important implications in Public Health.

PO3.76

Seroprevalence of Toxoplasma gondii in Moose and Roe Deer in Sweden

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Toxoplasma gondii is one of the more common parasitic zoonoses in the world. In young children and immunocompromised persons infection can lead to severe disease and death. Ingestion of undercooked meat and handling of raw meat are important infection routes in man. In Sweden game meat is widely available and 7 out of 10 households serve game meat at least once a year. Moose is the most important game species and hunting of roe deer is also common. The aim of this study was to investigate the seroprevalence of *T. gondii* in Swedish moose and roe deer in order to estimate their role as potential disease carriers to humans. Blood samples were collected from 425 moose (*Alces alces*) and 235 roe deer (*Capreolus capreolus*) during 1990 to 2007 and analysed for presence of *T. gondii* antibodies by a direct agglutination test. Antibodies were detected in 87 (20%) and 77 (33%) of the moose and roe deer samples, respectively. In moose the infection was more common in northern than in southern Sweden, whereas there was no regional differences in roe deer. There was an increase in seroprevalence with age in both species. The results show that *Toxoplasma* infection is widely spread in the Swedish moose and roe deer populations. Precautions should therefore be taken when handling internal organs and carcasses of harvested cervids. It is also important to prevent the risk of toxoplasmosis from game meet by cooking it well before serving. Freezing the meat greatly reduces chance of infection.

PO3.77

Risk Assessment for Toxoplasma gondii in the Danish Pig Industry

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Toxoplasmosis may have serious consequences for pregnant women or immuno-compromised patients. Contact with infected cats and litter, contaminated soil and infected meat are risk factors for toxoplasmosis. Although the prevalence of Toxoplasma in pig production has declined significantly during the past 30 years, it was suggested that a large part of human toxoplasmosis cases may be ascribed to meat, including pork. Moreover, perinatal screening of pregnant women and infants for Toxoplasma has proven to be of limited value. This has raised the question how to survey for Toxoplasma: in humans or meat? Therefore, the role of meat, including pigs and pork, as a risk factor for human toxoplasmosis was assessed. The release assessment showed that outdoor-reared pigs as well as sows and boars were at higher risk of Toxoplasma infection. Consumption of mildly cured pork products and inadequately heat-treated pork were associated with increased risk. Knowledge on elimination or survival of Toxoplasma in cured pork products is sparse, which is unsatisfactory given current trends toward lower salt content and lower cooking temperatures. It was concluded that, aside from consumption of raw pork, certain mildly cured ready-to-eat pork products, that have not been heat-treated, may constitute a risk for toxoplasmosis, if not frozen prior to manufacturing. Information on the effects of curing on survival of Toxoplasma in meat is sparse. However, most of the pork used for manufacturing in Denmark originates from pigs raised indoors and for logistic reasons is frozen prior to processing, thereby reducing the risk for human toxoplasmosis.

PO3.78

The Identification of *Entamoeba* Spp. in Wild African Green Monkeys (*Chlorocebus Sabaeus*) on St. Kitts, West Indies

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Entamoeba spp. are often diagnosed in the stool of non-human primates and inhabitants of the tropics. Although the majority is considered to be harmless, care should be taken when *E. histolytica* is involved. Infection by this gastrointestinal parasite may cause hemorrhagic dysentery, extra-intestinal pathologies, such as liver abscesses, and even death. A pilot study indicated that *Entamoeba* spp. (75%) were the most prevalent parasites in wild African green monkeys (*Chlorocebus sabaeus*) on the island of St. Kitts, West Indies. However, prevalence data of *E. histolytica* are not available and the possible reservoir function of these animals for zoonotic transmission remains unclear. Therefore, the objectives of this study were to study the occurrence of *E. histolytica* and to estimate the zoonotic reservoir function of African green monkeys on St.Kitts. To this end, *Entamoeba* samples previously collected from 40 animals from nine locations were re-examined. Two polymerase chain reaction protocols targeting the *small subunit ribosomal DNA* gene were used for the identification of *E. histolytica*in all samples. In addition, other *Entamoeba* spp. were also identified using a reverse line hybridisation blot protocol. The molecular identification revealed the absence of *E. histolytica*, but the presence of *E. dispar, E. hartmanni, E. coli* and *E. chattoni*. These findings suggest that African green monkeys at St. Kitts are not a potential reservoir for *E. histolytica* infections.

PO3.79

Seroprevalence of Toxoplasmosis(*Toxoplasma gondii*) in Pigs, Goats, Cattle, Dogs and Cats in Peninsular Malaysia

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Toxoplasma gondii is a species of parasitic protozoa in the genus Toxoplasma. The definitive host of *T. gondii* is the cat, but the parasite can be carried by the vast majority of warm-blooded animals, including humans. Toxoplasmosis can cause severe disease in many species of animals, including embryonic death and resorption, fetal death and mummification, abortion, stillbirth and neonatal death in goats and sheep. Therefore in this study, antibodies towards the protozoan parasite, *Toxoplasma gondii* were assayed in sera of 200 goats, 100 pigs, 126 cattle from various states of Malaysia. A total 135 dogs and 55 cats around Ipoh, Malaysia were also screened. The indirect fluorescent antibody test (IFAT, cut-off titer 1:200) was used. Our findings showed that antibodies were found in 35.5% of goats, 14.5% cats, 9.6% dogs, 7.9% local cattle and 4% yellow cattle but none in pigs. Therefore, we can conclude that toxoplasmosis infection is most prevalent in goats and can be commonly found in domestic animals in Malaysia.

PO3.80

Age-Related Infection and Transmission Pattern of Human Cysticercosis in Southern Ecuador

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Background: Human neurocysticercosis is recognised as an important but neglected cause of epilepsy in developing countries where the parasite occurs. Data on the transmission dynamics of the parasite in endemic areas are scarce. Individuals living in these areas are likely to be highly exposed to the parasite, but relatively few of them develop active infections.

Objectives: The present community-based study aimed to describe changes of antibody responses and infection patterns related to age and/or gender in a south Ecuadorian rural population by combining antibody and antigen serological data with demographic characteristics.

Findings: In 25% of the population antibodies to T. solium cysticerci were detected while 2.9% had circulating parasite antigens. The proportion of antibody positives increased significantly till the age of 40 years to become stable in older individuals. A rule-based simulation model was developed to explain these variations and to reflect the dynamics of exposure to, and transmission of the parasite. In contrast, the proportion of people presenting active infections (antigen positives) was significantly higher in people older than 60 years. Immunosenescence could explain such an observation since a weaker immune system in the elderly would facilitate the establishment and maintenance of viable cysticerci in comparison with fully immunocompetent younger individuals.

Conclusions: This work points out the role of the immune system in the development of cysticercosis inside an exposed population and highlights new essential issues in the understanding of the transmission dynamics of the parasite, its incidence and the resulting immunological response at population level.

PO3.81

Determination of Morphologic Characters of Intestinal Helminthes in Dog

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Human infection especially with dog's helminthes parasites is an emerging health issue, as the human environment is increasingly shared with infected animals, either pets or wild life. In this study, the intestinal content of 83 stray dogs, collected from the Kordestan,West Azarbaijan, and Kermanshah provinces in Iran. Following autopsy of the animals, their small intestine were removed, slit open and the epithelium of the intestine scraped into a jar. Recovered helminthes were fixed in alcohol and the cestodes were stained with carmine. their morphological characters as the size of body(length and width), esophagus, tail, spicule, buccal cavity, bursal sac, gubernaculums, egg, hooks, rostellum, cirrus sac, Para uterus, scolex, mature and gravid proglotides and number of testes, hooks, proglotides, spines, eggs in each sac, were measured and recorded.The parasites were identified according to the keys and guidelines given by Yamaguti (1961), Anderson (1992) and Khalil et al. (1994) finally. The different species recovered from these animals are listed as follows:

Toxocara canis, Toxascaris leonina, Ancylostoma caninum, Oxynema sp., Rictularia affinis, Taenia hydatigena, Taenia ovis, Taenia multiceps, Echinococcus granulosus, Dipylidium caninum, Mesocestoides lineatus and Macracanthorhynchus hirudinaceus.

PO3.82

Prevalence of Specific Antibodies Against Toxoplasma gondii in Sheep in the Czech Republic

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Toxoplasmosis in sheep herds have spread globally. The infection causes abortions. Meat of infected animals contains tissue cysts that can be the source of infection for humans.

The aim of this study was to identify and evaluate the prevalence of specific antibodies against T. gondii in sheep in the Czech Republic.

From February 2006 to August 2007, 155 samples of blood serum from clinically healthy sheep were examined. Herds with farming conditions typical for the Czech Republic were selected (50 to 200 animals grazing in the pasture most of the year). Cats, definitive hosts of Toxoplasma gondii, often occur near sheep farms, as well as conditions in the Czech Republic are favorable for a long-term survival of T. gondi oocysts.

Sheep blood serum samples were examined by IFAT for specific antibodies against Toxoplasma gondii. Out of a total of 155 sheep, 121 were positive (78 %). Detected specific IgG antibodies against Toxoplasma gondii ranged between 1:40 and 1:2560. Low titers (40 – 160) were detected in 78 % positive sheep. Titers exceeding 1000 were detected only in 8 % positive animals. No positive animals showed clinical symptoms (including abortions).

Latent toxoplasmosis does not pose a health risk for sheep, but tissue cysts contained in meat of animals with circulating antibodies play an important role in food safety.

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PO3.83

Genotyping of *Toxoplasma gondii* isolates in capybaras (*Hydrochaeris hydrochaeris*) from São Paulo state, Brazil

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Toxoplasma gondii is a worldwide protozoan, but the genotype distribution of the isolates varies across the continents with a higher genetic diversity in South America than in Europe, USA and Africa. The greatest diversity was observed among isolates from Brazil. However, little is known about genetics of *T. gondii* isolates from wild mammals in Brazil. The aim of this study was to determine genotypes of *T. gondii* isolates in capybaras (*Hydrochaeris hydrochaeris*) from São Paulo state and if the allele types at marker CS3 are associated with mouse-virulence. Genotypes of 36 *T. gondii* isolates from capybaras from six counties were determined. Sixteen genotypes were identified using 11 genetic markers including SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, Apico and CS3. No classical clonal Type I and Type II isolates were found, confirming other findings that these lineages are rare in Brazil. Eight of these 36 isolates were grouped into the common clonal lineages in Brazil, previously designed as Types BrI, BrII and BrIII. Seven of the 16 genotypes were reported for the first time in this study. Three of the 36 isolates showed mixed infections. Analysis of mortality rates in infected mice indicated that Type BrI is highly virulent, Type BrII is intermediately virulent and Type BrIII is non-virulent, which is in agreement with previous report. The allele types at the CS3 locus are strongly linked to mouse-virulence of the parasite. These genotyping results support previous findings that the *T. gondii* population is highly diverse in Brazil.

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