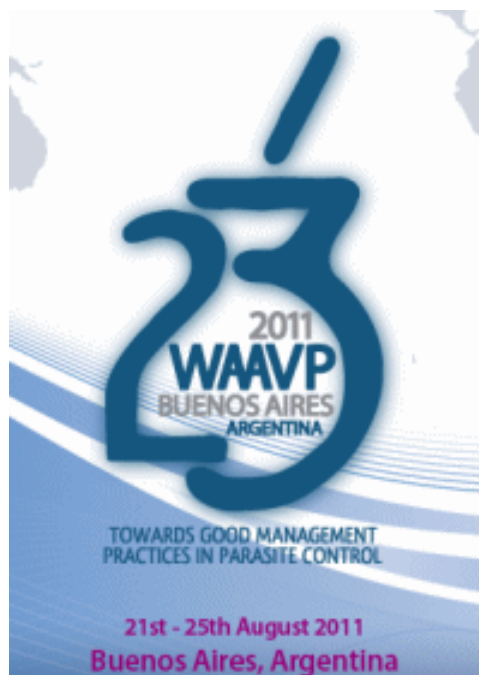


The 23rd International Conference of the
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“Towards Good Management Practices in
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Towards sustainable parasite control practices in livestock production with emphasis in Latin America

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Endo and ectoparasites of domestic ruminants directly or indirectly contribute to reduce sustainability affecting Food Security in subsistence or small scale farming systems, Food Safety (Food Borne diseases and pesticide residues), Environment (pesticides pollution and ecotoxicity) and farmer's equity (limited or uneven access to relevant technical information/training). This is especially true for some regions of Latin America where there still are huge areas of natural grazing land for cattle, sheep and goats. Sustainable parasite control is not an absolute concept given the different regions and productive systems of the world and therefore, could have different level of farmer's adoption and impact. This article develops a conceptual framework to better understand how far is each region or country from attaining a reasonable increase in animal production while preserving sustainability. Within this context the capacity to prioritize the target parasites species for control according to local epidemiology and production system, the early diagnosis and monitoring of parasite resistance as well as the availability of well trained field professionals acquire here a major role creating an enabling environment for present and future decision support systems approach. Until new and different ways of controlling parasites become available; the challenge is to utilize Good Animal Husbandry Practices and IPM principles in a pragmatic way allowing the rational use of pesticides.

Nanomedicine: novel approaches in human and veterinary therapeutics

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Background: Nanomedicine can be defined as the application of nanotechnology to the prevention and treatment of diseases as well as for diagnosis purposes. In this context, the development of various types of drug-carrier nanodevices offers new strategies for targeted drug delivery.

Methods: This review is divided in two different parts. The first one summarizes the main types of nanomedicines developed in the past few decades, including drug nanocrystals, polymer therapeutics, lipid-nanosized drug delivery systems and polymeric-nanosized drug delivery systems. The second part of our review is devoted, more specifically, to the presentation of polymeric nanoparticles. Here, we discuss various aspects of nanoparticle formulation, characterization, behaviour in the body and some of their potential applications.

Results: More particularly we present some approaches for the treatment of cancer, treatment of infectious diseases and the potential of these nanoparticles as adjuvants for vaccination purposes. The rationale of using nanoparticles for tumor targeting is based on the deliver of the anticancer drug in the vicinity of the tumor, minimising the secondary effects and the toxicity associated to drug widespread to healthy organs or cells. On the other hand, the control of infectious disease is a key area in health, and consequently very good considered in the nanomedicine praxis. For treatment, pathogens may reside in different habitats, intracellular, extracellular or both and this aspect will condition the rational for the use of any type of nanoparticles. For prophylaxis, nanotechnology offers new alternatives to produce new adjuvants, more convenient to use, safer and easy to administer than the classical ones. Furthermore, the new adjuvants based on nanoparticles may be susceptible to be used even through mucosal routes.

Summary: The introduction of nanotechnology in the pharmaceutical field has revolutionized the possibilities for drug delivery, increasing the possibilities for the emerging of new treatments with a higher specificity and lower secondary effects.

Plenary: Molecular diagnosis of infections and resistance in veterinary and human parasites

P. W. Hunt

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Since 1977 >2000 research papers described attempts to detect, identify and/or quantify parasites, or disease organisms carried by ecto-parasites, using DNA-based tests and 149 reviews of the topic were published. Despite this, only a few DNA-based tests for parasitic diseases are routinely available, and most of these are optional tests used occasionally in disease diagnosis. Malaria, trypanosomiasis, toxoplasmosis, leishmaniasis and cryptosporidiosis diagnosis may be assisted by DNA-based testing in some countries, but there are very few cases where the detection of veterinary parasites is assisted by DNA-based tests. The diagnoses of some bacterial (e.g. Lyme disease) and viral diseases (e.g. tick borne encephalitis) which are transmitted by ecto-parasites are more commonly use DNA-based tests, and research developing tests for these species makes up almost 20% of the literature.

Other important uses of DNA-based tests are for epidemiological and risk assessment, quality control for food and water, forensic diagnosis and in parasite biology research. Some DNA-based tests for water-borne parasites, including *Cryptosporidium* and *Giardia*, are used in routine checks of water treatment, but forensic and food-testing applications have not been adopted in routine practice. Research, including epidemiological research, makes the widest use of DNA-based diagnostics, delivering enhanced understanding of parasites and guidelines for managing parasitic diseases.

Despite the limited uptake of DNA-based tests to date, there is little doubt that they offer great potential to not only detect, identify and quantify parasites, but also to provide further information important for the implementation of parasite control strategies. For example, variant sequences within species of parasites and other organisms can be differentiated by tests in a manner similar to genetic testing in medicine or livestock breeding. If an association between DNA sequence and phenotype has been demonstrated, then qualities such as drug resistance, strain divergence, virulence, and origin of isolates could be inferred by DNA-based tests. No such tests are in clinical or commercial use in parasitology and few tests are available for other organisms.

Why have DNA-based tests not had a bigger impact in veterinary and human medicine? To explore this question, technological, biological, economic and sociological factors must be considered. Additionally, a realistic expectation of research progress is needed. DNA-based tests could enhance parasite management in many ways, but patience, persistence and dedication will be needed to achieve this goal.



SESSION A

Keynote Lecture

- **Challenges of nematode control in ruminants: Focus on Latin America** - M. B. Molento

Oral Communications

- **Estimating the diagnostic parameters specificity and sensitivity for faecal egg count reduction tests** - R.J. Dobson
- **Epidemiological study of gastro-intestinal nematodes of sheep in Uruguay Prevalence and seasonal dynamics** - D. Castells
- **The epidemiological consequences of resistance to *Teladorsagia circumcincta* in grazing sheep as assessed using a mathematical model** - Y. Laurenson
- **Effect of parasitism on bodyweight and reproductive performance in adult sheep** - W. E. Pomroy
- **Parasite control in organic fattening system in a temperate area of Argentina** - C. Entrocasso
- **Investigating the feasibility of targeted selected anthelmintic treatment in first season grazing calves on Scottish organic and conventional dairy farms** - A. Jackson
- ***Cooperia punctata*: Effect on cattle productivity?** - B. Stromberg
- **Treatment and Progression of Nematode Parasitisms in Feedlot Cattle** - T. A. Yazwinski

Posters

- **Dynamic of inhibition-desinhibition of *Ostertagia ostertagi* in a natural grazing production system** - C. A. Fiel
- **Administration of long-acting moxidectin to pre-pubertal ewe-lambs results in improved reproductive performance** - G.C. Fthenakis¹
- **Coprological prevalence of gastrointestinal helminths in South American camelids from the Northwest of Argentina** - M. M. Cafrune
- **Longitudinal Study of Gastrointestinal Worm Burdens in First Season Grazing Calves on Scottish Organic and Conventional Dairy Farms** - A. Jackson
- **Anthelmintic efficacy of 2.5% w/w quebracho condensed-tannins (CT) extracts in sheep infected with gastrointestinal nematodes** - J. Schapiro
- **Dynamics of Fecal Eggs Shedding of *Trichostrongyles* Nematodes in Cow Herds of the Southeast of Córdoba (Argentina)** - C. O. Descarga
- **A Preliminary Study Using Effective Microbes for Worm Control in Goats in Malaysia** - P. Chandrawathani
- **ParaCalc® – a novel tool to evaluate the economic importance of worm infections on the dairy farm** - J. Charlier
- **Sheep infected with gastrointestinal nematode parasites consume more medicated feed block** - F. Fishpool
- **Ovicidal and larvicidal activity of the crude extracts from *Phytolacca icosandra* against *Haemonchus contortus*** - M.M. Hernández-Villegas
- **Gastrointestinal parasite control measures in use within the Central Tablelands of New South Wales** - A. Carr
- **Parasites in buffaloes – Corrientes, Argentina** - O. Racioppi
- **Controlling resistant nematodes in dairy sheep: a balance between drug efficacy and milk residues** - J. Sallovitz
- **Phenotypic characterisation of two nematodes isolates obtained from an anthelmintic resistance survey on Scottish cattle farms** - D. Bartley
- **Strategic drenching program in Organic Farming in the Czech Republic** - E. Vernerova
- **Efficacy of an energy block containing *Duddingtonia flagrans* in the control of gastrointestinal nematodes of sheep** - M. F. Sagüés
- **Soy protein polymers as controlled release system of fungi nematophagous to the control nematode para sites in ruminants** - M. F. Sagüés
- **Study of inhibition-desinhibition of *Ostertagia ostertagi* under simulated grazing periods in the Centre - Eastern Province of Buenos Aires** - J. F. Gélvez
- **Detection of the β -tubulin Phe200Tyr allele in *Haemonchus placei* populations isolated from cattle in Minas Gerais, Brazil** - B. S.A.F. Brasil
- **Nematode egg counts during the first postpartum month is a useful tool for milk production prediction in grazing Holstein cows** - M. E. Mejía
- **Circulating IGF-1 levels and mammary gland development in antiparasitic treated and untreated prepubertal Holstein heifers** - A. Perri
- **Interactions between gastrointestinal nematodes and subclinical mastitis in low-input dairy sheep management systems in Greece** - N. Tzanidakis
- **The effect of the ambient temperature on the development and survival of the infective stage larvae of *Cooperia oncophora*** - C.W. Saueremann
- **Larval challenge does not cause production loss in sheep given continuous anthelmintic treatment** - G. Kelly
- **Accuracy and Precision of Sampling for Worm Egg Count Estimates** - L. Kahn

- **The effect of four-weekly eprinomectin treatment on milk production in seasonally-calving pasture-based dairy cattle** - W. A. Mason
- **Influence of the order of evaluation of sheep and goat conjunctiva by people under training on FAMACHA System** - F. Rosalinski-Moraes
- **Targeted selective treatments of first season grazing cattle** - J. Höglund
- **Ivermectin and Moxidectin Resistance Factor in Cooperia spp. in Beef Cattle in Mato Grosso do Sul, Brazil** - F. de A. Borges
- **Risk factors in the development of bovine anthelmintic resistance in the Pampeana Region of Argentina** - S. L. Cristel
- **Gastrointestinal parasitism in small ruminants on small farms, in the Caatinga biome of the state of Bahia, Brazil** - M. A. Ornelas-Almeida
- **Soil moisture influences the moisture content of sheep faeces** - K. Saad
- **Control of the periparturient rise in faecal Teladorsagia circumcincta egg output of ewes using moxidectin 2% w/v injection** - D. J. Bartram
- **Efficacy of Zolvix (monepantel) in sheep against gastro-intestinal parasites resistant to currently available anthelmintics** - P. Rolfe
- **Efficacy and pharmacokinetics of Zolvix (monepantel) against gastro-intestinal parasites in goats** - P. Rolfe
- **Toxicity of Bacillus thuringiensis proteins against the blood-feeding nematode Haemonchus contortus** - M. E. López-Arellano
- **Re-evaluation of Efficacy against Nematode Parasites and Pharmacokinetic of Topical Eprinomectin in Cattle** - S. Rehbein
- **Effects of a protein supplement and anthelmintic treatment on communally grazed Tswana goats naturally infected with gastrointestinal nematodes** - F. R. Bakunzi
- **Monepantel against arrested gastrointestinal nematode larvae in sheep** - H. Sager
- **Parasite migration and mucosal antibody levels in two Canarian sheep breeds showing differential resistance to Haemonchus contortus** - J. F. González
- **Identification of Gastrointestinal Nematodes in Crossbreed Goats with Different levels of Resistance** - L. Giotto Zaros
- **Evaluation of methods of control gastrointestinal nematodes in sheep herd** - H. Rocha de Medeiros
- **Low and high FEC sheep productive performance under extensive grazing conditions** - M. V. Benavides
- **Alternanthera pungens and Colletia paradoxa, as Potencial New Anthelmintic Parasitics** - J. Saldaña
- **Effect of plant protein source on periparturient resistance to parasites in ewes** - P. Sakkas
- **Effect of feeding Santa Cruz sheep with nutritional pellets containing Duddingtonia flagrans chlamydospores in the reduction of Haemonchus contortus (L3) in feces** - P. Mendoza de Gives
- **Comparison of an individual versus a composite sampling method for counting gastrointestinal nematode eggs in sheep feces** - C. Guerrero-Molina
- **Prevalence of anthelmintic resistance in gastrointestinal parasites of South American camelids in the Southeastern United States** - B. Storey
- **Efficacy of avermectins against naturally acquired intestinal nematodes in Girolando calves from a commercial herd in Vale do Mucuri, southeastern Brazil** - M. do S. Veloso Leite Ferraz da Costa
- **Efficacy of a Limonen Formulation in Sheep Artificially Infected with Haemonchus contortus** - A. C. S. Chagas
- **Strategic integrated control of Haemonchus contortus in sheep in Passo Fundo, Southern Brazil** - M. I. B. Vieira
- **Correlation between Famacha and Packed Cell volume in Crossbred Goats with Different Levels of Resistance** - L. da Silva Vieira
- **Experimental cross infection of Haemonchus contortus from sheep to cattle** - M. Guzmán
- **Macrocyclic lactone resistance in an isolate of ovine Trichostrongylus colubriformis from North Queensland** - M. Knox
- **The use of Stylosanthes guianensis cv. Mineirão and Medicago sativa hays to control gastritestinal nematodes in feedlot lambs** - D. Sobreira Rodrigues
- **In vitro effect of Tagetes minuta and Mentha villosa decoctions on eggs and larvae of Haemonchus contortus** - I. T. F. Macedo
- **Development of real-time RT-PCR procedure to determine the relative expression of interferon gamma in response to Haemonchus contortus infection in Pelibuey lambs localized in tropical areas** - E. López-Arellano
- **Influence of Gastrointestinal Nematodes of quality lamb (Ovis aries) meat** - S. Valledor
- **Selection of ewes with low and high trichostrongylids eggs count in Argentina** - J. Romero
- **Evaluation of the eggs development of Trichuris vulpis at laboratory conditions** - M. L. de A. Rodrigues
- **Efficacy of Monepantel against multiple anthelmintic resistant nematodes of sheep in the subtropical area of Argentina** - P. Steffan
- **Safety and Endoparasitic Activity of a New Molecule (Aurixazole) in Cattle** - A. J. Costa
- **Risk factors for the development of bovine anthelmintic resistance in Argentina** - V. H. Suarez

Challenges of nematode control in ruminants: Focus on Latin America

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Gastrointestinal nematodes (GIN) of ruminants (cattle, sheep and goats) are ubiquitous and can cause severe injuries to infected animals and significant losses in farming revenues. GIN are able to survive severe environmental and host conditions, but mankind has developed a number of ingenious methods for parasite control. The commerce and use of modern anthelmintic drugs with a broad spectrum of activity has been a solid tool for nearly 40 years but the continuous use of these drugs, however, has led to the selection of populations of drug-resistant worms worldwide. At present, the ever-growing agricultural systems in Latin America are facing many challenges and cannot rely on the far-reaching objective of parasitic elimination from the host or the environment. The lack of extensive programs for monitoring drug resistance exacerbates the negative consequences of reduced efficacy, which is evident in some areas with the increase in mortality rate even after treatment. Experts agree that new schemes of parasitic control are needed and should be based on the strategy of targeted selective treatment where affected hosts are identified and treated accordingly. In this article, we will focus our discussion on the challenges for the control of GIN in Latin America by 2020 imposed by reduced drug efficacy. We will evaluate phenotypic and molecular markers, methods for single-animal evaluation, and the implementation of schemes for anthelmintic treatment that address parasites in refugia.

Estimating the diagnostic parameters specificity and sensitivity for faecal egg count reduction tests

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Background: The faecal egg count reduction test (FECRT) is the primary tool for estimating anthelmintic efficacy and diagnosing drug resistance. However, only one review of its ability, in sheep, to correctly diagnose susceptibility i.e. specificity (Sp) and correctly diagnose resistance i.e. sensitivity (Se) has been published.

Methods: Data sets, from experimental infections, where drug efficacy was determined by both controlled slaughter studies (the gold standard) and FECRT were used to estimate Sp and Se of the FECRT. There were 35 and 17 susceptible and resistant cases, respectively. Of these cases 24, 11, 4, 4 and 9 had efficacies of 100%, <100 to 95%, <95 to 90%, <90 to 60% and <60%, respectively. Nine of the sets were from cattle and the remainder from sheep, covering 10 nematode species. FECRT efficacy was estimated by (1) pre- and post-treatment egg counts of the same animals and (2) post-treatment egg counts of control and treated animals.

Results: For both FECRT methods Sp was 100% i.e. all susceptible cases were correctly diagnosed as susceptible. Correct diagnosis of resistance (Se) was 94% and 88% for FECRT methods (1) and (2) respectively.

Conclusions: The high Sp and Se observed here for the FECRT methods were based on experimental infections and probably represent the best possible values, in the field lower values could be expected. Similar estimates were made by McKenna (2006, N.Z. Vet. J. 54, 365–366) for FECRT in sheep. However, FECRT in cattle and horses from the field are likely to have reduced precision because of low counts, highly aggregated distributions of counts and small group sizes for horses; this will be discussed in relation to the best estimates of confidence limits for efficacy.

Epidemiological study of gastro-intestinal nematodes of sheep in Uruguay

Prevalence and seasonal dynamics

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Helminth parasites are one of the most important causes of productivity losses in sheep livestock in Uruguay. The previous epidemiological information was obtained partially during the 50's and a complete study carried out in 1974. The development of anthelmintic resistance, changes in production systems, global warming, among others, requires an update in the epidemiological inventories. The study was carried out in 6 farms distributed throughout the country from March 2007 to March 2009. Every 45 days, 12 helminth-free tracers lambs (n=192) grazing with the main flock of the farm and them were slaughtered for worm count. In two farms previous an after the grazing period the tracers were maintained in housed pens free of helminth infections to study hypobiosis. Cumulative mean worm burden was 3936 with maximum of 9824 (Variation Coefficient V.C. = 0.63) in May 2007 and minimum of 667 (V.C. = 0.60) in February 2008. Overall seasonal dynamics showed a clear peak of nematodes in autumn (2007 and 2008). *Haemonchus contortus* (35.1%) and *Trichostrongylus colubriformis* (31.9%) were the most abundant species (67% of total), followed by *Trichostrongylus axei* (10.3%), *Nematodirus spathiger* (7.7%), *Cooperia* sp. (7.5%) and *Teladorsagia circumcincta* (4.8%). There was a low intensity of *Strongyloides papillosus* (1.6%), *Trichuris ovis* (0.6%) and *Oesophagostomum venulosum* (0.5%). Highest counts of *H. contortus* were observed in autumn and the lower counts in winter, whilst the largest numbers of *T. colubriformis* and *T. axei* were observed in winter. In winter 35 to 75% of *H. contortus* were found as larvae stages (Hypobiotic). In conclusion, the relative abundance of the main gastrointestinal nematodes species of sheep an their seasonal dynamics, were updated in Uruguay. In agreement to the previous information *Haemonchus* sp. and *Trichostrongylus* sp. were the most abundant genera. Seasonal dynamics show a peak of *Haemonchus contortus* in autumn and *Trichostrongylus* sp. in winter.

The epidemiological consequences of resistance to *Teladorsagia circumcincta* in grazing sheep as assessed using a mathematical model

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Introduction: A mathematical model was developed to address the consequence of sheep nematode resistance on the epidemiology of parasite dispersion. The model was used to explore separately the impact of initial larval pasture contamination level and the impact of sheep resistance on subsequent pasture contamination.

Method: To investigate the impact of initial pasture contamination level, a population of immunologically naïve lambs was modelled grazing on a medium quality pasture, initially contaminated with 1000, 3000 or 5000 *Teladorsagia circumcincta* larvae per kg DM grass from weaning to slaughter. To explore the impact of resistance status, the 10% most (R) and the 10% least (S) resistant lambs from the modelled population were grazed separately on a pasture with an initial contamination of 1000 larvae per kg DM grass, and predicted pasture contamination was compared to that of the entire population grazed on a pasture with the same initial contamination level.

Results: As expected, the pasture contamination increased with increasing levels of initial larval contamination. For the 1000, 3000 and 5000 larval contamination levels the average predicted pasture contamination (larvae/kg DM grass) were 1670 (maximum = 2481, day 77), 2336 (maximum = 3218, day 56) and 2966 (maximum = 4068, day 50), respectively. For the investigation into sheep resistance, large differences were predicted for pasture contaminations between the R and S groups, and these differences increased as the grazing season progressed. The average pasture contamination predicted (larvae/kg DM grass) for R and S were 555 (maximum = 916, day 43) and 3365 (maximum = 5909, day 94), respectively, compared to 1670 (maximum = 2481, day 77) for the entire population. The predicted pasture contaminations (larvae/kg DM grass) at slaughter, for the same groups, were 137, 4697 and 1436, respectively.

Conclusions: Both initial pasture contamination level and sheep resistance have large impacts on predicted pasture contamination. Further, the effect of resistance status of sheep on pasture contamination increased with time.

Effect of parasitism on bodyweight and reproductive performance in adult sheep

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Background: The effects of parasitism in adult sheep in New Zealand are poorly understood.

Methods: To estimate the production benefits of targeted anthelmintic treatment of 'light' ewes, 500 'light', mixed-age ewes (average weight 50-51kg) were selected soon after weaning (January), in both 2008 and 2009. They were individually identified and 250 randomly selected ewes were then treated with a BIONIC® capsule (Merial NZ Ltd) which releases albendazole and abamectin in combination continuously over approximately 100 days. The 500 Romney cross-breed ewes were managed as part of a larger flock of 2500 ewes. Bodyweights and faecal egg counts (FECs) with pooled larval cultures by treatment group were measured at the beginning of the trial period (January), before mating (March) and at ultrasound pregnancy scanning (June). A different set of ewes were selected in 2009.

Results: In January the FECs were low (mean 120 eggs/g) in 2008 and zero in 2009 (reflecting recent anthelmintic treatment of all ewes). By March (whilst the capsule was still active) the mean FECs in the untreated control ewes had increased to 792 (range 0 - 8650) eggs/g in 2008 and 726 (range 0 - 12300) eggs/g in 2009. *Haemonchus* was the dominant genus. Low numbers of worm eggs were also found in the capsule-treated ewes, mean 41 eggs/g in 2008 and 36 eggs/g in 2009. By June (at least 60 days after expiry of these capsules) the mean FECs of all groups had reduced, although the capsule-treated ewes had slightly lower mean counts; (capsule-treated 49 eggs/g, control 91 eggs/g ($p<0.05$) in 2008 and capsule-treated 53 eggs/g, control 111 eggs/g ($p<0.01$) in 2009). Treating 'light' ewes in January, soon after weaning, using a combination anthelmintic capsule resulted in small gains in liveweight ranging from 1 – 2 kg. Whilst the pregnancy rate was similar between both groups in both years the scanning percentage was increased by 8% ($p=0.09$) in 2008 and by 13% in 2009 ($p<0.01$) reflecting an increase in multiple pregnancies in treated ewes.

Conclusions: These results highlight the loss of production that can be seen in these 'light' ewes that results from parasites and indicates that control of parasites by some sustainable means will likely improve productivity from these sheep.

Parasite control in organic fattening system in a temperate area of Argentina

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Gastrointestinal parasites are well known to produce severe losses in fattening cattle of Argentina. The excessive use of drug applied without epidemiological knowledge lead to an increase in anthelmintic resistance. Rational use of drugs avoid resistance and food-environmental contaminations. .

The aim of this work was to develop a parasite control program according with organic rules in a sustainable fattening cattle production, minimizing use of drugs.

A paddock of 18 ha was divided in two of nine (A and B), with degraded perennial pastures was used. Each paddock had four months of grazing and eight of resting, from August 2004 till December 2008. Excess of grass was harvested for hay No grazing animals during summers and grain supplementation in winter time was needed. This parasitological management was required after a sever clinical parasitosis appeared in winter 2004. The "agronomist judgment" on grazing system, used several years before, with many plot rotations and allowed to reach heavy larval burden. Paddock A were grazed from April to August, without plot rotations allowing pasture selection, and paddock B from August to December using few plot rotations in spring.

Forty to fifty weaned calves of six to eight month of age were used each year, depending on grass availability at start in April (autumn).

Strategic supplementation with grain and hay during winter, under organic rules, was applied.

Food quality, grass and larval availability (L 3/kgDM) and fecal samples (epg) from calves, were taken every 21-30 days. Anthelmintic treatment was applied based on risk evaluations with animal, parasite, grass dates and weather conditions. Statistic analysis was made using Glimix procedure of SAS, including year effect.

Heavy pasture infestations (9900 L3/kgDM) in paddock A and clinical parasitosis in calves occurred during winter 2004. Change in grass management with strategic treatment (levamisole) had to be applied in August with permanent parasite surveillance from there. No following problem was found, being significant less larval count ranking from 5 to 572 L3/kg DM during years 2005-2008.

The egg count (epg) in 2004 reach an annual average of 160 (0-784) and from 93 to 34 in following years (0-210). The grass and animals management was appropriated for good animal productions terms (500-600 gr/d/an) considering food offered with one treatment in five years.

Investigating the feasibility of targeted selected anthelmintic treatment in first season grazing calves on Scottish organic and conventional dairy farms

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Background: Adequate control of gastrointestinal parasitism can be achieved using highly effective anthelmintics and grazing management. However, there is interest in alternatives to the programmed use of anthelmintics in order to optimise efficacy and to reduce selection for resistance. Targeted selective treatment (TST) has been evaluated in small ruminants. TST is based on treating only a proportion of a group of animals that will benefit the most from anthelmintic treatment; thus leaving a pool of unselected parasites in an untreated group. In order for TST to be successful, there must be reliable markers or a system that identifies the individuals requiring treatment. This study aimed to investigate the feasibility of a TST approach for use on first season grazing calves (FSG) under both organic and conventional management.

Methods: All FSG (total n=105) from three dairy farms (2 organic, 1 conventional) were recruited in central and south west Scotland in 2010. At each visit calves were body condition scored and their weight estimated with girth bands. Blood and faecal samples were collected for measuring faecal egg counts (FEC) and serum pepsinogen. At 8 weeks post-turnout calves that had an average growth rate of <0.75kg/day since turnout were treated with eprinomectin topically. The other calves in the group were left untreated. At 12 weeks post-turnout calves that had an average growth rate of <0.75kg/day since the last visit were treated with eprinomectin only if they had not been treated previously. At the end of the grazing season, growth rate data were compared to calf performance on these farms over the previous grazing season.

Results: Across all farms, approximately 50% of FSG were treated at 8 weeks post turnout. At 12 weeks post turn out the percentage treated varied by farm. Following a TST approach resulted in improved live weight gain (LWG) with less anthelmintic treatments on both organic farms compared to previous years, however, end of season pepsinogen levels were high. FEC did not correlate with LWG. This study suggests that TST based on rate of daily live weight gain may have a useful role in organic livestock systems.

Cooperia punctata: Effect on cattle productivity?

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Cooperia spp. have become the most prevalent parasite in US cow/calf operations as observed in the NAHMS Beef Cow/Calf survey in 2008. This is at least in part due to the widespread use of endectocides that have minimal activity against these parasites. The effects of *Cooperia* spp. on cattle productivity are largely unknown, and this study was conducted to assess such effects. Two hundred calves (average weight 460 lb) were acquired from northwestern Arkansas and northeastern Oklahoma and were vaccinated and dewormed upon arrival. Animals were preconditioned for approximately one month and fed a standard growing ration throughout the study. At 4 weeks, all calves were dewormed a second time, re-vaccinated, moved to pens equipped with GrowSafe® system feed bunks and given an additional week to acclimate. Calves were randomly divided into two groups (n=80) and each group was further divided into two replicate pens (n=40). On day 0 and 14, two pens were orally inoculated with a gavage of 1×10^5 and 0.825×10^5 *Cooperia punctata* infective larvae, respectively, with the control pens receiving a similar volume of tap water. Data collected included biweekly fecal egg counts, daily individual feed consumption and weight gain over the 60 day test period. The presence of *Cooperia punctata* (>99%) was confirmed by necropsy on days 35 and 60 post infection (PI) in a subset of animals. Egg counts were positive by day 14 PI and remained at levels similar to values seen in field studies. The sham-treated group gained weight 7.4% more rapidly ($p = 0.02$) than inoculated animals (3.24 lb vs 3.0 lb ADG, respectively). The inoculated animals also consumed 1.5 lb (DMI) less compared to sham treatment ($p = 0.02$). Data suggested that *C. punctata* has a deleterious affect on both appetite and nutrient uptake or utilization. Mesenteric lymph nodes were increased in size and the small intestinal mucosa was thickened with an increased amount of mucus. The most prominent histological changes in the small intestine involved mild to moderate numbers of intraepithelial lymphocytes (IEL) and globule leukocytes as well as aggregates of eosinophils within the lower lamina propria. Treatment of one group (n=40) of infected calves with an endectocide did not remove the parasites (FECRT = 8.8%), while treatment of the other group with a benzimidazole was effective (FECRT = 98.1%).

Treatment and Progression of Nematode Parasitisms in Feedlot Cattle

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Nematode parasitisms of feedlot cattle is one of the least investigated conditions in large animal parasitology. Additionally, it is during this phase of production that the most expensive tenure of the animal is occurring; a time when parasitisms can be most economically detrimental and when parasiticide effectiveness should be of greatest spectrum and degree. To investigate the above, 100 animals were selected at random from approximately 2000 animals received during a 5 day period at a feedlot in east-central California. Also by random selection, 92 of the 100 animals received a generic, injectable preparation of ivermectin (0.2 mg kg⁻¹BW) combined with clorsulon (2.0 mg kg⁻¹BW). The remaining 8 animals were left as untreated controls. At two weeks post-treatment, the 8 control and 8 randomly-selected treated cattle were shipped to Arkansas for continued housing in dry lot; with animal harvest and nematode quantifications at approximately 6 weeks post-treatment. At the end of feedlot (25/Feb/2011), nematode quantifications were performed on 9 animals selected at random from the remaining 84. Fecal samples were obtained from the study animals at pre-treatment and again periodically post-treatment for the determination of EPG and coproculture L3 counts. Based on fecal egg count reductions, no evidence of anthelmintic effectiveness was observed for any patent nematode genus. For the animals sacrificed at 6 weeks post-treatment, nematocidal efficacies of 0%, 30% and 48% were recorded for adult burdens of *Cooperia (punctata and oncophora)*, *Ostertagia ostertagi* and *Haemonchus placei*. No significant infections by immature stages were present in the above animals. At the time of this writing, nematode counts had not been performed on the cattle harvested at the end of feedlot. At the time of delivery to the feedlot, 95% of the animals were patent for nematode infections (average EPG of 62). At 120 days into feedlot, only 35% of the animals remained patent (average EPG of 2); this, despite the relative lack of efficacy for the endectocide used at receiving.

Dynamic of inhibition-desinhibition of *Ostertagia ostertagi* in a natural grazing production system

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From September 2006 to March 2007, was determined the seasonal incidence of the most common gastrointestinal nematodes and particularly for *Ostertagia ostertagi* the period during which the inhibition-desinhibition was greater. A group of twelve calves called “permanent or collectors” were introduced to grazing conditions. The calves were kept under continuous grazing natural pasture and subsequently were withdrawn in monthly pair and were housed and fed hay free of parasites and water *ad libitum*. The calves in pairs were slaughtered on day 15 post housed period. At the time of slaughter animals were sampled for fecal egg count per gram (EPG), and were obtained aliquots of the contents from the abomasum and intestines for worms count and identification, extraction of abomasum mucosa for count and identification of hypobiotic or inhibited stages and blood for pepsinogen measurement. It was observed an initial inhibition around 60% in October, which was maintained in November, and then rise in December with values near to 75%. Two phases of desinhibition were observed, the first one between December and January when was observed a declination of inhibited larvae of about 40%, while that between February and March was observed the second and final one.

In permanent pasture, the cumulative effect of environmental conditions on larvae in the pasture would be the main cause of inhibition of *Ostertagia ostertagi*. There would not be a unique and massive larvae desinhibition, because two well defined phases are already showed.

Administration of long-acting moxidectin to pre-pubertal ewe-lambs results in improved reproductive performance

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Objective: Objective was the evaluation of reproductive effects after one administration of a long-acting antiparasitic (moxidectin 2% LA, dose rate: 1 mg per kg bodyweight) to pre-pubertal ewe-lambs at the beginning of the reproductive season. The study was performed in Greece (latitude N 36° 26') in a flock free from brucellosis, *Chlamydophila* infection and toxoplasmosis.

Methods: In total, 45 animals, naturally infected with trichostrongylids, were allocated into treated (n= 30, treatment with moxidectin 2% LA, carried out on the 21st June, [D0]) or control (n=15, untreated) group. Rams of confirmed fertility, were introduced from the 15th August [D55] to the 20th December [D182] into the ewe-lambs. Throughout the study, epg counts of ewes were monitored. Reproductive performance of ewes was assessed.

Results: Up to D112, arithmetic mean epg counts in treated animals were 0 epg; thereafter and up to D350, they were 23 to 473 epg. Respective figures for control animals throughout the study were 190 to 977 epg. Reproductive performance parameters were calculated as follows, respectively for treated and control animals; median '*Interval to first mating after ram introduction*': 36.5 d and 71.0 d ($P=0.04$); median '*Age at first mating*': 8.5 m and 10.0 m ($P=0.045$); '*Cycling rate*': 20.0% and 6.7% ($P=0.03$); '*Mating rate*': 86.7% and 66.7%; '*Return-to-oestrus rate*': 26.7% and 26.7%; '*Abortion rate*': 3.3% and 0%; '*Lambing rate*': 83.3% and 66.7%; '*Total lambs born per ewe*' and '*Liveborn lambs born per ewe*': 1.5 and 1.1 ($P=0.01$); '*Stillbirth rate*' 0% and 0% and '*Lamb bodyweight per ewe*': 5.0 kg and 3.8 kg ($P=0.005$).

Conclusions: Long-acting anthelmintic treatment of pre-pubertal ewes results to earlier reproductive activity and improved reproductive performance of the animals. Hence, it may be employed as a management strategy according to targets set in individual flocks.

Coprological prevalence of gastrointestinal helminths in South American camelids from the Northwest of Argentina

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Parasitic gastroenteritis of South American camelids (SACs) is caused by a variety of helminths. Some of them (all nematodes) appear to be fairly host-specific (i.e. *Graphinema aucheniae*, *Mazamastrongylus* (= *Spiculopteragia*) *peruvianus*, *Camelostrongylus mentulatus*, *Nematodirus lamae*, *Lamanema chavez*, *Trichuris tenuis*, *Capillaria* sp.) while others are shared with domestic ruminants (several species of Trichostrongylidae, *Fasciola hepatica*, tapeworms). This abstract reports the coprological prevalence of different helminths in domestic llamas and wild vicuñas from the Northwest of Argentina (NWA), a region of the country that supports almost 95% of the llama stock and about 75% of the vicuña population. Faecal samples were obtained from 708 adult llamas (89 herds) and 222 vicuñas (seven populations) from three Provinces of the NWA (Jujuy, Salta and Catamarca). Most vicuñas were maintained in semi-captive conditions. The majority of the llamas and wild vicuñas were raised without anthelmintic treatment. Samples were individually assessed by two methods: the modified MacMaster technique for Trichostrongylidae and *Strongyloides* eggs, and the sedimentation-flotation technique described by Cafrune *et al.* (Vet. Parasitol. 2009, 166: 321) for specific helminths of SACs and *F. hepatica*. Eggs of Cestoda were registered by both techniques. Individual prevalence was calculated as the number of SACs showing eggs of each helminth related to the total number of each host species sampled. *Trichuris* sp. shows the highest prevalence in llamas (38.8%) and vicuñas (47.3%), followed by *Lamanema chavez* but only in llamas (18.5%). Eggs of *Capillaria* sp. showed similar prevalence in both SAC species (7.3% and 7.2%) as it also occurs with *F. hepatica* (4.2% and 4.1%). *Nematodirus* sp. was found in 5.1% of the llamas and 2.3% of the vicuñas. Eggs of Trichostrongylidae were observed in 19.3% and 3.2%, and eggs of Cestoda in 9.8% and 2.7% of the llamas and vicuñas, respectively. *Strongyloides* sp. was found in 2.7% of the llamas and 0.5% of the vicuñas. As expected among SACs in their original habitats, eggs of host-specific nematodes appeared to be more prevalent than other helminths.

Longitudinal Study of Gastrointestinal Worm Burdens in First Season Grazing Calves on Scottish Organic and Conventional Dairy Farms

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Background: In the UK organic dairy industry the use of veterinary medicinal products for preventive treatment is prohibited; meaning that livestock have to be managed in such a way as to avoid the prophylactic use of anthelmintics. UK organic regulations state that where a disease risk is identified and the welfare of animals cannot be guaranteed by management practices alone, veterinary treatments may be administered. In practice, this means the farmer has to prove the presence of gastrointestinal parasites, usually by means of faecal egg counts (FEC) in order to treat with an anthelmintic. Conversely, conventional farmers can treat calves prophylactically with anthelmintics. There are limited data available on the effects of these two separate approaches to gastrointestinal parasite control on the calf health and productivity. In this study first season grazing (FSG) cattle in organic and conventionally farmed herds were monitored over a 12-month period to determine the patterns of helminth infection and animal over time.

Methods: Six dairy farms (n=3 organic and n=3 conventional) were recruited in central and south west Scotland in 2009 and FSG animals (n=15) were selected on each farm. Farms were visited four times during the grazing season. At each visit the sentinel animals were body condition scored, weighed, and blood and faeces were collected. FEC was conducted on faeces and blood samples were analysed for serum pepsinogen, fructosamine and *O. ostertagia* specific antibody concentration. Data were analysed to investigate associations between growth rate and markers of gastrointestinal parasitism by animal, farm and system.

Results: All organic farmers used anthelmintic treatments during 2009; in each case following concerns over poor performance and following FEC conducted outwith this study. Organically farmed calves had significantly lower growth rates compared to the conventionally farmed calves and significantly higher FEC and plasma pepsinogen levels.

Conclusions: These results suggest that gastrointestinal parasitism can impact organic dairy youngstock, leading to lower productivity and a higher risk of disease.

Anthelmintic efficacy of 2.5% w/w quebracho condensed-tannins (CT) extracts in sheep infected with gastrointestinal nematodes

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Control of gastrointestinal nematodes (GIN) in sheep is usually achieved by the use of synthetic anthelmintics. However, continuous use of these products has led to the development of anthelmintic resistance. A way to counteract the emergence of this problem is to minimise the use of synthetic anthelmintics by using new alternative treatments such as nutraceuticals. Therefore, we wanted to evaluate the anthelmintic efficacy of condensed-tannins (CT) extracts from quebracho (*Schinopsis balansae*).

The objective was to evaluate the anthelmintic efficacy of 2.5% w/w quebracho CT extracts when administered along with the food. Thus, to measure the reduction effect in faecal egg counts (FEC), larvae, and adults, eighteen nematode-free Corriedale sheep were randomly allocated by live weight in 3 groups. On day 0, each animal was orally infected with a single dose of 5000 L₃ of mixed GIN. While all the animals were fed throughout the experiment with alfalfa pellets, only animals from Group 1 and 2 received quebracho CT extracts along with the pellets (days -2 to 40 and 21 to 40, respectively). Faecal eggs counts were daily performed in the sheep of the 3 groups. By the end of the experiment, animals from all the groups were slaughtered to compare the average worm burden.

Control sheep had the highest FEC throughout the experiment, and its value was significantly higher than FEC from Group 1 and 2 (Group 1: 2006, Group 2: 1564, and Group 3: 5724; $P < 0.01$). A lower viability and number of larvae were recovered from faecal cultures of animals who received quebracho CT extracts (Group 1: 8267, Group 2: 12732, and Group 3: 36846; $P < 0.01$). Likewise, sheep that received quebracho CT extracts had lower worm burden than animals in control group (Group 1: 1823, Group 2: 1374, and Group 3: 6661; $P < 0.01$). However, no significant differences were observed between number of larvae and worms in sheep from Group 1 and 2.

These results suggest that quebracho CT extracts could be used in the control of GIN in sheep. However, further work is needed to improve the oral administration of these extracts.

Dynamics of Fecal Eggs Shedding of Trichostrongyles Nematodes in Cow Herds of the Southeast of Córdoba (Argentina)

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Introduction: The increasing intensification of the beef cow systems in the Pampas region require an update of their parasite epidemiological pattern. The objective of this study was the characterize the fecal worm eggs per gram (epg) and helminth genus dynamics in cows during the pregnant-lactation period.

Methods: From autumn to summer of 2009 and 2010, 4 groups (2009: I-II; 2010: III-IV) of 35 Braford cows (6-9 years) calving in July-September and belonging to four herds of Noetinger (Córdoba) were studied. The animals grazed lucerne in April and from October to December, while from May to September a ration based on sorghum silage with rye grass/oats was consumed. Monthly it was determined: epg, helminth genus and body condition (scale 1-9). The epg logarithm levels and their correlation between sampling was analyzed by a mixed lineal model (LSD, Fischer 5 %).

Results: The initial epg means were bellow 50 (negative: 26-43 %; < 50: 31-54 %; 50-100: 14-18 %; > 100: 6-15 %) and increased by July 2009 (I: 67 ± 59 ; II: 184 ± 249) and August 2010 (III: 91 ± 126 ; IV: 88 ± 175) The July (I, II), August (III) and September (I) values of epg were different ($p < .05$). The correlation for consecutive epg were: 0,65 (I), 0,69 (II), 0,59 (III) y 0,65 (IV). *Ostertagia spp.* was the most prevalent genus (I: 31-59 %; II: 23-44 %; III: 14-56 %; IV: 17-47%). *Haemonchus spp.* was important in herds I and IV (11-55 %) and *Cooperia spp.* in II and III (14-46 %). From May (I: 7.1 ± 0.5 ; II: 6.8 ± 0.5 ; III: 5.7 ± 0.7 ; IV: 6.0 ± 0.7) to October (I: 5.7 ± 1.1 ; II: 3.6 ± 0.7 ; III: 4.6 ± 0.7 ; IV: 3.5 ± 0.6) a severe waste in the body condition was observed.

Conclusions: The coincidence of increasing epg levels with the initial stage of the loss in body condition is significant. The high correlation of epg demonstrate the capability of adults cows to keep the shedding status along the time. The elevated participation of *Haemonchus spp.* and *Cooperia spp.* contribute to the fact that cows epg may be higher and more heterogeneous than expected.

A Preliminary Study Using Effective Microbes for Worm Control in Goats in Malaysia

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Anthelmintic resistance which is escalating in Malaysia and other countries is forcing farmers worldwide to look at alternative, green technologies to improve productivity and reduce diseases caused by helminths in livestock. One such method is the use of Useful Microbes or UM Technology for worm control. Therefore, a preliminary study was conducted to evaluate the anthelmintic effect of UM on nematode parasites of goat in a government farm. Fifteen Boer goats were randomly selected and fed with UM Bokashi mix (effective microbes in the form of granules) with pellet and UM fluid in drinking water. UM used in this study was produced by the Department of Veterinary Services, Penang by fermenting UM stock culture with fruit base and molasses. Faecal egg counts (FEC) using the modified McMaster technique was carried out weekly for 5 weeks. The results show that the UM used for animal feeding (5%Bokashi with commercial goat pellet) and ad lib UM fluid in drinking water, fed over a period of 1 month indicated an average Faecal Egg Count reduction from 3240 e.p.g to 677 e.p.g (79% reduction). Further studies are necessary to establish the systematic use of UM in smallholder farms as well as large commercial establishments and to determine its mode of action in the pathophysiological aspects in the face of common limitations such as poor nutrition and other stress factors.

ParaCalc[®] – a novel tool to evaluate the economic importance of worm infections on the dairy farm

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Although there is a plenitude of scientific literature describing the losses in productivity that are caused by infections of cattle with gastrointestinal (GI) nematodes and the liver fluke (*Fasciola hepatica*), only few attempts have been made to convert these production losses to an economic cost. In addition, these economic assessments typically estimate the global cost of infection in a specific region. The value of such studies is limited when the purpose is to offer economic guidance in making decisions at the farm-level. The objectives of this paper were 1) to develop a tool for the veterinarian/herd advisor to estimate the herd-specific costs of GI nematode and liver fluke infections in dairy herds; 2) to apply the tool on Belgian dairy herds and 3) to evaluate the user experiences from a selected number of veterinarians. The developed tool consists of a standardized spreadsheet model with 6 steps: (1) identification of animals 'at risk' of production-limiting helminth infections; (2) assessment of the helminth infection status of the herd by measurement of serum pepsinogen and indirect ELISA results; (3) assessment of anthelmintic treatment usage and related costs; (4) assessment of the effects on production of helminth infections; (5) estimation of the monetary value of production outputs and (6) aggregation of the results. The tool was applied on data from Belgian dairy herds. Unlike previous studies that provide only a global estimate, this approach enabled us to assess the potential costs for a farmer and propose a minimum attainable disease cost. This could be used as a target value for farmers. The users evaluated ParaCalc[®] to be a useful tool to raise the farmer's awareness on the costs of worm infections, offering added value for their services. Future improvements and developments are discussed.

Sheep infected with gastrointestinal nematode parasites consume more medicated feed block

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Targeted selective treatment is the allocation of anthelmintic to those animals most affected by gastrointestinal nematodes (GIN). Selective treatment may also arise if consumption of a medicated feed block (MFB) containing an anthelmintic is principally by animals most affected by GIN. This study aimed to determine if voluntary intake of a MFB was higher in sheep with GIN infection.

Twenty four, 2 year old, Merino wethers were allocated to receive GIN infection or remain uninfected. Prior to treatment allocation negative worm egg count (WEC) was confirmed in all sheep. GIN infection was established by giving an oral dose of 10,000 *Trichostrongylus colubriformis* L3 (McMaster strain; anthelmintic susceptible) and 3,000 *Haemonchus contortus* L3 (Kirby strain; anthelmintic susceptible). Uninfected animals received a Cydectin LA (1 mg/kg moxidectin; Virbac (Australia) Pty. Ltd, Australia) injection at the recommended rate. Animals were adapted to paddock (2 ha) and had access to a non-medicated feed block for 5 weeks after treatment. The feed block was changed to a MFB containing fenbendazole (1.5 g/kg) at day 36 post-treatment. Blood samples (10 ml; K₂-EDTA) were collected via jugular venipuncture starting on day 36 and then every 2 days until day 50. Faeces were sampled on days 36, 43 and 50 to determine WEC. Plasma samples were analysed for fenbendazole metabolites by HPLC. Treatment effects on plasma concentrations of fenbendazole metabolites were analysed using general linear models with separation of least squares means by a protected Student t-test.

Mean MFB intake was higher in infected sheep compared to uninfected sheep ($p=0.012$) when averaged over time intervals. MFB intake differed among sampling times ($p<0.001$) with infected sheep consuming more MFB than uninfected sheep at day 38 ($p = 0.016$). Thereafter, MFB intake differences between treatments did not differ ($p > 0.05$). WEC of infected sheep decreased from an average of 2165 epg (day 36) to 120 epg (day 50). Uninfected animals had negative WEC at all times.

This study showed that Merino wethers infected with GIN parasites initially consumed a greater amount of a MFB than their uninfected counterparts. That the differences in MFB intake between infected and uninfected groups declined as worm egg count decreased provides additional support for the existence of 'self-medication' in grazing sheep.

Ovicidal and larvicidal activity of the crude extracts from *Phytolacca icosandra* against *Haemonchus contortus*

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Background: The development of anthelmintic resistance has impacted on the success of conventional anthelmintics (AH) for the control of gastrointestinal nematodes in grazing/browsing sheep and goat. Medicinal plants from the traditional herbology in Mexico may provide new candidates as alternative sources of AHs for ruminants. This study evaluated the leaf extracts from *Phytolacca icosandra* against infective L₃ larvae and eggs from *Haemonchus contortus* collected from sheep.

Methods: Three extracts of different polarities were obtained from leafs using ethanol, *n*-hexane and dichloromethane as the solvents. The effectiveness of the AH activity *in vitro* was evaluated using larval migration inhibition (LMI) and egg hatch (EHA) assays.

Results: For the LMI assays, the ethanolic extract of *P. icosandra* showed a 55.4 % inhibition of larval migration at 2 mg/mL ($p < 0.05$). The dichloromethane extract of *P. icosandra* showed a 67.1% inhibition of migration at 3 mg/mL ($p < 0.05$) and a dose-dependent response with a LD₅₀ of 0.90 mg/mL. The *n*-hexane extract failed to show inhibition of larval migration at any concentration explored. In the EHA for the ethanol extract, the lowest concentration tested (0.15 mg/mL) resulted in inhibition of egg hatching greater than 72.6%. Therefore, the LD₅₀ could not be calculated for this extract. The LD₅₀ of the dichloromethane extract of *P. icosandra* was 0.28 mg/mL. An inhibition greater than 90% was observed with both the ethanolic and dichloromethane extracts when using a concentration of 0.90 mg/mL or higher. The *n*-hexane extract failed to show egg hatch inhibition at any concentration tested. The AH activity reported for *P. icosandra* could be attributable to the flavonoids, steroids, terpenoids, coumarins and/or saponins that were present in the ethanolic and dichloromethane extracts. A combination of more than one component may also explain the observed AH activity against the *H. contortus* life stages that were evaluated.

Conclusion: The ethanolic and dichloromethane extracts of *P. icosandra* showed clear *in vitro* AH activity against the *H. contortus* eggs and the L₃ larvae. The hexanic extract of the plant leaves failed to show any *in vitro* AH activity.

Gastrointestinal parasite control measures in use within the Central Tablelands of New South Wales

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Background : Gastrointestinal parasites can cause major growth and productivity losses in young beef cattle. While recommended drenching programs have been developed for use in beef herds in the Central Tablelands of New South Wales, the adoption of these programs by cattle producers is largely unknown. As part of a larger study into the efficacy of worm control strategies focussing on *Ostertagia ostertagi* over summer months and the expression of Type II Ostertagiasis in autumn, a survey was conducted to determine what gastrointestinal parasite control measures are in current use by producers within the Central Tablelands, including the anthelmintics used in these control programs.

Method : A questionnaire survey of 267 producers with greater than 250 cattle was conducted. Information on current drenching programs, anthelmintics used and the delivery method was collected and analysed.

Results : The survey response rate was 19.5% (52/267). In 2009, 88% of producers elected to give an anthelmintic treatment at weaning. Weaning occurred between February and May in spring calving herds, with 73% weaning in the March-April period (16/41 weaning in March and 14/41 weaning in April). The most dominant anthelmintic group used was the macrocyclic lactones (used by 80% of producers) and over 60% of producers preferred a pour-on preparation. The survey identified that 78% of producers utilised 'worm-safe' pastures (defined as pastures rested for 4 months, or grazed by adult stock, or grazed by sheep, or used for hay and silage making, or used for crop making) within their gastrointestinal control programs.

Conclusion : Meat and Livestock Australia currently recommends a drench at weaning (autumn) and in late winter, followed by a drench in spring if the winter drench was not a macrocyclic lactone and possibly a summer drench, for spring calving herds in the Central Tablelands. Only 24% of producers (10/41) are following these recommendations, and of these all but one producer is using a macrocyclic lactone.

Parasites in buffaloes – Corrientes, Argentina

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Introduction: During the last three decades, the authors have began working with small herds of buffaloes in Corrientes province, at the northeastern of Argentina. At present, this activity in constant/permanent development, is one of the most important production and complementary of the livestock holdings. It has still been expanded/spread to other provinces. Argentina has 90.000 km² (9.000 000 ha) of water surface which is not appropriate for cattle production. These conditions allowed the buffaloes to be increased from 1.128 heads in 1985 to 100.000 in 2010. This amount is annually growing due to the incorporation of new bellies and farmer, who believe and trust in this animal.

In 2004, the authors became working with buffaloes to find out which are the parasites occurring in Corrientes province, Argentina.

In the first four years, trematodes infestation has found, but in the last three years they determined parasitism by gastrointestinal nematodes, cestodes and coccidia and several ectoparasites arthropod.

2400 buffaloes of several ages, for beef and milk production, have been examined in 20 holdings.

The area where the investigations have been carried on, was very dried up, but rainfalls have fortunately started in 2010. At that time, drought buffaloes shared the area with bovine livestock.

Methods: Gastrointestinal parasites were removed by rectal and recent stools, identifying the samples and conditioning in polyethylene bags to be taken to the laboratory, to be processed.

To determine *trematoda* species, a special sedimentation coprology technique, was used (Dennis, Stone & Swanson). In case of *nematode* and *cestode* eggs and *coccidia* oocysts it was used the flotation method with sugar solution. Larval cultures were used to identify genres by direct observation for arthropods.

Results: The population diagnosed parasitic Protozoa: *coccidia*.

Helminth, *Capillaria spp*, *Cooperia spp*, *Haemonchus spp.*, *Fasciola spp* *Moniezia spp*.

Arthropods: *Dermatobia hominis*, *Haematobia irritans*, *Haematophinus tuberculatus*. *Bohophilus microplus* and *myiasis*

Conclusions: Parasitism is similar to the one described in the literature of buffaloes.

Controlling resistant nematodes in dairy sheep: a balance between drug efficacy and milk residues

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Extra-label use of the macrocyclic lactones (ML) to control endo- and ecto-parasites in dairy sheep and goats is rather common in an attempt to increase milk production. The intensive use of these compounds led to the worldwide development of drug resistant in nematode populations. Different pharmacological strategies have been assessed to improve the efficacy of ML against resistant nematodes. However, the pattern of milk residues must be considered when a parasite control program is established in lactating dairy animals. The aims of the work were to simultaneously evaluate the comparative anthelmintic efficacy, the plasma-milk exchange and milk residual profiles for eprinomectin (EPM) and moxidectin (MXD) obtained after their oral administration to dairy sheep naturally infected with resistant nematodes. Twenty-four (24) milking ewes naturally infected with ivermectin-resistant gastrointestinal nematodes (mean faecal nematodes eggs count: 1324) were used. Experimental groups were (n=8): untreated control, EPM and MXD orally administered at 0.2 mg/kg. A topical EPM formulation was given orally (as an experimental extra-label use). Individual fecal samples were collected at 0 and 15 days post-administration to run the faecal nematodes eggs count reduction test (FECRT). Blood and milk samples were collected over 15 days post-treatment. Drug concentrations in plasma and milk residues were measured by HPLC. A higher efficacy, indirectly estimated through the FECRT, was obtained after MXD administration (98.8%) compared to the EPM treatment (71.4%). The third-stage larvae differentiation study demonstrated that *Haemonchus spp* was the only nematode genus surviving after both treatments. Higher plasma concentration profiles and greater milk residues were measured after MXD compared to the EPM treatment. MXD persisted for prolonged time both in the bloodstream and milk. Considering the established MRL, milk collected from MXD-treated dairy ewes should be discharged during 3 days post-treatment. However, the low plasma-milk exchange of EPM permitted to derive the milk from treated sheep without a withdrawal period, in spite of the extra-label use by the oral route. The parasite control scheme to be used in dairy small ruminant production should be carefully selected according to parasite susceptibility and the risk of milk residues over the permitted maximum levels.

Phenotypic characterisation of two nematodes isolates obtained from an anthelmintic resistance survey on Scottish cattle farms

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In the United Kingdom little is known about the anthelmintic sensitivity of bovine gastrointestinal parasites to anthelmintics and which, if any, worm species are implicated in resistance. Over the last decade, sporadic reports of ivermectin resistance in *Cooperia* in UK cattle have occurred, but as yet, no regional prevalence studies have been conducted. An ongoing study is examining ivermectin sensitivity against naturally acquired infections on Scottish cattle farms using the faecal egg count reduction test (FECRT), whilst thiabendazole (TBZ) resistance is also being assessed using the egg hatch test (EHT).

Thus far, FECRTs have been performed on four farms (n = 6 - 16 animals per farm) using an injectable formulation of ivermectin (Ivomec Super[®], Merial Animal Health Ltd) at the manufacturer's recommended dose rate of 0.2µg IVM /kg body weight (BW). At 14 days post treatment, the mean percentage reductions in trichostrongyle egg counts of the four herds, as assessed using the formula $[(T_1 - T_2 / T_1) \times 100]$ where T_1 and T_2 are the FEC of animals pre- and post treatment respectively, were 100%, 84%, 82% and 72%. The results indicate a lack of efficacy to injectable ivermectin at three of the sites i.e. <95% reduction in egg count. Results from the EHTs, indicate sensitivity to TBZ on all four establishments. The predominant species present post treatment was *Cooperia*.

Infective larvae (L_3) derived from excess faecal material from two of the field isolates were passaged through two donor animals in order to generate material for further characterisation. The efficacy of IVM pour-on (IVM-PO; 0.5mg/kg BW, Ivomec Classic Pour-On for Cattle, Merial Animal Health Ltd), moxidectin pour-on (MOX-PO; 0.5mg/kg BW, Cydectin 0.5% Pour-On for Cattle, Pfizer AH) and injectable IVM (IVM-INJ; 0.2mg/kg BW) were assessed. For each isolate 20 six-month-old male parasite naive calves were each challenged with 50, 000 L_3 , treated on day 28 post-infection (PI, n=5 per treatment) and slaughtered seven days later (day 35PI).

Seven days post-treatment faecal egg counts for the IVM-PO, IVM-INJ and MOX-PO, were reduced by 20%, 37%, 99.8% and 99.1%, 8% and 99.7% respectively for the two isolates when compared to the untreated controls. The results of the artificial challenge study confirmed the field findings and provide information on treatment options for the future.

Strategic drenching program in Organic Farming in the Czech Republic

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Organic farming is an integral part of the agriculture policy of the Czech Republic, which has been developed in the Czech Republic since 1990. The preparation of the "Action Plan (No 236 of 17 March 2004) of the Czech Republic for the Development of Organic Farming" was initiated by the ministry of Agriculture. This plan is a strategic document of the Czech Republic related to the development of organic farming until 2010 covering the areas as a research, an education and a consulting for farmers.

The goal of our research was promotion of cooperation with sheep and goat farmers at organic farms, assistance with proposal of dehelmintization strategy in these farms, also providing farmers with possibility to discuss parasitic diseases in their farm. Samples of faeces of animals in herd (average 40-80 animals) were taken from rectum of each animal. The high prevalence of gastrointestinal nematodes and cestodes was estimated in the model farms, but in individual cases, low degree of infection was frequently determined. Because the faeces were taken individually, the target therapy could be recommended to animals with middle or higher infection or to animals with clinical symptoms. This group consisted mostly of young animals which will gradually acquire ability to produce proper immunity against parasitic infections and they would not need dehelmintization or only some cases for targeted dehelmintization occur.

Efficacy of an energy block containing *Duddingtonia flagrans* in the control of gastrointestinal nematodes of sheep

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New alternatives for the control of gastrointestinal nematodes affecting livestock production worldwide have been studied for some time. Amongst them, biological control is one of the most promising strategies due to its ecological sustainability characteristic. Of all natural antagonists studied to this day, the one with the best possibilities to control gastrointestinal nematodes in livestock is the nematode-trapping fungus *Duddingtonia flagrans*. The efficacy of the nematode-trapping fungus *D. flagrans* incorporated into an energy block was evaluated for the control of gastrointestinal nematodes in sheep. Four naturally parasitised sheep with average nematode egg counts of 2,470 egg per gram grazed by pairs on two similar parasite-free paddocks for 30 days. During that period, one pair of sheep (treated animals, T1) received an energy block containing chlamydospores of *D. flagrans*, while the second pair (control animals, C1) received a fungus-free energy block. The animals in both groups were taken off the paddocks after contaminating the pastures with either nematode eggs plus fungal chlamydospores (T1) or nematode eggs alone (C1). Twelve parasite-free sheep were divided into two groups of six animals each. The treated group (T2) was placed on the paddock previously contaminated with parasites and fungus, while the control group (C2) was placed on the parasite-only paddock. These two groups grazed on their respective paddocks during 30 days, after were slaughtered in order to determine the parasite burden present in each animal. Results showed that animals in T2 harboured significantly less nematodes than their counterpart in group C2. The efficacy of *D. flagrans* was 100% against *Haemonchus contortus* and *Teladorsagia circumcincta*, 89.9% against *Trichostrongylus colubriformis*, 87.5% against *Cooperia onchopora*, and 90% against *Trichostrongylus axei*. No efficacy was detected against *Nematodirus spathiger*, *Trichuris ovis* and *T. skrjabini*. The energy block performed effectively as a vehicle for the administration *D. flagrans*, which lowered the number of infective larvae on pasture, thus decreasing the adult worm burden in sheep.

Soy protein polymers as controlled release system of fungi nematophagous to the control nematode para sites in ruminants

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Due to the rapid increase in cases of anthelmintic resistance as well as the increasing demand of safe food for human consumption obtained using environmentally-friendly technologies, it is imperative to find alternatives for the control of gastrointestinal parasitosis of ruminants. Among these, Biological Control (BC) is based on the natural organisms as *Duddingtonia flagrans*, a nematophagous fungi which has the ability to go through the gastrointestinal tract of livestock and maintaining intact of germination and predatory activity in the environment and reduce the number of infective larvae in faecal pats. Current research focuses on the obtention of formulations used as vehicles for the spores of nematophagous fungi. Soy proteins polymers (SPP) have a great potential as a controlled release system (CRS) for active compounds, such as nutrients, medicine and could be used therapeutically. SPP forms a stable polymer, very resistant to degradation because its ability to polymerise into different polymeric structures such as sheets or plastics. The purpose of this trial was evaluate the predatory activity of *D. flagrans* included in a SPP formulation such as CRS; determine the fungi release from polymers, the suitable physic structure for bucosophagic probe administration in sheep, and to observe the time of permanency of the SPP in the rumen of a cannulated sheep. Ten replicates were made. The SPP was extracted weekly for the observation of the physical structure and faeces were collected to observe the predatory activity of the fungus in Petri dishes with water agar 2 % and *Panagrellus spp* as baits. The result showed that polymer formulation not affected the predatory activity of *D. flagrans* and the fungi release was successful. The SPP remained in the rumen degrading slowly for 4 weeks and it liberated constant *D. flagrans* for faeces. Therefore the use of soy protein polymer as vehicle of fungi nematophagous for ruminant's nematode control could be the base for a potential biodegradable controlled release system.

Study of inhibition-desinhibition of *Ostertagia ostertagi* under simulated grazing periods in the Centre - Eastern Province of Buenos Aires

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From August to March 2006 the bovine trichostrongilidosis epidemiology with emphasis in the *Ostertagia ostertagi* inhibition-desinhibition phenomenon was studied, in order to determine the rates and monthly fluctuations of this complex and investigate the possible causes of these variations. Forty four Holando Argentino calves 4-5 months old free of gastrointestinal parasites were distributed in four monthly grazing periods (August / September, September / October, October / November, November / December). After each grazing period the animals were housed and fed hay free of parasites and water *ad libitum*. Every month a couple of each grazing period animals were slaughtered for worms count , intestinal and abomasal worms identification, fecal egg count and blood for pepsinogen measurement. The pastures were sampled to determine the herbage larval infectivity. The weather data were collected and analized. In all grazing periods were predominated *Ostertagia ostertagi* adult forms and inhibited stages. The highest percentages of *Ostertagia ostertagi* hypobiotic forms were observed in the mid to late spring, coinciding with those months where there was an increase in temperature and photoperiod. There were three phases marked desinhibition. Unlike hipobiosis, the environmental condition does not appear to be central in the process of *Ostertagia ostertagi* desinhibition it is more logical to assume that it is a parasite adaptation to extreme environmental conditions have influenced their action in the genus for many years, and probably due more to “desinhibition spontaneous” after certain period of inhibition rather than the action of some external stimulus on the animal that could start the process.

Detection of the β -tubulin Phe200Tyr allele in *Haemonchus placei* populations isolated from cattle in Minas Gerais, Brazil

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Background: Parasitic nematodes of the genus *Haemonchus* infect a range of ruminant hosts and are of major veterinary importance. After years of widespread use of drugs for parasitic infection control, the increasing prevalence of resistant nematodes threatens livestock production in several parts of the world. In *Haemonchus contortus*, resistance to the antihelminthic drugs benzimidazoles (BZ) has been shown to be due to mutations β -tubulin genes, mainly in the codon 200 (Phe200Tyr) of isotype-I. In this study we evaluate the frequency of the β -tubulin Phe200Tyr mutation in *Haemonchus placei* isolated from cattle in Brazil.

Methods: Thirty animals from a cattle herd raised in the city of Teófilo Otoni, Minas Gerais (Brazil) had their stool samples collected and analyzed for the presence of trichostrongylid eggs. The property evaluated has a historical of regular use of BZ for nematode control. Positive samples had their eggs counted and submitted to culture. Genomic DNA was isolated from single larvae and used to evaluate β -tubulin Phe200Tyr allele (R) frequency through allele specific PCR (ASPCR). In addition, the Internal Transcribed Spacer (ITS2) of larvae DNA was sequenced for species identification.

Results: Five animals (~17%) presented trichostrongylid eggs in stool, with counts ranging from 600 to 2200 eggs per gram. β -tubulin Phe200Tyr allele presence was detected in all of the samples analyzed and its frequency varied from 0,05 to 0,15 (N=30 larvae/host). Although no homozygous resistant individual (RR) was found, heterozygous individuals (RS) frequency ranged from 0,1 to 0,3 (Average = 0,16). In order to confirm nematode morphological identification, the ITS regions of 2 larvae/host were sequenced and presented 100% identity to *Haemonchus placei* (gi219938533).

Summary/Conclusions: Our study reveals the presence of the BZ resistant β -tubulin Phe200Tyr allele in *Haemonchus placei* populations isolated from cattle in Minas Gerais (Brazil). Although, still at low frequency (Average = 0,076) continuous selective pressure provided by the use of BZ might rapidly increase its prevalence. This study also reinforces the usefulness of molecular methods for monitoring antihelminthic resistance occurrence and predicting fluctuations in its frequency, thereby enabling rational management of drug use.

Nematode egg counts during the first postpartum month is a useful tool for milk production prediction in grazing Holstein cows

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The onset of anthelmintic drugs resistance in cattle makes essential to design alternative control strategies with integrated approaches including parasite burden and production parameters. It is critical to identify target animals to be treated, and times of treatment, avoiding massive drug use. To this respect a study comparing three nematode infection diagnostic methods for milk production prediction in dairy cows was performed. Grazing cows from the Experimental School of Inchausti, 25 de Mayo, Buenos Aires, Argentina (35°36' S, 60°32' W) that calved in a year (N=150) were individually sampled for rectal feces, coccigeal blood and milk during their first month after calving. Nematode eggs (EPG) were counted in feces by the Mc Master method adapted by INTA. Pepsinogen (P) was measured in serum by the colorimetric method of Ross et al. and expressed as Tyrosine mU/ml. Anti Ostertagia antibodies concentration was evaluated in milk by the Svanova ELISA kit and Optical density ratio (ODR) calculated according to manufacturer instructions. Individual daily milk production was recorded by means of the Alpro-Milking computerized system and lactation curves were performed for each cow. Cut-off levels were established for each measured nematode parameter to separate cows in two groups (with high or low parasite indexes) for each method. Monthly milk yield was compared between groups by two way ANOVA for repeated sampling and total milk production was compared by t-Test for each diagnostic method. Only when cows were separated by the EPG method (EPG = 0 vs EPG > 0) a difference in total milk production was found ($P < 0.05$), and productivity differed during the first five month of lactation ($P < 0.05$). Milk production from groups of cows separated by Anti-Ostertagia levels in milk expressed as ODR (ODR ≤ 0.5 vs ODR > 0.5), or by serum pepsinogen expressed as Tyrosine units (Tyr ≤ 1000 vs Tyr > 1000) did not differed. We conclude that EPG counting during early lactation in grazing dairy Holstein cows may be a useful tool to identify the animals susceptible to have impaired production due to nematode burden.

Circulating IGF-1 levels and mammary gland development in antiparasitic treated and untreated prepubertal Holstein heifers

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We have previously showed that gastrointestinal nematode parasites delayed puberty onset and decreased circulating IGF-1 levels in dairy cattle. As this growth factor is essential to mammary parenchyma development during prepuberty, we wish to investigate whether anthelmintic treatment in naturally infected grazing dairy heifer calves can increase IGF-1 levels and improve mammary development. Forty female Holstein calves were randomly assigned, at birth, to an untreated control group (C) or to the treated group (T) which received monthly, from birth onward, the following drugs: ivermectin (0.63 mg/Kg), fenbendazol (7.5 mg/Kg) and/or levamisol (10 mg/Kg). All the heifers were raised together on infected pastures, and supplemented with milk, concentrate or corn according to age. At 20, 30, 40 and 70 weeks of age blood and fecal samples were taken for serum IGF-I determination by RIA and nematode egg counting (EPG) in feces. Mammary biopsies were taken to 6 heifers in each group, using a Tru-Core® Biopsy Needle (Medical Device Technologies, Inc.), for histological and immunohistochemical studies. EPG counts in feces decreased with age ($P = 0.027$) and were higher in C than T heifers ($P = 0.020$). IGF-1 levels increased with age ($P = 0.003$) and were higher in T than C heifers ($P = 0.042$). Mammary slices stained with Hematoxylin-Eosin and immunolabeled with PCNA and Estrogen receptor alpha antibodies (monoclonal anti PCNA clon PC10 - Santa Cruz Sc 56 and monoclonal anti Estrogen receptor Clon 1D5 Bio Genex, respectively) were analyzed with the Image Pro Plus 3.0 software. Two way ANOVA for repeated sampling was performed to compare image parameters between groups. Mammary samples from T heifers had higher ratio of parenchyma/total area ($P = 0.036$), PCNA index ($P = 0.037$) and expression of estrogen-receptor alpha ($P = 0.006$), compared with C heifers; no effect of age was evidenced for any of those parameters. We conclude that lowering parasite burden to naturally infected prepubertal dairy heifers induces a higher mammary parenchymal development and cell proliferation, as well as a higher density of parenchymal estrogen receptors, in association with increased circulating IGF-1 levels.

Interactions between gastrointestinal nematodes and subclinical mastitis in low-input dairy sheep management systems in Greece

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Background: Parasites (especially gastrointestinal nematodes, GIN) and subclinical mastitis remain the most important animal health and welfare problems in sheep production. Management practices and the related abiotic stress factors are known to increase the susceptibility of sheep to intestinal parasites and mastitis. These problems are currently mainly controlled by regular chemical treatments, a practice which may result in resistance in parasite/pathogen populations and consumer concerns about residues loss and welfare practices in 'low-input' systems.

Aim of the study was to describe the abundance of parasitic and udder infections and identify possible interactions in sheep flocks under different 'low-input' dairy management systems.

Methods: This study included 10 extensive and 10 semi-intensive sheep flocks in Crete, Greece. From each flock faecal and milk samples were taken on a monthly basis from 20 'Sfakiano' breed ewes (10 from each different 2 lambing periods) for 9 consecutive visits (milking period). Faecal samples were processed with modified McMaster technique, in order to assess GIN EPG numbers while milk samples were analyzed for CFU (Colony Forming Units) and SCC (Somatic Cell Count). FAMACHA and Body Condition Score (BCS) were also recorded for each sheep.

Results: In total, low parasitic egg output was encountered in both systems (i.e. mean EPG values of 43.6 ± 2.3 and 31.3 ± 3.6 for extensive and semi-intensive flocks respectively). Late lambing ewes in extensive flocks showed significantly lower EPG numbers compared to the ones from the semi-intensive flocks. Ewes bred in semi-intensive systems showed a peak in EPG values during June-July. EPGs were weakly related to FAMACHA records ($r_s = -.037$, $p < .05$), while EPGs and BCS values had a more pronounced correlation ($r_s = .179$, $p < .01$). Farming system ($p < .01$), lambing period ($p < .01$) and their interactions significantly affected EPG numbers in sheep. EPGs showed no correlation with SCC and CFU values ($r_s = -.179$, $p < .01$).

Conclusions: Parasitic egg output was constantly low throughout the experiment, probably due to the semi-arid climate of the island of Crete and husbandry systems applied. Parasitic infection in late lambing ewes seems to be significantly lower in extensive systems while no difference was shown in early lambing ewes. We failed to demonstrate a correlation between nematode infection and subclinical mastitis possibly due to the low parasitic infection intensity.

The effect of the ambient temperature on the development and survival of the infective stage larvae of *Cooperia oncophora*

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Background: Data on different factors, especially those occurring outside the host, that influence the population dynamics of *Cooperia oncophora*, are limited. In order to clarify some aspects about the population dynamics of *C. oncophora* a series of experiments were undertaken.

Methods: Experiment 1 investigated the effect of temperature on the survival of third stage larvae ([L3]). These were individually cultured from the faeces of 5 different cattle. For each host approx 30-80 [L3] were placed with deionised water in 3 wells of a 24 well culture plate and stored at different set temperatures (8, 16, 20, 24, 28 or 37 °C). Viability was regularly assessed. Experiment 2 investigated the survival of [L3] in herbage. Grass strips were artificially contaminated with [L3] in water. On a regular basis the herbage was cut and the clippings examined for larvae. Experiment 3 investigated the development of *C. oncophora* eggs to the [L3] stage at different set temperatures. Faeces from 3 cattle were independently mixed with vermiculite to achieve a similar consistency regarding hydration and aeration. These were cultured at different set temperatures (8, 16, 20, 24, 28, 32 or 37 °C). The number of eggs and larvae at different stages were regularly counted using a floatation technique ("Flotac").

Results: In Exp 1 the 50% survival time at 37°C was only 7 days but increased at 16°C to 330 days. For Exp 2 under field conditions [L3] were only detected for up to 3 months. In Exp 3 the development time from egg to the maximum number of [L3] was only 9 days at 32°C but 26 days at 16°C. The development success increased from 15% at 16°C to 30% at 28°C.

Conclusion: The results from these experiments show a strong relationship between temperature and time for all parameters. There is an indication of variation in results between animals that were the source of eggs or larvae.

Larval challenge does not cause production loss in sheep given continuous anthelmintic treatment

Creating a worm-free sheep with a controlled release capsule

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Background: Measuring loss of production due to infection with gastrointestinal nematode parasites (worms) in the field poses significant difficulties in the provision of worm free controls. Past approaches have relied on frequent anthelmintic administration or the use of controlled release capsules (CRCs) to suppress worm burdens. These approaches do not prevent intake of L3 which may trigger a host immune response and contribute to the pathophysiology of worm infection. This study aimed to quantify the cost of larval challenge in sheep administered a CRC and thus determine the suitability of CRC treated sheep to be a proxy for worm-free sheep in grazing experiments.

Methods: The experiment was an incomplete 2x3 latin square with 81 Merino wethers allocated at random to receive either a thrice-weekly mixed oral infection (*Haemonchus contortus* 500 L3/week, *Trichostrongylus colubriformis* 2000 L3/week and *Teladorsagia circumcincta* 2000 L3/week) (INF) or no infection (UNINIL). Treatment was either nil (NIL), a CRC (albendazole, 2.3mg/day), or a short-acting anthelmintic administered once worm egg count (WEC) reached 1500 epg (TT). The experiment was conducted in two 63 day periods (separated by a 14 day washout period) with infection treatments swapped between periods. A subset of animals was killed at the end of each period.

Results: In CRC sheep, WEC was always negative and mean total worm count was seven. INF-NIL sheep recorded maximum WEC (8676 epg) on day 63 and mean total worm count of 17522. Liveweight gain and wool growth of INF-CRC did not differ from UNI-CRC sheep. In contrast, liveweight gain was reduced in INF-NIL (61g/d, $P<0.001$) and INF-TT (26g/d, $P<0.001$) when compared to their respective UNI groups. Blood eosinophil counts were raised in all infected groups, including CRC, though levels were low. Total intestinal antibody titre specific to *T. colubriformis* was higher ($P=0.005$) in INF-CRC and INF-TT groups compared to UNI-CRC.

Conclusions: Larval challenge is immunogenic in sheep administered a CRC but this challenge is not associated with production loss. CRCs provide a useful proxy for worm-free sheep in field studies investigating the impact of worm infection on productivity.

Accuracy and Precision of Sampling for Worm Egg Count Estimates

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Background: Reliance on faecal worm egg counts (WEC) as the basis for tactical anthelmintic treatment is a critical part of integrated worm control programs for sheep. The importance of WEC highlights the need for sampling that provides a good estimation of the true group mean. The typical approach used in Australia is the collection of ten faecal samples from a mob of sheep which could typically contain 200–1000 sheep. The collection of ten samples under these situations accounts for 1–5% of the mob. This study was conducted to test the hypothesis that ten samples may be insufficient to provide an accurate and precise estimate of the true group mean WEC.

Methods: Faecal samples were collected on four occasions from all sheep in a single mob of 500 adult Merino ewes in the Northern Tablelands of NSW, Australia. Sampling occurred in 2003 on the following dates: 14th August, 2nd September, 17th September and 2nd November. WEC was determined (1 egg = 60 epg) for each sheep and data entered into a spreadsheet. Ewes were treated with naphthalophos and levamisole at recommended rates on 17th September and lambed over a 5-week period starting 24th September. For each date, the requisite number of animals to achieve 4 (20 sheep), 8 (40) and 12% (60) sampling was randomly chosen *in-silico* and this process repeated a further 4 times to provide 5 random selections from each sampling date. The accuracy and precision of the WEC estimate for each selection group (i.e. 4, 8 or 12%) was calculated.

Results: Group mean WEC was 563, 1612, 2605 and 805 epg respectively for the four sampling dates with *Haemonchus contortus* contributing 43, 54, 70 and 74%. In relation to the true group mean, accuracy of WEC estimates increased from 69 to 73 and to 81% and precision improved from 19 to 17 and to 12% respectively for the three sampling levels.

Conclusions: Sampling of 10 animals from commercial mobs may not provide an accurate and precise estimate of the true group WEC mean. Increased level of sampling is more accurate and precise but may not always be practical. The choice of sampling 40 animals may be a practical compromise as it provides for a sampling level of 4–20% in most instances.

The effect of four-weekly eprinomectin treatment on milk production in seasonally-calving pasture-based dairy cattle

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Background: To quantify the effect intestinal parasitism has on milk production parameters in seasonally-calving, pasture-based dairy cattle.

Methods: Three seasonal-calving herds from the lower North Island of New Zealand were enrolled in this study with a key selection criteria being herd size > 450 cows. The study population consisted of all mature lactating cows between three and seven years of age that had calved by approximately eight weeks after the start of mating. In each herd, 90-116 cows were randomly allocated to the treatment group and matched to two control cows based on calving date and age. The treatment group comprised approximately 20% of adult dairy cows in each herd. A total of 319 cows were allocated to the treatment group and 604 cows to the untreated control group. Each treated cow was given four-weekly treatments of topical eprinomectin (0.05mg/kg; Ivomec Eprinex, Merial NZ Ltd) throughout at least the first two-thirds of lactation. Milk production data for two of the herds were obtained from bimonthly herd testing; daily milk production data were available for the final herd. Two sample t tests were used to compare the milk solid (milk fat + milk protein) production in the treated and control groups at the herd level. Multivariate linear regression was used to account for the effect of herd and to assess interactions. Pooled-milk from treated cows and bulk milk samples were analysed for *Ostertagia* antibody ELISA optical density ratios (ODR; Svanovir®).

Results: There was a significant treatment x herd interaction ($p=0.035$). Treatment improved 205 day milk solid (MS) production in one herd (364.5 kg MS/205 day lactation vs. 346.5 kg MS/205 day lactation; $p=0.003$), but no differences were detected in the other two herds. The mean milk *Ostertagia* ELISA ODR was 0.9 (range 0.6 to 1.3) with no differences detected between herds and treatments, although the power of the ODR analysis was low.

Conclusions: This study confirms previous findings of the significant variation in response to anthelmintic between herds. The reasons for these differences need to be explored. Despite ODR being high on all farms, a significant effect on productivity was only seen on one farm.

Influence of the order of evaluation of sheep and goat conjunctiva by people under training on FAMACHA System

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Background: The FAMACHA© System is based on the clinical evaluation of anemia by comparing the color of the conjunctiva with a five standard colors chart. To minimize the subjectivity of this examination, it is required the training of the evaluators. This practice is conducted with the evaluation of 20 sheep/goats of known hematocrit value disposed laterally and with the conjunctiva exposed to allow that all the participants be able to score each animal. All the participants are disposed in a queue, and it is not allowed to change the order of each person. As the color of the mucosa could be altered by exposure, this study was performed to assess whether there is a difference of the degree FAMACHA© awarded between the first and the last row of reviewers.

Methods: It was conducted eight training courses for farmers, students and technicians in different cities of Parana State, Brazil, from October 2009 to August 2010. For each practice, classes of 12 to 25 persons evaluated 20 sheep and/or goats. The degree awarded by each participant was compared with the hematocrit of each animal for assignment of errors. The log of penalized and not penalized errors were analyzed in a randomized block design in which treatments were the order of evaluation (first five evaluators x last five) and blocks were the different courses (n = 8).

Results: The average log of penalized error was 1.514 and 1.491 for the first and last raters, respectively. For the non-penalized error, these values were 1.491 and 1.493. The response among reviewers before logarithmic transformation had a high coefficient of variation (23.73% and 22.78% for penalized and not penalized errors), demonstrating high diversity in the ability to award correct FAMACHA scores among people who attend the courses. There was not effect of block or treatment ($p > 0.05$).

Summary/Conclusions: There was no significant difference in the evaluation of FAMACHA score between the first and last evaluators, indicating that exposure of the conjunctiva of sheep during the assessment by groups of 12 to 25 people does not result in significant change in the ocular mucosa.

Targeted selective treatments of first season grazing cattle

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Background: The effective management of diseases caused by gastrointestinal (GI) parasites in ruminants through targeted selective treatments (TST) has been addressed as a novel control strategy, which can prevent the development of anthelmintic resistance (AR). Another advantage of the TST approach is that it is acceptable also in organic herds, at least in Sweden. The aim of the present study was to validate the use of a weight-gain-based TST against major GI-parasites in first season grazing (FSG) cattle under field conditions in Sweden.

Methods: A three-year grazing trial was performed to compare animal performance and levels of parasite control in three grazing groups, each with 18-24 FSG grazing similar set-stocked pastures. The experimental groups were subjected to either: A) no parasite control, B) repeated doramectin (Dectomax[®]) injections, or C) targeted selective weight-gain-based anthelmintic treatments of individual calves, but only if their performance was inferior to the average of the poorer half of those in group B. Each year, weight gains and parasitological variables were measured at predetermined intervals for 22-24 weeks during the grazing season.

Results: In all years the dewormed calves in group B performed significantly better (0.42-0.61 kg/day) than those in the TST group C (0.33-0.51 kg/day), which in turn always exceeded the untreated group A (0.25-0.43 kg/day). In general, there were no differences either in faecal egg counts (FEC) or in serum pepsinogen levels between FSG in groups A and C. The average cumulative FECs in A were in the range of 1271-1953 ep_g and in C 1221-1968 ep_g. In contrast, parasite eggs were recorded rarely from group B and then only during the first two years (12-38 ep_g). The animals in B received 7 doses each year, whereas those in C received on an average 0.54 doses.

Conclusion: The TST approach tested here significantly decreased the numbers of anthelmintic treatments without being associated with major production losses. Still, the weight gains in animals subjected to TST were consistently (and significantly) lower than in those animals regularly dewormed. From this point of view the TST approach may be questioned, especially since there were no signs of selection for AR in group B despite a fairly intensive use of anthelmintics.

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Ivermectin and Moxidectin Resistance Factor in *Cooperia* spp. in Beef Cattle in Mato Grosso do Sul, Brazil

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Background: Sensible and reliable tests are necessary to detect small frequencies of resistance in a worm population, allowing preventive management to slow down selection pressure on that population. In Brazilian beef cattle, *Cooperia* has shown to be the major resistant nematode to macrocyclic lactones. The objective of this study was to determinate resistance factor (RF) of *Cooperia* field isolates to ivermectin (IVM) and moxidectin (MXD).

Methods: Eight field isolates, one susceptible and seven previously characterized as IVM-resistant by FECRT, were evaluated by a modifield larval migration in agar test.

Results: The values of IVM EC50 in the evaluated isolates were: IVM-Susceptible: 1.16 η Mol, Bom Senhor: 4.09 η Mol (RF= 3.53), Baunilha: 3.57 η Mol (RF= 3.07), Três Barras: 4.09 η Mol (RF= 3.52), Duas Irmãs: 2.50 η Mol (RF= 2.15), Bom Retiro: 11.35 η Mol (RF= 9.78), Soberana: 6.03 η Mol (RF= 5.20), Lucero Porã: 8.63 η Mol (RF= 7.44). These isolates showed to be much more susceptible to MXD than to IVM, with reduced EC50 and RF values: MXD-sensitive isolate: 0,75 η Mol, Baunilha: 0,93 η Mol (RF= 1,24), Três Barras: 0.36 η Mol (RF= 0.48), Duas Irmãs: 2.57 η Mol (RF= 3.42), Bom Retiro: 1.43 η Mol (RF= 1.90), Soberana: 1.087 η Mol (RF= 1.45), Lucero Porã: 0.49 η Mol (RF= 0.65). When comparing the results of previous FECRT for each *Cooperia* isolate to the the larval migration assay, it was observed higher sensitivity of the *in vitro* test to detect MXD resistance. The efficacy of MOX against the Duas Irmãs isolate was 100% (FECRT), while the FR was 3,42.

Conclusions: The *in vitro* test allowed phenotypic differentiation between sensitive and resistant isolates and has demonstrated to be a practical way and a possible tool for diagnosis of IVM and MOX resistance in field isolates of *Cooperia* spp. It was observed difference between susceptibility to MXD and IVM. One limitation for using this technique in field surveys is the need of monospecific nematode infections. Further studies are necessary to determine the discriminating-dose to differentiate between susceptible and resistant nematodes.

Risk factors in the development of bovine anthelmintic resistance in the Pampeana Region of Argentina

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In a regional study on anthelmintic resistance (AR) in cattle nematodes in the pampeana region of Argentina high levels of AR were recorded. The continue use of anthelmintics and some management practices have probably favored the appearance of AR. The aim of the present work was to determine the risk factors in the development of AR by studying differences between cattle production systems and different farm management practices. An observational study type case-control was used to identify risk factors. Fifty (50) cattle herds were surveyed, a group with AR (cases) and a group without AR (controls). Then were compared with respect to presence of the each hypothesized risk factor. The study of risk factors by means of surveys determined that the main management measure involved in the development of RA were the frequency of the treatments; three or more anthelmintic treatments applied throughout the year favoured the appearance of AR (*odds ratio* OR = 41.6). Likewise, predetermined strategic control treatments (OR = 4.6), summer treatments (OR = 3.7) and the absence or very low larvae in refugia (OR = 3.7) are associated with AR. These results show the importance of the correct use of the anthelmintics, applying control programs that consider the importance of the larvae in refugia, especially with summer treatments. To avoid the appearance of AR and to preserve the anthelmintic drugs efficacy, current cattle farm worm control programs require of an urgent re-formulation.

Gastrointestinal parasitism in small ruminants on small farms, in the Caatinga biome of the state of Bahia, Brazil¹

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A study of nematodes infecting small ruminants raised by smallholders in Caatinga (scrublands) biome of the state of Bahia, Brazil was performed in 11 goat and 37 sheep herds selected by non-probabilistic sampling. Herds were evaluated through egg counts (Gordon & Whitlock, 1939) and infective strongyle larvae faecal samples, body condition score (BCS, from 1 to 5) and FAMACHA® score determination from 116 goats and 474 sheep. Gastrointestinal nematodes (GIN) eggs were detected in 93.9% of goat faecal samples and 71.7% of the sheep samples. However eggs per gram (EPG) counts in 76.3% (450/590) of the samples were below the established cutoff point (800 EPG). The mean EPG was higher in the goats (1,243.5) than in the sheep (646.6), with a significant difference ($p < 0.001$) between the results. The sheep under 12 months old and those over 48 months were 2.57 ($p=0.03$) and 2.56 ($p=0.003$) times more likely to be infected, respectively, when compared with the other animals. There was no positive association between the positive rate of the fecal samples and the age of the goats. Most of the goats (89.7%) had a BCS less than or equal to three, but 57.8% of those classified as normal body condition (BCS=3) had the highest average EPG count (2,445). The nutritional state of the sheep was classified as normal (56.1%) and fat (21.5%) and the average EPG was below 800. None of the goats had FAMACHA® eye score 1, but scores 3 (48.3%) and 4 (42.4%) were observed respectively with average EPG counts of 1,042 and 1,831, without significant difference ($p>0.05$). FAMACHA® score in sheep ranged from 1 to 5, but scores 2 and 3 were the most frequent, at 30.1% and 40.7%, in both cases with significant differences ($p=0.04$ and $p=0.02$), respectively. In the anemic sheep, with scores 5 and 4, the EPG counts were 1,417 and 1,325. *Haemonchus* (49.6%; 66.8%), *Trichostrongylus* (43.44%; 29.2%), *Oesophagostomum* (4.31%; 2.51%) and *Cooperia* (2.14%; 0.79%) were found in goat and sheep faecal cultures respectively. Farmers need to integrate these methods aimed at minimizing ruminants exposure to gastrointestinal nematodes. Supported by FAPESB.

Soil moisture influences the moisture content of sheep faeces

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Background: Temperature and moisture are important variables controlling development of gastrointestinal nematode eggs to the infective larval stage (L3). Moisture effects development through rainfall amount and timing, but soil moisture may also be important. Larval development occurs within the faecal pellet and effects of moisture must occur through faecal moisture content (FMC). An experiment was conducted in a temperature-controlled incubator to determine the effect of soil moisture content and water application timing and amount on FMC.

Methods: A 3x3x2 factorial experiment was conducted with 3 starting soil moisture content (0, 10, 15%), 3 water application days (-1, 0, 3 relative to faecal deposition) and 2 water application amounts (12 and 24 mm) with 4 replicates. External to the design were unwatered controls. Temperature varied with daily minimum of 16.2 and maximum of 30.7°C and evaporation rate ranged from 2.3 to 5.0 mm/day. Sheep faeces (14g) were loosely placed on the surface of containers with a mixture of sterilised soil and aggregate. FMC on day 0 was 58.2%. Faecal pellets and soil were subsampled on days 1 – 4, 7 and 14 for dry matter determination.

Results: Mean FMC (all days) was greatest when soil moisture was 10 or 15% ($p=0.001$) and when water application occurred on day 0 ($p<0.001$). Water application amount increased FMC ($p<0.001$) but only on days 4 and 7. The interaction between the effects of soil moisture and application day reached significance ($p=0.003$) on day 4. Prior to this, water application provided on the day of deposition negated any effects of soil moisture on FMC. In contrast, FMC on day 4 was greater if soil moisture was 10 or 15% but only for those treatments which received water on day 0. Soil moisture on day 2 best predicted ($p<0.001$) FMC on days 1 – 3 ($R^2=0.29, 0.58$ and 0.55 respectively).

Summary: Soil moisture influenced FMC but this effect was negated if water application occurred on the day of deposition. Post deposition water application was most effective in the absence of starting soil moisture. Soil moisture on day 2 best regulates early FMC which is most important for development of L3.

Control of the periparturient rise in faecal *Teladorsagia circumcincta* egg output of ewes using moxidectin 2% w/v injection

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Background: On many UK farms, the development to patency of overwintered infective *Teladorsagia circumcincta* larvae ingested from pasture is the principal source of the periparturient rise (PPR) in faecal egg output. Anthelmintic treatments are required to suppress the PPR of ewes turned onto heavily L₃-contaminated pastures, to ensure adequate subsequent control of teladorsagiosis in their lambs. A field study was undertaken in a sheep flock in south-east Scotland with a history of ivermectin-resistant *T. circumcincta*, to determine the effects of treating newly-lambled ewes with a long-acting injectable formulation moxidectin 2% w/v (CYDECTIN®) (LA) versus an oral formulation of moxidectin 0.5% w/v (OD) on the PPR and the implications for the control of teladorsagiosis in their lambs.

Methods: The effects of treatment prior to turnout of newly-lambled ewes with LA (1 mg/kg bodyweight) ($n=73$) were compared with those of treatment with OD (0.2 mg/kg bodyweight) ($n=98$). Each group was grazed separately. Stocking density and pasture levels of overwintered *T. circumcincta* larvae were similar for each group. The efficacies of the treatment strategies were assessed using ewe and lamb faecal nematode worm egg counts (FWECs) and pasture larval counts measured at 14- to 28-day intervals from lambing (day 0) until weaning (day 109). A faecal egg count reduction test (FECRT) was performed at the end of the study to assess the ivermectin resistance status.

Results: Comparison of the areas under the FWEC-time curves indicates that OD-treated ewes shed approximately 3.5 times more *T. circumcincta* eggs between lambing and weaning than LA-treated ewes. This resulted in a higher level of pasture larval contamination by OD-treated ewes. Lambs co-grazed with OD-treated dams shed approximately 1.7 times more *T. circumcincta* eggs than lambs co-grazed with LA-treated dams. The FECRT demonstrated 22% (95% confidence interval, -67 to 63%) efficacy of oral ivermectin (0.2mg/kg bodyweight).

Summary/Conclusions: Treatment of periparturient ewes with a single dose of LA effectively controlled the PPR, even in the presence of ivermectin-resistant *T. circumcincta*. The resulting low levels of pasture larval challenge should lead to higher growth rates and reduced frequency of anthelmintic treatments in their lambs. The latter has potential to reduce selection pressure for anthelmintic resistance.

Efficacy of Zolvix (monepantel) in sheep against gastro-intestinal parasites resistant to currently available anthelmintics

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Background: Monepantel (Zolvix®; 25 g/L) has been developed to treat and control infections with gastro-intestinal parasites in sheep and, due to its unique mode of action, to manage the widespread resistance that affects the older classes of anthelmintics. Studies were conducted to confirm the effectiveness of monepantel against nematodes in sheep known to be resistant to these anthelmintics.

Methods: Twenty VICH compliant dose determination or dose confirmation studies conducted in Europe, New Zealand, Australia and South Africa assessed the efficacy of Zolvix (monepantel, 2.5 mg/kg) against natural and experimental (adult) or experimental (fourth-stage larvae) infections. Efficacy was determined by the reduction in worm burdens at necropsy in treated versus untreated animals. The resistant status of the isolates was confirmed within the studies by treatment of animals with the appropriate anthelmintic or in separate resistance characterisation studies. Three field studies were conducted on 34 sheep farms across New Zealand and Australia where the efficacy of Zolvix was compared to commercially available anthelmintics and assessed by fecal egg count reduction.

Results: In controlled slaughter studies, Zolvix was highly effective against a range of gastro-intestinal parasites resistant to the older anthelmintics (below). Resistance was present in *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus axei*, *T. colubriformis*, *Cooperia curticei*, *Nematodirus spathiger* and *Oesophagostomum venulosum* to benzimidazoles and imidazothiazoles. Resistance to macrocyclic lactones was present in *H. contortus*, *T. circumcincta*, *T. colubriformis* and *N. spathiger*. Resistance to salicylanilides and organophosphates was present in *H. contortus*. The degree of resistance varied from mild to very severe, and especially severe in *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus spp.* and often to dual, triple and quadruple combinations of different classes.

In the field studies, resistance was confirmed to the benzimidazoles, imidazothiazoles and macrocyclic lactone classes. Zolvix was highly effective at all sites.

Conclusion: Zolvix was highly effective at 2.5 mg/kg against adult and immature stages of a wide range of nematodes in sheep known to be resistant to the older anthelmintics. These results confirm the unique mode of action of this new anthelmintic class. Zolvix will be a useful tool in the management of intestinal parasitism, especially those parasites resistant to the older anthelmintics.

Efficacy and pharmacokinetics of Zolvix (monepantel) against gastro-intestinal parasites in goats

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Background: Monepantel (Zolvix®; 25 g/L) has been developed to treat and control infections with gastro-intestinal parasites in sheep and, due to its unique mode of action, to manage the widespread resistance that is present to currently available classes of anthelmintics. The same parasites are present in goats and arguably the resistance problem in that host species is more severe. A series of studies was conducted to determine and confirm the appropriate effective dose rate in goats against common adult and fourth-stage larvae of parasites and to also assess the efficacy of Zolvix when given under typical field conditions.

Methods and Results: In the dose determination study, treatment was given against fourth stage larvae at oral doses of 1.25, 2.5, 3.75 and 5 mg/kg. Zolvix was highly effective against all parasites at 3.75 and 5 mg/kg and against most nematodes at 1.25 and 2.5 mg/kg. Blood samples were analyzed for levels of monepantel and monepantel sulfone. The overall exposure to the sulfone metabolite was lower in goats than in sheep at the same dose rate. The shorter half life of the sulfone in goats might be explained by a higher clearance of the sulfone in this species. Another two studies were conducted to confirm the efficacy of Zolvix at 3.75 mg/kg in naturally or experimentally infected goats by the effect on worm counts. Zolvix was highly effective (>98%) against adult *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus axei*, *T. colubriformis* and *Chabertia ovina* and >99% effective against fourth-stage larvae of *H. contortus*, *Teladorsagia* spp *Oesophagostomum venulosum*. In field studies incorporating 10 separate field sites in France, Australia and New Zealand, Zolvix (at a minimum dose rate of 3.75 mg/kg) was highly effective in reducing fecal egg counts approximately 10 days after treatment.

Conclusion: It is concluded that Zolvix was effective at 3.75 mg/kg against adult and immature stages of a range parasites infecting goats and this new anthelmintic class will be a useful tool in the management of intestinal parasitism, especially those parasites resistant to currently available anthelmintics.

Toxicity of *Bacillus thuringiensis* proteins against the blood-feeding nematode *Haemonchus contortus*

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Bacillus thuringiensis synthesizes proteins that are used as biological control against different kind of pathogenic insects, which affect agricultural crops. Some proteins such as Cry and Cyt have been identified as the main proteins with lethal action; even some of them are used in the control of pests since long time ago. On the other hand, other *B. thuringiensis* strains have also shown lethal activity against parasitic nematodes, such as the blood-feeding parasite *Haemonchus contortus*. Recently, soluble proteins from *B. thuringiensis* IB-16 strain showed a nematocidal effect against *H. contortus* eggs and larvae stages. However, purified proteins might increase the nematotoxic effect of *B. thuringiensis*. The aim of the present study was to identify and to evaluate the toxicity of purified Cry and Cyt proteins from IB-16 *B. thuringiensis* strain against *H. contortus*. So, the amino acid sequence of *B. thuringiensis* IB-16 strain was analyzed in the Gene Bank data base and showed 100% homology with *Bacillus thuringiensis* subsp *israeliensis* (*Bti*) strain. Three main products Cyt1Aa, Cry4Ba and Cry11Aa from IB-16/*Bti* were selected and cloned in 407 acryalliferous *B. thuringiensis* strain. The lethal activity of the purified proteins was evaluated against the fourth larvae and adult stages of *H. contortus*. Lethal activity from 80% to 89% was observed in bioassays after 72 h of nematode and toxins exposure. Lethal toxin concentration (LC₅₀) was used at 0.125 and 0.25 mgml⁻¹ of protein. The main nematode damage caused by Cyt1Aa and Crystal (4Ba and 11Aa) proteins was identified on the nematode surface coat and midgut cells, where epithelial cells were swelling. Also, disintegrated somatic cells with some vacuoles between them were observed. In conclusion, this study showed the possible used of IB-16/*Bti* proteins as a nematocidal product against *H. contortus*, considered the main tropical nematode in the livestock industry. This project was partially financially by CONACYT, México, Num 23953

Re-evaluation of Efficacy against Nematode Parasites and Pharmacokinetic of Topical Eprinomectin in Cattle

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Background: Eprinomectin is the most recent commercialized member of the macrocyclic lactones in large animals and was developed as topical treatment for use against endo- and ectoparasites in cattle of all classes and ages in the 1990s. As macrocyclic lactones have been used for more than 25 years in bovines, this study was conducted to re-evaluate the efficacy of topical eprinomectin against contemporary nematode strains. In addition, the pharmacokinetic profile of eprinomectin following topical treatment of cattle that did not groom was determined in order to assess the extent of percutaneous absorption and to address the speculation that after topical treatment with macrocyclic lactones, oral ingestion was the main mechanism for the systemic exposure of cattle.

Methods: Sixteen 8-9 months old, male Brown Swiss calves (206-256 kg) experimentally infected with a range of recently isolated nematode parasites were blocked based on pre-treatment bodyweight and randomly allocated to control (untreated, n=8) or EPRINEX[®] Pour-on (Merial) at 0.5 mg eprinomectin/kg administered once on Day 0 (n=8). Calves were individually housed in a manner preventing them from physical contact with others and from self-grooming. Plasma samples collected between 1 and 21 days following treatment were analysed by HPLC methodology. Parasites were recovered using routine techniques at necropsy 21 days following treatment. Pharmacokinetic evaluations were performed using non-compartmental analysis in WINNONLIN[®] software.

Results: When compared with controls, efficacy of EPRINEX Pour-on treatment was >99% (p<0.05) against adult *Dictyocaulus viviparus*, *Bunostomum phlebotomum*, *Cooperia oncophora*, *C. surnabada*, *C. punctata*, *Nematodirus helvetianus*, *Oesophagostomum radiatum*, *Ostertagia ostertagi*, *O. lyrata* and *Trichostrongylus axei*, and inhibited *Ostertagia* and inhibited *Nematodirus* larvae. Main pharmacokinetic parameters were AUC(0-Tlast) 112 ± 22 day*ng/mL, T_{1/2} 5.2 ± 0.9 days, and C_{max} 9.7 ± 2.2 ng/mL; individual maximal eprinomectin concentrations were observed 3 to 7 days after treatment.

Conclusions: This study confirmed the continued high level of efficacy against a wide range of gastrointestinal and pulmonary nematodes. Plasma concentrations and pharmacokinetic parameters were comparable to those reported previously indicating that oral ingestion is not required to achieve efficacy following topical administration of eprinomectin.

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Effects of a protein supplement and anthelmintic treatment on communally grazed Tswana goats naturally infected with gastrointestinal nematodes

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This study was carried out to determine the effects of anthelmintic treatment and protein supplementation on health and productivity of indigenous Tswana goats. Thirty six goats of around 1 year old and of unspecified breeds were randomly divided into 4 groups of 9 animals each. The first group was treated with an oral dose of albendazole every three months. The second group was fed protein supplement daily, while the third group was drenched every three months and fed protein supplement daily. The fourth, control group was neither drenched nor fed supplement. The control group generally had higher eggs per gram (EPG) counts than the 3 treatment groups, with the highest peak in January. There was no significant difference in EPGs between the protein and the anthelmintic groups, while the protein supplemented animals had significantly lower mean egg counts than the controls. The anthelmintic/protein group had significantly reduced faecal nematode egg counts when compared to the other 3 treatments. The protein and the protein/anthelmintic groups gained significantly more weight than the anthelmintic group, while the anthelmintic group also gained significantly over the control group. The protein supplemented and anthelmintic- treated groups had significantly higher PCV values than the controls. No significant differences were observed for albumin among the groups. The additive helminth control effect of anthelmintic and protein supplementation combinations may go a long way in curbing the rampant use of anthelmintics that has the potential of promoting drug resistance.

Monepantel against arrested gastrointestinal nematode larvae in sheep

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The amino-acetonitrile derivatives (AADs) form a new anthelmintic class of which monepantel was the first molecule to be developed for use in sheep. The efficacy of this drug against normally developing fourth-stage larval and adult gastrointestinal nematodes has been described in several studies. Additionally, the AADs are known to control nematodes that are resistant to the classical anthelmintics due to their novel mode of action. However, it was unclear whether this drug would also be active against inhibited gastrointestinal nematodes.

Four studies were performed in Australia, Switzerland and the UK using naturally infected sheep. After grazing on pasture the animals were housed for 2 to 4 weeks prior to treatment in order to allow maturation of all non inhibited stages. All animals were allocated either to an untreated control group or to a group treated orally with monepantel at a dose of 2.5 mg/kg bodyweight. Efficacy was calculated based on worm counts. In addition to the standard washing procedure of the abomasum and the intestine, the mucosa from the washed organs was digested and the content examined for nematode larvae. The focus was on the inhibited forms of *Haemonchus contortus*, *Trichostrongylus axei* and *Teladorsagia* spp.

In all studies the efficacy of monepantel was >99% against adult stages as well as developing and inhibited larvae of *H. contortus*, *Trichostrongylus* spp. and *T. axei*. These results indicate that monepantel can be recommended for anthelmintic treatment of sheep also at the end of the pasture season.

Parasite migration and mucosal antibody levels in two Canarian sheep breeds showing differential resistance to *Haemonchus contortus*

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Background: We have demonstrated two Canarian breeds of sheep –Canaria Hair Breed (CHB) and Canaria Sheep (CS)– with differential resistance to *Haemonchus contortus* infection. Several abomasal immune cells (eosinophils and $\gamma\delta$ /WC1+T cells) were inversely correlated with parasitological parameters in the resistant CHB sheep and suggested an important potential role of these cells in immunity (González et al., Vet. Parasitol., 2011). Here, we extend these findings and determine the role of mucus and associated excreted antibody (IgA) in these two breeds.

Methods: Fourteen CS and thirteen CHB were infected with 20,000 L3 of *H. contortus*. Five animals of each breed were killed at 7 dpi and the rest at 28 dpi, *Haemonchus*-specific IgA in mucus using larval and adult antigens was quantified by (ELISA) at 7 and 28 days post-infection (dpi) in experimentally infected CS and CHB sheep including positive and negative mucus in each plate. The ability of abomasal mucus to affect the motility of the juvenile parasites was also assessed using a larval migration inhibition assay (LMIA).

Results: Mucus from resistant CHB sheep showed higher specific IgA against adult antigen (0.88 ± 0.309) and greater inhibition activity in the LMIA ($18,13 \pm 8.9$) than from mucus collected from susceptible CS (IgA ES: 0.519 ± 0.298 ; LMIA: 8.2 ± 3.9) at 28 dpi. In CS, mucus IgA against larval antigens correlated negatively with larval length (7 dpi) ($r = -0.9$; $p < 0.05$). In contrast, mucus IgA against adult antigens was negatively correlated with adult length ($r = -0.829$; $p < 0.05$) and fecundity ($r = -0.886$; $p < 0.05$) in CHB sheep. In addition, local immunoglobulins correlated positively with abomasal eosinophil ($r = 0.661$; $p < 0.005$) and $\gamma\delta$ /WC1+ T cells ($r = 0.9$; $p < 0.05$) only in the resistant breed.

Conclusions: Local production of parasite-specific IgA has been shown to modulate gastrointestinal parasite infectivity in several parasite species. CHB resistant sheep produced more local specific immunoglobulins, including IgA. The mucus from these animals had a greater inhibitory effect on larval movement. Negative correlations between parasite-specific antibody and parasitological correlates (larval motility, adult worm counts & fecundity) between and within the sheep breeds are discussed and suggest differential modulation of parasite establishment between the breeds.

Identification of Gastrointestinal Nematodes in Crossbreed Goats with Different levels of Resistance

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The aim of this study was to identify the species of nematodes from abomasum and small intestine of F2 crossbred goats (½Anglo-Nubian x ½ Saanen) naturally infected. Sixty young goats were kept together on pasture without anthelmintic treatment for 4 months. Based on the average of eggs per gram counts, twelve animals that presented the lowest EPG were classified as resistant and twelve animals that presented the highest EPG were classified as susceptible and slaughtered for recovery, identification and quantification of the species present in the abomasum and small intestine. The classification was based on morphological characters according to Ueno and Gonçalves method, including the length of the nematodes, length of the spicules of the males and the type of ovoider and length of females in both groups and submitted to Analysis of Variance using Statistical Analysis System. The number of parasites was lower in goats classified as resistant compared to that classified as susceptible (758.5 and 3653.5, respectively; $P < 0.05$). All parasites from abomasums were identified as *Haemonchus contortus*, with average length of females of 14.4mm (± 4.3 mm) and 16.3mm (± 3.5 mm), males 11.6mm (± 2.9 mm) and 12.4mm (± 2.2 mm), length of spicules of 410 μ m ($\pm 28\mu$ m) and 435 μ m ($\pm 21\mu$ m) and length of ovoider 471 μ m ($\pm 83\mu$ m) and 463 μ m ($\pm 89\mu$ m), to resistant and susceptible groups, respectively. In *H. contortus* females of resistant and susceptible groups, the predominant type of vulvar process was linguiform (47.56% and 49.33%, respectively). In the small intestine form resistant and susceptible animals *Trichostrongylus colubriformis* was identified, with average length of parasitic females of 6.87mm (± 0.71 mm) and 7.19mm (± 0.76 mm), males of 5.39mm (± 0.6 mm) and 6.55mm (± 0.7 mm), length of spicules of 147.9 μ m ($\pm 12.1\mu$ m) and 158.1 μ m ($\pm 12\mu$ m) and length of ovoider of 469.3 μ m ($\pm 49\mu$ m) and 464 μ m ($\pm 48 \mu$ m). It can be concluded that the infection caused by gastrointestinal nematodes in crossbred goats with different degrees of resistance was mixed, characterized by *Haemonchus contortus* and *Trichostrongylus colubriformis*. Study funded by CAPES, CNPq and Embrapa Caprinos e Ovinos.

Evaluation of methods of control gastrointestinal nematodes in sheep herd

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The administration of anthelmintics to control gastrointestinal nematodes can increase the production system costs and/or develop parasitic resistance. In this way, the aim of this work was evaluate three methods to control gastrointestinal nematodes: Famacha, strategic control based on albendazole administration and the eggs per gram (EPG) counts. It was used 240 sheep undefined breed, divided in three groups with 80 sheep each that represents the average characteristics of original herd and kept together on pasture naturally infected by gastrointestinal nematodes (85% of *Haemonchus* sp. in larvae cultures). In each group it was used a different method of worm control: I - FAMACHA®, II - strategic control (SC) based on albendazole administration and III - based in eggs per gram (EPG) counts, using the 500 EPG averages as a cutoff to anthelmintic administration. During eight months, each 14 days on rainy season and each 30 days in dry season, feces from 20 animals belonging to each treatment were collected to EPG counts. At the same day, the animals were submitted to FAMACHA method and only animals from group I that presented FAMACHA 3, 4 or 5 were medicated with albendazole. The EPG mean was higher in animals of groups I and II (570 EPG; $P>0.05$) and lower in group III (300 EPG; $P>0.05$). During the experimental period, the animals from group II were medicated with anthelmintic three times in a year and that from group III, once. The worm control by FAMACHA permitted to save 40% of costs regarding to strategic method. The FAMACHA and EPG counts permitted reduce the use of anthelmintics and, consequently, the lower costs than strategic control. However, to obtain similar results, it is necessary to have a book keeping records of the herd to help the decision of how method of control use and a team with knowledge in FAMACHA method and/or estimate the EPG counts in laboratory. Study funded by Funcap, CNPq and Embrapa Caprinos e Ovinos.

Low and high FEC sheep productive performance under extensive grazing conditions

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Southern Brazil is a sub-tropical region where *Haemonchus contortus* is predominant throughout most of the year. Under these conditions, grazing adult sheep harbour high FEC counts and it is not uncommon for flocks to be drenched an average of nine times/year. It is becoming harder for farmers to control sheep gastrointestinal nematode (GIN) infections due to parasite resistance to currently available drugs in the market. Exploring host resistance through faecal egg counts (FEC) measurements is a low cost alternative with potential to control GIN infections. The effects of FEC selection on flock production are being studied in a trial where low and high faecal egg count breeding ewes, with respective FEC<500 and FEC>1000, were allocated to contiguous paddocks at a commercial farm in Southern Brazil. Ewes were naturally challenged at adult age, and the FEC average was calculated based on two different challenge cycles, with 45 days interval. These two sub-flocks, having 60 breeding ewes each, were maintained at different paddocks at all times, except during mating season (45 days/year). The objective was to measure performance on numbers of drenches, mating and weaning weights of dams and their offspring, respectively. FEC averages for the low and high groups of dams were of 177 and 2287 (p=0.0001), respectively. A three-year period was analysed and results show an average increase of 2kg/year in weaning weight for the low FEC ewe's offspring compared to the high FEC group progeny (p=0.0086). Low FEC ewes were also 3kg heavier than high FECs at mating time (p=0.0009). An average reduction of 1.3 drenches/year for the low FEC group was observed, with significant year variation. Higher reductions in drenches were observed in more humid years. Although the low FEC group of dams showed egg count averages of 177 during natural challenge, it doesn't mean they were unable to show high egg counts after their challenge period, in fact these low FEC breeding ewes had to be drenched four times every year (two strategic drenches at the beginning of mating and of lambing). The results found here show that it is feasible for farmers to explore individual host resistance to GIN infections by keeping more resistant individuals. In doing so farmers would also successfully reduce the number of drenches in their flocks.

***Alternanthera pungens* and *Colletia paradoxa*, as Potencial New Anthelmintic Parasitics**

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Nematodes cause severe illness in ruminants, with high mortality rate and consequently, they produce important economic losses to producers. To fight them, synthetic parasitic like benzimidazoles, avermectins and isoquinoleines are applied yearly and their indiscriminate use had driven to frequent reports of worm resistance to these active substances. New efficient and safe therapeutic alternatives are always needed in order to keep the viability of cattle production. A seldom followed trend is to validate the ethnoveterinary knowledge, which can be either a valid alternative for allopathic medicine or a source for new leader compounds. Our group is trying since some years to develop Phytoveterinary medicine, in this particular case anthelmintic phytotherapeutics. After a screening of different natural species, two plants, *Alternanthera pungens* and *Colletia paradoxa*, have shown promising anthelmintic activity, so that can they can be employed as potential veterinary phytopharmaceuticals. The first one is widely employed in human medicine and there are reports on the former's antiparasitic properties.

To evaluate anthelmintic activity it was used an in vitro model with *Nippostrongylus brasiliensis* (L4) (already reported by us), reporting a lethal concentration 50 (CE₅₀), for each sample was serially diluted twice with 6 replicates each, and if necessary the test was repeated. Statistical analysis was performed using Student's test, a probability greater than 0.05 was not considered significant. Albendazol was employed as positive control (CE₅₀: 0,09µg/ml). Infusions and ethanolic extracts from each plant were assayed for biological activity. The more active one from *A. pungens* were further fractionated through liquid-liquid extraction and the anthelmintic activity of the resulting partitions were evaluated. Three raw extracts were obtained from *C. paradoxa*: ethanol, infusion and acidified water.

This is the first report on the anthelmintic activity of these two plants. The hydroalcoholic extract from *A. pungens* was the most active (CE₅₀: 63µg/ml), whereas the infusion had little activity (CE₅₀: 478µg/ml), solvent fractionation of the ethanolic extract cause a diminution in the overall activity of the extracts. The butanolic extract was inactive and the one from the AcOEt partition showed poor bioactivity. The aqueous remnant was the most active (CE₅₀: 91µg/ml) but with less bioactivity than the original extract. The aqueous extracts from *C. paradoxa* showed interesting activity: the acidified water had CE₅₀: 24,5µg/ml and whereas the infusion had a CE₅₀: 39µg/ml. The hydroalcoholic extract had poor activity: CE₅₀: 415µg/ml. *C. paradoxa* shows promising activity that can be developed as an anthelmintic phytoveterinary.

Effect of plant protein source on periparturient resistance to parasites in ewes

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Background: Effects of metabolizable protein (MP) supply on the degree of periparturient relaxation of immunity (PPRI) in sheep may be dependent on MP quality and thus protein source. Here we hypothesized that xylose-treated soybean meal (SoyPass) supplementation reduces the degree of PPRI more effectively than field bean supplementation, as the latter contain less digestible undegradable protein with lower levels of methionine.

Methods: Twenty-four multiple-bearing ewes were trickle infected with *Teladorsagia circumcincta* larvae from day₋₅₆ to day₊₃₁ relative to parturition date (day₀). From day₋₂₆ onwards, ewes were fed at either 0.8 (LP) or at 1.2 times their respective MP requirements using either SoyPass (HPS) or field beans (HPB). Ewe and litter body weight and ewe condition score were measured weekly. Ewe faecal egg counts (FEC) were assessed twice weekly, and transformed via log (FEC+1) for statistical analysis. Regularly collected blood samples were analysed for plasma pepsinogen.

Results: Mean log FEC during lactation was lower for HPS than LP ewes, whilst that of HPB ewes was intermediate (2.33 vs 2.63 vs 2.49; s.e.d. 0.12; P=0.083). By day₊₃₁, HPS ewes were heavier than LP ewes while HPB ewes were intermediate (63.1 vs 58.8 vs 61.3 kg; s.e.d. 1.50 kg; P<0.05). Similar effects were observed for litter body weight (27.33 vs 22.66 vs 25.88 kg; s.e.d. 1.7; P<0.05). HPS and HPB ewes had higher condition score than LP ewes during lactation (2.1 vs 2.1 vs 1.8; s.e.d. 0.07; P<0.005). LP ewes had higher plasma pepsinogen concentrations than HPS and HPB ewes, both during gestation (2.12 vs 1.17 and 1.43 mU; s.e.d. 0.28 mU; P=0.008) and lactation (1.96 vs 1.14 and 1.28 mU; s.e.d. 0.25 mU; P=0.008).

Summary/Conclusions: These results suggest that extra MP supply from field beans might be less effective in reducing the degree of PPRI than that from SoyPass. Whilst field beans also did not significantly improve lactational performance, both protein sources resulted in reduced pepsinogen concentrations. This suggests that additional MP from field beans was mainly directed to maintain abomasal wall integrity rather than immunity to parasites. Thus, dietary protein quality affects the effectiveness of additional MP to reduce PPRI in sheep.

Effect of feeding Santa Cruz sheep with nutritional pellets containing *Duddingtonia flagrans* chlamydospores in the reduction of *Haemonchus contortus* (L₃) in feces

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This research was aimed to evaluate the effect of feeding supplementation of Santa Cruz sheep with *Duddingtonia flagrans* chlamydospore nutritional pellets (DFCNP) in reducing the population of *Haemonchus contortus* infective larvae in feces. Two groups of six *H. contortus* artificially infected Santa Cruz sheep were formed. Group 1 (Control) was fed under a daily nutritional regime for 11 weeks, consisting in commercial sheep food (CSF), Molasses and dried and grinded alfalfa; group 2 (Treated) received a feeding supplementation with 10 nutritional pellets containing 7% sorghum, 51% Soya paste, 20% wheat bran, 18% molasses, 2% mineral salts and 2% lime and an amount of *D. flagrans* chlamydospores adjusted to 2×10^6 chlamydospores according to the live weight of the animals. Fecal samples were taken directly from rectum of each animal once a week to estimate the number of eggs eliminated per g of feces (epg) through the McMaster technique. Also, three fecal cultures (of 6 grams of feces) per animal were performed in three plastic Petri dishes. Live weight of sheep was recorded once a week. Results showed an important reduction in the *H. contortus* larvae population in fecal cultures of sheep supplemented with DFCNP in a range of 42.1-84.3% reduction percentage during the whole experiment, excepting the third sampling with no reduction recorded. Daily weight gaining was very similar in the two groups of sheep; being 136g for group 1 and 163g for group 2. The consumption of DFCNP offers a good alternative in terms of daily weight gaining compared with a CSF. Package of Cell Volume in group 2 showed lightly lower values than group 1; nevertheless, the gaining in live weight was not affected. The reduction in the larvae population in feces is a clear indicator about the efficacy of nematophagous fungi acting as biological control agents with good expectations as an alternative tool of control in integrated control and prevention programs.

Comparison of an individual versus a composite sampling method for counting gastrointestinal nematode eggs in sheep feces

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Introduction: The conventional method for estimating the average parasite egg count for a group of sheep is not economically convenient, as individual anthelmintic treatments are further more inexpensive than the cost for each test. This may limit the number of tests requested by the animal owners. In this study, individual samples were compared against composite ones to quantify gastrointestinal nematode eggs per gram of feces.

Methods: Grazing Suffolk and Dorset ewes raised in Hutzilac, Estado de Morelos, Mexico, located in a subhumid climate with a mean annual temperature of 10°C and a mean annual 1725 mm rainfall were included in the study. After grazing, sheep were kept overnight in pens. Thirty individual samples were taken and 30 composite samples of freshly released feces were collected from the floor at days -7, 0, and 14. Fenbendazole was administered on day 0. The McMaster, coproculture and Baermann techniques were carried out. Statistical analysis was performed using Bootstreat 1.2. Bootstrap resampling was used to estimate the difference of means, correlation and regression with 10,000 repetitions in each case.

Results: Regarding Suffolk sheep, the 95%confidence interval was (-1,485.0, 250.0), which indicates that the individual means and composite are the same. The correlation estimator 0.485 showed a regular association. The worst situation occurred when the lineal regression analysis result was $R^2=0.235$. On the other hand, for Dorset sheep, the 95%confidence interval for the difference of individual and composite means was (-13.0, 200.0) which is considered acceptable. When the correlation estimator was determined, an unfavorable value of -0.257 was assessed. Lineal regression analysis did not show a relation, as the determination coefficient was $R^2 = 0.06$. When breeds were combined. the 95% confidence interval for mean differences was (-173.0, 250.0) with a correlation coefficient $r=0.384$ and a $R^2 = 0.148$.

Conclusions: Evidence has been provided (Baldock 1990, Hood 2006, Morgan 2005, McKenna I 2007), that composite samples can replace individual ones. Tests designed in this preliminary study did not provide enough evidence to support such pronouncement. Hence further integration methods have to be evaluated, as well as more composite samples and statistical models to be in accordance with other authors.

Prevalence of anthelmintic resistance in gastrointestinal parasites of South American camelids in the Southeastern United States

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Background: Gastrointestinal nematode parasites, particularly *Haemonchus contortus*, are the greatest single health problem in small ruminants and camelids in the southeastern US. Anthelmintic treatment failures due to high levels of drug resistance are becoming commonplace in sheep and goats, and it appears that similar trends are occurring in camelids. The prevalence of anthelmintic resistance on goat and sheep farms in the southeastern United States is well documented; however, very little is known about the prevalence of anthelmintic resistance on camelid farms. The objective of this study was to evaluate the status of anthelmintic resistance in gastrointestinal parasites of South American camelids in the southeastern United States.

Methods: The DrenchRite[®] Larval Development Assay (LDA) was used to evaluate the presence of anthelmintic resistance on 32 farms (16 alpaca and 16 llama) in the southeastern United States. The DrenchRite[®] LDA utilizes a 96 well plate with doubling concentrations of benzimidazole, levamisole, and ivermectin anthelmintics, along with untreated control wells, to make inferences on the resistance status. Additionally, wells containing ivermectin are used to determine resistance to moxidectin.

Results: *Haemonchus contortus* was the primary species present on every farm, with *Trichostrongylus colubriformis* being the next most common. However, all farms but 3 had insufficient levels of *T. colubriformis* to quantify the presence or absence of resistance. Of the 32 farms evaluated for *Haemonchus contortus*, all 32 (100%) were resistant to benzimidazole, 7 (22%) were resistant to levamisole, 28 (88%) were resistant to ivermectin, and 7 (22%) were resistant to moxidectin. Twenty-eight farms (97%) demonstrated multi-drug resistance, 4 farms (13%) exhibited resistance to all 3 classes of anthelmintics, and resistance to all anthelmintics (total anthelmintic failure) was found on 1 farm (3%). Of the farms evaluated for *T. colubriformis*, resistance to one or more drugs was present on all 3.

Conclusions: Anthelmintic resistance is a serious problem on camelid farms in the southeastern United States, with a prevalence of resistance similar to that seen in sheep. Parasite control must become more evidence-based and include regular surveillance for anthelmintic resistance. In addition, management of refugia using FAMACHA should be implemented on camelid farms to reduce the selection for resistance.

Efficacy of avermectins against naturally acquired intestinal nematodes in Girolando calves from a commercial herd in Vale do Mucuri, southeastern Brazil

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Intestinal nematodes of cattle is still a great health and economic concern worldwide. The control of these parasites has been based on anthelmintic applications and its ongoing use has contributed to the development of drug-resistant parasites. The present study aimed to evaluate the efficacy of two nematode control programs based on the application of avermectins on beef calves. Eighty-four half-blood Gir x Holstein (F1) calves aged six months who were naturally infected with intestinal helminthes received two different anthelmintic treatments. Group A received the anthelmintic agent following the routine farm procedures (eight treatments with ivermectin 1%) and Group B received a strategic treatment with ivermectin 3.15% at the beginning and at the end of the rainy period. Data on weight gain, counts of eggs per gram of feces (EPG) and genus of larvae from fecal cultures were collected on a monthly basis during 21 months. The nematode species in the pasture were monitored using tracer calves. During the experimental period, the monthly average temperature varied between 22 and 33°C (except for July). There was no significant reduction ($p>0.05$) in EPG counts in any group after anthelmintic treatment. Age was the variable better correlated ($r=58$) with EPG counts. EPG counts were significantly ($p<0.05$) lower at 18–21 months of age. The genera identified in the stool cultures were *Cooperia*, *Haemonchus*, *Oesophagostomum* and *Trichostrongylus*. *Cooperia* was the most prevalent genus in the first five months of the trial and *Haemonchus* was the most prevalent in the following months. Tracer calves showed high nematode burden (>500 helminths) most months and the main species identified were *Cooperia punctata* and *Haemonchus contortus*. There was no significant ($p>0.05$) difference in EPG counts and weight gain between groups A and B. The study demonstrated that macrocyclic lactone acquired resistance in nematode parasites and the development of immunity was paramount to reduce nematode burden.

Efficacy of a Limonen Formulation in Sheep Artificially Infected with *Haemonchus contortus*

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It is necessary to investigate and scientifically validate phytotherapeutic alternatives for future use to control gastrointestinal nematodes in ruminants. This study aimed to evaluate the anthelmintic activity in lambs of a formulation based on the commercial plant isolate R-(+)-Limonen. Twenty-four Santa Inês lambs were housed and treated with anthelmintic to remove natural infection. They were then orally infected with 4,000 L₃ *Haemonchus contortus* Embrapa2010 isolate (CARS). Twenty-eight days after infection the lambs were weighed and the fecal egg count (FEC) was measured to obtain a similar average of the two parameters in the following treatments: 1) negative control (water), 2) positive control (levamisole 4.7mg/kg body weight (BW)), 3) R-(+)-Limonen (600 mg/kg BW in fasting) and 4) R-(+)-Limonen (500 mg/kg BW). The egg hatch test (EHT) was performed in the negative control and in the R-(+)-Limonen (500 mg/kg BW). On the eighth day after treatment there was a significant reduction in FEC. Treatment 3 and 4 attained efficacies of 81.3% and 98.7%, respectively. The average FEC on day zero was 4,458 and 3,875, falling to 833 and 50 on the day +8, respectively. There was egg hatch inhibition of 8.7% in the negative control and 63.4% in treatment 3 for day +8. In treatment 4, there were not enough eggs to perform the test, since the efficacy was 98.7%. In treatment 3, toxicological effects were observed, mainly lack of motor coordination, head shaking and lack of interest in food. These effects were much less severe in treatment 4, in which only head shaking for 15 minutes was observed. In treatment 4, the animals received a lower dose and were not denied food. According to others authors, maybe the food slowed the passage of the R-(+)-Limonen and increased the exposure time of *H. contortus*. In conclusion, the treatment at 500 mg/kg BW reached the efficacy recommend by WAAVP and the EHT results indicated the possibility of a reduction in the pasture contamination. However, we believe this formulation can be improved in the future with a smaller dose per mg/kg, to reduce toxicity.

Strategic integrated control of *Haemonchus contortus* in sheep in Passo Fundo, Southern Brazil

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Introduction: Sheep meat production is a profitable alternative for farmers, but gastrointestinal parasites are one of the main causes of production losses. The Famacha method arose from the need of a non-chemical approach that could identify animals with clinical haemonchosis and refer them for treatment. The use of Voisin grazing management is an alternative for the reduction of the number of larvae in pastures. The aim of this study was to assess these two methods using integrated control of *Haemonchus contortus* in meat sheep.

Material and methods: An experiment with 32 female Suffolk, Ile de France and cross-bred sheep divided into two groups of 16 animals was carried out from January 2009 at the Center for Extension and Animal Husbandry Research (Cepagro) of Universidade de Passo Fundo (UPF), Brazil. In group 1, only animals with eggs per gram (EPG) equal to or greater than 2,000 were medicated; in group 2, the animals were assessed by the Famacha method. Every 30 days, weight, microhematocrit, and fecal samples were assessed for all animals. The McMaster's technique and coproculture with larval identification were used for diagnosis (UPF) and microhematocrit determination was used to assess the degree of anemia. Behavioral variables and body parameters were measured at Cepagro.

Results: Mean live weight and mean EPG between the groups were not statistically significant on the F test ($P>0.05$). Microhematocrit determination showed that group 2 (31.91) had better results than group 1 (29.89) ($p=0.0130$). The score for body parameters (χ^2) revealed some differences between the two groups: 3.28 for group 2 and 3.18 for group 1 ($p=0.060$). The score for the qualitative and quantitative flight-time test obtained on the F test showed differences between the groups: 2.0 for group 2 and 3.1 for group 1 ($p=0.019$), and 2.1 for group 2 and 1.5 for group 1 ($p=0.065$), respectively. The genus *H. contortus* was the most prevalent in this study.

Conclusion: The use of strategic integrated control of *H. contortus* in an intensive meat sheep production system proved efficient and self-sustaining.

Correlation between Famacha and Packed Cell volume in Crossbred Goats with Different Levels of Resistance

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The aim of this study was to correlate the Famacha scores and packed cell volume (PCV) values of crossbred goats with different levels of resistance. For this purpose, 92 F2 crossbred goats ($\frac{1}{2} \times \frac{1}{2}$ Saanen Anglo-Nubian) were used. Every week, blood samples were collected and the Famacha method of worm control was performed. The estimated sensitivity, specificity, predictive positive and negative values were evaluated by chi square test, using the EPI-INFO version 6.0 software. Positive results were considered as in grades 3, 4 and 5 Famacha and PCV values $\leq 19\%$ as indicative of anemia. The results indicated a moderate sensitivity of the test, both for the animals in the resistant group (45.5%) as for the animals in susceptible group (50%), demonstrating that, in this study, the method was able to correctly identify 45.5% of the resistant and 50% of the susceptible animals that require anthelmintic treatment. The animals of the resistant group showed higher means of true negative and specificity values, showing that the method was effective in identifying non anemic animals. Comparing the values of PCV and Famacha, it appears that the obtained results, i.e., 97.6% of the resistant and 91.3% of the susceptible animals were truly negative (grade 1 or 2 and PCV $> 19\%$), which represents a negative Famacha score and no degree of anemia, while 83.3% of the resistant and 64.9% of the susceptible animals were false positives (grades 3, 4 or 5 and PCV $> 19\%$), meaning positive Famacha and absence of anemia. More research is demanded in using the method Famacha in an attempt to reach a common denominator in applying this method in goats. Study funded by FUNCAP, CNPq and Embrapa Caprinos e Ovinos.

Experimental cross infection of *Haemonchus contortus* from sheep to cattle

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Haemonchus contortus produces severe economic losses in sheep farms all over the world. The alternate or mixed sheep/cattle grazings have been used as an alternative to complement anthelmintic treatments. The aim of this study was to evaluate the capability of *H. contortus* to infect cattle. For this purpose, three isolates of *Haemonchus spp.* were obtained from natural infections in mixed sheep-cattle grazings of Argentina and named as Ayacucho, Cedive and Curuzú. A fourth one originates on a farm in Uruguay where only sheep have been grazing during the last 20 years and was named as Sul. Sixteen lambs and sixteen calves of 4 to 6 months old were dewormed, identified and allocated into four comparable groups of 4 animals each. The lambs and calves were each infected with 7500 and 30000 infective larvae (L3) respectively, as follows: G1 with Ayacucho isolate, G2 with Cedive, G3 with Curuzú and those in G4 with Sul isolate. Half of the animals of each group was necropsied at day 45 post infection (p.i.) and the other half at day 90 p.i. for counting and identification to determine rate proportions (R.P.: number of adults / number of L3 inoculated x 100) and speciation of the worms established in abomasums of lambs and calves. A function involving three measures of male spicules was used to discriminate between *H. contortus* and *H. placei* species. At day 45 p.i. the R.P. and speciation of established adult worm in the abomasums of lambs were: G1: 28.8% (92% *H. contortus*, 8% *H. placei*); G2: 37.5% (91% *H. contortus*, 9% *H. placei*); G3: 70.5% (71% *H. contortus*, 29% *H. placei*) and G4: 8.5% (98% *H. contortus*, 2% *H. placei*). At day 90 p.i. the R.P. was lower than 10% for G1, G2 and G4; the G3 showed a high R.P. (48.2%). At day 45 p.i. the R.P. and speciation in calves were: G1: 6.8% (92% *H. contortus*, 8% *H. placei*); G2: 0.04% (100% *H. contortus*); G3: 3.8% (*Haemonchus spp.*) and G4: 0.04% (*Haemonchus spp.*). At day 90 p.i. no worms were recovered from abomasums. It might be concluded that in mixed sheep-cattle grazings *Haemonchus contortus* may develop in cattle to adult stages in low proportions and with a shortened life span. These findings call for further field research to a better understanding of the phenomenon of cross transmission from sheep to cattle.

Macrocyclic lactone resistance in an isolate of ovine *Trichostrongylus colubriformis* from North Queensland

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Background: In Australia, anthelmintic resistance is commonly observed against the major chemical groups in the major pathogenic species of sheep *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Teladorsagia circumcincta*. Resistance of *T. colubriformis* to macrocyclic lactones (MLs) had only been confirmed once in an isolate from a Gold Coast goat farm. Although MLs are widely used in Australia, resistance of *T. colubriformis* to this anthelmintic group appears to be rare in sheep. Here we report the observation of a sheep isolate of *T. colubriformis* that may be resistant to MLs.

Methods: Larvae were collected from cultured sheep faeces collected from a farm near Mackay in North Queensland. Two worm free sheep in pens were infected with the larvae and faecal egg count (FEC) monitored from day 21-35 after infection. Both sheep were then dosed orally with 200 µg/kg moxidectin (Cydectin, Fort Dodge Australia Ltd) and FEC undertaken at days 42, 44 and 50. A larval development assay for ivermectin (IVM), thiabendazole (TBZ) and levamisole (LEV) anthelmintics compared this isolate, Mackay (MKY), against susceptible McMaster (McM) and resistant Gold Coast goat (Mox-R) *T. colubriformis* isolates. Microscopic examination of larvae and adult worms confirmed MKY was *T. colubriformis*.

Results: Comparison of FEC before and after treatment with moxidectin indicated no significant reduction in FEC. For the larval development assays, comparison of resistance ratios (RR) for IVM showed that MKY had the same resistance ratio as that for Mox-R (RR of 21 and 19, respectively) when compared to McM. For both TBZ and LEV, the MKY isolate appeared to more resistant than the Mox-R isolate with RR of 42 vs 27 and 7.8 vs 1.0 for MKY and Mox-R, respectively, compared to the McM isolate.

Summary/Conclusions: An isolate of multi-drug resistant *T. colubriformis* was identified from sheep on pasture in tropical North Queensland. This isolate appears to be highly resistant to ML, BZ and LEV anthelmintics. This is the first confirmed instance of this level of resistance in this species from sheep in Australia and sounds a warning for other areas where *T. colubriformis* is a major pathogen and current control is reliant on ML anthelmintics.

The use of *Stylosanthes guianensis* cv. Mineirão and *Medicago sativa* hays to control gastrintestinal nematodes in feedlot lambs

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The gastrintestinal nematodes parasitism is considered one of the most important limitation to the sheep breeding. Some reports have shown that the consumption of tanniniferous plants reduces the faecal egg counts (FEC) and the worm burdens in ruminant. The aim of this study was to investigate the influence of diets containing *Stylosanthes guianensis* cv. Mineirão and *Medicago sativa* hays on the FEC in feedlot lambs. For this study were used three groups (G1, G2 and G3) of 13 crossbred Santa Inês lambs, weighing between 20 and 34kg and aged six to seven months. Only healthy males were used and as they come, were treated with Sulfaquinoxaline sodium for three days and drenched. The experiment started thirteen days after an acclimate period. The groups were reared in 03 indoor enclosures for six weeks with water and mineral salt ad libitum and 03 different feeding protocols. The dairy diet for each group was: 3 Kg *Medicago sativa* hay, 29,7 Kg sugarcane plus elephant grass (1:1) and 3,6Kg of concentrate (30% soybean meal, and 70% corn) for G1; 3,9 Kg *Stylosanthes* hay, 29,7Kg sugarcane plus elephant grass (1:1) and 3,6Kg of concentrate for G2 and 39,6Kg sugarcane plus elephant grass (1:1) and 7,2kg of concentrate for G3. During the trial faecal samples were collected every week to evaluate the FEC and the data were compared by Kruskal-Wallis nonparametrical statistical test. The FEC means and standard deviations from day zero were: 1282±1515 for G1, 854±869 for G2 and 1055±982 for G3. The FEC means and standard deviations observed were: 1078±1421 for G1, 462±819 for G2 and 1424±2645 for G3. The FEC from G2 were significantly smaller than the others and there was no significant difference between G1 and G3. According to available researches *Stylosanthes guianensis* cv. Mineirão has higher levels of condensed tannins than *Medicago sativa*, moreover this trial shows that this *Stylosanthes* variety could be useful to control gastrintestinal nematodes in sheep.

In vitro effect of *Tagetes minuta* and *Mentha villosa* decoctions on eggs and larvae of *Haemonchus contortus*

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Gastrointestinal nematodes control is based on the administration of synthetic anthelmintics, however the development of resistant populations requires the search for control alternatives. Popular reports relate *Tagetes minuta* and *Mentha villosa* as plants with anthelmintic activity. The aim of this study was to evaluate the effect of *T. minuta* decoction (TMD) and *M. villosa* decoction (MVD) on eggs and larvae of *Haemonchus contortus*. The plants decoctions were prepared by boiling 400g of leaves in 4000 ml of distilled water for 2 hours. The decoctions were frozen and lyophilized. For egg hatching test (EHT), 100 eggs of *H. contortus* were incubated for 48h with TMD and MVD at concentrations of 0.31 to 5 mg/ml. In larval exsheathment test (LET), *H. contortus* 1,000 L3 were incubated for 3 hours with TMD (620 µg/ml) or MVD (310 µg/ml). After incubation, the larvae were washed and centrifuged three times in PBS and exposed to artificial exsheathment by contact with sodium hypochlorite solution. The kinetics of exsheathment was monitored for 1h by regular observation at intervals of 10 minutes. Both tests were accompanied by controls. The data were subjected to analysis of variance and compared by Tukey ($p<0.05$) and Kruskal-Wallis ($p<0.05$) tests in EHT and LET, respectively. In EHT the concentration of the decoction which prevented 50% of larvae hatching (EC50) was calculated using SPSS 8.0. For the TMD, the inhibition percentage of egg hatching at the concentrations of 0.31, 0.62, 1.25, 2.5 and 5 mg/ml was 12.1, 44.6, 85.8, 96.8 and 100%, whereas the MVD efficacy was 24.7, 62.7, 90, 94.3 and 94.4%, respectively. The EC50 were 0.66 and 0.5 mg/ml for TMD and MVD, respectively. In LET, *H. contortus* exsheathment was blocked by two decoctions, during 1h. The decoctions of *T. minuta* and *M. villosa* showed inhibitory activity on egg hatching and larval exsheathment. Thus TMD and MVD may have potential as anthelmintic for the control of *H. contortus* if *in vivo* investigations indicate useful effects.

Development of real-time RT-PCR procedure to determine the relative expression of interferon gamma in response to *Haemonchus contortus* infection in Pelibuey lambs localized in tropical areas

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The aim of this study was to determine the expression of *inf-γ* on infected Pelibuey lambs with the blood-feeder nematode *Haemonchus contortus* based on real time RT-PCR procedure. Nineteen Pelibuey-breed lambs from four to five months old were selected base on natural hemonchosis resistance phenotypes traits. White- blood cells were taken to extract RNAt (100 ng/μl) and cDNA (100 ng/μl) from infected lambs at 0, 1, 2, 7, 14 and 28 days after infection. The upstream and downstream of *inf-γ* primer sequences were 5'Tgg gcT cTc ATc TcT AgT cTT c-3' and 5'gAT ATA TAc ccA TAT TAT gcc cAT c-3', respectively. Positive control such as *gapdh* and *β-actin* genes were also used. Real-Time PCR (qPCR) was performed with a Rotor-Gene Q with SYBR Green I using a final volume of 10 μl with specific primer mix. Quantitative PCR was performed in 35 cycles with the following parameters: 95°C for 5 min of pre-incubation, 10 s of denaturation at 95°C, 63°C for 15 s and 72°C for 8 s. Melting curve analysis was carried out to ensure single product amplification. Data expression recorded as RG mode (take off and amplification) by REST software programme. Differences of mRNA abundance were observed for the *inf-γ* expression ($P<0.01$). *inf-γ* was up-regulated in resistance lambs after one and two days after infection. In comparison to control genes, which showed 1.65 media amplification with 23 take off. In contrast, infected lambs tolerance to *H. contortus* showed >1.8 amplification media and around 28 take off. Melting curve analysis showed a single product at 81 Tm to *inf-γ* and 85 Tm and 90 Tm to *gapdh* and *β-actin* genes, respectively. These results suggest that hemonchosis infection in resistant as compared to susceptible lambs elicits a modified Th1-type immune response through the first 48 h, characterized by overexpression of *inf-γ*. Differential regulation of Th1 cytokines between Pelibuey lambs may be partially responsible for differences in parasite resistance.

Influence of Gastrointestinal Nematodes of quality lamb (*Ovis aries*) meat

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The production of quality sheep meat arises as an economic alternative in national sheep systems. It is based on the wintering of the lambs, who are exposed to different genes of gastrointestinal nematodes (NGI) infecting larvae. The aim was to describe the presence and action of the NGI regarding the live weight gain in lambs that were intended for such production. It worked with 360 lambs (Corriedale), corresponding to three producers of heavy lambs estates. In each establishment the sample was subdivided into two groups, Control and Treatment (GC and GT) with anthelmintic broad-spectrum. Remained under infection natural of NGI, individually recorded every 21 days the live weight, was extracted to stool and treated to GT. Samples of stool fecal, was established as a parasitic genus with greater distribution on the *Haemonchus contortus* (51.4%), followed by *Trichostrongylus spp* (40.7%), although seasonally (autumn and winter), are presented *H. contortus* 82.3%, *Trichostrongylus spp* 7.97%, and *H. contortus* 20.4% *Trichostrongylus spp* 73.3% respectively. In the descriptive statistical analysis weight variable behaved with distribution next to normal, while the eggs per gram (HPG) of stool with a Poisson distribution. In each establishment, treatment and sex, using the method of ordinary least squares was estimated, through the analysis of regression weight per day, daily gain (GD) individual and this was his analysis of variance. The difference between GT and GC was significant in establishing A ($Pr > 0.0043$ F *), with half of GD 36 and 73 g respectively, in B and C were not significant ($Pr > F$ 0.3068) and ($Pr > 0.1813$ F), with averages of GD for GC and GT - 0.004 and 0.006 g. and - 0.003 and 0.003 g respectively, representing void winnings (maintenance level). It was concluded that at this stage of growing and fattening the pathogenic genus of NGI most prevalent are *H. contortus* and *Trichostrongylus spp*. Statistical analysis based to estimating a single GD showed significant differences in setting A and given that there were differences between the GC and GT for the HPG variable, in the three shows that food was restricted, not reflecting the HPG control in the GD except in A setting, but in the other two the trend was the same, also in establishments B and C males presented a better performance in the production of meat.

Selection of ewes with low and high trichostrongylids eggs count in Argentina

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Introduction: Ewe's selection could be a reliable tool to improve genetic resistance to parasite in farms, either complementary of the introduction of selected rams, or useful by itself. We propose a field procedure.

Methods: In years 2005-2006, 470 (ov-05) and 136 (ov-06) Corriedale ewe lambs, belonging to a commercial farm from Corrientes, Argentina (30°03'09"S; 58°15'47"W) were weaned, dewormed, and identified. Monthly, 15 faecal samples were taken, searching for natural infection trends, measured by FEC. Once high FEC were detected, all lambs were sampled and drenched, and then, this schedule was performed over again until get two sampling events of the whole flock, in which the 3rd quartile values were distinct to the median. At the end of each year, animals were grouped as high (h) or low (l) eggs count; depending on they belonged to the 4th or 1st quartile of distribution, in all samplings.

Ewe's mating was performed in February of 2007 and 2008 for ov-05, and in 2008 for ov-06. After that, were dewormed and keep grazing together until the next mating. FEC values of groups "h" and "l" were compared before and after lambing by Kruskal-Wallis test.

Results: Sixty three "b" ewes were selected in ov-05 (13,4%), from which 30 lambed in 2007, and 23 in 2008. In addition, 54 "a" ewes (11,5%) were also selected, from which 21 and 9 lambed in 2007 and 2008, respectively.

Regarding ov-06, 20 "b" (14,7%) and 15 "a" (11,0%) ewes were selected, from which 19 and 9 respectively lambed in 2008. The FEC's averages comparison of "h" and "l", showed for the ov-05 group 1490 ("h") vs. 544 ("l") in prepartum, and 726 ("h") vs. 340 ("l") in postpartum of 2007, whereas in 2008 were 100 ("h") vs. 24 ("l") in prepartum, and 800 ("h") vs. 233 ("l") in postpartum. Ov-06 showed 325 ("h") vs. 90 ("l") in prepartum, and 1100 ("h") vs. 356 ("l") in postpartum. Differences among "h" and "l" were always highly significative ($p < 0,05$)

Conclusion: Ewes selected as either "h" or "l", in at least two whole sampling after weaning, repeated their same condition in peripartum. This procedure allowed the selection of 11-14,7% of the flock in both senses (susceptible or resistant).

Evaluation of the eggs development of *Trichuris vulpis* at laboratory conditions

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Background: *Trichuris vulpis* is a nematode parasite of dogs' cecum and is highly prevalent worldwide. In literature there are reports of *T. vulpis* causing the syndrome of visceral larva migrans (Sakano et al., 1980), a disease previously attributed only to *Toxocara* sp.. *T. vulpis* eggs are highly resistant to environmental conditions and can survive for long periods. The presence of the nematode is evidenced by finding of eggs in faeces by stool examinations. In Brazil, few is known about the biology of this nematode and to aim expand this knowledge, began a study to 1- the fecal eggs counts (EPG) from two naturally infected dogs 2- observed at the different stages of development eggs of *T. vulpis* kept under refrigeration ($\pm 9^{\circ}\text{C}$) and temperature ($\pm 25^{\circ}\text{C}$).

Methods: In March 2010, a dog naturally infected with OPG early 4400 was donor stool and held the EPG of the samples using the technique McMaster (Gordon; Withlock, 1939), and recovery of eggs by the modified flotation technique (Rodrigues and Honer, 1984). The recovered eggs were kept in Petri dishes at room temperature and refrigerator for observation of developmental stages.

Results: The eggs of *T. vulpis* kept at room temperature and refrigeration ($\pm 9^{\circ}\text{C}$) were evaluated each 48 hours and to observed the larval formation. In March of 2010 (summer), mean temperature 28.2°C , we observed the complete larvae development in 13 days and in May 2010 (autumn) mean temperature of 22.4°C the larval development occurred after 26 days. In January 2011 (summer), temperature mean of 35°C , the larvae development occurred in 10 days. The eggs kept under refrigeration do not developed.

Conclusion: It was observed that the temperature influences in the development of eggs of *T. vulpis*, which is of great importance, for the biology of the nematode. The viability of infections was not evaluated. This study is ongoing and more information will expand knowledge of this dog nematode at tropical regions.

Efficacy of Monepantel against multiple anthelmintic resistant nematodes of sheep in the subtropical area of Argentina

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A controlled test efficacy trial with monepantel (ZolvixTM) against multiple anthelmintic resistant nematodes of sheep naturally infected in the subtropical area of Corrientes province (Argentina) was carried out. The nematode populations on the farm had previously been tested for susceptibility to anthelmintics and results indicated a high level of resistance to ivermectin, benzimidazole derivatives, levamisole and closantel with *Haemonchus* spp and *Trichostrongylus* spp. mainly involved.

Fourteen female weaned lambs, of Polwerth breed, weighing 29 kg on average were selected from the flock according to the number of nematode eggs in the faeces (e.p.g.). The animals were weighed, identified with numbered ear tags and divided at random into two comparable groups of seven animals each of which were treated as follows: G1 of animals (average e.p.g.: 2574) treated orally with monepantel at the dose rate of 2.5 mg/ kilo body weight (k.b.w.) and G2 of animals (average e.p.g.: 2520) that received saline solution in a volume equivalent (1 ml/ 10 k.b.w.) and was the nontreated control group. G1 and G2 remained separated on concrete floored pens and fed lucerne pellets and fresh water ad libitum up to necropsy. Faecal samples were collected on day +4 after treatment for e.p.g. counts and all animals were necropsied on day +8 after treatment. Digestive tracts were removed for worm recovery, identification and counting from treated and non treated lambs to determine the absolute efficacy of monepantel. The efficacy of monepantel was 100% against *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus axei*, *Trichostrongylus colubriformis*, *Cooperia pectinata* and *Cooperia punctata* ($P < 0.05$). E.p.g counts in G1 were negligible at day 4 after treatment onwards ($P < 0.05$).

These results indicate that monepantel showed a high efficacy against multiple anthelmintic resistant nematodes of sheep, being specially recommended to be used in a rotational scheme of drugs.

Safety and Endoparasitic Activity of a New Molecule (Aurixazole) in Cattle

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Background: Development new molecules to control helminthes in ruminants are a measureless need mainly by increasing prevalence and severity of resistance to major groups of anthelmintics used today. Aurixazole is a disofenolato of levamisole solution which was developed by the Brazilian Company Ourofino Animal Health Ltd. A new formulation containing 24% Aurixazole administered orally was evaluated on two studies in cattle: safety assessment (study I) and anthelmintic efficacy (study II).

Methods: In study I, 20 healthy cattle were distributed in two groups of 10 animals each, one treated and other as an untreated control. All animals were individually examined pre- and post-treatment daily until 14 days post-treatment (DPT) and any adverse events, whether related to the treatment or not, were identified and recorded. Haematological and serum biochemistry variables (urea, creatinine, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase and creatine kinase) were analyzed on days -7, 0, 3, 7 and 14. For anthelmintic efficacy (study II), a controlled test was conducted using 32 naturally infected calves and randomly allocated by nematode EPG in four groups of eight animals each: untreated control (GI), Aurixazole (GII), Albendazole (GIII) and Fenbendazole (GIV). All animals were necropsied after 7 DPT and parasites were recovered, identified and counted.

Results: No adverse events or changes in haematological and biochemistry parameters were observed after treatment or compared with control group. Aurixazole was 100% efficiency against *Haemonchus placei*, *Cooperia punctata*, *C. spatulata*, *C. pectinata*, *Oesophagostomum radiatum* and *Trichuris discolor*. Similar efficacies were observed in GII and GIII, but were ineffective against *T. discolor*.

Conclusion: The results showed that the new molecule Aurixazole is clinically safe and provides a promising endoparasiticide for cattle.

Risk factors for the development of bovine anthelmintic resistance in Argentina

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A high level of anthelmintic resistance (AR) was recorded in the central cattle producing area of Argentina. Risk factors associated with AR on bovine ranches (BR) of this region were studied from data derived from a telephonic or personal interview survey of 50 ranch owners who had conducted a faecal egg-count-reduction test no more than three years before. The questionnaire contained 35 questions including descriptors of the ranch cattle management and gastrointestinal nematode control. The BR were placed in the following regions: Humid Pampeana (n=15), Subhumid Pampeana (n=24) and Semiarid Pampeana and North Patagonian (n= 11). Avermectin (AVM) resistance was present in 25 herds and benzimidazole (BZD) resistance in 11 herds. General AR to both AVM and BZD was present in 10 herds. Associations were evaluated between the binary outcome variable (AR versus no AR) and a variety of risk variables registered from the questionnaire. Variables with $P < 0,05$ or Odds Ratio (OR) > 2 were offered to a multivariate logistic regression model. These categorical variables were anthelmintic treatment strategy (X^2 9,7, $P < 0,002$; OR 7,0), Nov.-Jan. treatment (X^2 5,1, $P < 0,02$; OR 3,7), use in the past more than 75% of AVM (X^2 3,9, $P < 0,05$; OR 3,2) and quantitative categorized were number of total annual treatments (X^2 19,7, $P < 0,0001$; OR 41,6), AVM total annual treatments (X^2 15,7, $P < 0,0001$; OR 12,6), refugia index (X^2 6,4, $P < 0,01$; OR 5,0), number of Nov.-Jan. treatments (X^2 3,86, $P < 0,05$; OR 3,5). Contributory main effects for general AR in the final model were number of total annual treatments (OR= 8,29), use in the past more than 75% of AVM (OR= 17,2), number of Nov.-Jan. treatments (OR= 2,7), and for AVM resistance were AVM total annual treatments (OR=11,5), number of AVM Nov.-Jan. treatments (OR= 5,8) and use in the past more than 75% of AVM (OR= 4,4). Results showed that treatment frequency, the year period of treatment and the past treatment frequency with an only drug were the main risk factors implicated in the AR development.



PROCEEDINGS
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ABOUT WAAVP AUTHORITIES PLENARY LECTURES SESSIONS

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- **Targeted Selective Treatment For Sustainable Sheep Nematode Control: Studies In Western Australia** - R. B. Besier

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Virtual microscopy for learning veterinary parasitology

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Background: Virtual microscopy has been widely used in pathology due to its ability to promote teamwork, feedback, and improved academic performance.

Methods: A virtual microscope was designed for studying parasitology in order to quantitatively assess its effectiveness on knowledge gains by comparing mean scores of a final laboratory test with identical exams given over the past 5 years, when no virtual microscope was available. Additionally, at the end of the Veterinary Parasitology course, a survey was carried out among students to evaluate the virtual slides in a side-by-side comparison with the conventional microscope laboratory.

Results: The average score for the 2010 exams was 10–15 points higher than for any prior exam, a result that is statistically greater than the mean for all years between 2005 and 2009 (overall $F = 15.34$, $P < 0.001$ for all years versus 2010 via the Bonferroni multiple comparison test). Moreover, survey results revealed that the virtual microscope was enthusiastically received, highlighting improved time and increased student collaboration.

Conclusions: The purpose of this study was to evaluate which studying system is of more value for parasitology students in diagnostic skills training. According to results, it is tempting to assume that virtual microscopy is better as a learning tool than is the traditional microscope for the identification of parasite fixed specimens, especially in institutions where large classes attend school and microscopes are scarce and expensive. On the other hand, the implementation of virtual microscopy immortalizes collections of glass slides, particularly those that include specimens that are hard to collect or process. Many students have explicitly urged the faculty to apply virtual microscopy to other courses offered in the DVM curriculum in our institution, as they enjoyed studying using this technology. Virtual microscopy proved to be an effective method for learning veterinary parasitology in a country where parasitic diseases are sometimes underestimated due to the lack of diagnostic skills. Nevertheless, the combination of both viewing methods should be encouraged as students must be competent in the use of regular light microscopes.

A computer-based teaching program for the coproscopical identification of parasite stages in domestic animals

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A CD-ROM-based teaching tool was developed to train the skills of veterinary students in the identification of parasite stages in faecal samples of domestic animals. Facing a substantial reduction of practical teaching hours in Veterinary Parasitology in many faculties, the program is aiming to support the students during the learning process, but the tool is not meant to replace the practicals. The program covers the most relevant parasite stages excreted with faeces of dogs, cats, horses, cattle, sheep, goats, pigs and poultry in Western Europe. Following an introducing image every object is presented by a number of other pictures of the same parasite stage to give credit to the situation in routine diagnosis, where objects may be located between debris and are visible from different angles. In addition, coccidial oocysts are presented in an unsporulated and sporulated stage. After a diagnosis has been made by the user, this decision can be verified. Each parasite stage is linked with the relevant diagnostic technique(s) and the taxonomy section. For every animal species images from routine samples are presented in which more than one parasite stage is present. Here, all clearly visible stages are linked with the correct diagnosis, which can be shown with a mouse click. The E-learning program was first offered to the veterinary students at the Vetsuisse-Faculty, University of Zurich in 2010. According to an evaluation at the end of the term the feedback from the students was found to be very positive. In the case of sufficient request, the generation of an English version of the program would be a viable option.

The Prevalence of Parasitism in Non-Treated US Beef Herds Demonstrating Shifts in Parasite Populations

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Background: A US cattle parasite database was established by Intervet/ Schering-Plough Animal Health [ISPAH] in 1999. Over 18,000 fecal samples from non treated cattle have been collected and the fecal egg counts recorded.

Methods: Four private parasitology laboratories receive fecal samples from ISPAH sales representatives, veterinarians, distributors and nutritionists. Sample submission forms delineate the owner, sponsor, sales rep, age group and containment. Treatment is not revealed. The number of eggs per 3 gm sample and the parasite type, based on egg morphology, is recorded. The form is returned to the sender and treatment is added. The farm manager receives the completed form and a copy is provided for the database administrator. Data can be analyzed by the total worm egg count and by the type of parasite identified. A summary can be generated for a state or region. Reports can be produced for age groups, confinement and treatments.

Results: Thirty six thousand samples from 39 states have been processed and added to the database. Data includes samples from over 2,000 farms or ranches. A total of 18,829 of the 36,000 samples were from non-treated cattle. The average egg count was 44 egg/3gm. This also represents a typical parasite burden for the location. The data shows that the prevalence of parasites such as tapeworms (*Moniezia*), *Nematodirus* and whipworms (*Trichuris*) was greater than expected. Nationwide 42.6% of the cow/calf herds and 40.2% of the yearlings were positive for tapeworms. *Nematodirus* infections were present in nursing and early weaned calves from 61.8% of the herds while 52.6% of the yearlings were positive for *Nematodirus*. Whipworms were found in nursing and weaned calves from 20.6% of the cow/calf herds and in 16.3% of the yearlings.

Summary/Conclusions: The data demonstrates that a tactical treatment of non treated cattle in the US would be cost effective and a strategic deworming program is justified. In addition the use of macrocyclic lactones may have played a role in allowing parasites commonly missed by macrocyclic lactone treatment to become more prevalent with possible increase in economic significance to the producers.

An effective molecular approach for the diagnosis of strongylid nematode infections in sheep – implications and prospects

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We assessed a real-time PCR method for the diagnosis of naturally acquired strongylid nematode infections in sheep (n = 470) in a temperate climatic zone of south-eastern Australia, employing a panel of 100 'control' samples from sheep known not to harbour parasitic nematodes. We compared the diagnostic specificity (100%) and sensitivity (98%) of this method with a conventional faecal flotation method. We established a system to rank the contribution of particular strongylid nematodes to the faecal egg counts (FECs). The testing of faecal samples showed that *Teladorsagia circumcincta* (80%) and *Trichostrongylus* spp. (66%) were most prevalent, followed by *Chabertia ovina* (33%), *Oesophagostomum venulosum* (28%) and *Haemonchus contortus* (1%). *Te. circumcincta* and *Trichostrongylus* spp. represented the largest proportion of strongylid eggs in faecal samples from most sheep. The ability to rank the contribution of strongylid nematodes to mixed infections represents a major advantage over conventional coprodiagnostic methods. This PCR tool has the potential to replace the conventional technique of larval culture. Our current focus is on comparing the performance of PCR approach in relation to 'total worm counts' and 'larval culture', and on assessing its suitability for the detection of drug resistant nematodes, in conjunction with conventional faecal egg count reduction testing.

The economic effects of blanket versus selective anthelmintic treatment strategies in dairy cows

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Background: Gastrointestinal nematode infections (mainly those with *Ostertagia ostertagi*) continue to be a major cause of production losses in dairy cattle. Anthelmintic treatments are a cornerstone of the current control strategies, but there is an increasing pressure to use the available anthelmintics selectively in order to slow down the development of anthelmintic resistance and prevent residues in the food chain. However, selective treatment approaches involve additional costs for diagnostics and labour and it has not been evaluated whether they could be economically competitive. The objectives of this study were (1) to investigate the economic effects at farm level of blanket vs. selective anthelmintic treatment strategies in dairy cows and (2) to determine how these effects depend on level of infection or herd size.

Methods: A conceptual model was developed where the complex process of nematode control was split up in parts small enough to understand independently. The variations or uncertainty in each step were taken into account by Monte Carlo simulation. A total of 6 different treatment strategies were compared with the baseline situation where no treatments were applied. These treatment strategies included different blanket or selective treatments at calving or at housing. Input data were created based on literature and expert opinion. A univariate sensitivity analysis was conducted to evaluate the effects of a changing herd size, level of infection and economic parameters.

Results: In general, all evaluated control strategies resulted in an expected economic benefit compared with the baseline situation. However, the large variation in expected benefits indicated that on the individual farm this benefit is not guaranteed. The strategies applying treatment at calving outperformed the strategies with treatment at housing. Selective treatment strategies performed at least as well (or better) than blanket control strategies. A higher level of infection of a herd resulted in higher expected benefits for the different control strategies. Increases in herd size reduced the variation and thus the risk for the producer when a certain strategy is applied.

Conclusion: Selective anthelmintic treatment in dairy cows holds potential as an economically competitive alternative for blanket treatment strategies.

The efficacy of Rabon (tetrachlorvinphos) as a control method for trichostrongylosis in small ruminants

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Gastrointestinal nematodes are a leading cause of economic losses in small ruminant production. The advent of the development of resistance to traditional anthelmintics by gastrointestinal nematodes warrants the development and use of alternative methods of control. One possible method is Rabon® (Tetrachlorvinphos 7.76%), a chemical pesticide used to prevent the development of flies in cattle feces. The purpose of this study is to evaluate the helminthocidal potential of Rabon® on trichostrongyle nematodes of small ruminants by determining the ability of Rabon® to reduce larval and adult trichostrongyle counts both in vitro and in vivo. To assess in vitro larvicidal ability, increasing concentrations of Rabon® (17.5mg, 35mg, 70mg, 140mg, 210mg, and 1000mg) were added to fecal cultures of nine katahdin lambs and the trichostrongyle eggs present were allowed to develop to the L3 stage and were counted. To assess in vivo helminthocidal activity, five katahdin lambs were administered Rabon® at the recommended dosage (70mg/100lb body weight) for cattle in the United States via mineral mix while five lambs were fed normal mineral mix for two weeks. Blood and fecal samples were obtained tri-weekly for identification and enumeration of larval cultures, determination fecal egg count [EPG], and packed cell volume [PCV]. ANOVA was used for comparisons of larval counts in the in vitro study while a repeated measures ANOVA was used for comparisons of larval counts, EPG, and PCV over time in the in vivo study. The data from the in vitro study showed an overall significant difference between treatment groups with all but the lowest concentrations (17.5mg, 35mg) having significantly reduced larval counts when compared to control ($p < 0.001$). The in vivo study showed a decreasing trend in the larval counts of treated animals compared to control animals but the difference was not statistically significant ($p = 0.052$). Additionally, there was no difference in EPG and PCV between treatment and control groups over time indicating no effect on adult worms. This suggests that Rabon® is not an effective method of control for trichostrongylosis at the concentration allowed for use in the United States; however, higher concentrations of Rabon® may prove effective.

Targeted Selective Treatment For Sustainable Sheep Nematode Control: Studies In Western Australia

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Background: Sustainable nematode management programs aim to prevent parasitic disease and minimise animal production loss, without exacerbating anthelmintic resistance. Resistance management in sheep based on the “refugia” concept requires that some nematodes not recently exposed to anthelmintics are deliberately allowed to survive, to dilute resistant worms surviving treatment. “Targeted selective treatment” (TST) aims to provide refugia by avoiding anthelmintic treatment to individuals apparently coping with worm infections when a flock treatment is given, so these animals continue to contaminate pasture with eggs from less-resistant worms. Key research questions are whether this risks significant parasitism; what proportion of a flock should remain untreated to provide significant refugia in different situations; and what is the most appropriate and practical index for sheep treatment selection. Field trials in Western Australia tested an index based on worm egg counts to indicate the proportion of a flock requiring treatment, and sheep body condition scores as the basis of individual-animal treatment decisions.

Methods: A TST strategy based on the worm egg count-body condition score index was compared with a traditional worm control program for adult Merino ewes on three properties. Over a period of 10–12 months, the TST groups received 60%, 53% and 73% of the number of drenches administered to the normally-treatment groups (all sheep drenched). The main worm genera present in this trial were *Teladorsagia* and *Trichostrongylus*.

Results: No clinical signs of parasitism or adverse effects on reproduction occurred in the TST groups. Wool weights and bodyweight gains were nearly 0.3 kg and 2 kg, respectively, lower in two of the TST groups than in the normal treatment groups, but these differences were not significant, and body condition scores of the TST groups were consistently greater than optimal production recommendations. The non-treated sheep excreted significantly more worm eggs onto pasture than did the treated sheep, indicating a useful refugia potential.

Summary/Conclusion: The TST strategy has potential as an easily-implemented approach to sustainable drench management and would reduce the costs of worm control. The format tested has specific application in adult sheep, and where *Haemonchus contortus* is not a major risk. Studies are required to develop decision indices for various environments to ensure a balance between worm control efficiency and sheep production.



SESSION B

Keynote Lecture

- **Neosporosis in animals-the last five years** - J.P. Dubey

Oral Communications

- **Cytokine gene expression profiles in peripheral blood mononuclear cells from Neospora caninum naturally infected dams throughout gestation** - S. Almería
- **Toltrazuril treatment of congenitally acquired Neospora caninum-infection in newborn mice and their off-spring (2nd generation)** - B. Gottstein
- **Immune Response To Neospora Caninum Native Antigens And Recombinant Proteins Formulated With Immune Stimulating Complexes In Heifers** - Y. Hecker
- **Prevalence of Eimeria bovis and Eimeria zuernii in German cattle herds and factors influencing oocyst excretion** - B. Bangoura
- **Neospora caninum and Besnoitia besnoiti infection in South Australian cattle** - M. P. Reichel
- **An inter-laboratory comparative study of serological tools employed in the diagnosis of Besnoitia besnoiti infection in bovines** - L. M. Ortega-Mora
- **First case report of Equine Protozoal Myeloencephalitis in Argentina** - G. Moré
- **A genome project for Sarcocystis neurona, the cause of equine protozoal myeloencephalitis** - D. K. Howe

Posters

- **Abortion in Pregnant Buffaloes Experimentally Infected with Neospora caninum** - A. L. Chryssafidis
- **Population Genetics of Trypanosoma evansi from Camel Hosts in Eastern Africa** - B. Salim
- **Epidemiology of Eimeria and associated risk factors in goats of Punjab, Pakistan** - T. Rehman
- **Effect of coccidiosis on weight gain of calves and preventative control with toltrazuril, diclazuril and decoquinate** - A. Deniz
- **Diversity and seasonal occurrence of Eimeria species in a mixed flock of communally reared sheep and goats in Mafikeng in the North West Province, South Africa** - F. R. Bakunzi
- **Efficacy of sulfonamides and Baycox® against Isospora suis in experimental infections of suckling piglets** - H. C. Mundt
- **Control of coccidiosis in stabled calves in Germany by different treatment schedules** - A. Dausgies
- **Efficacy of toltrazuril 5% suspension against Eimeria bovis and Eimeria zuernii in calves and observations on the associated immuno-pathology** - N. N. Jonsson
- **Transmission of Neospora caninum to sheep by transfer through semen** - S.S. Syed-Hussain
- **Patterns of Cryptosporidium oocysts shedding by calves from a dairy farm of Italy** - G. Capelli
- **In vitro and in vivo effects of the arylimidamide DB745 against Neospora caninum** - F. Barna
- **Dynamics of bovine Besnoitia besnoiti (Apicomplexa, Sarcocystidae) infections: within and between farms spreads** - E. Liénard
- **Detection of antibodies against Neospora caninum and Toxoplasma gondii in armadillos (Chaetophractus villosus) Argentina** - M. Kin
- **Anti-neosporal effect of HPLC fractions of Sophora flavescens and Torillis japonica extracts against Neospora caninum on dogs and goats** - H. J. Youn
- **Anti-neosporal effect of HPLC fractions of Sophora flavescens and Torillis japonica extracts against Neospora caninum on mice** - H. J. Youn
- **Cryptosporidium spp. in a Brazilian Beef Cattle Herd** - R. P. Heckler
- **Recrudescence of Neospora caninum infection in chronically infected mice** - E. Jiménez-Ruiz
- **Evaluation of protective effect of NcGRA7, NcSAG4, NcBSR4 and NcSRS9 formulated in Poly-e-Caprolactone against Neospora caninum infection in mice** - E. Jiménez-Ruiz
- **Systemic immune response in heifers vaccinated with different Tritrichomonas foetus vaccines** - L. Fuchs
- **Neospora caninum seroprevalence in dairy cattle from Valle de Lerma, Salta** - R. Neumann
- **Evidence of Different Pathogenicity among Neospora caninum Strains Nc-Bahia and Nc-1** - A. L. Chryssafidis
- **Studies on potential intermediate and definitive host species of Besnoitia besnoiti** - W. Basso
- **A review of the economic impact of Neospora caninum** - M. P. Reichel
- **Cutaneous bovine and equine leishmaniasis due to a yet non-specified Leishmania organism** - B. Gottstein
- **Evaluation of the protection conferred by a naturally-attenuated Neospora caninum isolate against congenital neosporosis in mice** - L. M. Ortega-Mora
- **Immune Response In Neospora caninum Seropositive Calves Experimentally Suppressed With Dexametasone** - Dadín P. Moore
- **Ocurrence Of Antibodies Against Neospora Caninum In Sheep In The Humid Pampas** - Y. Hecker 1
- **Dynamics of Parasitaemia, Stress Profiling and Acute Phase Protein Expression in Caprine Trypanosoma evansi Infection** - R. S. K. Sharma
- **Length of infection in heifers immunized with experimental Tritrichomonas foetus vaccines and challenged** - L. Fuchs
- **Experimental infection with Neospora caninum in pregnant buffaloes (Bubalus bubalis) in Argentina** - J. Konrad

- **Effect of NC-1 strain of Neospora caninum inoculated experimentally in pregnant buffaloes (*Bubalus bubalis*)** - J. Konrad
- **Serology, isolation and genotype characterization of *Toxoplasma gondii* from naturally infected pregnant dairy cows (*Bos taurus*)** - J. L. Garcia
- **Eimeria species in Danish dairy cattle - preliminary data from an ongoing study** - H. L. Enemark
- **Bovine neosporosis in the South of Romania: seroprevalence of *Neospora caninum* infection in dairy cattle** - M. Ionita
- **Sexual transmission of *Toxoplasma gondii* (Nicolle & Manceaux, 1909) in caprines** - L. F. Santana
- **Comparative study of the techniques Bioassay in mice and Polymerase Chain Reaction (PCR) to detect *Toxoplasma gondii* in tissues of female goats** - L. F. Santana
- **A case report of *Trypanosoma* spp. in bulls in Corrientes, Argentina** - J. D. Alvarez
- **Parasitic and rickettsial infections of goats and cattle in Angónia District, Tete, Mozambique** - M. A. Ornelas-Almeida
- **Seroprevalence and risk factors to *Neospora caninum* infection in ewes of Planalto Catarinense, Santa Catarina State, Brazil** - A. Barbosa de Moura
- **Cytokine expression in pregnant dairy cows naturally infected with *Neospora caninum*** - M. Rambeaud
- **Sanitary surveillance of bovine encephalitis: *Neospora caninum* as causative agent** - C. Del Fava

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Neosporosis in animals-the last five years

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Neospora caninum is a protozoan parasite of animals. Until 1988, it was misdiagnosed as *Toxoplasma gondii*. Since its first recognition in 1984 in dogs and the description of a new genus and species *Neospora caninum* in 1988, neosporosis has emerged as a serious disease of cattle and dogs worldwide. Abortions and neonatal mortality are a major problem in livestock operations and neosporosis is a major cause of abortion in cattle. This review is focused on current status of neosporosis in animals based on papers published in the last five years. Worldwide seroprevalences are tabulated. Strategies for control and prevention are discussed.

Cytokine gene expression profiles in peripheral blood mononuclear cells from *Neospora caninum* naturally infected dams throughout gestation

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Background: *Neospora caninum* is a major cause of abortion in cattle but the reasons why some animals abort and not others remain unclear. An essential role for Th1 cytokines, such as IFN- γ and IL-12, in protective immunity against *N. caninum* has been indicated. However, little is known about the cytokine patterns in *N. caninum* infected pregnant cattle, where a considerable level of immune modulation may exist, and to our knowledge, only a previous study has been performed in natural infections.

Methods: In this study, the cytokine gene expression for IFN- γ , IL-12, IL-10, IL-4 and TNF- α was analyzed by real time RT-PCR TaqMan in peripheral blood mononuclear cells in *N. caninum* naturally infected cows throughout pregnancy. Samples were collected from 18 cows (13 *N. caninum* seropositive and 5 *N. caninum* seronegative) at days 45, 90, 120, 150, 180 and 210 of pregnancy or until occurrence of abortion.

Results: Four seropositive animals aborted. *Neospora caninum* naturally infected dams showed up-regulated levels of Th1 cytokines, IFN- γ in addition to TNF- α and IL-12p40 mRNA, and up-regulation of the Th2- regulatory cytokine, IL-10 expression compared to seronegative animals. On the contrary, expression levels of IL-4 (Th2 cytokine) were not significantly different among groups throughout the study period. The present study shows clear differences at cytokine gene expression levels in peripheral blood in animals naturally infected with *N. caninum* compared to seronegative control animals throughout pregnancy.

Summary/Conclusions: To our knowledge, this is the first study focusing on cytokine gene expression of Th1, Th2 and regulatory cytokines in *N. caninum* naturally infected pregnant cows throughout pregnancy.

Toltrazuril treatment of congenitally acquired *Neospora caninum*-infection in newborn mice and their off-spring (2nd generation)

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Background: *Neospora caninum* is one of the most important causative agent of abortion in cattle. One potential approach to interrupt the diaplacentally dictated transmission mode is to treat congenitally infected off-spring animals. This approach was preliminarily studied in two generations of experimentally infected mice. Toltrazuril, a symmetrical triazinone, is registered under the original name Baycox for the control of coccidiosis in poultry, piglets, calves and lambs.

Methods: In our study, C57BL/6-mice were infected with *N. caninum* tachyzoites during pregnancy, yielding a transplacental infection of developing fetuses. Subsequently, congenitally infected newborn mice were perorally treated with toltrazuril/Baycox 5% suspension (or placebo) 31 mg/kg on days 3, 10, 17 and 24 post partum. Infection status was monitored serologically (ELISA) and post-mortem by molecular analyses (PCR) of brain and other organs. Seropositive animals of all groups (vero- and placebo-treated infected and non-infected animals) were mated thus as to generate a second generation of off-spring. These were parasitologically and serologically identically monitored as the first generations.

Results and Conclusions: 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. 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. The number of diseased and *Neospora*-positive pups was markedly reduced after toltrazuril-treatment when compared to other groups. Treatment also resulted in the highest antibody response. All animals of the second generation that were obtained from congenitally infected first generation mothers demonstrated a seropositive status post partum, but diaplacental transmission did not occur, either in off-spring derived from treated or from placebo-treated mothers. In conclusion, we could show that treatment with toltrazuril had a high impact on the course of infection in the first generation of congenitally *N. caninum*-infected newborn mice. The murine model, however, appeared not suitable to study second generation effects, as the parasite was not efficiently transmitted congenitally into these animals as shown by negative PCR results in all groups. Long-term studies in calves (several generations) are necessary to clear the questions if successive treatment schedules will lead to parasite clearance in breeding lines.

Immune Response To *Neospora Caninum* Native Antigens And Recombinant Proteins Formulated With Immune Stimulating Complexes In Heifers

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The aim of this study was to compare the immune responses to live *Neospora caninum* tachyzoites, native antigens and recombinant proteins formulated with immune stimulating complexes (ISCOMs) in seronegative pregnant heifers. To prepare the either the live tachyzoites or the native antigens, the Nc6 strain was used. Twenty seronegative pregnant heifers were involved: 4 were inoculated intravenously with 1×10^8 live tachyzoites (Group A); 4 were inoculated with native antigens obtained from tachyzoites (750ug/dose) formulated with ISCOMs (Group B); 4 were inoculated with a mix of four recombinant proteins (SAG₁, PIs, Hsp20, GRA7 (30ug of each protein/dose)) also formulated with ISCOMs (Group C); 3 received ISCOM-MATRIX (Group D) and 3 were controls receiving PBS (Group E); all these last four groups were inoculated twice by subcutaneous via with interval of 21 days previous mating. The recombinant proteins were engineered to be expressed in *Escherichia coli* and purified in nickel resine. The animals were bled weekly to assess immune parameters. After vaccination and mating, the animals will be challenged using Nc1 strain at day 70 of gestation. The humoral immune response was evaluated by indirect fluorescence antibody test (IFAT). Whole blood samples were stimulated to assess IFN- γ production by using a commercial ELISA. The cellular immune response will be also evaluated by flow cytometry. Preliminary serological results showed that in week 2 after first dose, there were *Neospora* specific antibodies developed in animals from Groups A and B but antibody responses were higher in Group A compared with Group B. However, after the second dose, the animals in group A antibody titles ranged between 3200 to 6400 as the animals of group B. Therefore, after second dose, antibody titers developed by the group A and B were similar. On the other hand, the animals in group C did not develop antibodies. The other experimental groups did not show titles to IFAT. Currently, cellular immune response is being characterized. After euthanasia, tissues will be obtained for further histological and PCR analysis.

Prevalence of *Eimeria bovis* and *Eimeria zuernii* in German cattle herds and factors influencing oocyst excretion

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Background: The present study was designed to investigate the prevalence of the pathogenic coccidia species *E. bovis* and *E. zuernii* in shed-reared animals in German dairy and fattening facilities.

Methods: Samples were obtained from 65 cattle farms distributed randomly across all the regions of Germany regardless of the occurrence of clinical problems. The samples were obtained rectally. Faecal consistency and the total number of oocysts per gram of faeces (OPG) were determined, along with the OPG values for *Eimeria (E.) bovis* and *E. zuernii*. A questionnaire was completed for each farm to record information about herd size and management, along with individual animal data.

Results: *Eimeria* oocysts were detected in 62 of these farms, which gives a prevalence of 95.4%. The farm prevalence of the pathogenic species was 76.9% for *E. bovis* and 83.1% for *E. zuernii*. The number of oocysts excreted could not be correlated significantly with farm type or farm management, but depended on the floor type, the age of the calves and the time after rehousing. Furthermore, there was a positive correlation between OPG and the observation of diarrhoea. *E. zuernii* had a greater influence on the occurrence of diarrhoea than *E. bovis*.

Summary/Conclusions: This study confirms that herd management frequently does not meet the requirements of effective coccidia control despite the fact that the pathogenic coccidia *E. bovis* and *E. zuernii* are ubiquitous in German cattle populations.

***Neospora caninum* and *Besnoitia besnoiti* infection in South Australian cattle**

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Background: Both, *Neospora caninum* and *Besnoitia besnoiti* are protozoan parasites that infect cattle. The role of *N caninum* in abortions in cattle world-wide is well established, while *B besnoiti* appears to be emerging as a significant pathogen of cattle in Europe. Prevalence information about both of those parasites is lacking for South Australia.

Methods: A serological survey of South Australian beef and dairy cattle was conducted for the presence of antibodies to those protozoans. In general, eight sera were tested from each of the heifer and mature cow cohort on 9 dairy and 51 beef properties in South Australia. Tank milks from 122 dairy farms were also available for testing. Two commercially available ELISA kits (HerdChek and PrioCheck® *Besnoitia* Ab2.0) were used for the detection of antibodies and performed and interpreted according to manufacturers' specifications.

Results: For *N caninum* the antibody ELISA indicated an overall sero-prevalence at the individual animal level of 2.70% (95% CI; 1.65% to 3.74%). At the herd level, 29.4% (95% CI; 16.9 to 41.9%) of beef, and 32.5% (95% CI; 12.0% to 76.9%) of dairy cattle herds showed antibodies, as did 3 out of 122 (2.54%; 95% CI -0.28% to 5.36%) of the tank milk samples. The maximum within-herd sero-prevalence detected was 20% for beef herds and 25% for dairy herds.

Testing for *B besnoiti* antibodies the ELISA identified 18.4% (95% CI: 15.8% - 21.0%) of 869 individual cattle sera as positive, 18.8% (136/723) (95% CI: 16.0% - 21.7%) of beef and 16.5% (22/133) (95% CI: 10.2% - 22.9%) of dairy cattle. At the herd level, 91.8% (45/49) (95% CI: 84.2% - 99.5%) of beef properties and all dairy properties showed evidence of *Besnoitia* antibodies.

Conclusions: The sero-prevalence of *N caninum* in South Australian cattle appears to be in general agreement with other reports from cattle populations around Australia. Although *N caninum* infection is low at the individual animal level, it is widespread across a third of the cattle properties. Antibodies to *B besnoiti* appear to be widespread throughout the state, with close to 20% of individual cattle showing antibodies, yet clinical cases appear to be absent. Further validation of that ELISA is on-going.

An inter-laboratory comparative study of serological tools employed in the diagnosis of *Besnoitia besnoiti* infection in bovines

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Background: Bovine besnoitiosis is considered an emerging chronic and debilitating disease in Europe. The causative agent is the cyst-forming parasite *Besnoitia besnoiti*. Many infected animals remain subclinical and the only sign of disease is the presence of parasitic cysts in the sclera and conjunctiva. Serological tests are useful to detect asymptomatic/sub-clinical cattle for control purposes since there are no effective drugs or vaccines. In this sense diagnostic tools need to be further standardized. The aim of this study was to compare the serological tests available in Europe in a multi-centered study.

Methods: A coded panel of 401 well characterized sera from infected and non infected bovines was provided by all the participants (SALUVET group -UCM; FLI, Wusterhausen; LP, ENV, Toulouse; IPB, Berne). The tests evaluated were: an in house ELISA developed by SALUVET group, 2 commercial ELISAs (PrioCHECK *Besnoitia* Ab V2.0, INGENASA), IFAT and Western blots (tachyzoite and bradyzoite extracts under reducing and non reducing conditions). Two different definitions of a gold standard were used: i) the decision of the majority of tests (Majority); ii) pre-test information based on clinical signs. WinEpiscope 2.0 was employed to estimate sensitivity (Se), specificity (Sp) and agreement, expressed as Kappa (κ)-values.

Results: Relative to the gold standard "Majority" almost 100% Se and Sp were obtained with 3 out of four bradyzoite based Western blots regardless the conditions and laboratory. A slight variability was observed when tachyzoite based Western blots were compared. However all Western blots showed almost perfect agreement. Regarding ELISAs, the in-house ELISA, PrioCHECK and INGENASA showed very good diagnostic characteristics (95-100% Se and SP). IFAT FLI Wusterhausen performed better than IFAT SALUVET-UCM with 100% Se and 96% Sp. Relative to the gold standard "pre-test info", Sp significantly decreased as expected due to the existence of subclinical infections.

Conclusions: In house, PrioCHECK *Besnoitia* Ab V2.0 and INGENASA ELISAs performed very well and are suitable tools to be used in epidemiological studies. However Western blot tests based on bradyzoite extracts performed better and could be employed as a *posteriori* test to re-test doubtful results in valuable samples for control purposes.

First case report of Equine Protozoal Myeloencephalitis in Argentina

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Equine protozoal myeloencephalitis (EPM) is frequently caused by *Sarcocystis neurona* and generates significant economic losses in American horses. In Argentina antibodies to this protozoan have been detected but no clinical cases have been reported. EPM is characterized by neurological signs corresponding with multifocal lesions of the central nervous system (CNS). Serological tests, histopathology, immunohistochemistry (IHC) and polymerase chain reaction (PCR) are recommended methods for specific diagnosis.

A 12-year-old, mix breed, female horse, from a farm in Buenos Aires Province was received with asynchronous walking and bilateral stringhalt. The mare was treated with phenytoin, B1 vitamin, NSAIDs and subsequent tenectomy, without positive results. The clinical course was progressive with depression, involvement of cranial nerves V and VII, atrophy of the muscles of the back, loin and pelvic limbs, dysmetria and gait instability, generalized tremors, poor response to stimuli, severe dragging of hooves and tetraparesis. Considering the prostration, the horse was euthanized and necropsied. At necropsy no gross lesions were observed in the CNS. Microscopically, lesions were observed mainly in thalamus, brainstem and spinal cord, and consisted in small hemorrhagic multiple foci and small foci of chronic inflammation conformed by histiocytes, multinucleated cells and occasional foam cells. Neuronal necrosis was also observed together with satelliosis, perivascular mononuclear infiltrates, and endothelial swelling in the spinal cord and scattered areas of axonal degeneration. The IHC revealed the presence of intracellular *S. neurona* meronts and free merozoites in brain and spinal cord samples. By PCR-RFLP using the primers JNB33-JNB54 digested with HinfI and DraI, and primers JNB25-JD396 digested with HinfI and HindIII, a specific restriction pattern of *S. neurona* was observed in brain samples.

This is the first case report of EPM in Argentina, confirmed by histopathologic, immunohistochemical and molecular studies. The previous detection of antibodies to *S. neurona* in horses, the abundance of opossums (*Didelphis albiventris*) in our environment and the specific confirmation in this case, suggest that the disease may be widespread in Argentina. It is necessary to establish an EPM surveillance program in order to evaluate the importance of this disease in Argentina, as well as to establish control strategies.

A genome project for *Sarcocystis neurona*, the cause of equine protozoal myeloencephalitis

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Sarcocystis neurona is the leading cause of neurologic disease in horses and an emerging pathogen of marine mammals. This organism is a member of the Apicomplexa, which encompasses a broad group of intracellular parasites that cause significant morbidity and mortality in man and animals. The Apicomplexa are phylogenetically placed with ciliates and dinoflagellates, many of which contain a plastid organelle acquired by secondary endosymbiosis. The evolution of this clade remains somewhat enigmatic, so the *S. neurona* genome and plastid sequences will help resolve uncertainties regarding the phylogeny of these organisms. Of equal importance, a genome sequence for *S. neurona* will be a valuable resource for gene discovery and utilization of current investigative technologies (e.g., genomics, proteomics, etc.). A 454 pyrosequencing/Sanger hybrid approach has been utilized to sequence and assemble the *S. neurona* genome. To aid gene annotation, 21,716 ESTs have been produced from the extracellular merozoite stage of the parasite, and additional transcriptome analyses are planned from intracellular schizonts. The current *S. neurona* genome assembly is comprised of 4889 contigs that come together into 272 scaffolds and suggests an approximate genome size of 124 Mb. The genome assembly is being annotated, and phylogenomic analyses will compare the *S. neurona* genome with other members of the Apicomplexa. cursory BLAST searches have revealed the expected “housekeeping” genes (heat shock proteins, transcription/translation-associated proteins, metabolic enzymes, etc.) along with orthologues of numerous rhoptry (ROP) and microneme (MIC) proteins from *T. gondii*. However, matches to *T. gondii* dense granule (GRA) proteins were much less common. As expected, multiple surface antigen (SAG/SRS) orthologues were identified, but the number of *S. neurona* paralogues appeared to be far fewer than the approximately 160 SAG/SRS loci that have been observed in *T. gondii*. Importantly, the *S. neurona* genome sequences and their annotations will be deposited in SarcoDB and EuPathDB so that the information can be freely accessed by the research community.

Abortion in Pregnant Buffaloes Experimentally Infected with *Neospora caninum*

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The objective of this study was to verify the outcome of neosporosis in pregnant buffaloes, on the first trimester of pregnancy. Four buffaloes, seronegative to *Neospora caninum* and other abortive agents, were submitted to OvSynch protocol, with successful insemination at the same time. By the 70th day of gestation, three were inoculated endovenously with *N. caninum* (Nc-1 strain) and one remained as control. The fetal viability was verified ultrasonographically and fetal death was detected 28 days after inoculation in the infected animals, as the control remained healthy. Serum samples were collected weekly and both infected animals were positive in ELISA anti-*N. caninum* by 14 days p.i. After slaughtering, fragments of brain and heart from those dead fetuses, as well as tissues from the mothers, were analyzed by PCR, and DNA of the parasite was detected in mothers and fetuses. This is the first confirmation of abortion in pregnant buffaloes due to *N. caninum* infection.

Keywords: *Neospora caninum*, abortion, buffalo, PCR

Population Genetics of *Trypanosoma evansi* from Camel Hosts in Eastern Africa

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Background: Trypanosomiasis due to *Trypanosoma evansi* is a widely distributed disease of livestock, affecting specially camelids and equines and is transmitted by biting flies. The disease is of great concern to many developing countries such as Sudan, where its large camel population estimated at over 4 million heads is at risk. It is generally believed that *T. evansi* has evolved when camels infected with *T. brucei* moved to tsetse free areas, and the parasite has recently been suggested to be considered as a subspecies of *T. brucei* complex.

Methods: We have investigated genetic variation at 15 microsatellite loci of *T. evansi* isolated from camels in Sudan and Kenya to evaluate the genetic information partitioned within and between individuals and between sites.

Results: We detected a strong signal of isolation by distance across the area sampled. The results also indicate that either, and as expected, *T. evansi* is purely clonal and structured in small units at very local scales and that there are numerous allelic drop out in the data, or that this species often sexually recombines without the need of the “normal” definitive host, the tsetse fly. Though the first hypothesis is the most likely, discriminating between these two incompatible hypotheses will require further studies at much localized scales.

Conclusion: If we do not assume full clonality for *T. evansi* our genetic data are difficult to interpret and in contradiction with what is expected from this strictly mechanically transmitted trypanosome. This is an important issue as these different hypotheses (full clonality and strong viscosity versus sexual reproduction) have not the same consequences in term of spread of parasite resistance to trypanocidal drugs.

Epidemiology of Eimeria and associated risk factors in goats of Punjab, Pakistan

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A cross-sectional study was carried out to determine the prevalence, species characterization and associated risk factors with Eimeria in goats of district Toba Tek Singh from April, 2009 to March, 2010. Of the total 486 faecal samples examined for Eimeria, 271 (55.76%) were found infected with five species of Eimeria. Amongst the identified species of Eimeria, *E. ninakohlyakimovae* was the commonest one (53.14%) followed in order by *E. arloingi*, *E. alijevi*, *E. caprina* and *E. hirci* with prevalence of 47.97, 33.58, 28.04 and 21.77 percent respectively. Peak prevalence was observed in August. Wet season was found favourable for Eimeria. Kids had significantly higher prevalence ($P<0.05$) of Eimeria than adults while higher prevalence of Eimeria was observed in female goats. Beetle breed were found at highest risk of Eimeria (62.61%, $\chi^2=4.9911$, $P=0.0255$) followed in order by Desi, Dera Din Panah and Teddy breeds of goats. Among management and husbandry practices; watering system, housing system, floor type and herd size strongly influenced the prevalence of Eimeria. Coccidiosis was more prevalent in close housing system, non-cemented floor type, pond watered animals and larger herds ($P<0.05$) as compared to open housing system, partially cemented floor type, tap watered animals and smaller herds respectively. Feeding system and body condition of animals were not found risk factors ($P>0.05$) influencing prevalence of Eimeria.

Effect of coccidiosis on weight gain of calves and preventative control with toltrazuril, diclazuril and decoquinate

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Coccidiosis affects all types of animals. It is an intestinal protozoal disease impacting the gut health of the animals and causes watery, hemorrhagic or pasty diarrhoea depending on the species of *Eimeria* sp. involved. *Eimeria bovis*, *Eimeria zuernii*, *Eimeria alabamensis* are the most well-known pathogenic species involved in calf coccidiosis. The first ones *E. bovis* and *E. zuernii* can also cause hemorrhagic diarrhea and complications. Significant negative affect of coccidiosis on weight gain development was already reported (Stockdale et al. 1981; Bangoura and Dauschies, 2007). The economic impact of the disease in cattle in the US is thought to be around \$100 mio. or more annually. Oocysts excretion of two pathogenic species '*E. zuernii* and *E. bovis*' was controlled excellently longer time by the preventive treatment of toltrazuril (Baycox 5%) in cattle compared to diclazuril prevention (Mundt et al. 2007). Compared to untreated and infected group of calves, prevention of calves with toltrazuril (Baycox 5%) at risk of coccidiosis provided 139 g/day significantly more weight in 5 weeks study period (Deniz et al. 2009). Recent comparative studies in cattle investigated the efficacy of controlling of coccidiosis on weight gain development in calves revealed that treatment of calves two weeks postweaning before oocyst peak with toltrazuril (15 mg/kg) or diclazuril (1 mg/kg) and starting postweaning onwards with in-feed decoquinate (0,5 mg/kg) up to day 98 preventatively resulted in 5,26 kg and 7,11 kg more weight in toltrazuril group of calves compared to diclazuril and decoquinate respectively (Veronesi et al. 2010a). Long-term effect of controlling coccidiosis in calves at risk was studied in a comparative study. In 40 weeks time period, treatment with toltrazuril of calves provided better body weight (around 8 kg more) and body condition score and better control of oocysts excretion compared to diclazuril prevention (Veronesi et al. 2010b). Conclusively, coccidiosis compromises body weight development of calves even long-term, and treatment with toltrazuril provides better prevention of body weight compromises even long-term.

Diversity and seasonal occurrence of *Eimeria* species in a mixed flock of communally reared sheep and goats in Mafikeng in the North West Province, South Africa

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Diversity and seasonal occurrence of coccidia in a communally reared mixed flock of sheep and goats at Mafikeng, North West Province, South Africa, was determined between March 2008 and February 2009. Faecal specimens were collected directly from the rectum of the animals and the number of oocysts per gram of faeces (opg) determined. The mean monthly opg for goats (774) was significantly higher than that for sheep (544). Higher oocyst counts were observed during the hot, rainy season than during the cold, dry season. The highest mean values for both the sheep (862.5 opg) and goats (1200 opg) were recorded during March. Six species (*Eimeria crandallis*, *E. bakuensis*, *E. weybridgensis*, *E. ahsata*, *E. intricata*, and *E. ovinoidalis*) were recovered from sheep, with *E. crandallis* and *E. bakuensis* occurring most frequently. The last 2 species, together with *E. ahsata*, are considered among the most pathogenic species in sheep. In goats, 7 species (*E. arloingi*, *E. jolchijevi*, *E. caprina*, *E. alijevi*, *E. caprovina*, *E. christenseni* and *E. hirci*) were recovered, with *E. arloingi* and *E. jolchijevi* occurring most frequently. Up to 5 *Eimeria* species were recovered from individual specimens in goats while up to 4 were recovered in sheep. No cross-infections between goats and sheep were recorded and no clinical coccidiosis was noted during the study. It is increasingly becoming evident that the pathogenic *E. arloingi* is one of the most commonly occurring *Eimeria* species in goats in South Africa.

Efficacy of sulfonamides and Baycox® against *Isospora suis* in experimental infections of suckling piglets

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Sulfonamide treatment of piglets against neonatal coccidiosis has frequently been suggested in the literature. In order to evaluate the efficacy of sulfonamides against experimental *Isospora suis* infections in suckling piglets (oral infection with 1,500 sporulated oocysts of *I. suis* per piglet on the 4th day of life) two trials were conducted. In trial I oral sulfadimidine (group Sulfa-Oral) was applied in doses of 100 mg/kg of body weight (BW) one day before infection and 75 mg/kg BW daily for the following 5 days, and sulfamethoxypyrimidine (SMP) was applied parenterally in daily doses of 75 mg/kg BW for the same time period. In trial II, SMP was applied parenterally in doses of 75 mg/kg BW (a) from the day of infection daily for seven days (SMP-Standard), (b) for two days starting on the day of infection (SMP-Early), (c) for three days starting two days post infection (d.p.i.) (SMP-Middle), (d) for two days starting 5 d.p.i. (SMP-Late), and (e) every other day from the day of infection until 6 d.p.i., as well as (f) orally in doses of 75 mg/kg BW from the day of infection for seven days (SMP-Oral). The sulfonamide-treated groups were compared to a toltrazuril-treated group (single oral treatment with Baycox® 5% suspension, 20 mg/kg BW 2 d.p.i.) and to a water-treated Control group. Each group consisted of 7-9 piglets. The parameters evaluated were oocyst excretion and faecal consistency/diarrhoea from four to 15 d.p.i. Sulfa-Oral, SMP-Early and SMP-Late had no significant effect in reduction of oocyst excretion and diarrhoea, whereas treatment for 3-7 days with SMP reduced both parasite shedding and diarrhoea significantly. Oral treatment with SMP was comparable to parenteral application. Baycox® had the most pronounced effect and completely suppressed oocyst excretion and diarrhoea during the examination period. The repeated application of sulfonamides can in principle be used to control piglet coccidiosis under a known infection condition; however, the amount of work required is considerable and the practicability is poor. Due to the short half-life of sulfonamides and the lack of predictability of the infection condition a reliable control of piglet coccidiosis under field conditions is unlikely. Baycox®, on the other hand, applied once during prepatency has a sustainable effect and is used to control *I. suis* most effectively.

Control of coccidiosis in stabled calves in Germany by different treatment schedules

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Background: The impact of different treatment schedules on the course of coccidiosis in calves and in farms with different patterns of *Eimeria* spp. infection was investigated.

Methods: The investigations were carried out in a controlled, blinded, randomised, multicentre field study in four farms with a total of 228 animals. All farms had a known history of regular clinical stable coccidiosis. The observation period lasted 56 days starting with the housing of the calves in the respective stable units. Three different treatment schedules and two anticoccidial drugs were compared to a sham treated control. Baycox® bovis (toltrazuril, 15 mg/kg body weight) or Vecoxan® (diclazuril, 1 mg/kg body weight) were administered either on study day (SD) 0 (stabling), SD 7 or on SD 14 after housing of the respective group of calves. To assess the efficiency of treatment, the time period after treatment was divided into two equal cycles, each of which corresponded to the duration of one complete life cycle of *Eimeria* spp. Oocyst excretion, quantified as oocysts per gram of faeces (opg), and faecal consistency were assessed regularly.

Results: It could be shown that coccidia displayed an important cause of diarrhoea. Relevant oocyst excretion (defined as at least 500 opg of *Eimeria bovis* or *Eimeria zuernii*) was significantly linked to the occurrence of diarrhoea. Treatment with both anticoccidials resulted in a reduction of the intensity and extent of oocyst excretion. Treatment with toltrazuril proved to be superior both to sham (placebo) and diclazuril treatment in respect of the intensity of oocyst excretion over all treatment time points for both the first and the second parasitic life cycle. Thus, a good development of immunity against *Eimeria* spp. after treatment with toltrazuril is concluded.

Summary/Conclusions: Generally, treatment with the anticoccidials on SD 7 and SD 14 controlled the outbreak of clinical coccidiosis in cattle efficiently. Nevertheless the investigations proved there may be different ideal time points for treatment depending on the respective farm. A positive, lasting beneficial impact is less likely the further away from the ideal application time for a particular farm a product is administered, especially in the case of diclazuril treatment.

Efficacy of toltrazuril 5% suspension against *Eimeria bovis* and *Eimeria zuernii* in calves and observations on the associated immuno-pathology

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16 calves were infected with suspensions containing approximately 230,000 *Eimeria bovis* and 70,000 *E. zuernii* oocysts and either treated (n = 8) with toltrazuril at 15 mg/kg or with a placebo 14 days after infection. Observations were made on the clinical condition, faecal score, liveweight of calves daily from one day post infection (PI) until 24 d PI when all calves were euthanased and examined post mortem. Samples were collected from ileum and colon for histological, immunohistochemical and gene expression studies. The study demonstrated a high efficacy of toltrazuril for the treatment of *E. bovis* and *E. zuernii* infections in calves reaching 99% (based on arithmetic mean oocyst counts in faeces) within three days of treatment and remaining at or above this level for six days. Toltrazuril did not have a significant effect on the pattern and extent of immune cellular infiltration in the mucosa of ileum and colon but the expression of the genes coding IL-2, IL-10 and TNF- α in the ileum and TNF- α in the colon were elevated in calves treated with toltrazuril. Higher levels of oocyst shedding were significantly associated with lower expression of genes coding for IL-2, IL-10 and higher IP-10. It is concluded that toltrazuril is effective for the treatment of coccidiosis due to *E. bovis* and *E. zuernii* in calves and enables the development of a normal or enhanced immune response to infection.

Transmission of *Neospora caninum* to sheep by transfer through semen

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Recent reports from New Zealand indicate *Neospora* has a possible role in causing abortions in sheep. This study aimed to investigate if horizontal transmission occurs through semen. In Part 1, six month old crossbred ram lambs (n=32), seronegative to *N. caninum*, were divided into 4 equal groups. Group 1.1 remained uninoculated whilst the remainder were inoculated with *N. caninum* tachyzoites intravenously as follows: Group 1.2 - 50 tachyzoites; Group 1.3 - 10^3 tachyzoites; Group 1.4 - 10^7 tachyzoites. Semen samples were collected weekly for 8 weeks for the detection of *N. caninum* DNA and quantified using a high resolution melt PCR (HRM-PCR). Plasma collected 1 month post-inoculation was subjected to ELISA (IDEXX CHEKIT) and western blot. For Part 2, 32 seronegative ewes were divided into 2 equal groups. Group 2.1 ewes were mated with 3 rams from Group 1.1 (uninoculated) while Group 2.2 ewes were mated with 3 rams from Group 1.4 (from 9 days post inoculation). Mating occurred over 2 oestrus cycles. Ewe sera 1 and 2 months post-mating was tested for seroconversion by ELISA and western blot. All experimentally infected rams seroconverted and at 1 month their IgG S/P % values ranged from 11-36.5% in Group 1.2, 12-39.5% in Group 1.3 and 40-81% in Group 1.4. However, none of the ewes mated with the experimentally infected rams seroconverted. For the western blot, responses towards immunodominant antigens (IDAs) were observed directed against proteins at 10, 17, 21, 25-29, 30, 31, 33 and 37 kDa. Rams in Group 1.2, 1.3 and 1.4 were noted to have at least 3 IDAs present. None of the ewes showed any of the 8 prominent IDAs except for the one at 21 kDa which was seen in most ewes in both Group 2.1(90%) and 2.2 (80%). *N. caninum* DNA was detected intermittently in the ram's semen up to 5 weeks post-inoculation with the highest concentration of DNA at 1-2 weeks post-inoculation. All positive semen samples were screened for micro-satellites to confirm the genotype was that of the inoculum. These results suggest that though *N. caninum* DNA can be found in the semen of experimentally infected rams, the transmission of *N. caninum* via natural mating is an unlikely event.

Patterns of *Cryptosporidium* oocysts shedding by calves from a dairy farm of Italy

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Cryptosporidium spp. is considered to be an important agent of diarrhoea in young calves. The aims of the study were a) to evaluate the oocysts shedding in infected calves and b) to correlate faecal consistency with oocysts emission.

Methods: Calves borne between January 2009 and May 2010 were examined once a week during the first month of life with a copro-antigen ELISA test for Rotavirus, Coronavirus, *Escherichia coli* k99 and *Cryptosporidium* spp. Forty-seven calves positives for cryptosporidiosis were selected and screened by Immunofluorescence (IF) test on all four faecal samples and oocysts counted. Faecal consistency was scored for correlation with oocyst emission.

Results: A total of 185 faecal samples were screened. IF detected 86 (46.5%) positive samples, 31 more than ELISA test. *Cryptosporidium* positivity was recorded from the first to the fourth week of life, with the majority of animal positives at the second sampling (71.4%; age range 11-18 days) and/or at the third (55%; 15-25 days). Nine-teen calves (40.4%) were positive only once, 18 (38.3%) twice and 10 (21.3%) for three consecutive weeks. Abnormal faecal consistency was recorded from 36 animals (76.6%). In particular, 25 calves (53%) had abnormal stools only once in a month, six calves (13%) twice, four (8.35%) three times and one calf all the four weeks. Seven-teen faecal samples (20%) showed low oocysts per gram (opg) emission (range 100-1000 opg), 24 (28%) medium (1000-50000 opg) and 45 (52%) high emission (>50000 opg). Abnormal faecal consistency was significantly ($p < 0.01$) more reported in animals with single *Cryptosporidium* infection (28 samples, 55%) respect to animals co-infected with Rotavirus (10 samples, 19%), or with rotavirus single infection (4 samples, 7.5%). Excluding faecal samples positives for other infectious agents, low oocysts shedding was associated to normal stools (16/17, 94%), while medium-high oocysts shedding (> 1000 opg) was equally distributed among normal (55%) and abnormal stools (45%).

Conclusion: Cryptosporidiosis was found to be associated with abnormal faecal stools more often than other infectious agents. Low oocysts shedding (< 1000 opg) was never related to abnormal faecal samples, however medium to high *Cryptosporidium* oocysts shedding, estimated by IF, can not be used to predict the severity of the infection.

In vitro* and *in vivo* effects of the arylimidamide DB745 against *Neospora caninum

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Neospora caninum (Apicomplexa: Eimeriina: Sarcocystidae) is considered to be the main cause of bovine abortion in Europe and the USA. The main consequences of *Neospora caninum* infection in cattle are of considerable financial impact. Losses are caused directly by abortions or indirectly through breeding of less viable calves. Due to the lack of effective chemotherapy against bovine neosporosis, there is a need to develop new antiprotozoal compounds which would either eliminate the parasite or avoid its transmission.

In our efforts to identify promising novel drug candidates against neosporosis, 25 arylimidamides were screened *in vitro* by employing a *N. caninum* tachyzoite culture system expressing beta-galactosidase as a reporter gene. Arylimidamines, a class of drugs previously referred to as “reversed” amidines, have been shown to be highly effective against other intracellular protozoan parasites such as *Trypanosoma cruzi* and *Leishmania*. Against *N. caninum*, DB745 was shown to be the most promising compound, with an IC₅₀ of 80nM in 3-day growth assays, affecting both intracellular parasite proliferation as well as host cell invasion. No host cell toxicity (human fibroblasts) was noted up to 1.25 µM. However, long-term growth assays and TEM observations indicated that DB745 did not act entirely parasitocidal.

This was confirmed by an *in vivo* experiment employing the murine cerebral infection model. DB745 was first administered orally or intraperitoneally at day 14 post-infection, treatment continued for 14 days. There was no difference in the numbers of animals succumbing to experimentally induced neosporosis in control- and treatment groups. Real-time PCR assessment of the cerebral parasite burden showed only a moderate decrease in DB745-treated mice. These findings suggest that DB745 is highly active against *N. caninum* infection *in vitro*, but not in the murine model for cerebral neosporosis.

Dynamics of bovine *Besnoitia besnoiti* (Apicomplexa, Sarcocystidae) infections: within and between farms spreads

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Background: *Besnoitia besnoiti* (Apicomplexa, Sarcocystidae) is the causative agent of bovine besnoitiosis. This parasite is thought to be mechanically transmitted by biting flies, medical devices and potentially direct animal contact. Most infected animals are asymptomatic but *B. besnoiti* infection can produce an acute disease with fever, oedema, abortion and orchitis. Chronically affected animals develop lichenified and alopecic skin, emaciation and bulls may become infertile. Up to 1990, bovine besnoitiosis was geographically limited in France to four “departments” (administrative divisions) in the South West of the country. Since the 1990s, besnoitiosis outbreaks have been reported in about 35 southern “departments”. In addition, bovine besnoitiosis is spreading in Europe and was declared “emergent disease in cattle” by the European Food Safety Authority in 2010.

Methods: Clinical examinations and serological analyses (Western blots and PrioCheck *Besnoitia* ELISA) were performed in enzootic and epizootic areas of Southern France. The dynamics of *B. besnoiti* infections within a farm was assessed by longitudinal studies in infected farms during two years. Between farms diffusion was evaluated in two different management systems: i) extensive breeding with transhumance in the French Pyrenees and Alps and ii) intensive or semi-intensive breeding conditions with poor commercial exchanges.

Results: Moderate (10-20%) to high (20-30%) annual incidence rates were observed within farms located in enzootic and epizootic areas respectively. According to serological data, most of the new infections occurred during the pasture season however some were observed indoors in relation with stable flies' activity. Most of the clinical cases were observed in summer, period of horse flies' activity. Mortality rates were usually low in enzootic areas and high in areas of emergence (up to 5-10%). Between farms diffusion was low in intensive breeding systems (mainly associated with trade) and high in transhumant breeding systems with high serological prevalences (50 to 80%) in a majority of farms.

Conclusion: Within farms diffusion of cattle besnoitiosis seems to be intense and rapid whereas between farms diffusion is slow except in transhumant context. Trade and mixed grazing conditions are thought to be the main risk factors.

Detection of antibodies against *Neospora caninum* and *Toxoplasma gondii* in armadillos (*ChaetophRACTUS villosus*) Argentina

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Neospora caninum (*N. caninum*) and *Toxoplasma gondii* (*T. gondii*) are two apicomplexan parasites that can cause neurologic disease and abortions in their intermediate hosts and less frequently cause disease in their definitive hosts. Little is known about the importance of armadillos, as reservoirs for parasites of zoonotic or veterinary importance.

Serum samples from 150 armadillos, *ChaetophRACTUS villosus*, were collected in La Pampa- Argentina during years 2007 to 2010. The sera were analyzed for antibodies to *N. caninum* by an enzyme-linked immunosorbent assay (CIVTEST *Bovis Neospora*, Laboratories HIPRA, S.A. Spain) and for antibodies to *T. gondii* by a latex agglutination (Toxotest látex, Wiener Laboratories S.A.I.C. Rosario, Argentina) and an indirect hemagglutination test (Toxotest HAI, Wiener Laboratories S.A.I.C. Rosario, Argentina).

Antibodies to *Neospora caninum* were detected in 48 (32%, with a probability of 95% and a confidence interval (CI) of 25-40%) animals and for *Toxoplasma gondii* were detected in 37 (25%, 95% CI 18-32%) animals. Only 9 (19%) of the 48 armadillos that were seropositive for *N. caninum* had also antibodies to *T. gondii*. Seventy-five of the armadillos did not have antibodies to neither of the two parasites.

Our findings provide the first serological evidence of *N. caninum* and *T. gondii* infection in *ChaetophRACTUS villosus*, supporting the hypothesis that these armadillo can play a role in the life cycle of both parasites.

Anti-neosporal effect of HPLC fractions of *Sophora flavescens* and *Torillia japonica* extracts against *Neospora caninum* on dogs and goats

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In order to evaluate the anti-neosporal activities of the HPLC fractions of medicinal herb extracts against *Neospora (N.) caninum* in vivo, we selected two HPLC fractions of herbal extracts of *Sophora (S.) flavescens* and *Torillia (T.) japonica* having good anti-neosporal activities against *N. caninum* in the previous study (Youn et al., 2004). Korean native goats and suckling dogs were infected with Korean *Neospora* (KBA-2) and inoculated with these HPLC fractions to evaluate the anti-neosporal activities. We investigated the clinical signs, survival rates, abortion rates and histo-pathological lesions. The pregnant goats were infected with *N. caninum* and tested anti-neosporal efficacy of these HPLC fractions. Two goats infected with *N. caninum* but not treated with any drug showed abortion at 27 days after infection, and multi-focal necrotic encephalitis was found in fetus brain. Six goats out of 8 pregnant goats that were treated high dose of *S. flavescens* (7.125 ng/ml) and *T. japonica* (7.125 ng/ml) showed abortion. But there is difference in average abortion days. Average abortion days of high dose of *S. flavescens* (7.125 ng/ml), low dose of *S. flavescens* (2.850 ng/ml) and high dose of *T. japonica* (7.125 ng/ml), low dose of *T. japonica* (2.850 ng/ml) were 51 days, 45 days, 78.5 days, 50.5 days, respectively.

The suckling dogs were infected with Korean *Neospora* (KBA-2) to dogs, but it could not be evaluated the anti-neosporal efficacy because pathogenicity of *Neospora* (KBA-2) was very low. HPLC fractions of *S. flavescens* and *T. japonica* showed good efficacy in protection of *Neospora*, and improved survival rates in mice. We could found out that *S. flavescens* and *T. japonica* could delay abortion days in pregnant goats but it could not prohibit *Neospora*, infection or treat neosporosis.

Through this experiment, HPLC fractions of *S. flavescens* and *T. japonica* showed good anti-neosporal efficacy. Further research on the herbal materials should be carried out.

Anti-neosporal effect of HPLC fractions of *Sophora flavescens* and *Torillia japonica* extracts against *Neospora caninum* on mice

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In order to evaluate the anti-neosporal activities of the HPLC fractions of medicinal herb extracts against *Neospora (N.) caninum* in vivo, we selected two HPLC fractions of herbal extracts of *Sophora (S.) flavescens* and *Torillia (T.) japonica* having good anti-neosporal activities against *N. caninum* in the previous study (Youn et al., 2004). Mice were infected with Korean *Neospora* (KBA-2) and inoculated with these HPLC fractions to evaluate the anti-neosporal activities. We investigated the clinical signs, survival rate and histo-pathological lesions. The BALB/c mice infected with *N. caninum* showed rough hair, anorexia and motor ataxia from 14 days and all of them were dead during 17 to 25 days after infection. Necrotic or non-suppurative inflammations were found in brain, pancreas, lung, liver and spleen. Especially necrotic encephalitis index was 8.15 that means severe lesion. There was no difference between control group and the group treated with HPLC fractions of herb extract in average survival days, but survival rate in HPLC fraction treated mice improved more than that of control group. Especially the mouse which was treated with high dose of *S. flavescens* (7.125ng/ml) and *T. japonica* (7.125ng/ml) showed improved survival rate to 25% and 41.7%, respectively and showed decreased brain lesion score to 1.81 and 2.23, respectively.

Through this experiment, two HPLC fractions of *S. flavescens* and *T. japonica* showed good anti-neosporal efficacy. Further research on the herbal materials should be carried out.

***Cryptosporidium* spp. in a Brazilian Beef Cattle Herd**

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Background: *Cryptosporidium* spp. (Apicomplexa: Cryptosporidiidae) is an opportunistic protozoa that may parasite humans and animals causing usually asymptomatic infections. The role of this parasite as the etiologic agent of diarrhea in Brazilian cattle is still unknown, whereas is considered an emerging parasite problem in other countries. The objective of this study was to diagnose cryptosporidiosis as the cause of a diarrhea outbreak in a beef cattle herd not responding to antibiotic treatment and to determine possible risk factors for this parasitic disease.

Methods: The study was carried out in a herd of Nelore beef cattle (*Bos indicus*) with 4,000 animals in Mato Grosso do Sul State, Brazil. Fecal samples were obtained from 85 animals randomly distributed (n) into these groups: male (52) or female (33); with (25) or without diarrhea (60); 0-1 (27), 1-4 (38), 4-12 (10) or 12-24 month-old (10). Each sample was freshly smeared on two microscope slides and stained by a modified Ziehl-Neelsen technique to detect *Cryptosporidium* oocysts. Frequency tables were constructed and associations between occurrence of *Cryptosporidium* and diarrhea and sex were verified by the chi-square test ($P < 0.05$) and age by the Fisher's exact test

Results: Among the 85 animals analyzed, 54 (63.52%) showed oocysts of *Cryptosporidium* spp.s in the feces. The variable sex did not influenced ($P = 1.00$) the frequency of infection by *Cryptosporidium* in the evaluated animals. It was observed that 63.46% of the male and 63.63% of the female cattle were positive. The presence of diarrhea was not associated to the parasitism ($P = 0.62$), so other etiologic agents may be involved. When analyzing the age categories by the Fisher's exact test, the animals with less than one month-old showed higher frequency of this parasite. 81.48% of the calves in this category were positive, while lower frequencies were observed in the other categories: 1-4 (55.26%), 4-12 (60%) and 12-24 month-old (50%). The genotypic characterization of this isolate will be useful for the *Cryptosporidium* specie identification.

Conclusions: This study shows the high frequency of *Cryptosporidium* in a beef cattle farm in Brazil. The most susceptible category was the 0-1 month-old calves.

Recrudescence of *Neospora caninum* infection in chronically infected mice

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Background: Transplacental transmission (TT) of *Neospora caninum* may lead to abortion or birth of congenitally infected calves. Endogenous TT (EnTT) is the most common route of infection in cattle and occurs as a consequence of a recrudescence of a chronic infection. Up to now cerebral mouse models have been developed in an attempt to study *N. caninum* associated chronic infection and pregnant mice models to study acute infection associated to exogenous transplacental transmission. In the present study, a recrudescence mouse model was developed.

Methods: A first generation of 8 week old BALB/c mice was infected subcutaneously with 10^6 tachyzoites of Nc-Sp7 (G1) and Nc-Sp3H (G2), high and low virulent *N. caninum* isolates, respectively. After 54 days, blood samples were collected and specific antibodies were evaluated to monitor the infection. At day 90 post-infection (p.i.) seropositive mice were mated. After pregnancy, pups (second generation) were maintained until day 50 post partum (p.p.), when blood samples were collected for serological analysis in order to identify seropositive mice. The second generation seropositive mice were also mated and their pups represented the third generation and were sacrificed at day 50 p.p. Dams (first and second generation) were sacrificed at day 30 p.p. Morbidity and mortality rates were recorded. Humoral immune response and TT throughout two consecutive generations were evaluated.

Results: In the first generation, higher humoral immune response ($p < 0.0001$) and higher morbidity and mortality rates ($p < 0.0001$) were observed in NcSpain7 infected mice (G1) before mating compared to the group inoculated with NcSp3H (G2). In the second generation low neonatal mortality rates were observed although vertical transmission was recorded in both infected groups. Survivor pups from G1 showed higher levels of specific antibodies compared to G2 ($P < 0.05$). Finally the third generation of both G1 and G2 groups did not develop specific antibodies and morbidity and mortality rates were almost 0%.

Conclusions: In the present study the recrudescence of *N. caninum* infection was induced through gestation of chronically infected dams emulating the EnTT described in pregnant cattle. The recrudescence of the infection occurs in the first pregnancy and seems not to take place in further generations. An experimental recrudescence mouse model has been successfully developed, which may be useful in testing efficacy of vaccine formulations to prevent EnTT.

Evaluation of protective effect of NcGRA7, NcSAG4, NcBSR4 and NcSRS9 formulated in Poly- ϵ -Caprolactone against *Neospora caninum* infection in mice

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Background: *Neospora caninum* has been recognized as a major cause of infectious bovine abortion world-wide. Vaccination has been suggested to be cost-effective in controlling neosporosis although no drugs or efficient vaccines are still available. The use of parasite proteins expressed during the chronic phase of the disease, and of other proteins, possibly associated with virulence, together with the use of new vaccine delivery systems based on encapsulation could improve the immunostimulatory properties of traditional vaccines. Thus, protective efficacy of the recombinant antigens rNcGRA7 (found in both tachyzoite and bradyzoite stage), rNcSAG4, rNcBSR4 and rNcSRS9 (bradyzoite-stage specific proteins) encapsulated in Poly- ϵ -caprolactone nanoparticles against neosporosis was evaluated in a Balb/c pregnant and non pregnant mouse model. This is the first time that rNcBSR4 and rNcSRS9, have been evaluated as vaccine candidates.

Methods: rNcGRA7, rNcSAG4, rNcBSR4 and rNcSRS9 were expressed in *Escherichia coli* and encapsulated within Poly- ϵ -Caprolactone (PEC) nanoparticles. Groups of mice were immunized with nanoencapsulated proteins singly or in combinations and then were challenged with *N. caninum*. Parameters evaluated were: immune response prior challenge, morbidity and mortality and parasite presence.

Results: High IgG1 and IgG2a antibody levels anti-*N. caninum* and specific antibodies directed against recombinant proteins were developed by all immunized and challenged groups. However, a low production of IFN- γ was detected, which indicate a failure in a correct balance of Th1/Th2 response to fight against acute neosporosis during pregnancy, except for the group vaccinated with encapsulated rNcSRS9. In addition, high morbidity and mortality rates were observed in dams. Furthermore, in pups vertical transmission was not prevented, and high neonatal mortality rates occurred with no significant differences among groups. Despite the global absence of efficacy, the best result was obtained in encapsulated rNcSRS9 immunized group, where a slight delay in mortality was observed.

Conclusions: Any formulations tested showed protection against vertical transmission of *N. caninum*. However promising results obtained with rNcSRS9 protein might be improved if an experimental model for recrudescence is employed to test vaccine formulations based in bradyzoite stage specific proteins.

Systemic immune response in heifers vaccinated with different *Tritrichomonas foetus* vaccines

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Experimental whole killed cells vaccines against *Tritrichomonas foetus* (*Tf*) with different adjuvant were tested. A total of 90 heifers were assigned in nine groups of ten animals each one: Group (G) 1: received an experimental vaccine containing *Tf* and Quil A adjuvant, G2: *Tf* and Al(HO₃) gel adjuvant, G3: *Tf* and a mix of Quil A and Al(HO₃) gel adjuvant, G4: *Tf* and an oil adjuvant, G5: *Tf* and an adjuvant containing arqueosomas, G6: *Tritrichomonas suis* and a mix of Quil A and Al(HO₃) gel adjuvant, G7: a commercial vaccine and G8 and G9 were Control Infection and Control No Infection, respectively. Heifers of Groups 1 to 7 were subcutaneously vaccinated with two doses and a third dose was applied into vaginal submucosa. The females were vaccinated at 3-week intervals. After two weeks of the last vaccine dose, heifers were exposed to *T. foetus* by intravaginal instillation of 3 x10⁶ organisms. Serum samples for ELISA diagnostic were obtained at 0, 20 and 40 days post-first-vaccine dose (dpfv) and at 14, 28, 42, 56, 70, 84, 98 and 112 days post-challenged. Levels of serum antibodies measured as Elisa value (EV) of all vaccinated Groups were higher than the Control Group (p= 0.05). Since 20 dpfv, G7, G3, G4 and G1, showed higher EV than G2, G5 and G6 establishing significant differences (p= 0.05). All groups showed the peak EV at 40 dpfv but G7, G3, G4 and G1 showed higher EV than the others groups. EV for vaginal Mucus was higher in vaccinated heifers than in Control heifers (p=0.05). In conclusion, vaccines of G1, G3, G4 and G7 induced a better systemic immune response than the others vaccines.

***Neospora caninum* seroprevalence in dairy cattle from Valle de Lerma, Salta**

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Neosporosis is a parasitic disease causing reproductive failure in cattle worldwide including Argentina. The disease has been characterized in the humid pampas, however, there are no current data about neosporosis in the province of Salta, where the dairy cow population reaches 5,600 animals. A cross-sectional study was carried out in dairy herds to determine the prevalence of neosporosis in Valle de Lerma (S-24,975; W-65,506) Salta province, northwest of Argentina between July 2010 and November 2010. Blood samples were randomly collected from 667 cows in 20 herds. *N. caninum* antibodies were detected by immunofluorescent antibody test. The serum was screened and serological titers $\geq 1:200$ were considered positive. Antibodies to *N. caninum* were detected in 239 dairy cows (35.8%) and all herds (20/20) had at least one seropositive animal. This study included 51.3% of all Valle de Lerma dairy herds. The seroprevalence found in this region was higher than that recorded in the humid pampas (16.6%). This information will help to characterize the regional bovine health problems.

Evidence of Different Pathogenicity among *Neospora caninum* Strains Nc-Bahia and Nc-1

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The objective of this study was to verify differences among the experimental infection of pregnant cattle with two strains of *Neospora caninum*. Eight cows, seronegative to *N. caninum* and other abortive agents, were selected for the study. Five animals were infected with Nc-Bahia strain (group 1), two with Nc-1 strain (group 2) and one remained as control (group 3). The females from groups 1 and 2 were inoculated endovenously with 5×10^8 tachyzoites, at 70 days of gestation. Serum samples were collected weekly, and all animals from groups 1 and 2 were seropositive at 21 days p.i. (ELISA). The fetal viability was verified ultrasonographically. Fetal death was detected 35 days p.i. in both animals from group 2, while all others remained viable at that time. Group 2 was then slaughtered and tissue samples were collected. No fetal death was detected in group 1, by ultrasound examinations, and those females were slaughtered only at 42 days p.i., when one fetus from group 1 was found dead. The DNA of the parasite was detected in tissues from mothers and fetuses of both groups, even in those which were alive. The results of this experiment indicate a difference of pathogenicity between these strains of *N. caninum*, although the congenital transmission has been detected with both strains.

Keywords: *Neospora caninum*, strain, pathogenicity, abortion

Studies on potential intermediate and definitive host species of *Besnoitia besnoiti*

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Background: The definitive host of *Besnoitia besnoiti*, the cause of bovine besnoitiosis, is unknown, and information on potential intermediate hosts is incomplete.

Methods and Results: In order to investigate potential definitive and intermediate hosts for European isolates of *B. besnoiti*, dogs, cats, rabbits, guinea pigs, gerbils (*Meriones unguiculatus*), common voles (*Microtus arvalis*) and NMRI-mice were inoculated with *B. besnoiti* isolated from naturally infected German cattle. *B. besnoiti* tachyzoites or tissue cysts from the skin of a naturally infected cow were fed to cats and dogs, or were inoculated subcutaneously into rabbits and rodents. Non-inoculated animals were monitored as negative controls. Feces from dogs and cats were daily examined by a sedimentation–flotation technique, but no *B. besnoiti* oocysts were identified during at least 11 weeks. Specific antibodies to *B. besnoiti* tachyzoites were detected by the indirect fluorescent antibody test (IFAT) and immunoblot in 2 out of 3 cats fed tissue cysts since 5–7 weeks post inoculation (wpi), and in all rodent species and rabbits inoculated with either tachyzoites or bradyzoites since 3 wpi until euthanasia (15–25 wpi). Dogs, and cats fed tachyzoites did not seroconvert. Control rabbits and rodents, inoculated with inactivated bradyzoites remained seronegative (IFAT titer <50). The inoculated rabbits presented transient fever, serous conjunctivitis and swelling of the testes 2–3 wpi. No clinical abnormalities were noticed in the other animal species. *B. besnoiti*-DNA was detected by PCR in blood from some rabbits, gerbils and voles at 9 days post-inoculation, and in skin, heart, lung, striated muscle and kidney tissues from voles at 19–21 wpi.

Conclusions: Domestic dogs and cats were not confirmed to be definitive hosts of *B. besnoiti*. However, it appears that the parasite may infect feline tissues orally, as cats seroconverted after feeding on *B. besnoiti* tissue cysts. The molecular and serological results from this study suggest that European *B. besnoiti* isolates may infect cats, rabbits, guinea pigs, gerbils, mice and voles. A persistence of the parasite in the tissues was demonstrated only in voles.

A review of the economic impact of *Neospora caninum*

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Background: *Neospora caninum* is invariably rated as one of the most important infectious causes of abortions in cattle world-wide. In Australasia, where bovine brucellosis is absent now, it is usually reported to be the cause of 30-40% of all diagnosed abortions, particularly in dairy cattle. Despite that, there seems to be evidence now of a waning in interest in the disease, possibly because the economic impact of the infection has not been clearly communicated to primary producers and veterinary community.

Methods: A search was conducted on PubMed using *cattle* and *Neospora* as search terms. As of March, 10th 2011, this yielded 753 publications whose abstracts were initially screened individually for suggestions of economic relevant information (abortion incidence, prevalence and risk, serological data, impact on milk production and reproductive parameters etc.). Relevant papers were subjected to a meta-analysis.

Results: Out of the 753 publications initially selected in the database search, only about 10% contained economically relevant information (concentrated in less than ten countries). Most contained case reports from mostly individual herd events, or results of (sometimes large-scale) serological surveys. The analysis of available data from the publications that contained economically relevant data showed the most prominent economic impact of *N. caninum* infections to be that of abortion events. The total annual cost was estimated to range from NZ \$1.468 million in the New Zealand beef industry to an estimated total of US\$ 423 million impact *per annum* in the US dairy population. The total annual costs in just those countries alone, was approaching \$ 1 billion *per annum*.

Conclusions: A review of *Neospora* and cattle-related literature yielded limited published information on the economic impact of infection. Most of the qualifying articles dealt with individual case or herd reports, with limited controls or were manuscripts that established sero-prevalence data only. Nevertheless, a meta-analysis of the published literature did put the economic impact of *N. caninum* infections in cattle world-wide at in excess of one billion US dollars annually.

Cutaneous bovine and equine leishmaniasis due to a yet non-specified *Leishmania* organism

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Background: Recently, a novel etiological agent of cutaneous leishmaniasis was identified in a Swiss cow and in horses that, for most cases, sporadically appeared as autochthonous infections in geographically distant regions of Germany and Switzerland.

Methods: The infection was initially diagnosed upon clinical and immunohistological findings, complemented by electron microscopy. Conventional *in vitro* cultivation of the *Leishmania* organisms failed so far. Comparative sequence analysis of *Leishmania*-PCR products obtained from the internal transcribed spacer 1 (ITS1) of ssrRNA allowed to preliminarily classify the detected *Leishmania* organisms.

Results and Conclusions: The histopathology of the bovine cutaneous lesions revealed that the epidermis of the animal was severely ulcerated and covered by a thick serous-fibrinous crust and, focally, few parakeratotic lamellar cell sheets. The dermis was diffusely infiltrated by numerous eosinophils and foamy macrophages. Few plasma cells, lymphocytes and a moderate fibroblast proliferation were visible. Multiple foci of cells filled with typical *Leishmania*-shaped organisms were visible. Immunohistology using an anti-*L. infantum* hyperimmune serum yielded a clear positive reaction. Electron microscopic visualization of these organisms again confirmed the presence of *Leishmania*-specific structures and organelles. Subsequent comparative sequence analysis of *Leishmania*-PCR products (GeneBank™ accession numbers for the cow GQ281282, for the horses GQ281278, GQ281279, GQ281280, and GQ281281) classified the respective *Leishmania*-isolates as neither Old World nor New World *Leishmania* species. However, the PCR-typed isolates exhibited a close phylogenetic relationship to *Leishmania* sp. *siamensis*, an organism recently identified in a visceral leishmaniasis patient from Thailand (Sukmee et al., 2008). The cow was euthanized approximately one year after diagnosis and subjected to pathological and parasitological investigations. While all cutaneous lesions had disappeared, PCR was able to detect parasite DNA in a lymph node. *In vitro* cultivation again failed. The potential transmitting vectors for all these cases have not yet been identified. Future investigations will have to elucidate the veterinary-epidemiological relevance of this etiological agent, as well as biological parameters such as transmission mode and geographical origin and distribution.

Evaluation of the protection conferred by a naturally-attenuated *Neospora caninum* isolate against congenital neosporosis in mice

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Background: The parasite *Neospora caninum* is an important abortifacient agent in cattle world-wide and the predominant route of transmission is considered to be transplacental. The development of an effective and safe vaccine is of great importance today. Recently, a new isolate of *N. caninum* (Nc-Spain 1H), obtained from the brain of a healthy congenitally infected calf, showed low virulence in both mouse and bovine models. We therefore hypothesized that Nc-Spain 1H may be a suitable candidate for a live vaccine.

Methods: We investigated the safety and efficacy of the immunization with live Nc-Spain 1H tachyzoites to prevent transplacental transmission using a well-established pregnant BABL/c mouse model. In a first experiment, the efficacy of the immunization with 5×10^5 tachyzoites was studied and in a second experiment, a set of titrations from 5×10^5 to 5×10 tachyzoites was performed to determine the effective dose-50 (ED-50). In both experiments, BALB/c mice were immunized subcutaneously before pregnancy twice at 3-week intervals and challenged at mid gestation by inoculation of 2×10^6 Nc-Liv tachyzoites. Before challenge, safety was determined by analyzing the parasite presence in dam brains. To assess efficacy, neonatal mortality and vertical parasite transmission were evaluated.. In experiment 2, in order to calculate the ED-50, a protection rate against congenital infection was determined considering as protected mice those whom pups survived healthy until the end of the experiment and no parasite presence was detected in their brains. In addition, immune response was studied.

Results: The immunization with 5×10^5 tachyzoites significantly reduced neonatal mortality to 2.4% and vertical transmission from 92.8% to 2.8%. Regarding the safety of the live vaccine, results showed no parasite-DNA presence in dam brains before challenge. In experiment 2, all the doses displayed protection rates above 60%. Since no groups showed protection under 50%, the ED-75 was calculated, resulting in $5 \times 10^{4.08}$ Nc-Spain1H tachyzoites/dose. These results were associated to a Th1-type immune response.

Conclusions: These results confirm the safety and the efficient protection conferred by Nc-Spain 1H against congenital neosporosis even when mice were immunized with low parasite doses.

Immune Response In *Neospora caninum* Seropositive Calves Experimentally Suppressed With Dexametasone

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The aim of this study was to characterize the immune response to *Neospora caninum* in seropositive calves experimentally immunosuppressed by administration of dexamethasone (Dx). A total of 16 beef breed cattle 8-10 months of age were used divided into 4 experimental groups: Group A: 4 seropositive animals treated with Dx; Group B: 4 seropositive animals treated with placebo; Group C: 4 seronegative animals treated with Dx; and Group D: 4 seronegative animals treated with placebo. Dx treatment consisted of a dose of 0.1 mg/kg/day intramuscularly for 5 consecutive days. Clinical examination including rectal temperature was performed at one day interval. Blood samples with heparin were obtained for counting leukocytes and characterizing its formulae. Serum was obtained weekly to evaluate total IgG by indirect fluorescent antibody test. Leukocytes were separated from whole blood to assess PCR. Not clinical signs were observed through the experiment time, however few animals from all experimental groups had fever during a short period of time. Leukocytopenia was recorded in all animals receiving Dx (Groups A and C) at the 3rd and 14th day after giving the first dose. Similarly, neutrophilia and lymphopenia was recorded at the 3rd and 14th days, respectively. Interestingly, there were two peaks of IgG titers (mean reciprocal antibody titers: ≥ 500) in animals from Group A compared with those in animals in Group B (mean reciprocal antibody titers: ≈ 275). All animals from Groups C and D remained seronegative through the experimental period. Two weeks after giving 1st dose of Dx all four calves in Group A had PCR positive results on the leukocytes. Parasitemia and seroconversion was induced in *N. caninum* seropositive calves after experimental immunosuppression by administration of dexamethasone.

Occurrence Of Antibodies Against *Neospora Caninum* In Sheep In The Humid Pampas

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Neosporosis is an important infection in young and adult sheep, leading to low production and abortion in other countries around the world. There are about 3,000 and 2,400,000 sheep involved milk and meat production in the humid pampas, respectively. The aim of this work was to describe the occurrence of antibodies against *Neospora caninum* in sheep from humid pampas, Argentina. A serological survey of *Neospora caninum* was carried out in 850 sheep from 11 herds. An indirect fluorescent antibody test (IFAT) was performed and serological titers $\geq 1:50$ were considered positive. Data regarding to sex, categories and type of exploitation were available and associations with serostatus were tested. Antibodies to *N. caninum* were detected in 22 sheep (2.6%) at titers 50: 7 animals (31.8%); 200: 4 animals (18.2%); 800: 5 animals (22.7%); 1,600: 2 animals (9.1%) and 3,200: 4 animals (18.2%). At least 7/12 (58.3%) herds had one seropositive animal. There was no effect of the production system, sex and status on the prevalence of the disease (Fisher's Exact Test, $p > 0.05$). Currently, serology for *Toxoplasma gondii* is being assessed. This is the first report of *N. caninum* exposure in sheep from Argentina; however this infectious agent should not play an important role as an important abortifacient agent sheep.

Dynamics of Parasitaemia, Stress Profiling and Acute Phase Protein Expression in Caprine *Trypanosoma evansi* Infection

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Background: *Trypanosoma evansi* is widely distributed and infects a range of domestic livestock. Goats appear to be resistant to the disease and may serve as potential reservoirs, but the infection dynamics is still poorly understood. The objectives of this study are to investigate the dynamics of *T. evansi* parasitaemia in goats, and to determine the stress response and acute phase protein expression during infection.

Methods: Twelve female Boer goats 8-10 months of age were divided into two equal groups, and were kept in adjacent pens in a fly-proof house. One group was inoculated with 1×10^4 *T. evansi* intravenously while the other remained as a control. The goats were bled every alternate day from day 0 to day 62 post-infection (pi), and the infected animals were treated with diminazene diaceturate on day 62. Blood was collected on three occasions post-treatment, at six day intervals. Blood samples were examined for levels of cortisol, neutrophils, lymphocytes, haptoglobin and serum amyloid-A. Parasitaemia was quantified using the haematocrit centrifugation technique and a haemocytometer.

Results: The first and highest peak of parasitaemia was detected on day 6 pi (2.5×10^6 parasite/ml), followed by the second lower peak on day 14 and a third minor peak on day 28. Parasite levels then declined gradually over time. Following treatment, the parasitaemia dropped with no detectable levels after 6 days. The infected group showed three main peaks of cortisol increment corresponding to the initial parasitaemia peaks. Neutrophil counts were significantly lower in the infected group throughout the infection period and resumed to normal values upon treatment. Significant lymphopaenia was observed in the infected goats only during the active phase of parasitaemia. Haptoglobin and serum amyloid-A revealed an acute and chronic stress response to parasitaemia, respectively. Haptoglobin levels were significantly higher during the active parasite multiplication phase.

Conclusion: *T. evansi* exhibits active waves of parasitaemia during the early stages of infection in goats. Parasitaemia persists as a chronic low grade infection making them suitable reservoirs. The stress response in goats may cause chronic immunosuppression that may influence productivity.

Length of infection in heifers immunized with experimental *Tritrichomonas foetus* vaccines and challenged

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Experimental inactivated whole cells vaccines against *Tritrichomonas foetus* (*Tf*) with different adjuvant were tested. A total of 90 heifers were assigned in nine groups of ten animals each one: Group (G): G1, received an experimental vaccine containing *Tf* and Quil A adjuvant, G2: *Tf* and Al(HO₃) gel adjuvant, G3: *Tf* and a mix of Quil A and Al(HO₃) gel adjuvant, G4: *Tf* and an oil adjuvant, G5: *Tf* and an adjuvant containing arqueosomas, G6: *Tritrichomonas suis* and a mix of Quil A and Al(HO₃) gel adjuvant, G7: a commercial vaccine and G8 and G9 were control infection and Control no Infection, respectively. Groups 1 to 7 were subcutaneously vaccinated with two doses and a third dose was applied into vaginal submucosa. The females were vaccinated at 3-week intervals. After two weeks of the last vaccine dose the heifers were exposed by intravaginal instillation of *T. foetus* with 3 x10⁶ organisms. Cervico-vaginal mucus (CVM) for culture diagnostic was obtained at 14, 28, 42, 56, 70, 84, 98 and 112 days post-challenged (dpc). The mean length of infection for G8 was of 64 days. G3, G1, G7 and G4 quickly cleared the infection at 26, 33, 33 and 38 days, respectively. Heifers from G6, G2 and G5 had a mean length of infection of 45, 47 and 70 days, respectively. In conclusion, systemic priming heifers immunized from G3 vaccine and challenge resulted in faster clearance of *T. foetus* infection.

Experimental infection with *Neospora caninum* in pregnant buffaloes (*Bubalus bubalis*) in Argentina

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Seroprevalence of *Neospora caninum* in buffaloes (*Bubalus bubalis*) was characterized by our research group and a high prevalence was proved in the Northeast of Argentina. To characterize the pathogenesis of the disease an experimental infection was carried out with *N. caninum* in pregnant buffaloes. Twelve Mediterranean buffaloes ranged from 4 to 14 years old and seronegative to *N. caninum* were used. The buffaloes were kept in the ranges under extensive systems until slaughter. The female buffaloes were oestrus synchronised using the Ovsynch protocol. After that they were kept in natural service for 72 hours with 3 male buffaloes clinically and genitally fit. The pregnancy was confirmed by ultrasound. Buffaloes of 70 (n = 3) and 90 (n = 6) days of gestation (dg), respectively were inoculated intravenously with 10⁸ tachyzoites of Nc-1 strain; and 2 animals were not inoculated. The buffaloes were slaughtered at 28 days post inoculation (dpi) with 98 and 118 dg (n = 8), and then at 42 (n = 4) dpi with 132 dg. No abortions were observed during those periods. The fetuses were necropsied and tissue samples and fetal fluids from cavities were obtained. 1/10 fetuses was not viable at the time of autopsy and presented moderate autolysis. DNA was extracted (Qiagen® kit) from tissues and detection of parasite DNA was performed by a nested-PCR on the ITS-1 region of *N. caninum* and amplification product was visualized in the in 2% agarose electrophoresis gel. The fetal cavity fluids were analyzed by indirect immunofluorescence (IFI) to dilutions 1/10, 1/16 and 1/25. The presence of DNA from *N. caninum* inoculated in 10/10 fetuses was confirmed. The 2 fetuses from non inoculated female were negative. At 132 days of gestation, the IFI fetal fluid was positive in all dilutions in only 2/4 inoculated fetuses. The higher gestational age fetuses were confirmed positive by IFI fetal immunocompetence. The negative result of the control animals suggests the absence of horizontal transmission. In this study, the pathogenic effect of *N. caninum* and the transmission of the strain used in females pregnant buffaloes inoculated intravenously at 70 and 90 days of gestation were determined.

Effect of NC-1 strain of *Neospora caninum* inoculated experimentally in pregnant buffaloes (*Bubalus bubalis*)

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Neospora caninum is an Apicomplexan parasite which has shown a high seroprevalence in buffaloes in Argentina. While there are descriptions of lesions in bovine fetuses produced by experimental inoculation with *N. caninum*, there is little information in buffaloes fetuses. The aim of this study was to characterize the pathogenic effect of NC-1 strain of *N. caninum* in fetus when inoculated in pregnant buffaloes. 12 Mediterranean pregnant buffaloes, seronegative to indirect immunofluorescent (IFI) were used for this purpose. Ten cows were inoculated with 10⁸ viable NC-1 strain tachyzoites intravenously, and two control cows were inoculated with placebo. Pregnant cow buffaloes were challenged at 70 (n=3) and 90 (n=7) days of pregnancy (DOP), and then slaughtered. The genital tract was recovered, 98 DOP and 28 days post inoculation (DPI) (n=3), 118 DOP and 28 DPI (n=3), 132 DOP and 42 DPI days (n=4). Control fetuses were 98 and 118 DOP. Twelve fetuses were recovered; one of them was dead at the time of slaughtering. It belonged to an inoculated female buffalo of 70 DOP and slaughtered 28 DPI. The fetus showed an advanced degree of autolysis and a small size (length=9 cm). At necropsy, samples from placenta, central nervous system (CNS), heart, lung, liver, kidney, the skeletal muscle, tongue and the intestine were fixed in 10% buffered formalin and stained with hematoxylin and eosin. There were no macroscopic changes in organs or tissues of viable fetuses. Multiple mononuclear inflammatory foci were observed in different fetuses' organs from inoculated mothers: peribronchiolar interstitial pneumonia in 10/10, myocarditis in 3/10, epicarditis in 6/10, interstitial nephritis in 6/10, interstitial myositis in 6/10, hepatitis periportal in 7/10. CNS lesions were focal gliosis 1/10, meningitis 1/10 and focal necrosis 2/10. Placental lesions (9/10) were moderated mononuclear infiltration in the placentoma and allantoides. Control fetuses showed neither macroscopic nor microscopic alterations. The histopathological lesions observed in this work were similar to those identified in bovine fetuses of similar age; however, the lesions were less severe. The finding of a dead fetus inside the inoculated buffaloes shows the susceptibility of this species when inoculated early in pregnancy.

Serology, isolation and genotype characterization of *Toxoplasma gondii* from naturally infected pregnant dairy cows (*Bos taurus*)

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Toxoplasma gondii is a worldwide parasite recognized as one of the main zoonosis in human beings. The present study aimed to isolate *Toxoplasma gondii* strains from pregnant dairy cows and their fetuses slaughtered in an abattoir for human consume. Blood (with EDTA) from 60 pregnant dairy cows, and blood and tissue samples (brain, lung, heart, and liver) from their fetuses were collected and used for mouse bioassay. The presence of *T. gondii* was confirmed through indirectly by immunofluorescence assay (IFAT) considering positive animals with titers ≥ 50 for cows and ≥ 25 for fetuses. Isolates were genotyping by PCR-RFLP using SAG1, SAG-2, alt. SAG-2, SAG3, BTUB, L358, GRA6, C22-8, C29-2, PK1, Apico, CS3 markers. Antibodies against *T. gondii* were observed in 55% (33/60) of cows and in 3.7% (2/54) of fetuses. Thirteen fetuses (21.6%) and six (10.0%) cows had tissues and blood detected as positive in the mouse bioassay. Brain cysts from *T. gondii* were observed in two mice, one of them infected with blood sample from a cow (N. 53) with more than 4 years old in the 6th month of pregnancy, and in a mouse infected with blood sample from a fetus (N.103) in the 6th month of gestation. Genotyping characterization of both strains showed genotype II for all PCR-RFLP markers tested. This is the first genotype II isolation in cattle from Brazil. The potential role of beef in epidemiology of *T. gondii* for human beings is yet enigmatic, and more studies are necessary to elucidate the real risk of this food for consumers, including the potential risk for meat workers once that blood samples were detected as positive.

***Eimeria* species in Danish dairy cattle – preliminary data from an ongoing study**

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Contrary to the majority of European countries, antiparasitics are on prescription only in Denmark, thus treatment requires a proper diagnosis made by a veterinarian, and therefore relies on adequate diagnostic procedures. This study was performed to obtain information about presence of *Eimeria* spp. in Danish cattle herds, and secondly to improve awareness and proper diagnosis of these infections.

Collection of samples was initiated in October 2010 from dairy herds with ≥50 cows and known diarrhea problems among calves. From each herd individual faecal samples were taken once from approximately 10 calves aged 3 weeks to 6 months. Veterinarians were instructed to collect samples 3-4 weeks following relocation to common pens, and from groups with reduced growth, uneven appearance and diarrhea.

Oocyst excretion was analyzed using a modified McMaster technique. *Eimeria* spp. were identified based on morphology, and oocysts from highly positive specimens were sporulated for additional species verification. Furthermore faecal consistency was scored on a scale from 0 (firm) to 5 (watery with blood and/or mucus).

Currently (March 2011) 42 herds and a total of 356 calves have been analyzed. *Eimeria* spp. were detected in 95.2% of the herds, and 90.5% were positive for the known pathogenic spp. *E. bovis* and/or *E. zuernii*. Of the individual calves 56% were shedding oocysts at the time of sampling, and 39% were excreting *E. bovis* and/or *E. zuernii*. A total of 11 different spp. have been identified so far. Of the faecal samples included in the study 7% had a firm/normal consistency, 81% were soft to liquid, and 12 % were watery with blood and/or mucus. Oocyst excretion above 5000 oocysts per gram (OPG) was found in 6.5% of the calves, whereas 12.0% excreted 500-5000 OPG. Clinical coccidiosis was diagnosed in 11 of 42 herds based on detection of pathogenic *Eimeria* spp. and the following criteria: one or more samples with ≥5000 OPG; mean OPG >2500; one or more samples with >2500 OPG and clinical signs. Moderate to massive excretion of the less studied species *E. subspherica* and *E. auburnensis* was in several cases correlated to diarrhea. These cases however were not diagnosed as coccidiosis.

The results warrants further pathogenicity studies of the different *Eimeria* spp. In addition, it was shown that correct diagnosis of coccidiosis is a challenge and knowledge of the management system is essential for interpreting laboratory findings. In many cases these findings do not support diagnosis of coccidiosis either because there is no real coccidiosis problem or because faecal samples were obtained at the wrong time.

Bovine neosporosis in the South of Romania: seroprevalence of *Neospora caninum* infection in dairy cattle

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Background: *Neospora caninum*, a coccidian parasite closely related to *Toxoplasma gondii*, causes severe economic losses by reproductive disorders, particularly by abortion in cattle worldwide. In Romania, infection with *N. caninum* in cattle has been recently reported, but studies are limited in the North and Center of the country. Therefore, considering the importance of the parasite in bovine reproduction and the lack of information on spreading of the disease in many regions of country, the aim of this study was to obtain additional sero-epidemiological data on infection with *N. caninum* in dairy farms from the South of Romania.

Methods: The study was conducted in September-October 2010, in nine counties from the South of Romania. A total of 230 blood samples were collected from cows (age 2.5 to 10 years) from nine dairy farms, one farm for each county. The presence of specific IgG antibodies against *Neospora caninum* in serum was determined using an indirect ELISA test.

Results: The average seroprevalence of *N. caninum* antibodies in dairy cows was 41.73% (96/230), but the within-herds prevalence ranged between 13.46% and 80%. Out of the positive samples, 75% (72/96) had a high positive reaction (S/P ratio more than 1.0), while 25% (24/96) had a low positive reaction (S/P ratio between 0.5 and 1). Statistically, there was a significant difference between these two categories of positive samples ($p=0.007$).

Conclusions: This study indicates that *Neospora* infection is widespread in the South of Romania, and this fact could be one of the causes of abortions registered in some herds in the studied area. However, a serological screening across the country is planned in order to assess the actual national prevalence of *N. caninum* infection, followed by implementation of a prevention and control program.

Sexual transmission of *Toxoplasma gondii* (Nicolle & Manceaux, 1909) in caprines

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The aim of this study was to confirm the sexual transmissibility of *Toxoplasma gondii* in experimentally infected caprines. For doing that, two male caprines, free of *T. gondii* antibodies, were inoculated with 2,0 x 10⁵ P strain oocysts (A) and 1,0 x 10⁵ RH strain tachyzoites (B), respectively. A third male was maintained as control (C). After inoculating the males, 12 reproducing females, serologically negative for *T. gondii* had their rutting periods synchronized and were divided in three groups (GI, GII and GIII) and naturally mated with males A, B and C. Five females were put together with male A (GI), other five with male B (GII) and two with male C (GIII). At the end of the gestational period, or in case of abortion, females were euthanized and tissue samples from the uterus, vagina, ovary, skeletal and cardiac muscles, brain, liver and spleen were collected for searching of *T. gondii* using bioassay in mice and Polimerase Chain Reaction (PCR) techniques. Serum samples were collected at 14, 7 and 1 day before natural mating, and also at 3, 6, 9, 11, 14 days after natural mating, and weekly until the end of the gestational period. Antibodies against *T. gondii* were searched in all serum samples by Enzyme Linked Immunosorbent Assay (ELISA). Additionally, bioassay in mice and PCR techniques were applied in seminal and tissue samples of males, tissue samples of females and their respective “products” (fetus or stillbirths). All females from groups I and II presented specific antibodies against *T. gondii* after natural mating. By bioassay and PCR, it was possible to identify *T. gondii* in seminal samples of several experimental dates when natural mating occurred. Moreover, by these two techniques, it was possible to identify this coccidian in tissue samples of females mated by infected males, as well as in the gestational “products”. In all infected males and females was not possible to realize any clinical sign of toxoplasmosis. Therefore, this work was successful in proving the sexual transmission of *Toxoplasma gondii* in the caprine species with a consequent vertical transmission. It is worth mention this is first time in literature that the sexual transmission of this parasite in caprine species was reported.

Comparative study of the techniques Bioassay in mice and Polymerase Chain Reaction (PCR) to detect *Toxoplasma gondii* in tissues of female goats

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The aim of the present study was to compare the sensibility of Polymerase Chain Reaction (PCR) and bioassay in mice techniques for the detection of *Toxoplasma gondii* in tissue samples of female caprines sexually infected by experimentally inoculated male caprines. In order to do this, two male caprines, free of *T. gondii* antibodies, were inoculated with $2,0 \times 10^5$ P strain oocysts (A) and $1,0 \times 10^5$ RH strain tachyzoites (B), respectively. A third male was maintained as control (C). After inoculating the males, 12 reproducing females, serologically negative for *T. gondii* had their rutting periods synchronized and were divided in three groups (GI, GII and GIII) and naturally mated with males A, B and C. Five females were put together with male A (GI), other five with male B (GII) and two with male C (GIII). During the experimental period, all females were serologically evaluated by the indirect immunofluorescence reaction (IFI), aiming at diagnosing the natural post-mating toxoplasmic infection. At the end of the gestational period, or in case of abortion, females were euthanized and tissue samples from the uterus, vagina, ovary, skeletal and cardiac muscles, brain, liver and spleen were collected for searching of *T. gondii* using bioassay in mice and PCR techniques. Seminal samples from males A and B, collected on the dates of natural mating, were positive for *T. gondii* in both techniques. All 10 females mated with males A and B presented specific antibodies against *T. gondii* three weeks after natural mating ($IFI \geq 1/64$). *T. gondii* was not detected in any of the 16 tissue samples collected from the two females mated with male C in any of adopted techniques. On the other hand, 24 out of the 80 tissue samples collected from 10 females (GI and GII) were positive (30%) for *T. gondii* in the bioassay technique. Meanwhile, 70 out of the 80 tissue samples (87.5%) were positive by PCR technique. According to these results, the PCR technique is more suitable for the diagnosis of caprine toxoplasmosis.

A case report of *Trypanosoma* spp. in bulls in Corrientes, Argentina

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We describe a case of bovine trypanosomiasis in the town of Curuzú Cuatia, province of Corrientes, Argentina (S Lat. 28°49' "W Long. 58°03'"). The same becomes important because it is the southernmost geographical point of the Americas, where it was described to this parasite.

M and M: Two Hereford bulls had low body condition, weight loss and anemia. For diagnosis, thick smears were made on whole blood centrifuged in the area of liquid-solid interface. The smears were stained with Giemsa, without fixation with methyl alcohol. Visualized the presence of n = 22 *Trypanosoma vivax* (Ziemann, 1905) with Leitz Dialux 20 EB microscope with 100X objective and 12X eye piece with scale. The measurement method was the comparison described by Coffin (1977) and took a multiplication factor 1.875. The parasites were defined biomorphometric study.

Results: The information of the 22 *Trypanosoma vivax* are presented as average (μm)(X), standard deviation (SD), coefficient of variation (CV) %; were: Kinetoplast-edge= X 7,7; DS 1,8 and CV23,2 ; Kinetoplast Rear-End= X 0,6; DS 0,3 CV 46,5.; Core to edge Kinetoplast= X 5,6.; DS 2.; CV35,2.; Nucleus, edge to edge= X3,8; DS 1,5.; CV 38,3 ; Swipe : flagellum length= X 12,5.; DS 5.; CV 40,6 ; length and width of the parasite X 29,5.; DS 10,7.; CV 34,7 and X 3 ; DS 1,1.; CV 36,0 respectively. This finding calls for a retrospective anamnestic study. Because the bulls had been recently acquired, it is possible that hatch trypanosomiasis when leaving the cattle ranch origin. This situation would place the outbreak, further north in San Luis del Palmar in the province of Corrientes (Lat. S 27 ° 31 "Long. O. 58° 34"). Also, it is to contemplate another possibility would be that trypanosomiasis has been incurred in establishing Curuzú Cuatia. In this case of the native cattle, trypanosome may be asymptomatic like animals in enzootic areas, affecting animals only introduced in it. In Argentina, *Trypanosoma vivax* was exotic up to 2008 and veterinarians and diagnostic laboratories are unfamiliar with the symptoms and morphology of the pathogen. The predominant clinical findings are anemia, weight loss and body condition. The size of *Trypanosoma vivax* is of 29.5 μm and it places itself among the largest bovine hematic protozoa, a description that coincides with those reported by other authors of *Trypanosoma vivax*. This finding confirms the presence of *Trypanosoma vivax* at the furthest points in South America described so far.

Parasitic and rickettsial infections of goats and cattle in Angónia District, Tete, Mozambique

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The Tete province is located in the centre-west area of Mozambique, holding one of the largest goat and cattle herds, which are raised under traditional productive systems, in communal pastures and extensive grazing system. In order to identify the main parasites of ruminant in this province, 115 fecal samples of Landim goat of 4 to 36 months old and 198 Angone cattle of 8 to 56 months old, male and female, were collected in 13 and four localities in Angonia District, respectively. *In this District the cattle and goat population* is estimated to be about 18000 and 13650 head, respectively. **Herds were selected by non-probabilistic sampling.** The Strongyloidea infection was detected in 83.4% of the goats and 77.8% of the cattle, with averages of EPG counts 528.4 (0 to 1275) and (0 to 1975) respectively. *Haemonchus* (94%; 28%), *Trichostrongylus* (1%; 70%) and *Oesophagostomum* larvae (5%; 2%) were identified in goat and cattle faecal cultures, respectively. *Strongyloides* (15.0% and 2.0%), *Trichuris* (1.9% and 1.0%) and *Moniezia* (13.5% and 5.1%) eggs were also observed in goat and cattle samples of respectively. At Ulongué area, *Fasciola gigantica* frequency was 83.3% in cattle, with averages of egg counts ranging from 1.90±1.83 to 3.42±3.36, while *Paramphistomum* genus was found in 6.7%, with one to two eggs per faecal sample. For protozoan infections, 48.5% of the cattle samples and 95.8% of the goat presented *Eimeria* sp. oocysts in the feces. Mean oocysts counting's for gram of feces was 316.7±388.4 for cattle and 22011.3±39110.2 for goats. Frequency of anti-*Neospora caninum* IgG was evaluated in 331 and 146 cattle and goat serum samples, respectively, using indirect immunofluorescent assay. Antibodies were detected in 27 cattle samples (8.15%) and positive reaction ranging from 1:200 to 1:800, while in goat, five (3.4%) of the samples were positive at 1:50 dilution. Blood smears from 31 goats were examined and 14 (45.2%) were positive for *Anaplasma ovis* and five (16.1%) for *Theileria* sp. This study was supported by CNPq.

Seroprevalence and risk factors to *Neospora caninum* infection in ewes of Planalto Catarinense, Santa Catarina State, Brazil

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Neosporosis occurs in sheep and is associated with reproductive failure. The aim of this study was to verify seroprevalence for neosporosis in ewes, identify risk factors and correlation with reproductive disorders. Serum samples from 1308 ewes from Planalto Catarinense, Santa Catarina State, Brazil, were tested using the indirect fluorescent antibody test (IFAT) for detection antibodies against *Neospora caninum* ($\geq 1:50$) and an epidemiologic questionnaire was applied in order to correlate with risk factors of neosporosis. These data were then tabulated and statistically analyzed by the Fisher's exact and χ^2 tests ($P < 0.05$). Antibodies to *N. caninum* were observed in 92 (7%) of the animals, with titers ranging from 50 to 3200. In 39 (42.4%) of the 92 farms there was at least one positive animal for *N. caninum*. An association between seroprevalence for *N. caninum* and breed ($P = 0.0249$, OR = 0.59, IC 95% = 0.36-0.94), and contact with bovines ($P = 0.0491$, OR = 0.57, IC 95% = 0.32-1.04) was observed. There was no association between seropositivity to *N. caninum* and reproductive disorders.

Cytokine expression in pregnant dairy cows naturally infected with *Neospora caninum*

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Neospora caninum, a major cause of abortion in cattle worldwide, can be transmitted horizontally by ingestion of oocysts or vertically from the infected dam to the fetus via the placenta. The precise mechanism of fetal loss and vertical transmission is poorly understood, but it seems likely that immune factors influence both. The objective of this study was to evaluate cytokine expression in pregnant dairy cows naturally infected with *N. caninum*. Peripheral blood mononuclear cells (PBMC) from seropositive (IFAT titer ≥ 400) and seronegative (control group; IFAT titer < 25) pregnant dairy cows (n=8 each) from a commercial dairy farm bled at 5 months gestation were isolated and stimulated with Concanavalin A (ConA; 5 μ g/ml), *N. caninum* tachyzoite lysate (5 μ g/ml), or PBS. After 20-24 hr incubation, PBMC RNA was extracted, reverse transcribed and evaluated by real time quantitative PCR for expression of Pol II (used as reference gene) IL-2, IL-4, IL-10, IL-12 and interferon gamma (IFN γ). IL-2, IL-4 and IFN γ were upregulated similarly in ConA-stimulated PBMC from seropositive and seronegative cows. No changes in cytokine expression were observed in tachyzoite-stimulated PBMC from seronegative cows; however IL-4 and IFN γ expression was upregulated in tachyzoite-stimulated PBMC from seropositive cows. Further studies are underway to evaluate cytokine expression in late pregnancy as well as the relationship of cytokine expression to vertical transmission. Better understanding of these events may aid in designing immune modulation strategies for avoiding abortion and/or vertical transmission by *N. caninum*.

Sanitary surveillance of bovine encephalitis: *Neospora caninum* as causative agent

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Neospora caninum is a protozoan that causes encephalitis in cattle, but its impact as a causative agent of neurological syndrome has been poorly studied. This study investigated the frequency of occurrence of *N. caninum* in the central nervous system (CNS) of cattle with neurological syndrome using histopathology techniques by hematoxylin-eosin staining in paraffin embedded tissue (HE), immunohistochemistry (IHC) and nested PCR using primers from the Nc5 region of the genomic DNA and primers from the ITS1 region of the ribosomal DNA. It was evaluated a convenience sample consisting of 302 bovine with neurological syndrome, aged from 0-18 years-old, from different herds of 10 Brazilian states, which were referred to the *Centro de Pesquisa e Desenvolvimento de Sanidade Animal do Instituto Biológico* for the differential diagnosis of encephalitis from January 2007 to April 2010. In the HE evaluation, 24.0% (72/302) CNS samples presented lesions of non-purulent meningoencephalitis, and tested negative to IHC. All samples submitted to nested PCR using primers from the ITS1 region of the ribosomal DNA were negative (0/302). The analysis of nested PCR using primers from the Nc5 region of the genomic DNA revealed two positive cases 0.66% (2/302), a 20-month-old male and a 72-month-old female, both from São Paulo state. Only the male presented severe degree of multifocal necrotizing encephalitis associated to mononuclear cell infiltration, a pathognomonic histopathologic lesion caused by protozoan parasites belonging to the family Sarcocystidae, while the other showed a slight edema and congestion of the meninges and neuropil. Considering histopathological and biomolecular analysis only the 20-month-old bovine presented encephalitis caused by *N. caninum* due to severe inflammatory lesions of non-purulent multifocal necrosis, thus representing 0.33% (1/302) of positivity. However, despite the low occurrence of neosporosis positivity found in the present work, future studies that include a representative sampling of animals aged 0-2 years-old should be performed to elucidate the real occurrence of *N. caninum* in Brazilian herds, contributing to the sanitary surveillance of bovine encephalitis.



SESSION C

Keynote Lecture

- **Reducing the future threat from (liver) fluke: realistic prospect or quixotic fantasy?** - I. Fairweather

Oral Communications

- **New insight in the Lymnaeids intermediate host of Fasciola hepatica in Belgium** - Y. Caron
- **Intermediate hosts of Fasciola hepatica in Sweden** - A. Novobilsky
- **Prevalence and seasonal dynamics of antibodies against Fasciola hepatica in dairy herds in Germany** - C. Strube
- **The prevalence of Fasciola hepatica and IBR in pasture-based dairy herds in Ireland** - Y. Y. G. Bloemhoff
- **Genetic relationship between Fasciola spp. in Asian countries** - M. Ichikawa
- **The search for "hidden antigens" in the liver fluke, Fasciola hepatica** - H. Mc Dougall
- **Evaluation of a coproantigen reduction test to determine flukicide efficacy on Scottish sheep farms** - P. J. Skuce
- **An experimental study in sheep, to confirm triclabendazole resistance of Fasciola hepatica in Patagonia, Argentina** - F. V. Olaechea

Posters

- **A Survey to determine the population characteristics of Fasciola hepatica in cattle livers in Autumn and Winter in the United Kingdom and Ireland** - F. MacGillivray
- **Epizootiology of the emergent lancet liver fluke (Dicrocoelium dendriticum) within a multi-host community in Alberta, Canada** - D. D. Colwell
- **Potential epidemiological threat of Myocastor coypus on the transmission of fascioliasis in Uruguay** - V. Gayo
- **Fasciola hepatica: the infectivity of an Argentinean isolate in three French populations of Galba truncatula** - R. Sanabria
- **A 3 year study in Argentina of the sheep infection by Paramphistomum leydeni: climatic factors and seasonality** - R. Sanabria
- **Presence of Fasciola hepatica in animals from Tinogasta department, Catamarca, Argentina** -
- **Spatial analysis and risk of Fasciolosis-Paramphistomosis in the rolling pampas Argentina** - C. Giudici
- **Comparative systemic availability of triclabendazole after administration of different oral formulations in dairy cows** - P. Ortiz
- **Triclabendazole and metabolites accumulation in liver flukes recovered from treated sheep** - L. Alvarez
- **Genetic analysis of Fasciola isolates from cattle in Korea based on second internal transcribed spacer (ITS-2) sequence of nuclear ribosomal DNA** - S. W. Kang
- **Use of satellite remote sensing and geographic information systems to determine the potential distribution and abundance of Fasciola hepatica in northern Patagonia** - F. Raffo
- **An optimized DNA extraction and multiplex PCR for the detection of Fasciola sp. in lymnaeid snails** - Y. Caron
- **Seroprevalence of bovine trematodosis in the area of Salto (Uruguay)** - J. Sanchís
- **Paramphistomum leydeni: experimental infections in lambs and characterization of the rDNA ITS markers from flukes and snails** - J. Romero

Reducing the future threat from (liver) fluke: realistic prospect or quixotic fantasy?

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The liver fluke remains an economically significant parasite of livestock and is emerging as an important zoonotic infection of humans. The incidence of the disease has increased in the last few years, as a possible consequence of changes to the World's climate. Future predictions suggest that this trend is likely to continue. Allied to the changing pattern of disease, reports of resistance to triclabendazole have appeared in the literature, although they do not all represent genuine cases of resistance. Nevertheless, any reports of resistance are a concern, because triclabendazole is the only drug that has high activity against the migratory and damaging juvenile stages of infection. How to deal with the twin problems (of increasing incidence and drug resistance) is the overall theme of this session on "Trematodes: *Fasciola hepatica* epidemiology and control" and of this talk to introduce the session.

Greater knowledge of fluke epidemiology and population genetics will highlight those regions where surveillance is most required and indicate how quickly resistant populations of fluke may arise. Models of disease risk are becoming increasingly sophisticated and precise, with more refined data analysis programmes and Geographic Information Systems (GIS) data. Recent improvements have been made in our understanding of the action of triclabendazole and the ways in which flukes have become resistant to it. While microtubules are the most likely target for drug action, tubulin mutations do not seem to be involved in the resistance mechanism. Rather, upregulation of drug uptake and metabolism processes appear to be more important and the data relating to them will be discussed. The information may help in the design of new treatment strategies or pinpoint potential molecular markers for monitoring fluke populations. Moreover, various "-omics" technologies are beginning to make an impact on the identification of novel targets for drugs and vaccines and they will become increasingly important in these areas. One aspect of disease management that remains inadequate, however, concerns the diagnosis of resistance. This has led to erroneous reports of resistance, which is hampering effective control of fasciolosis, as farmers may (be tempted to) switch to less effective drugs. A number of diagnostic tests are under development and progress with them will be reviewed. Finally, some recommendations with regard to strategic dosing programmes will be made.

New insight in the Lymnaeids intermediate host of *Fasciola hepatica* in Belgium

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The present work was carried out in the frame of a Belgian Science Policy project (PONDSCAPE -“Towards a sustainable management of pond diversity at the landscape level”). During the summer 2008, 7103 lymnaeids snails were collected from 125 ponds distributed in 5 clusters of 25 ponds each. Each cluster was located in a different geological region of Belgium. These snails belonged to the following species or genus: 2474 *Galba truncatula* (the main intermediate host of *Fasciola hepatica* in Belgium) and 4629 *Radix* sp. Moreover, several biological (presence of trampling and dungs, snail size ...) and non biological factors (pH, depth, fences ...) were also registered from the different biotopes. DNA was extracted based on Chelex® technique. Then the snail DNAs were screened through a multiplex PCR that amplifies the lymnaeid internal transcribed spacer 2 sequence (500-600 bp) (acting as an internal control) and a 124 bp *Fasciola* sp. sequence. Lymnaeid snails were found in 93 biotopes (66%). Thirty *Galba truncatula* (1.31%) and 7 *Radix* sp. (0.16%) were found positive for *Fasciola* sp. A classification and regression tree (CART) analysis was performed in an attempt to better understand the relative importance and relationships among the different recorded factors. For example, the geographic localization is one of the best explanatory variables for the abundance of the different snail species and the presence/absence of faecal material is the best explanatory variable for the presence of specific *F. hepatica* DNA material in the different screened snails.

Intermediate hosts of *Fasciola hepatica* in Sweden

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Background: *Galba truncatula* is generally considered as the main intermediate host (IMH) of the common liver fluke *Fasciola hepatica* in Europe. Other lymnaeid snails have also been referred as incident IMH of the fluke but their role in epidemiology is often questioned. In Sweden, neither the transmission nor the seasonal dynamics of fasciolosis have been studied yet. Hence investigation of freshwater snails as IMH of *F. hepatica* and an improved understanding about the transmission patterns of the fluke under Swedish conditions is required.

Methods: Several freshwater snail species were examined for the presence of *F. hepatica* larval stages both in the field and under experimental conditions. Investigations of freshwater snails in the field began in summer 2010 and will continue for the next two years. Snails were collected from 7 different farms (6 beef cattle farms, 1 sheep farm) with a previous history of fasciolosis. *Galba truncatula*, *Stagnicola palustris*, *Omphiscola glabra* and *Radix* sp. were collected and examined microscopically. In addition, some non-lymnaeid snails, such as *Aplexa hypnorum*, *Succinea putris*, and *Bithynia tentaculata* were also examined because these species were found in the same habitats as the lymnaeid snails. Laboratory maintained populations of *O. glabra* and *S. palustris* were established experimentally and exposed to miracidia of *F. hepatica*. Snails were then dissected at 30 days post exposure and examined by microscopy. Furthermore, the infection status of the snails was determined by the use of a PCR method.

Results: So far no naturally *F. hepatica* infected snails were observed. Also following experimental exposure, all snails (n=100) of *O. glabra* remained uninfected at dissection. In case of *S. palustris*, daughter rediae and undeveloped cercariae of *F. hepatica* were observed in 40 % (n=20) of exposed snails. This was also confirmed by PCR method.

Conclusions: First results from experimental infections suggested *S. palustris* could act as IMH of *F. hepatica* in Sweden. In contrast, *O. glabra* which was previously reported as susceptible IMH in France seems to be resistant to *F. hepatica* infection.

Prevalence and seasonal dynamics of antibodies against *Fasciola hepatica* in dairy herds in Germany

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Background: The liver fluke *Fasciola hepatica* is one of the most important parasites in cattle farming causing economic losses due to reduced milk yield and growth rates besides condemned livers. In the presented study the nationwide fasciolosis situation in Germany as well as seasonal and yearly fluctuations of *F. hepatica* antibody pattern in a north-western German region were investigated.

Methods: About 23,000 bulk tank milk (BTM) samples were collected in November 2008 from all German federal states to investigate the prevalence of antibodies against *F. hepatica* with an E/S-ELISA. Furthermore, between 691-1012 BTM samples collected in January, September and November 2008 as well as 2010 in the north-western region East Frisia were analyzed for seasonal variations.

Results: The nationwide study showed that 23% of the dairy herds across Germany were positive for antibodies against *Fasciola hepatica*. Highest prevalences were found in the federal states Schleswig-Holstein (37%), Lower Saxony (29%), Mecklenburg-Western Pomerania (24%) and Bavaria (19%). The lowest prevalence was found in Thuringia with only 2 % positive BTM samples. The seasonal and yearly fluctuation of anti-liver fluke antibodies for 2008 showed an increase of positive samples from January to September from 49% to 59% and a decrease to 54% in November. In 2010, the prevalence of 44% in January increased to 50% in September with a similar percentage of 49% in November.

Summary/Conclusions: Fasciolosis is especially common in the north-western part of Germany and the southern region (Bavaria). This is because the majority of dairy cows in these regions is kept on pastures, and the geographical conditions are well suited as habitats for the snail intermediate host. Concerning seasonal fluctuations antibody titers increased from January to September due to newly acquired infections during the grazing season. The lower percentages in November can be interpreted as already decreasing titers after housing. There was a slight decrease in the prevalence data from 2008 compared to 2010. This might reflect a lack of awareness about the importance of *Fasciola* control or be the consequence of restrictions to use fasciolicides in dairy cows due to food safety regulations. A subsequent GIS-analysis will correlate the ELISA results with geographical characteristics of the farms and help to identify areas with a high risk of fasciolosis.

The prevalence of *Fasciola hepatica* and IBR in pasture-based dairy herds in Ireland

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Background: *Fasciola hepatica* has a major economic impact on livestock in the world and in addition to direct production losses, infected animals may also be more susceptible to other infections, e.g. salmonellosis. (Piedrafita, et al; 2010)

The objective of this study was to determine the farm prevalence of *F. hepatica* and Infectious Bovine Rhinotracheitis (IBR) using milk ELISA s and determine the level of co-infection

Methods: Bulk milk samples were collected from 319 dairy farms located throughout Ireland over a twelve-month period (March 2009 to March 2010). Farms were located in the following regions, Border(1), Midland(2), West(3), Dublin and Mid-East(4), Mid-West(5), South-East(6), South-West(7). Milk samples for *F. hepatica* were processed, then analysed using a commercially available ELISA kit (BoFinn Diagnostics Ltd., Ireland): herds were classified as 'None' (0-15 S/P ratio), 'Low' (15-25 S/P ratio), 'Moderate' (25-75 S/P ratio) and 'High' (>75 S/P ratio). Duplicate milk samples were tested for IBR antibodies and herds were also classified as negative (<=25 S/P ratio), low positive (25-50 S/P ratio) and high positive (>=50 S/P ratio). Statistical analysis was conducted using Excel to calculate the prevalence of *Fasciola hepatica* and IBR.

Results: Preliminary results show that *F. hepatica* was widely distributed in Ireland amongst the participating herds, with prevalence ranging from 53.0% in March to 74.2% in November, whereas IBR had a more clustered distribution. Over time between 43.5% and 60.1% of the farms had antibodies to both *F. hepatica* and IBR. Over 54% of these farms have high antibody levels against both infections. In the Border and West regions over 75% of farms were positive for both *F. hepatica* and IBR in November.

Summary/Conclusions: These results show that *F. hepatica* and IBR are substantially present on dairy herds in Ireland, with a large proportion of farms having antibodies to both infections. There were some regional differences in the prevalence of co-infections. Further analyses will be undertaken to determine the interactions between the two infections.

References: Piedrafita, D., Spithill, T.W., Smith, R.E., Raadsma, H.W., Improving animal and human health through understanding liver fluke immunology. Parasite Immunol 32, 572-581.

Genetic relationship between *Fasciola* spp. in Asian countries

A molecular study of *Fasciola* flukes collected in Thailand and Myanmar

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Background: *Fasciola hepatica* (Fh), *F. gigantica* (Fg) as well as parthenogenetic *Fasciola* sp. which include no sperm in their seminal vesicles are distributed in Asian countries. Recently, we revealed that parthenogenetic *Fasciola* sp. originated in China. However, phylogenetic information on *Fasciola* spp. in Southeast Asian countries was available only in Vietnam. Thus, this study was designed to analyze *Fasciola* flukes from Thailand and Myanmar.

Materials and Methods: One-hundred and forty-six and 87 flukes were collected in Thailand and Myanmar, respectively. Hematoxylin-Carmine staining was conducted on anterior parts of the flukes for checking presence of sperm in the seminal vesicles. Total DNA was extracted from posterior parts of the flukes, and nuclear ribosomal internal transcribed spacer 1 (ITS1) region and mitochondrial NADH dehydrogenase subunit 1 (ND1) gene were amplified. DNA types were distinguished by nucleotide sequences. Phylogenetic analyses were conducted on ND1 haplotypes with Asian *Fasciola* flukes from Japan, Korea, and Vietnam, which were available in GenBank.

Results and Discussion: In Thailand, 128 flukes were identified as *F. gigantica* since they had abundant sperm in the seminal vesicles and displayed Fg type in ITS1. The remaining 18 flukes were considered as parthenogenetic *Fasciola* sp. since they had no sperm in the seminal vesicles. Similarly, in Myanmar, 80 flukes were identified as *F. gigantica* and the remaining 7 flukes were parthenogenetic *Fasciola* sp. On the other hand, ND1 sequences of parthenogenetic *Fasciola* sp. from the both countries were identical to that of parthenogenetic *Fasciola* sp. which have been reported in Asian countries, thus, we considered the parthenogenetic flukes detected in this study also originated in China. Moreover, 29 and 19 ND1 haplotypes were distinguished in *F. gigantica* from Thailand and Myanmar, respectively. Phylogenetic analyses revealed that *Fasciola* populations of the both countries were well differentiated, suggesting that Thailand and Myanmar have unique history in ancient domestication of ruminants which serve as definitive hosts.

The search for “hidden antigens” in the liver fluke, *Fasciola hepatica*

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Background: *Fasciola hepatica* is responsible for substantial economic losses and animal welfare issues within the agricultural sector worldwide. The increasing incidence of fasciolosis coupled with the emergence of flukicide resistance makes vaccination an attractive alternative control strategy. Hidden antigens have provided protection against infection with blood-feeding parasites, most notably the gastrointestinal nematode, *Haemonchus contortus* and the cattle tick, *Rhipicephalus (Boophilus) microplus*. Here, we attempt to identify hidden antigens in *F. hepatica*.

Materials and methods: Histological sections of adult *F. hepatica* were screened with a panel of lectins to identify those with an affinity for the parasite's gut. Those lectins which showed an affinity for the gut were used to purify glycoproteins from a crude somatic extract by affinity chromatography. The resultant fractions were separated on an SDS-PAGE gel by electrophoresis and the protein profiles analysed by mass spectrometry.

In parallel, the proteolytic activity of a somatic extract of adult *F. hepatica* was investigated. The ability of the fraction to digest haemoglobin, a potential food source, was measured in the presence/absence of class-specific inhibitors

Results: Two lectins, which had shown an affinity for proteins located on the surface of the adult *F. hepatica* gut, were selected to fractionate the somatic extract by affinity chromatography. The profile of proteins enriched by this method included an aspartyl proteinase in addition to several other proteins.

The class-specific inhibition of the proteolytic digestion of haemoglobin by somatic extracts of *F. hepatica* showed that proteolysis was due to the action of both cysteine proteases, and aspartyl proteinases.

Discussion: While cysteine proteases within liver fluke extracts have previously been characterized and identified as vaccine candidates, other components of the proteolytic machinery have been described herein using a combined approach of lectin-affinity chromatography and enzymology with class specific inhibitors and substrates. In particular the description of an aspartyl proteinase by these methods may have identified a novel hidden antigen for fluke control.

Evaluation of a coproantigen reduction test to determine flukicide efficacy on Scottish sheep farms

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Background: Over the past 10-15 years, there has been a significant increase in the incidence of liver fluke infections in Scottish sheep and a marked geographic spread of disease into previously fluke-free areas. This could be as a result of recent climatic changes and/or animal movements but there is also mounting anecdotal evidence of flukicide treatment failure, especially to triclabendazole (TCBZ), the most widely used flukicide in sheep. This study was initiated to investigate TCBZ efficacy in Scottish sheep naturally infected with *Fasciola hepatica*, and to compare two methods of determining treatment success, namely the standard faecal egg count reduction test (FECRT) and a new test, the coproantigen reduction test (CRT), based on sensitive detection of fluke antigen in faeces.

Methods: Two sheep farms were selected on the basis of previous fluke history and concerns over TCBZ efficacy. Ewes were pre-screened by fluke egg count (FEC) and divided into groups of ~25 on each farm, then treated with either TCBZ (Fasinex®) or closantel (Flukiver®) at the manufacturers' recommended dose rate and based on accurate weight determinations. Individual faecal samples were taken at day 0, 7, 14, 21 and 56 post-treatment. FECs were performed using a standardized egg sedimentation protocol and fluke coproantigen determined using the commercial BIO K201 fluke antigen ELISA (BioX Diagnostics, Belgium).

Results: Preliminary results indicate a good correlation between faecal egg counts and coproantigen ELISA readout. The CRT appears to be a better and earlier indicator of treatment outcome than the FECRT, as a proportion (~10%) of animals were still FEC +ve but coproantigen -ve 21 days post-treatment. This study clearly indicates the presence of TCBZ resistant fluke on both farms, with TCBZ efficacies of 15.2% and 57.8%, respectively, 14 days post-treatment. In contrast, closantel remained efficacious on both farms, with efficacies of 96.0% and 97.9%, respectively.

Summary/Conclusions: Results would support the use of FECRT and CRT in diagnosing flukicide resistance and/or treatment success in the field, with the CRT being the preferred option based on ease of use, sample storage and potential for high throughput.

An experimental study in sheep, to confirm triclabendazole resistance of *Fasciola hepatica* in Patagonia, Argentina

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The aim of the present experimental study in sheep was to compare the efficacy of TCBZ against the resistant liver fluke strain described in the study of Olaechea et al. (2011) against a susceptible field strain. A group of 10 lambs were infected each with 200 metacercariae of the resistant (R) strain, and were compared to 10 naturally infected sheep, known as susceptible (S) *F. hepatica* strain. Eleven weeks after infection, five lambs of the resistant group (RT) and five sheep of the susceptible group (ST), were treated with triclabendazole (10 mg/kg). The remainder lambs and sheep were used as untreated controls (RC and SC). The parameters studied were: GLDH, GGT, ELISA measuring antibodies against recombinant cathepsin-L1 and eggs per gram of faeces (epg). Lambs were slaughtered 16 weeks after infection and sheep 4 weeks after treatment and the number of flukes was counted. The GLDH, GGT serum levels and the OD value of the ELISA indicated that the infection was well established in all lambs and had not decreased as a result of the treatment in group RT. Patent infections were observed in all animals of groups RT, RC and SC. In group ST, no eggs were found after treatment and no flukes at necropsy, showing 100% efficacy of TCBZ. The percentages of flukes collected in artificially infected lambs were 15% in RC and 14% in RT. These results corresponded to efficacies of 9% in the resistant strain. Histological sections of flukes recovered from treated lambs (RT), showed normal morphology, with no damage attributable to the TCBZ treatment. Since the resistant strain was isolated from a cattle farm, it confirms the existence of triclabendazole resistance in Patagonia, Argentina.

A Survey to determine the population characteristics of *Fasciola hepatica* in cattle livers in Autumn and Winter in the United Kingdom and Ireland

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Background: In the UK and Ireland, animals that are housed during winter months are typically brought inside between October and December. The aims of this study were to identify the intensity of fluke infection in cattle in the autumn/winter and determine the fluke stages present by post mortem examination of cattle livers. The information provided could be useful in determining the most appropriate time to treat with a flukicide when cattle are housed.

Methods: Between August and December 2010, 126 cattle livers which had been condemned at slaughter were examined for the presence of fluke. They were sourced from local abattoirs accepting cattle from North East England (37), Ireland (49) and South Wales (40). The age of each animal was unknown but ranged from 18 months and above. The total number of fluke recovered from each liver was recorded and their size measured. Based on a combination of size and sexual dimorphism, fluke were either classed as 'adult', 'early immature' or 'late immature'.

Results: Over 99% of livers examined in Ireland (48) and South Wales (37), and 62% of livers examined in the North East of England (23), carried a fluke burden. Of those livers which did not contain fluke, many had evidence of previous fluke infection, with varying degrees of peri-biliary fibrosis.

There was a predominance of adult stages in all livers examined over the study period, with an average of 96% in Ireland, 98% in South Wales and 98% in North East England.

Summary/Conclusions: The results from this study indicate that the majority of liver fluke were present as adults during the autumn and winter in 2010. If this is indicative of a typical pattern of infection, then treatment of cattle with a flukicide at housing would be more straightforward in relation to the choice of products and the timing of treatment.

Epizootiology of the emergent lancet liver fluke (*Dicrocoelium dendriticum*) within a multi-host community in Alberta, Canada

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Background: The emergence of parasites and pathogens is a globally-recognized concern. A key shortcoming of models that attempt to forecast the spread and impact of emerging parasites, especially those that are host generalists, is that little data exists on the relative contribution of sympatric host species to the transmission of infective stages. The problem is acute in grazing systems where several species of potential definitive hosts share pasture. The lancet liver fluke, *Dicrocoelium dendriticum*, was identified as an emerging parasite in wapiti, white-tailed deer, mule deer, and beef cattle in Cypress Hills Provincial Park (CHP), Alberta around 1990. This study evaluated the circulation of the *D. dendriticum* among potential definitive hosts within the CHP. Most individuals of the four studied host species have been identified as infected through necropsy, with worm intensities in individuals varying by four orders of magnitude.

Methods: To estimate the relative contribution of each species of definitive host to overall transmission of eggs onto pasture, we evaluated worm burdens in the livers of hunter-shot definitive wild hosts and cattle, evaluated parasite egg output, and compared the two with host population size. Daily parasite egg output was evaluated in two ways. First, faecal samples from definitive hosts were analyzed for parasite eggs per gram and combined with estimated total daily faecal output and mean adult worm count. These data allowed a crude estimation of per capita worm fecundity in each individual. Second, live adult worms were extracted from yearling bovine livers and 50 individuals were randomly selected from two hosts. Live worms were incubated in RPMI medium for 24 hours. Eggs released by each adult were then counted. The mean egg output was calculated and combined with adult worm counts. When combined with estimates of host population size, we calculated and compared the relative contribution of each host species to the transmission of eggs according to the two methods of evaluating daily faecal egg output.

Results/Conclusion: Our preliminary results indicate beef cattle yearlings contribute approximately 70% of the eggs to pasture, while a small number of very heavily-infected deer (adult worm counts of 4000+) are the highest individual contributors to the transmission of *D. dendriticum* eggs onto pasture.

Potential epidemiological threat of *Myocastor coypus* on the transmission of fascioliasis in Uruguay

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Introduction: Liver fluke disease caused by *Fasciola hepatica* is distributed worldwide and is known to cause severe economic losses. This trematode is capable of infecting not only ruminants, but a number of mammalian herbivores. *Myocastor coypus* is a semi aquatic rodent original from South America. It has already been diagnosed as infected with *F. hepatica* in Uruguay. An attempt in understanding the importance of such rodent on the epidemiology of the disease was performed by comparing between artificial infection of sheep with metacercariae from *M. coypus* origin and from sheep origin.

Methods: *F. hepatica* eggs from the gallbladder of infected *M. coypus* and sheep were counted and incubated at 23°C for 14 days, after which they received light stimulation for hatching. Two to three miracidium were used to infect each of 60 *Lymnaea viator* snails; 30 with miracidium of *M. coypus* origin and 30 of sheep origin. Nine sheep were divided in 3 groups: six sheep were infected with 200 metacercariae each (Group A: *M. coypus* origin, Group B: sheep origin) and 3 were kept uninfected. 80 days after infection every sheep was slaughtered and every liver examined.

Results: The amount of eggs found in the *M. coypus* gallbladder was 1.400.500, with 23 adult *F. hepatica* in the liver. The sheep used for the experiment was found infected with 34 adult parasites, and 140.500 eggs were recovered from the gallbladder. The hatching rate of *M. coypus* origin eggs was of 18%, whereas the sheep ones had a 36% hatching rate. 35 days after infection all snails started shedding cercariae. When slaughtered, Group A sheep had 110, 62 and 46 adult *F. hepatica* each, Group B sheep had 94, 46 and 30 parasites each and none were found in the 3 uninfected sheep.

Conclusions: *M. coypus* is not only capable of completing the life cycle of *F. hepatica*, but is a wild reservoir that must be considered in the epidemiology of this parasite. Not only because of the amount of eggs found, the parasites recovered after slaughter, but because of its habits and colonising capacity (it has been nominated as one of the world's worst invaders by ISSG (Invasive Species Specialist Group). Special attention should be paid to it when designing control programmes.

Fasciola hepatica*: the infectivity of an Argentinean isolate in three French populations of *Galba truncatula

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The allopatric infections of snails with *Fasciola hepatica* are more efficient for parasitological production, as seen for Spanish and Moroccan strains of this parasite. The same species from South America, where a possible divergent evolution has occurred for several centuries, may give some insight on the capacity of *F. hepatica* to invade new areas. Experimental infections of preadult *Galba truncatula* with an Argentinean isolate of cattle-derived miracidia (2/snail) were carried out to compare their infectivity in three French populations of snails differing by their susceptibility to *F. hepatica* (two highly susceptible and one poorly susceptible to French miracidia). Controls were constituted by snails belonging to the same populations and infected with French miracidia according to the same protocol. Compared to infected controls, the prevalence of infection in snails infected with the Argentinean isolate was significantly lower (16.4% to 34.5% instead of 58.6% to 72.1%). In contrast, the length of the patent period was significantly longer in lymnaeids infected with Argentinean miracidia (76.2 and 85.9 days in highly susceptible populations instead of 18.2 and 20.7 days, and 69.9 days in the poorly susceptible population instead of 6.4 days). As well, the mean number of shed cercariae was higher in the Argentinean groups (651.1 and 897.3 per snail in highly susceptible populations instead of 166.8, and 222.1, and 236.5 per snail in the third population instead of 70.7). The Argentinean isolate of *F. hepatica* was more productive in terms of cercariae than French miracidia for the three populations of *G. truncatula*. Owing to longer patent periods, the Argentinean isolate seemed less aggressive for these snails. As redial burdens were comparable in snail groups infected with Argentinean or French miracidia (27-36 rediae in snail cadavers), the higher number of shed cercariae noted in the Argentinean groups might be explained by a continuous production of morulae in rediae throughout their life (instead of a degeneration of intraredial morulae which generally occurred when snails infected with French miracidia contained free cercariae within their body).

A 3 year study in Argentina of the sheep infection by *Paramphistomum leydeni*: climatic factors and seasonality

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We intended to relate climatic factors as mean monthly temperature, rainfall and soil water excess (SWE), to *Paramphistomum leydeni* infection, estimated by eggs per gram (EPG). Altogether 722 sheep faecal samples were collected from 2005 to 2007 in a farm located in the south of Entre Rios province (32°52'30"S; 59°26'06"O). Since sampling was not performed monthly we could not use time series statistics and other analyses were performed. A k-means analysis was done on climatic data to identify "meteorological" groups. Three of them were identified: a low temperature reduced amount of rainfall and SWE (respectively 13 °C; 25 mm, 0.73; n=11 average samples); a high temperature, medium rainfall and SWE (20.5°C; 96 mm; 8.91; n=15) and lastly a high temperature associated with high level of rainfall and SWE (19.7 °C; 179 mm; 61.4; n=8). Prevalences of infection were not significantly different among the 3 groups (66 to 76%), whereas there was an increase of the arithmetic or geometric averages from group 1 to 3. Thus arithmetic average increased from 4.1 to 7.1 and 11.6 respectively from group 1 to 3. However, EPGs from group 1 were significantly different from group 3 (Kruskal and Wallis test, p=0.05). We related individual values to the monthly EPG using a power model ($\ln(\text{Inf}) = -1.4 + 0.43 \ln(\text{Rainfall})$; p=0.000); despite the high significance, the explanatory power of the regression was modest ($R^2=0.05$). This regression interestingly did indicate that rainfall was a limiting factor up to less than 90 mm and further the EPG increase in relation to rainfall was small. To conclude, we demonstrate that, periods when rainfall is much below than 100 mm/month with associated temperature of 13°C are not favourable to infection.

Presence of *Fasciola hepatica* in animals from Tinogasta department, Catamarca, Argentina

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Background: Fasciolosis is a food-transmitted zoonosis caused by *Fasciola hepatica* (locally named "unca" or "uncaca"), a parasite that causes hepatobiliary pathology. As infected cases can be asymptomatic, they are usually subdiagnosed. In order to know about the epidemiology of the disease in the area and due to human positive serological data, we work on its presence of domestic and wild animals, and snails that behave as intermediate hosts.

F. hepatica is a common parasite in cattle and sheep, and is also found in goats, south american camelids and horses. Microscopic detection of eggs in feces is a useful, cheap and easy to perform diagnostic technique.

The aim of this work was to get information on the prevalence of the disease in different host species in the north area of Tinogasta department, Catamarca province, Argentina.

Methods: We studied animals born and grown in the area, living near the houses during 2010. Stool samples (n=349) were collected individually, preserved in 10% formalin saline solution, and concentrated by fast sedimentation based on modified Lumbrera technique. The direct diagnosis was confirmed by the identification of oval, yellowish, operculated eggs of about 150 x 60 µm. Simultaneously we collected snails (n=1294) in Abaucan river and its affluents for species identification.

Results: Stools samples were considered positive by the finding of at least one egg. Positive samples were 78/349 (22,35%) corresponding to 18/46 for cattle (39.13%), 4/18 for swine (22.22%), 18/70 for sheep (25.71%), 14/64 for goats (21.87%), 3/19 for llamas (15.78%), 9/68 for horses 17.64%) and 12/64 for donkeys (18.75%). No statistically significant differences were found between species. Snails were identified as *Lymnaea viatrix*.

Conclusions: Further studies should be carried out in order to correlate this findings to human cases. There are not confiscation registers for the local slaughter. The infection percentage that was found in animals shows that this is an endemic area of fasciolosis requiring important control measures. Snail identification demonstrate the presence of intermediate hosts.

Spatial analysis and risk of Fasciolosis-Paramphistomosis in the rolling pampas Argentina

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Fasciola hepática was considered in Argentina the enzootic bovine parasite that live in environments with soils lows, waterlogged and kindness for the development of *Limnea viatrix*, such as cattle breeding of Corrientes, north of Santa Fe, Patagonia, and Buenos Aires. Currently, the advance of agriculture into areas "high" of traditional farming, resulted in a shift of production of meat to paleoglens suitable for agriculture, as found in the region of the rolling pampas of southern and northwestern Santa Fe Buenos Aires. The situation that prompted a search in the mentioned region of parasite life cycle that are associated with low settings, so from January 2008 to December 2010 in order to predict risk situations in the health of animals, 806 faecal samples were collected from 18 cattle farms located in glens sweet basin streams of Santa Fe southern and northern of Buenos Aires, which were analyzed quantitatively by means of sedimentation filtration technique in 10 grams of fecal matter, with to determine the presence of eggs of trematodes. To measure the epidemiological risk, spatial cluster analysis as discrete distribution of Poison was performed with SaTScan, considering each field as an epidemiological unit. Coprological analysis were positive for *Fasciola hepatica* and *Paramphistomum Leidy*: The first parasite was found with a range of 1 to 30 eggs per gram of faeces, in 219 samples distributed in 12 fields, with relative risk levels (observed cases / expected cases) in the field n ° 9 of 4.25 ($p < 10^{-16}$). *P. leidy* was found with a range of between 1 and 118 eggs per gram of feces in 9 fields with a relative risk of 5.77 ($p < 10^{-16}$) in the second cluster with radius of 13.1 km in the fields No. 5, 12, 13, 14, 15 and 18. For the observed risk levels, especially for *F. hepática*, which is a pathogenic parasite which severely compromises the physiological status of infected animals, we conclude that it is necessary to increase antiparasitic strategies aimed at reducing this problem that involves human and animal health for pastoral systems located in areas of creeks in the region studied.

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Comparative systemic availability of triclabendazole after administration of different oral formulations in dairy cows

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A high level of infection with the liver fluke *Fasciola hepatica* in cows (78% prevalence) has been described in the Cajamarca valley, Peru. Its control is heavily dependent on the use of the flukicidal compound triclabendazole (TCBZ). Several oral TCBZ formulations for use in cattle are available in the Peruvian pharmaceutical market. However, there is a lack of information on quality control and on how this could affect drug absorption among “generic” formulations. Relative bioavailability studies allow comparison of the systemic exposure (drug concentration profiles) of a test drug product to that of a reference (pioneer) drug preparation. The experimental goal was to assess the comparative drug systemic exposure of a reference (RF) and four test (T1, T2, T3 and T4) TCBZ formulations in dairy cows. Thirty (30) Holstein dairy cows were randomly distributed in five groups (n=6) as follows: RF group, treated with the reference formulation (Fasinex[®]) and other groups treated with either T1, T2, T3 or T4, each representing a different commercially available TCBZ formulation. All treatments were orally performed at 12 mg/kg. Blood samples were obtained before and up to 168 h post-treatment. The plasma concentrations of TCBZ metabolites were quantified by HPLC. TCBZ-sulphoxide (TCBZSO) and TCBZ-sulphone (TCBZSO₂) were the only analytes recovered in plasma. There was no significant differences ($P>0.05$) in the peak plasma concentration (C_{max}) of TCBZSO between the RF and the different test formulations (ranging between 8.3-14.3 µg/mL). However, the TCBZSO area under the concentration vs time curve (AUC) after the T2 treatment (268.9 µg.h/mL) resulted significantly lower compared to that obtained for the RF group (418.1 µg.h/mL). The AUC value for this metabolite was higher ($P<0.05$) for T3 (619.9 µg.h/mL) and T4 (683.4 µg.h/mL) formulations, compared to the RF group. Based on the currently available bioequivalence criteria, the only test formulation under evaluation that could be considered equivalent to the RF was the T1, which demonstrated an equivalent systemic exposure for the active TCBZSO metabolite. The current comparison of TCBZ pioneer and test formulations in cows seems to greatly contribute to raise awareness on the need of further quality control for drug approval in the veterinary pharmaceutical field.

Triclabendazole and metabolites accumulation in liver flukes recovered from treated sheep

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The flukicidal compound triclabendazole (TCBZ) has a complex metabolic pattern that includes the systemic presence of sulfoxide (TCBZSO) and sulphone (TCBZSO₂) derivatives, as well as the hydroxi-TCBZ metabolites usually recovered from the bile of treated animals. The aim of current work was to evaluate the pattern of *in vivo* TCBZ/metabolites accumulation into adult *Fasciola hepatica* specimens recovered from infected sheep. Twelve (12) healthy Corriedale sheep were orally infected with one hundred (100) metacercariae of a TCBZ-susceptible *F.hepatica* isolate. Sixteen (16) weeks after infection, animals were intraruminally treated with TCBZ (10 mg/kg). At 3, 24, 48 and 60 h post-treatment, animals were sacrificed (n= 3) and samples of blood, bile, liver tissue and adult *F.hepatica* specimens were collected. TCBZ/metabolites concentrations were measured by HPLC. TCBZ-sulfoxide (TCBZSO) and sulphone (TCBZSO₂) were the only molecules recovered in the bloodstream with peak plasma concentration of 10.8 (TCBZSO) and 12.6 (TCBZSO₂) µg/mL. The same metabolites were also the main analytes accumulated within the adult flukes, reaching peak concentrations between 6.35 (TCBZSO) and 13.9 (TCBZSO₂) µg/g at 24 h post-treatment, which was coincident with the time where the maximum plasma concentration was attained. Low TCBZ concentrations (0.14 µg/g at 24 h) were measured within *F.hepatica*. TCBZ parent drug and its sulpho- and hydroxi-derivatives were recovered in bile collected from treated sheep between 3 and 60 h post-treatment. Although relatively high concentrations of hydroxi-TCBZ (ranging from 0.86 to 10.6 µg/mL) were measured in bile, this metabolite was not recovered within the flukes at any time post-treatment. Finally, TCBZ parent drug was the main compound accumulated in liver tissue over the 60 h post-treatment period. The drug concentration patterns observed within the adult liver fluke after TCBZ treatment followed a similar trend to those found in plasma. Overall, the data reported here confirm that the oral ingestion is a main route of drug entry into the trematode *in vivo* exposed to TCBZ/metabolites. However, the presence of TCBZ within the adult fluke (absent in the systemic circulation) may be related to some degree of transtegumental diffusion from bile or by a direct oral ingestion from portal blood.

Genetic analysis of *Fasciola* isolates from cattle in Korea based on second internal transcribed spacer (ITS-2) sequence of nuclear ribosomal DNA

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Nuclear ribosomal DNA sequence of the second internal transcribed spacer (ITS-2) has been used efficiently to identify the liver fluke species collected from different hosts and various geographic regions. ITS-2 sequences of 19 *Fasciola* samples collected from Korean native cattle were determined and compared. Sequence comparison including ITS-2 sequences of isolates from this study and reference sequences from *Fasciola hepatica* and *Fasciola gigantica* and intermediate *Fasciola* in Genbank revealed seven identical variable sites of investigated isolates. Among 19 samples, 12 individuals had ITS-2 sequences completely identical to that of pure *F. hepatica*, five possessed the sequences identical to *F. gigantica*-type, whereas two shared the sequence of both *F. hepatica* and *F. gigantica*. No variation in length and nucleotide composition of ITS-2 sequence was observed within isolates belonged to *F. hepatica* or *F. gigantica*. At the position of 218, five *Fasciola* contained single-base substitution (C>T) formed a distinct branch inside the *F.gigantica*-type group which was similar to those of Asian-origin isolates. Phylogenetic tree of the *Fasciola* spp. based on complete ITS-2 sequences from this study and other representative isolates in different locations clearly showed that pure *F. hepatica*, *F. gigantica*-type and intermediate *Fasciola* were observed. The result also provided additional genetic evidence for the existence of three forms of *Fasciola* isolated from native cattle in Korea by genetic approach using ITS-2 sequence.

Use of satellite remote sensing and geographic information systems to determine the potential distribution and abundance of *Fasciola hepatica* in northern Patagonia

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The present study describes an analytic tool to identify different risk areas for sheep and cattle fasciolosis in northern Patagonia, based on Normalized Difference Vegetation Index (NDVI) obtained through remote sensing.

Composite 16 day NDVI images of 250 meters resolution from the MODIS series were used to create a ten year (2000 – 2009) range mean NDVI value, which was scaled from 0 to 100. The study area, located at the east of the city of Bariloche, province of Río Negro, has a total surface area of 2,700,000 ha, of which 2% are wetlands areas in which intermediate hosts of *Fasciola hepatica* develop.

Sheep density in this region varies from 0.2 to 0.33 livestock units (sheep per ha).

Fasciolosis prevalence data was obtained by coprological techniques and recorded in a database located at the Parasitological Laboratory of the EEA INTA Bariloche, during the same study period. The estimated prevalence corresponds to 25 farms (0% to 100% prevalence), which range between 500 to 78.000 ha, and cover a total surface area of 290.900 ha (ca. 10.7% of study area).

For each farm, the mean NDVI values corresponding to areas of wetlands were identified and extracted. These values were reclassified into three classes: C I (NDVI <31.15) for low-risk, C II (NDVI from 31.15 to 38.7), medium risk and C III (NDVI > 38.7) high risk of fasciolosis.

The results of the correlation between the prevalence (dependent variable) and the area occupied by each class of NDVI (independent variable) are statistically significant ($P < 0.05$) and shows a strong relationship between C I and zero prevalence ($R = 0.791$), and between C II and 30% prevalence ($R = 0.711$).

The main advantage of using the MODIS NDVI series is its high temporal resolution, which ensures image availability regardless of weather. On the other hand, the disadvantage we note is that for the application at farm level, wetlands may be too small (less than one pixel NDVI MODIS resolution) which limits its inclusion in the study.

This work suggests that the proportion occupied by the different classes of NDVI can be used to predict the risk level of occurrence of fasciolosis in cattle and sheep. This tool can also prove useful to develop a Geographic Information System (GIS) allowing us to define the distribution and abundance of *F. hepatica*. Yet for this, it is still necessary to continue the studies in order to obtain a better correspondence between the variables, mainly at high prevalence infection levels.

An optimized DNA extraction and multiplex PCR for the detection of *Fasciola* sp. in lymnaeid snails

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This study deals with the development and validation of an original PCR protocol to assess the presence of *Fasciola hepatica* in *Galba truncatula* its main intermediate host in Western Europe. In the present study two DNA extraction techniques are compared and a new multiplex PCR is described. The Chelex® DNA extraction technique showed to be more appropriate than the classical Phenol/Chloroform/Proteinase K based method because of the absence of toxic organic solvent, shorter duration and lower cost, and a higher reproducibility regarding DNA concentrations and wavelength ratios. The multiplex PCR was set up to amplify the lymnaeid internal transcribed spacer 2 sequence (500-600 bp) that act as an internal control and a 124 bp *Fasciola* sp. sequence that is repeated more than 300,000 times in fluke whole genome. Ninety six snails were pooled and 6 snails (6.25%) found positive for *Fasciola* sp. The limit of detection is lower than the minimal biological infestation unit (one miracidium). DNA extracts from *Paramphistomum daubneyi*, *Dicrocoelium lanceolatum*, and *Fascioloides magna* didn't cross react.

Seroprevalence of bovine trematodosis in the area of Salto (Uruguay)

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Background: Wet areas with vegetation and mild temperatures offer a suitable environment for the survival of lymnaeid amphibious snails, the intermediate hosts for two parasitic trematoda affecting mainly ruminants, *Fasciolidae* and *Paramphistomidae*, which allows the occurrence of mixed infections also.

Methods: Blood samples were individually collected from 402 cattle in Salto (Uruguay). The IgG humoral immune response was checked against excretory/secretory antigens from adult *Fasciola hepatica* or *Calicophoron daubneyi* specimens. The seroprevalence of fasciolosis was tested in microtitre plates coated with 5 µg/ml antigen, sera were added at 1/20 and horseradish peroxidase-conjugated anti-bovine IgG was used at a 1/1000 dilution. An antigen concentration of 2 µg/ml protein, sera at 1/40 and horseradish peroxidase-conjugated anti-bovine IgG at 1/1500 were utilized for the detection of paramphistomosis (*C. daubneyi*).

Data were analyzed regarding some intrinsic (breed, age, gender) and extrinsic (aptitude) factors.

Results: Sixty-seven percent of the cattle was positive to the FhES-ELISA, 27% to the CdES-ELISA. The highest seroprevalence of fasciolosis was obtained in the Angus (88%), whereas Friesians achieved the highest values of paramphistomosis (46%). By taking into account the gender, cows showed the highest percentages of seropositivity. Cattle younger than 1.5 yr exhibited elevated percentages of sensitization against the two trematoda (86% fasciolosis and 43% paramphistomosis). Bovines with a double aptitude meat-milk reached the highest percentages of seropositivity to *F. hepatica*, and dairy animals reacted to *C. daubneyi*.

Conclusions: Cattle in the region of Salto (Uruguay) have a very high risk for exposure to the liver trematode *F. hepatica*, especially animals younger than 2 years. The management conditions of bovine livestock in the area of study enhances the risk of infection by gastric trematoda also. There is an urgent need for the application of procedures on the environment to reduce the habitats where the intermediate hosts can survive.

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***Paramphistomum leydeni*: experimental infections in lambs and characterization of the rDNA ITS markers from flukes and snails**

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Two intermediate hosts (IH) of *Paramphistomum leydeni* have been found in Argentina:

Drepanotrema depressissimum (Dd) and *Lymnaea viatrix* (Lv). Two lambs were experimentally infected with 50 metacercariae each, obtained from naturally infected Dd, and another four lambs were inoculated with metacercariae (250 each) recovered from experimentally infected Lv. Only those lambs that received metacercariae from Dd resulted infected and adult flukes were recovered. DNA was extracted from these flukes, from rediae and cercariae obtained from Lv and from *P. leydeni* eggs collected from adult flukes in order to compare with *P. leydeni* adult flukes from sheep naturally infected. For this purpose a PCR-RFLP assay was performed using the ITS2+ marker and endonucleases *HhaI* and *HinfI*.

Simultaneously, the same procedure using the ITS1+ segment as target restricted by *DdeI* enzyme was applied to characterize the infected snails: two Lv strains (U and SP) and one of Dd.

All the paramphistome samples given ~500 bp bands, and all restriction products resulted in 220 and 280 bp fragments for *HhaI*, and 130, 170 y 200 bp fragments for *HinfI*.

Regarding snails, the PCR for ITS1+ segments results in fragments of ~600 bp for Lv strain SP, and ~650 for Lv strain U and Dd. Restriction products were also different, being 120, 130 and 400 bp for LvU; 80, 130 and 390 for LvSP; and 280 and 370 for Dd.

Differences between paramphistomids using ITS2+ marker were not detected, but, differential infection patterns were recorded, depending on the infection source (Dd or Lv). Differences between both Lv strains may suggest that more than one *Lymnaeids* species or subspecies could be implicated in the life cycle development. Despite of this the specific influence of the IH in metacercarie infectivity remains unknown.

P. leydeni has emerged over the last 10 years, for the east-central region of Argentina, may be due to an adaptation to local snails, like Dd or Lv. However, some reports found that the IH genotype could get influence in the subsequent development of the parasite into the definitive host. Additional molecular markers and sequences analysis could be helpful to improve characterization of amphistomes and snails species and allows a better knowledge of the biology of these parasites.



SESSION D

Keynote Lecture

- **Harnessing 'omic technologies in a wormy world** - R. B. Gasser

Oral Communications

- **Progress of the Haemonchus contortus genome project and its application to study anthelmintic resistance** - J. Gilleard
- **Genomic Mapping of Resistance to Haemonchus contortus Using the High-density OvineSNP50 Beachchip** - G. Sallé
- **Gene expression in a triple-resistant isolate of Haemonchus contortus: comparison with a susceptible isolate** - A. J. Wolstenholme
- **Detection of benzimidazole resistance in the sheep nematode, Haemonchus contortus, using pyrosequencing on pools of eggs collected from sheep on farms in Ontario, Canada** - V. Barrere
- **Mptl-1 molecular monitoring in field H. contortus isolates** - R. Kaminsky
- **Whipworm (Trichuris spp.) infections in animals and man: Genetics of beta-tubulin and susceptibility to benzimidazoles** - T. V. A. Hansen
- **Teladorsagia circumcincta: Further characterisation of a P-glycoprotein gene associated with ivermectin resistance** - F. Turnbull
- **Levamisole and Pyrantel receptors from Haemonchus contortus: Functional reconstitution and resistance molecular mechanism** - C. L. Charvet

Posters

- **The use of a high -density SNP array for identifying genetic regions controlling nematodes resistance in sheep** - J. E. Miller
- **Polymorphism F200Y Frequency in Field Populations of Haemonchus contortus in São Paulo State, Brazil** - M. Beltrão Molento
- **Identification of Haemonchus contortus Using ITS-1 and ITS-2 Regions From Long Period Conserved Adult Individuals** - F. Ramos
- **Transcriptome analysis of Toxoplasma gondii using massive parallel sequencing method** - J. Yamagishi
- **Molecular characterization of an insulin receptor-encoding gene, Hc-daf-2, from the parasitic nematode Haemonchus contortus** - M. Hu
- **Molecular characterization of Ss-rio-3 - an atypical protein kinase encoding gene from the parasitic nematode Strongyloides stercoralis** - J. L. Zhao
- **Assessment of the expression of Glycoprotein P gen in a novel Fasciola hepatica isolated triclabendazole resistant-strain in Argentina** - S. Scarcella
- **Genetic Variability of Haemonchus contortus Revealed by Molecular Markers** - F. Ramos
- **Random Amplification of Polymorphic DNA, a simple tool for the detection of triclabendazole resistant strains in Fasciola hepatica** - S. Scarcella
- **Genotype characterization of strains of Haemonchus contortus susceptible or resistant to benzimidazole treatments in Argentina** - M. Guzmán
- **Characterization of the Parascaris equorum Transcriptome** - J. Krücken

Harnessing 'omic technologies in a wormy world

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Parasites cause devastating diseases, affecting literally billions of humans and animals worldwide. Unlocking the genomes of these neglected pathogens using next-generation technologies will yield critical and profound knowledge of their molecular biology on a massive scale. We have embarked on a program to sequence the transcriptomes and genomes of a number of neglected parasites of major socioeconomic importance and are developing strategic partnerships in this effort. The goals are to provide a significant advance in our fundamental understanding of parasites to support the development of new anti-parasitic drugs, vaccines and diagnostic tests, and to deliver a knowledge- and skills-base for the future molecular research into some important parasitic diseases.

Progress of the *Haemonchus contortus* genome project and its application to study anthelmintic resistance

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The relatively large genome sizes (300-500Mb) and typically high levels of genome polymorphism has made the production of high quality reference genome sequences both expensive and difficult. This has been a limiting factor for research aiming to tackle complex biological problems such as the molecular basis of anthelmintic drug resistance. The *Haemonchus contortus* genome project was initiated several years ago utilizing capillary sequencing technology. This has generated a large amount of sequence that has been made publically available and has been well utilized by the research community. However, the assembly of this sequence has been problematic, limiting our ability to undertake genome-wide annotation or to apply post-genomic technologies. However, the rapid progress in next-generation sequencing and associated technologies (which continues to increase exponentially) is now changing this situation. Deep sequencing of the *H. contortus* genome has now been undertaken on a variety of paired-end genomic libraries using Illumina and 454 sequencing technologies. This has significantly improved the genome assembly parameters. We are now applying an integrated in silico genefinding and RNAseq approach to annotate the assembled genome. In parallel to this work we have undertaken genetic analysis and a serial backcrossing approach whereby we have introgressed ivermectin resistance genes from two different resistant strains into the genetic background of the susceptible genome reference strain (MHco3(ISE)). We are currently performing Illumina sequencing of the parental and backcross strains to identify regions of the genome which contain major resistance conferring loci. We will present the latest information of the *H. contortus* genome assembly and annotation and the analysis of the backcross strains.

Genomic Mapping of Resistance to *Haemonchus contortus* Using the High-density OvineSNP50 Beadchip

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Background: Emergence of anthelmintic resistance and arising consumers concern about drug residues in food tend to make parasitic infection a curse for the sheep meat industry. Exploiting genetic variation in resistance to nematodes is a feasible alternative way of control. For instance tropical Martinik Black-belly (MBB) breed is more resistant than composite Romane (RMN) breed (Aumont et al., 2003). Exploiting this susceptibility variation in a first backcross design, Moreno et al. (2006) mapped 14 QTL for resistance against *Haemonchus contortus*. Using this latter design and additional data from newly born lambs of the same families, this paper reports the first results of a whole-genome QTL detection study using the OvineSNP50 Beadchip.

Methods: Four F1 MBB*RMN sires were backcrossed to RMN ewes to produce 1000 lambs in two subsets (BC1 and BC2) that were experimentally infected in 2 successive infections. Resistance was assessed for the whole flock by faecal egg counts (FEC) and resilience by packed-cell volume. Extreme susceptible and resistant BC1 lambs were measured at the end of the 2nd infection for worm burden, female worm length, IgG concentrations. BC2 lambs were measured for pepsinogen concentrations variation in both infections. Every lamb was genotyped with the Illumina OvineSNP50 Beadchip. A within-family analysis was performed by a likelihood estimation every 0.01 Megabase-pair and a joint linkage and association analysis was also carried out to refine QTL positions.

Results: Linkage analysis mapped 46 QTL on 19 chromosomes. The major QTL was found on OAR12 that carried two QTL with strong effect on FEC in each infection. Two additional regions strongly affecting FEC were mapped on OAR13 and on OAR7. Multiple QTL with low to medium effect were also found on OAR5 for both FEC and BC1-specific traits while OAR16 carried several 2nd infection related QTL. No QTL was found on OAR3 and two regions were mapped on OAR20. QTL effects were moderate with a mean effect of 0.3 phenotypic standard deviation.

Conclusion: A strong candidate region has been mapped on OAR12 while two other interesting regions have been found on OAR5 and OAR16.

Gene expression in a triple-resistant isolate of *Haemonchus contortus*: comparison with a susceptible isolate

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Background: Anthelmintic resistance is a major problem in small ruminants. The molecular basis of resistance to the benzimidazoles is well-established, however, no markers for levamisole and macrocyclic lactone resistance are described, though several candidate resistance genes have been proposed. We sequenced and measured the expression levels of many of these candidate genes in a highly triple-resistant isolate (3XR) of *Haemonchus contortus* isolated from sheep at the University of Georgia, comparing the results with those obtained using a fully susceptible laboratory-passaged isolate.

Methods: RNA was isolated from L3 of both the resistant and susceptible isolates. Gene expression was assessed using qPCR, with actin as the house-keeping gene. For sequence comparisons, cDNAs were amplified and sequenced directly.

Results: The 3XR isolate was fully resistant to benzimidazole, levamisole, ivermectin and moxidectin in the Drenchrite® larval development assay. We amplified and sequenced β -tubulin isotype 1 cDNA from both isolates. In 3XR, we found a 'BZ-resistance' allele frequency of ~40% at codon 167 and almost 100% at codon 200. We did not detect any 'resistant' alleles in the sensitive isolate. No changes were observed at codon 198. The resistant isolate also expressed much higher (>100 fold) levels of a transcript encoding a truncated nicotinic acetylcholine receptor subunit, *Hco-acr-8b*, than the sensitive isolate, where this transcript was virtually undetectable. The resistant isolate also expressed lower levels of *Hco-unc-29.3* than the sensitive worms. When we examined candidate resistance genes for macrocyclic lactone resistance, we observed small but significant decreases in the expression of *Hco-glc-3*, *Hco-glc-5*, *Hco-pgp-1* and *Hco-pgp-3*, and significant increases in the expression of *Hco-pgp-2* and *Hco-pgp-9* in the 3XR isolate.

Conclusions: Our results confirm the amino-acid changes associated with benzimidazole resistance in *H. contortus*, and suggest that detecting the truncated *Hco-acr-8b* transcript, or the polymorphism that causes it, may be a good marker for levamisole resistance. Changes in gene expression across many genes seem to be associated with macrocyclic lactone resistance, and it is unclear whether these are due to multiple polymorphisms, or to a single change that alters the expression of multiple genes. Further experiments are in progress to clarify this, and to develop a useful genetic test for levamisole resistance.

Detection of benzimidazole resistance in the sheep nematode, *Haemonchus contortus*, using pyrosequencing on pools of eggs collected from sheep on farms in Ontario, Canada

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Haemonchus contortus is a hematophagous gastro-intestinal nematode which occurs in sheep. The benzimidazole group of drug has been widely used to treat sheep for nematode infections. However, resistance towards benzimidazoles has been observed in various regions of the world. The gold standard test for detecting resistance is the Fecal Egg Count Reduction Test (FECRT) which is expensive and time consuming. Recently, a new method of detecting benzimidazole resistance has been described; it uses three genetic markers on the beta-tubulin isotype 1 gene. The three polymorphisms are Single Nucleotide Polymorphisms (SNPs) at positions 167, 198 and 200. Using pyrosequencing it is possible to detect the frequency of resistant alleles in pools of eggs. In 2010, a research project was established to determine the frequency of anthelmintic resistance in Ontario sheep flocks. Twenty five flocks across Ontario were enrolled in the study and their level of parasitism was monitored monthly throughout the grazing season by analysing 15 fecal samples from grazing lambs for each farm. When the average fecal egg count (FEC) reached 200 eggs per gram, ivermectin was supplied to producers for a drench check, and the reduction in average FEC 14 days after treatment was calculated. In those flocks with ivermectin drench failure, benzimidazole efficacy was assessed by FECRT. In addition, DNA was extracted from pool of eggs isolated from the feces and used for PCR. The *H. contortus* beta-tubulin gene was amplified. Using pyrosequencing, the frequencies of the resistant SNPs at positions 167, 198 and 200 were measured. A high frequency of resistant allele (mainly higher than 40-50%) at SNP 167 and 200 was observed in pools indicating benzimidazole resistant genotypes. The molecular results agreed with the FECRT results for the benzimidazole treatment. We conclude that benzimidazole resistance can be reliably detected by genotype analysis and this can be easily accomplished using pyrosequencing. Pyrosequencing can be used to evaluate the level of benzimidazole resistance on pooled egg samples from feces before treatment to ensure that appropriate treatment is selected.

Mptl-1 molecular monitoring in field *H. contortus* isolates

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Gastrointestinal nematodes (GI), *Haemonchus contortus* in particular, are a global threat to animal farming worldwide and cause substantial economical losses to the sheep and cattle industry. Even though alternative treatments have been developed, the use of anthelmintics has been central in the control programs of these parasites for decades but today their efficacies are hampered by the development of anthelmintic resistance (AR). It is essential to maintain the efficacy of old drugs and to manage any new anthelmintic like monepantel tightly to preserve animal productivity gains. There is thus an urgent need to develop tools to detect resistance genes while allele frequencies are still low. Detecting resistance before it becomes clinically apparent (< 1-2%) will permit implementation of changes in control strategies to preserve the effectiveness of that drug. The aim of the present study was to assess if current monepantel naive *H. contortus* isolates express a susceptible genotype and to monitor the natural rate of mutation occurring in *Hc-mptl-1*. We screened a panel 29 different isolates from 4 different countries and obtained a full length sequencing of the *Hc-mptl-1* gene for a total of 480 individual males. A panel of 257 single nucleotide polymorphisms (SNP) occurring in the open reading frame (ORF) were discovered with a total of 27 non-silent polymorphisms. Their possible implication in the development of monepantel resistance in the future will be discussed and analyzed. The monitoring of emergence of monepantel resistance in the futur using molecular diagnostic is thought to be extremely helpful to avoid a fast and uncontrolled development of AAD resistance and will help to prolong the life span of this new class of anthelmintics.

Whipworm (*Trichuris* spp.) infections in animals and man: Genetics of beta-tubulin and susceptibility to benzimidazoles

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Background: A persistent problem in the control of whipworm (*Trichuris* spp.) infections in man and a range of animals is the relatively low efficacy of treatment with a single application of benzimidazoles (BZ).

The presence of single nucleotide polymorphisms (SNPs) in codon 167, 198 and 200 in the beta-tubulin gene have been associated with BZ anthelmintic resistance in intestinal nematodes of veterinary importance. Recently it has been shown that SNPs at codon 200 are present in *Trichuris* populations of humans previously unexposed to BZ and it was hypothesised this species/genus therefore might be tolerant to BZ by nature.

Objectives: To confirm the presence of single nucleotide polymorphisms (SNPs) in the beta-tubulin gene in *Trichuris* spp. from a range of animals and man.

Methods and materials: DNA was extracted from a total of 180 *Trichuris* spp. adult worm specimens obtained from 7 different host species (pig: 24, deer: 21, lamb: 19, mouse: 15, dog: 19, baboon: 50, human: 29). In order to amplify the beta-tubulin fragments which covered codons 167, 198 and 200 of the gene, degenerate primers were designed. The sequences obtained were used to design species specific primers and used to amplify a 479 bp fragment of the beta-tubulin gene. The PCR products were sequenced, analysed and evaluated.

Results: We did not identify SNPs associated with BZ resistance in codon 167, 198 or 200 in any of the studied *Trichuris* spp. The phylogenetic analysis showed close evolutionary relationship between *Trichuris* spp. from pig, baboon and human; from lamb and deer; and from mouse and dog.

Future plans: To sequence the beta-tubulin gene in *Trichuris* spp. from camels and moose since *Trichuris* spp. infections in these hosts are notoriously difficult to treat.

***Teladorsagia circumcincta*: Further characterisation of a P-glycoprotein gene associated with ivermectin resistance**

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Background: Throughout the world, control of parasitic nematodes in livestock has been compromised by the emergence and spread of anthelmintic resistance. In most cases the genetic factors which underlie resistance to broad-spectrum anthelmintics are still poorly understood.

Recent work conducted independently at the Hopkirk Research Institute (New Zealand) and the Moredun Research Institute (Scotland) has implicated the involvement of a particular P-glycoprotein (Pgp) gene in multiple-anthelmintic resistance in *Teladorsagia circumcincta*. In the Hopkirk study, an analysis of allele frequencies across several different candidate genes in near-isogenic anthelmintic resistant and susceptible strains of *T. circumcincta*, revealed a strong linkage between the presence of particular alleles of the above Pgp gene and the resistant phenotype. Furthermore, resistance was found to be associated with an increase in the copy number of this gene. In the Moredun study, increased expression of the same Pgp gene was observed in a multiple resistant UK field isolate.

The focus of this study is to further characterise this Pgp gene and its possible role in ivermectin (and multi-drug) resistance in UK isolates of *T. circumcincta*.

Methods: Two populations of *T. circumcincta* were examined: MTci2 which is susceptible to anthelmintics, and MTci5 which is resistant to 3 broad spectrum anthelmintics. The coding sequence of the Pgp gene was PCR amplified from pools of larvae (L₃) from each isolate using the coding sequence of the New Zealand isolate as a reference.

Results: Full-length coverage of the Pgp coding sequence has been generated by assembling partial sequences into one contiguous sequence. The 3.822kb, full-length transcript has recently been amplified in its entirety from cDNA derived from both UK isolates. Preliminary sequence comparisons reveal significant polymorphism between the MTci2 and MTci5 sequences, although no non-synonymous SNPs have been identified to date. The UK isolates also share 97% identity to the Hopkirk isolate despite being geographically distant.

Summary: The role of this Pgp gene in ivermectin resistant *T. circumcincta* remains to be determined and whether there is any commonality of genetic mechanism underpinning resistance in different isolates. Future work will focus on the functional role, if any, of this Pgp gene in *T. circumcincta*.

Levamisole and Pyrantel receptors from *Haemonchus contortus*: Functional reconstitution and resistance molecular mechanism

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Background: The cholinergic agonists levamisole and pyrantel are widely used to treat parasitic nematode infestations. These anthelmintic drugs paralyze worms by activating the acetylcholine receptors (AChRs) expressed in nematode muscle cells. However, levamisole and pyrantel efficacies have been compromised by the emergence of drug resistant parasites, especially in the gastrointestinal nematode *Haemonchus contortus*, a major parasite pest of sheep. A detailed understanding of the composition of parasite AChRs and of the genetic and molecular changes causing anthelmintic resistance has been missing so far. Here, we report the first functional reconstitution and pharmacological characterization of *H. contortus* Levamisole and Pyrantel-sensitive AChRs in a heterologous expression system.

Methods: In the free-living nematode *Caenorhabditis elegans*, five AChR subunit and three ancillary protein genes are necessary *in vivo* and *in vitro* to synthesize a levamisole-AChR. We have cloned the *H. contortus* orthologs of these genes and expressed them in the *Xenopus* oocyte heterologous system for functional and pharmacological characterisation. We reconstituted two types of *H. contortus* L-AChRs with distinct pharmacologies by combining different receptor subunits and addressed the effect of a truncated form of *Hco-unc-63* subunit identified in a levamisole-resistant isolate.

Results: We show that the Hco-ACR-8 subunit plays a pivotal role in the selective sensitivity to levamisole and pyrantel. In addition, similar to *C. elegans* L-AChRs, the functional expression of both *H. contortus* receptors requires the ancillary proteins Hco-RIC-3, Hco-UNC-50 and Hco-UNC-74. Using this experimental system we could demonstrate that a truncated Hco-UNC-63 L-AChR subunit, which was specifically detected in a levamisole-resistant *H. contortus* isolate but not in levamisole-sensitive strains, hampers the normal function of L-AChRs when co-expressed with its full-length counterpart. **Conclusions:** We have characterized for the first time the functional levamisole and pyrantel expressed receptors of Clade V parasitic nematode. Moreover, we provide the first insight into a possible molecular mechanism involved in levamisole resistance in any parasitic nematode. This work advances the understanding of ACh receptor structure-function relationships in parasitic nematodes and opens the way not only for deciphering cholinergic anthelmintic drug resistance, but also for molecular screening for novel antiparasitic pharmaceuticals.

The use of a high –density SNP array for identifying genetic regions controlling nematodes resistance in sheep

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Gastrointestinal parasites have a profound detrimental effect on sheep production. To reduce these economic losses, it would be advantageous to identify sheep that are genetically resistant to parasites. As previously reported, a sheep flock segregating for genes controlling parasite resistance has been created at Louisiana State University. The flock includes 378 F2 offspring of five F1 sires produced from Gulf Coast Native (resistant) and Suffolk (susceptible) crosses. Fecal egg counts (FEC) for *Haemonchus contortus* and packaged cell volume (PCV) measurements were taken on all lambs at three time-points (at weaning, after 5 weeks on pasture, and 6 weeks after experimental challenge). In addition, all offspring, as well as their parents and grandparents, were genotyped with the Illumina Ovine SNP50 BeadChip. Preliminary results using PLINK software revealed significant associations ($P < 1.73 \times 10^{-9}$) on OAR1 (4 SNPs), OAR3 (7 SNPs), OAR4 (3 SNPs) and OAR6 (5 SNPs) for PCV and FEC at Measurement 1 (weaning). Significant associations ($P < 9.26 \times 10^{-6}$) were also found for single SNPs on OAR1, OAR2, OAR14 and OAR18 for PCV and FEC at Measurement 2 (pasture). No significant associations were detected for Measurement 3 (experimental challenge). These results are being further investigated using R/qtl software (<http://www.rqtl.org/>).

Polymorphism F200Y Frequency in Field Populations of *Haemonchus contortus* in São Paulo State, Brazil

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Haemonchus contortus is the most prevalent and pathogenic nematode of sheep and it is responsible for economical losses and health problems. Among several genetic polymorphisms associated to anthelmintic resistance, F200Y on isotype 1 of beta-tubulin gene is one of the most studied. F200Y is a T to A transversion that results in amino acid substitution (phenylalanine to tyrosine) in position 200 and is correlated to genetic resistance to benzimidazole. Most studies have focused on the determination of F200Y frequency in benzimidazole resistant populations of *H. contortus*. The objective of this study was to determine F200Y frequency in field populations of *H. contortus* from 33 sheep farms in São Paulo State, Brazil. Ten animals were submitted to faecal egg reduction count test (FERCT) using albendazole, closantel, ivermectin, levamisole, or moxidectin, and a control group. Third stage larvae (n=10/herd) were used for F200Y genotyping from control groups. DNA of single larvae was extracted with phenol:chloroform, then a region of beta-tubulin gene was amplified by nested-PCR, and the F200Y polymorphism was genotyped by ARMS-PCR. According to FERCT, *H. contortus* was resistant (efficacy < 80%) to albendazole, ivermectin, moxidectin, closantel, and levamisole in 93.1, 92.6, 82.1, 65.4, and 38.5% of the herds, respectively. Anthelmintic efficacy superior to 90% was only observed for levamisole (46.1% farms), closantel (19.2%), and moxidectin (7.2%). The frequency of allele A (associated to resistance) varied from 0.09 to 0.74, and the AA resistance genotype frequency varied from 0 to 66.7%. Statistical analyses did not reveal ($P > 0.10$) association between FERCT results and the F200Y resistance genotype. Also, most populations (81.8%) were in Hardy-Weinberg equilibrium suggesting that the F200Y polymorphism was not under selection. It is possible to conclude that due to the high prevalence of multidrug resistance parasites in the field, polymorphisms other than F200Y may be associated with genetic resistance in the studied populations of *H. contortus*.

Identification of *Haemonchus contortus* Using ITS-1 and ITS-2 Regions From Long Period Conserved Adult Individuals

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Haemonchus contortus is the most economically important nematode affecting sheep, causing significant economic losses. Accurate species identification of a parasite is essential for the diagnosis of infection in veterinary parasitology. It is also important for studying parasite epidemiology and the control of helminth related disease. Helminths have been identified by methods based on the morphology and morphometry of specimens. Molecular biology techniques now make it possible to develop new tools to identify all parasite stages. The majority of these methods are based on the polymerase chain reaction (PCR) from nuclear-encoded rRNA genes. It is critical to be able to recover DNA information from individuals conserved for a long time. The objective of this work was identify *H. contortus* by molecular methods using the ITS-1 and ITS-2 regions from conserved adults. After 12 months of conservation in 70% ethanol at 4 °C, DNA was extracted from *H. contortus* individuals by K proteinase in a buffered solution and the ITS-1 and ITS-2 from the nuclear-encoded rDNA genes were amplified using PCR. In the literature some interference of the ethanol in the PCR amplification using tissues obtained from *H. contortus* has been described. In the present study the ethanol did not produce any effect over the PCR amplification, probably due the process of drying the samples before the PCR procedure. Both PCR amplifications can be carried out under the same conditions, but the Tm was 1.5 °C lower than described in the literature. The PCR products were visualized in 1.5% agarose gels showing a 600 bp for ITS-1 and 300 bp for ITS-2. These results showed variability between Brazilian *H. contortus* individuals. The next step will be a development of a PCR duplex for fast identification of this nematode as well as the sequencing of the ITS regions, for comparison with existing sequences of *H. contortus* worldwide.

Transcriptome analysis of *Toxoplasma gondii* using massive parallel sequencing method

Comparison among tachyzoites, bradyzoites and sporozoites

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Background: Morphological variation of *Toxoplasma gondii* through their life cycle is one of the unique features of this parasite. Besides, this is tightly related to the survival strategy of this parasite. For example, in intermediate hosts, fast growing form called tachyzoites (Tz) spread in early infection and slow growing form called bradyzoites (Bz) develop under increasing immune pressure resulted in chronic infection. On the other hand, sporozoites (Sz) are generated by sexual proliferation in definitive hosts to keep genetic diversity. To investigate the molecular mechanism behind the stage conversion, we focus on transcriptome analysis with next generation sequencers. In particular, we utilized a method named TSS-seq which enable to elucidate more than millions of transcription start sites (TSSs) at once.

Methods: Parasites were cultured *in vitro* with human foreskin fibroblast cells. Tz were purified by filter treatment. Bz were induced *in vitro* by high pH treatment (pH 8.1) and purified with density-gradient centrifugation. Purity was validated by indirect fluorescent antibody test using SAG1 and BAG1 anti-body. Purified Sz were kindly provided by Dr. Dubey and his colleagues and subjected to excystation. Purified RNAs were subjected to the TSS-seq analysis using an illumina GA. Mapping of obtained short read sequences was executed by ELAND (illumina) allowing up to two mismatches. Following analyses were executed by in-house scripts described in Ruby.

Results: We obtained 3,299,041, 1,552,201 and 20,011,846 short read sequences for *T. gondii* from Tz, Bz and Sz specimens, respectively. Based on the transcriptome, 238, 525 and 664 genes were estimated to be transcribed stage specific manner, respectively. Besides, adjacent TSSs were clustered which resulted in identification of 5,961, 6,204 and 7,278 putative promoter regions, respectively. Among them, 1,443, 1,691 and 2,920 were activated in the stage specific manner, respectively. Distribution of short nucleotide sequences in each promoter region was examined. As a result, it was observed that CCAGTG was frequently distributed in Bz specific promoters. This finding implies that the sequence was one of the DNA cis-motif involved in the stage specific transcriptional regulation.

Molecular characterization of an insulin receptor-encoding gene, *Hc-daf-2*, from the parasitic nematode *Haemonchus contortus*

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It is postulated that insulin-like signaling plays a crucial role in the development of both the free-living nematode *Caenorhabditis elegans* and in parasitic nematodes. Although the structure and function of the insulin receptor-like gene, *daf-2*, from *C. elegans* has been well studied, nothing is known about its ortholog from any parasitic nematode. In the present study, a full-length cDNA (designated *Hc-daf-2*) inferred to encode an ortholog of *daf-2* was isolated from the parasitic nematode *Haemonchus contortus*. The full-length cDNA sequence of *Hc-daf-2* was 5462bp in length containing a 5'-UTR of 697bp, a 3'-UTR of 550bp and 4215 bp of protein-coding region, encoding a protein of 1405 amino acids. BLASTx analysis revealed that the predicted protein sequence had 48 – 63% identities/similarities to *daf-2* ortholog of *Ascaris sum*, *Brugia malayi*, *C. elegans*, *Loa loa*, *Parastrongyloides trichosuri* and *Trichinella spiralis*. Comparison of *Hc-DAF-2* with predicted amino acid sequences of *daf-2*, the insulin receptors of humans and *Drosophila* revealed that *Hc-DAF-2* has a putative signal peptide, a cysteine-rich region in the putative ligand-binding domain, a proteolysis site, a transmembrane domain and a tyrosine kinase domain. Phylogenetic trees constructed based on predicted amino acid sequences of *Hc-DAF-2* and *daf-2* orthologs from 27 other animal species including 7 nematode species for which full-length cDNA sequences are available in GenBank showed that *Hc-DAF-2* is grouped with strong bootstrap support (99%) within a nematode cluster excluding non-nematode species. Analysis by RT-PCR detected transcription of *Hc-daf-2* in all developmental stages and both sexes of *H. contortus* including eggs, L1, L2, L3, female L4, male L4, female adult and male adult. This study provides the first insight into an insulin receptor-encoding gene of parasitic nematodes and a sound basis for future functional genomic work.

Molecular characterization of *Ss-río-3* – an atypical protein kinase encoding gene from the parasitic nematode *Strongyloides stercoralis*

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Protein kinases are key enzymes that regulate a wide range of cellular processes, including cell-cycle progression, transcription, DNA replication and metabolic functions. Based on their structure, these enzymes can be classified into eukaryotic protein kinases (ePKs) and atypical protein kinases (aPKs). Among aPKs, there is a group of enzymes called RIO kinases which contain three kinases, namely RIO1, RIO2 and RIO3. These RIO kinases are considered essential for life, but almost nothing is known about them for multiple organisms. In the present study, a full-length cDNA (*Ss-río-3*) encoding a RIO3 protein kinase (*Ss-RIO-3*) was isolated from parasitic nematode *Strongyloides stercoralis*. The full length cDNA sequence was 1738 bp in size containing a 5'-UTR of 179 bp, a 3'-UTR of 38 bp and 1521 bp of protein coding region encoding a protein of 506 amino acids. BLASTx analysis revealed that the predicted protein sequence (*Ss-RIO-3*) had 39 – 69% identities/similarities to RIO3 orthologs of *Ascaris sum*, *Caenorhabditis elegans*, *C. briggsae*, *C. remanei*, *Loa loa* and *Haemonchus contortus*. Comparison of *Ss-RIO-3* with other RIO3 orthologs revealed the amino acid sequence identity of *Ss-RIO-3* was most pronounced in the ATP-binding motif, active site and metal binding loop. Phylogenetic trees constructed based on predicted amino acid sequences of *Ss-RIO-3* and RIO3 orthologs from 15 other animal species including 6 nematode species for which full-length cDNA sequences are available in GenBank showed that *Ss-RIO-3* is grouped with strong bootstrap support within a nematode cluster excluding non-nematode species. Analysis by RT-PCR detected transcription of *Ss-río-3* in different stages of *S. stercoralis* including postparasitic L1 larvae, free-living mixed male and female, post-free-living L1, infective L3 and parasitic adult female. This study provides the first insight into RIO3 protein kinase of *S. stercoralis* which represents an interesting group of parasites and a foundation for further investigations into the biochemical and functional roles of this molecule in the biological processes of parasitic nematodes.

Assessment of the expression of Glycoprotein P gen in a novel *Fasciola hepatica* isolated triclabendazole resistant-strain in Argentina

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Fasciolosis is a zoonotic disease caused by the trematode *Fasciola hepatica*. Its control is mainly based on the use of triclabendazole (TCBZ), a halogenated benzimidazole thiol derivative which shows efficacy against juvenile and adult stages. The intensive use of TCBZ has resulted in the development of resistant liver flukes. In *H. contortus* the resistance to the benzimidazoles is caused by genetic changes in β -tubulins. In *F. hepatica* TCBZ resistant were not detected these genetic changes for which the resistance obtained should be from other genetic or metabolic changes. In this case, the liver fluke could evade drug antiparasitic effects by overexpression of efflux transport pumps and/or overexpression of metabolic enzymatic systems. The P-glycoprotein (PgP) is a cell membrane transport protein and is known to participate in the expression of multiple drugs resistance (MDR) in different species. The objective of the present work was to identify the PgP gene and demonstrate its expression by RT-PCR in sensible and resistance adult flukes obtained to the efficacy test by Olaechea et al. (2011). Furthermore, the current knowledge on the role of PgP in molecular mechanisms of drug resistance in the parasitic *F. hepatica* is discussed.

To identify the PgP gene were used specific primers. Were obtained a fragment of 338 bp which presented 96% homology with *Schistosoma mansoni* in both strains. In the expression of mRNA from both strains no significant difference was observed. These results confirm the presence of PgP in the fluke and there was no difference in mRNA expression between different strains analyzed.

Genetic Variability of *Haemonchus contortus* Revealed by Molecular Markers

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Haemonchus contortus is the most important gastro-intestinal nematode affecting sheep, causing significant economic losses. This nematode is the main blood-feeder nematode localized in the abomasal tissue of domestic ruminants. The accurate species identification of this parasite is essential for the diagnosis of infection in veterinary parasitology. The use of molecular biology techniques now make it possible to develop new tools to identify all parasite stages to generate complete informations related to its identification. The majority of these methods are based on the polymerase chain reaction (PCR) of the nuclear-encoded rRNA genes. In addition, molecular DNA markers are able to reveal the genetic variability of the individuals found in the abomasal stomach compartment of affected sheeps. The objective of this work was to analyze the genetic variability of *H. contortus* by molecular markers. *H. contortus* individuals collected from the abomasal of a sheep had DNA extracted by K proteinase in buffered solution and the ITS-1 and ITS-2 of the nuclear rDNA genes and RAPD markers were obtained. In the literature is described some interference of the ethanol in the PCR amplification using tissues obtained from *H. contortus* but, in this work the ethanol did not produce any effect over the PCR reactions, probably due the drying process of the samples before the procedure. It was developed a PCR duplex using primers to the ITS-1 and ITS-2 regions of the nuclear-encoded rRNA genes. These PCR products were visualized in 1.5% agarose gels showing a 600 bp to the ITS-1 and 300 bp to the ITS-2. Besides, the five RAPD primers produced several amplification products which revealed high genetic variability among the individuals collected from the abomasal region of a sheep used as model of study. These informations suggest high genetic variability related to the Brazilian *H. contortus* individuals. The next step will be the identification of resistance genes to chemicals in the Brazilian individuals of *H. contortus*

Random Amplification of Polymorphic DNA, a simple tool for the detection of triclabendazole resistant strains in *Fasciola hepatica*

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Anthelmintic resistance in parasitic of livestock is a chronic problem in the world. Fasciolosis is a zoonotic parasitic disease caused by *Fasciola hepatica*. Its control is mainly based on the use of triclabendazole (TCBZ), a halogenated benzimidazole thiol derivative which shows excellent efficacy against both juvenile (immature) and adult stages. Benzimidazoles are effective, broad-spectrum anthelmintics that bind to β -tubulins and selectively disassembles microtubules. The intensive use of TCBZ has resulted in the development of resistant liver flukes. In *H. contortus* and other helminths the resistance to the benzimidazoles is caused by genetic changes in genes encoding β -tubulins. In the case of *F. hepatica* TCBZ resistant were not detected these genetic changes on the tubulin molecule for which the resistance obtained is from other genetic or metabolic changes. The Random Amplification of Polymorphic DNA (RAPD-PCR) technique involves the enzymatic amplification of DNA fragments, using primers of arbitrary sequence to hybridize loci randomly distributed throughout the genome. This reveals the existence of polymorphisms that are used as genetic markers and taxonomic all kinds of organisms. RAPD-PCR technique allows to comparatively evaluating the presence of genetic variations among strains of same species. The aim of the present work was to evaluate different strains of TCBZ-susceptible and TCBZ-resistant flukes of several places in Argentina and Peru using RAPD-PCR. The flukes were compared against controls pure strains TCBZ-susceptible (*Cullompton strain*) and flukes TCBZ-resistant (*Sligo strain*) both of the same geographic region. Were analyzed field strains flukes of Catamarca (northwest of Argentina), Chubut (south of Argentina) and Cajamarca (north of Peru). The primer used detected differences between Cullompton and Sligo strains and allowed us to detect probable resistant strains in the flukes field strains tested in the same geographic region. These preliminary results may be useful to better understand the mechanisms underlying the phenomenon of resistance at TCBZ in *Fasciola hepatica*. Further work on this relevant area is required.

Genotype characterization of strains of *Haemonchus contortus* susceptible or resistant to benzimidazole treatments in Argentina

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The resistance of worm populations to anthelmintics is a spread phenomenon in sheep farms all over the world. Resistance of trichostrongyles to benzimidazole derivatives (BZ) has a high prevalence being the mutation in β -tubuline genes the most frequent change observed in populations of worms in small ruminants. The aim of this study was to characterize the genotype of two *Haemonchus contortus* strains, which had been shown to be susceptible or resistant to benzimidazole treatments. The Amplification Refractory Mutation System- Polymerase Chain Reaction (ARMS-PCR) was used to detect eventual mutations in the 200 site of isotype 1 β -tubuline. For this purpose two strains of *H. contortus* resistant or susceptible to BZ treatments and named as Ayacucho and Cedive respectively, were utilized. From adult male worms recovered from the abomasums of infected lambs, the genomic DNA was obtained by use of a phenol-chloroform protocol for each individual. For ARMS-PCR technique four primers were used to amplify two different alleles in a single PCR reaction. Two primers HcF1 (forward) and HcR1 (reverse) direct the amplification of the control band (296 bp) from isotype 1 β -tubulina. The forward primer HcF1 with reverse primer HcSu direct to amplification of the region without mutation (146 bp) in susceptible to BZ strains. The reverse primer HcR1 with forward primer HcRT direct the amplification of the region containing mutation (198 bp) in resistant to BZ strains. Control band was observed either in resistant or susceptible to BZ strains. Results indicate that only bands of 198bp were observed (100%) in Ayacucho strain which suggest a complete dominance of resistant genes (homozigote) to BZ compounds. On the other hand Cedive strain shown that only 25 % of the worms were homocigote resistant whereas the other 75% of the population was heterocigote; this may account for the susceptibility to BZ derivatives treatment. The knowledge of the proportion in which genes have mutated to resistance to BZ derivatives might be of a great and useful help to implement rational control programs in the nearly future.

Characterization of the *Parascaris equorum* Transcriptome

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Background: The equine roundworm *Parascaris equorum* causes severe, sometimes even fatal disease in particular in foals. Throughout the last years, reports of treatment failure with macrocyclic lactones have increased considerably. To identify suitable new drug targets or vaccine candidates, a more comprehensive knowledge of its gene repertoire would be highly desirable. Moreover, *P. equorum* shows extensive somatic cell chromatin diminution and has been used as a model to study this intriguing developmental process.

Methods: Using RNA isolated from one male and one female worm, normalized cDNA libraries were prepared and sequenced using GS FLX Titanium chemistry on a Roche 454 sequencer. Sequence assembly was performed with CD-HIT before contaminants were removed using megaBLAST against equine and prokaryotic GenBank entries. Then, homologs were identified by BLASTx searches against *Caenorhabditis elegans*, nematodes and the complete protein database before BLAST2Go was exploited for Gene Ontology, InterProScan, enzyme code, and KEGG pathway annotation.

Results: The more than 2.2×10^6 reads (mean length=332 bp) were assembled to 33,739 contigs (after removal of contaminants) larger than 100 bp with a mean contig size of 578 bp. The largest contig obtained was 10,125 bp encoding a titin giant muscle protein. BLASTx identified homologs for 12,543 contigs (37%) in *Ascaris suum* and for 14,949 contigs (44%) in nematoda with *A. suum* being the top species hit for 32% of all contigs. While automatic annotation of contigs is not completed yet, manual inspection of the dataset already identified contigs covering more than 90% of the open reading frames of several potential drug targets such as slo-1, five different β -tubulins, and several neurotransmitter receptors but also of genes implicated in drug resistance such as P-glycoproteins.

Summary/Conclusions: *P. equorum* transcriptome data will allow to rapidly compare drug susceptible and resistant populations in terms of drug target sequences and expression levels. The data will also provide information on genetic variability (single nucleotide polymorphisms) to follow drug selection under field conditions. Moreover, comparison of *P. equorum* and *A. suum* and the filarial parasite transcriptomes will help to identify candidate genes involved in ascarid-specific developmental processes such as chromatin diminution or in filarial parasite-specific metabolic aspects as the reliance on endosymbiotic *Wolbachia*.



SESSION E

Keynote Lecture

- **Helminth parasites in pigs: challenges and current research highlights** - A. Roepstorff

Oral Communications

- **Influence of toltrazuril treatment on parasitological parameters and health performance of piglets in the field** - A. Joachim¹,
- **Immunological interactions between Trichuris suis and Oesophagostomum dentatum in pigs** - A. Andreasen
- **Confirmation of a QTL Region Associated With Susceptibility to Ascaris suum Infections in Pigs** - P. Skallerup
- **Survival and development of Ascaris suum and Trichuris suis eggs in deep-litter on an organic pig farm** - H. Mejer
- **Identifying Humane Endpoints in Chickens Artificially Infected with Coccidiosis** - M. Fisher
- **Evaluating the Effectiveness of an Insect Growth Regulator (Diflubenzuron 10%) to Control Flies (Musca Domestica) in High Posture Hens Manure Under Laboratory Conditions** - M. F. Iglesias
- **Localization of Ascaridia galli larvae in chickens 3 days post infection** - L. A. L. Olivares
- **Strongyloides spp. infections in domestic fowls in Chonburi, Thailand** - N. Taira

Posters

- **Comparison of three faecal egg counting methods for recovery of Ascaris suum eggs** - P. Nejsum
- **Intestinal parasites in European organic pigs** - A. Roepstorff
- **Swine mange in southeast Spain: a neglected and costly disease** - E. Berriatua
- **Long-term survival of Ascaris suum and Trichuris suis eggs in relation to pasture management** - H. Mejer
- **Occurrence of gastrointestinal helminths in pigeons (Zenaida auriculata), captured in Londrina City, Parana State, Brazil** - J. L. Garcia
- **New methodology to evaluate chemical compounds in poultry hens infested by mites (Acari, Analgoidea)** - E. C. Tucci
- **Faecal egg counts from free range hens in the UK** - G. Coles
- **Coccidian oocysts as type specimens: long-term storage in aqueous potassium dichromate solution preserves DNA** - R. N. Marshall
- **Control of Ornithonyssus sylviarum (Canestrini & Fanzago, 1877) (Acari: Macronyssidae) with vegetable oils** - N. M. Soares¹;
- **A new porcine model to advance understanding of scabies immunopathology** - K. Mounsey

Helminth parasites in pigs: challenges and current research highlights

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Helminths in pigs have generally received little attention from veterinary parasitologists, despite *Ascaris suum*, *Trichuris suis*, and *Oesophagostomum* sp. being common worldwide. The present paper presents challenges and current research highlights connected with these parasites.

In Danish swine herds, new indoor production systems may favour helminth transmission and growing knowledge on pasture survival and infectivity of *A.suum* and *T.suis* eggs indicates that they may constitute a serious threat to outdoor pig production. Furthermore, it is now evident that *A.suum* is zoonotic and the same may be true for *T.suis*. With these 'new' challenges and the economic impact of the infections, further research is warranted. Better understanding of host-parasite relationships and *A.suum* and *T.suis* egg ecology may also improve the understanding and control of human *A.lumbricoides* and *T.trichiura* infections.

The population dynamics of the 3 parasites are well documented and may be used to study phenomena, such as predisposition and worm aggregation. Furthermore, increased knowledge on larval establishment has provided tools for quantifying parasite transmission. Thus, an on-going study using helminth naïve tracer pigs has surprisingly demonstrated that soil infectivity with *A.suum* and *T.suis* increases during the first 2-3 years after pasture contamination.

Though all 3 species stimulate the Th2 arm of the immune system, *Oesophagostomum* seems weakly immunogenic, perhaps via specific modulation of the host immune system. *Ascaris suum* and *T.suis* potentially modulates the host immune response, up-regulating Th2 and down-regulating Th1. Resultingly, *A.suum* may compromise vaccination efficacies, whereas *T.suis*, which establish short-term in humans, is a favourite candidate for down-regulating autoimmune Th1-related diseases in man.

Some research areas have yielded potential sustainable control measures. For example, the heredity of host resistance to *A.suum* is so high that breeding for resistant genes may be a possibility. Experimental studies have demonstrated that fermentable dietary carbohydrates have an antagonistic effect on *Oesophagostomum* and to a lesser extent on *T.suis* and *A.suum*, whereas egg-destroying microfungi may be used to inactivate the hard-shelled *A.suum* and *T.suis* eggs in the environment.

Helminth control in Denmark has previously relied solely on anthelmintic treatment in herds with low helminth transmission. When indoor transmission rates increases and especially in outdoor herds, medication has to be combined with sustainable control measures, such as selected pig genomes, bioactive forages, and egg-destroying microfungi.

Influence of toltrazuril treatment on parasitological parameters and health performance of piglets in the field

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Background: Porcine coccidiosis caused by *Isospora suis* is one of the leading causes of neonatal diarrhea in suckling piglets. Currently the only registered drug for metaphylaxis is toltrazuril. The aim of the study was to evaluate the effect of toltrazuril treatment on piglet health and oocysts excretion.

Methods: Litters from 7 Austrian farms without and 8 Austrian farms with toltrazuril application were examined for oocyst excretion (including determination of oocysts per gram of feces; OPG), diarrhea (fecal score FS 1-4 with 3 and 4 being diarrhea), and general health (health score HS 1-4 with 3 and 4 describing poor health). Both groups included farms with different levels of hygiene. Samples from 265 litters without treatment, comprising 1588 individual samples, and 1548 samples from 258 treated litters were taken twice (around the 14th and the 21st day of life, respectively), examined by autofluorescence and, if positive, by McMaster counting.

Results: In both groups animals had less diarrhea and lower health scores during the second sampling but the treated piglets were always significantly healthier and had less diarrhea. The percentage of weaned piglets was higher in treated animals although this was not significant ($p = 0.052$). In the first round of sampling 17.8% of the individual samples from untreated piglets were positive for oocysts (with a maximum prevalence on the 12th to 15th day of life) while in the treated piglets only 0.4% shed oocysts. At the second sampling only 2.1% of the untreated animals and none of treated piglets excreted *I. suis*. Positive animals shed up to 8×10^3 OPG. There was an increased risk for infected piglets to develop diarrhea (odds ratio: 4.73) and poor health (odds ratio: 5.05) in untreated piglets, and poor hygiene without disinfection was identified as a risk factor for poor health, diarrhea and oocyst excretion.

Conclusions: The risk of poor health and diarrhea was also increased for piglets under poor hygienic conditions receiving toltrazuril, so both metaphylaxis of coccidiosis and good hygiene are necessary to effectively control neonatal diarrhea. The costs of treatment are considerably lower than the estimated financial production losses. Therefore, treatment is recommended for farms where clinical coccidiosis is diagnosed.

Immunological interactions between *Trichuris suis* and *Oesophagostomum dentatum* in pigs

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Background: The aim of this study was to identify possible interactions between *Trichuris suis* and *Oesophagostomum dentatum* in pigs with regard to worm population dynamics and host immune responses. *T. suis* is known to elicit a strong immune response leading to rapid expulsion, while *O. dentatum* induces relatively low levels of protective immunity.

Methods: Forty-eight helminth naïve pigs were allocated into 4 groups in a 2-factorial design. Two groups were trickle inoculated with either 10 *T. suis* eggs/kg/day (Group T) or 20 *O. dentatum* L3/kg/day (Group O) throughout the study. One group was trickle infected with same levels of both *T. suis* and *O. dentatum* (Group OT) and the remaining group served as an uninfected control (Group C). In each group, six pigs were necropsied 35 days post first inoculation (p.i.) and the remaining 6 pigs 71 days p.i. Quantitative Real-Time PCR was used to measure the expression of immune function related cytokine, antibody and receptor genes in tunica mucosa of the proximal colon (PCM) and from the ileo-caecal lymph node. The levels of *T. suis* and *O. dentatum* specific IgG1 and IgG2 serum antibodies were quantified by an in-direct ELISA using E/S-antigens.

Results: Worm burden data demonstrated a strong interaction between the two helminths: *T. suis* significantly reduced the *O. dentatum* numbers in co-infected pigs, while *O. dentatum* tended to increase the *T. suis* burdens. The gene expression data confirmed a strong Th2-dominated response to *T. suis* (e. g. marked increase in IL-13, ARG1 and CCL11) and a clearly weaker, or delayed, response to *O. dentatum* in the single infected groups. Statistical interactions were found between the two nematodes with regard to expression of several cytokine genes e.g. an increase in IL-13 observed in Group T was absent in Group OT (p=0.054, PCM, 35 p.i.). Also highly significant interactions were identified for *O. dentatum* specific IgG1 (p<0.0001) and IgG2 (p<0.0006) serum antibodies.

Summary: Some of these immune response related interactions may support, or even partially explain, the observed interactions between the two worm populations in co-infected animals.

Confirmation of a QTL Region Associated With Susceptibility to *Ascaris suum* Infections in Pigs

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Intestinal helminth infections cause health and welfare problems in both human and animal populations. Heritability analysis of a resource population of pigs infected with *Ascaris suum* (large roundworm) and *Trichuris suis* (whipworm) has shown high heritabilities for parasite burdens of both parasites. A number of putative QTL regions have been identified through a genome scan using a 7K porcine SNP-chip. To validate a QTL associated with *Ascaris* worm load on SSC4, we have genotyped two of the most promising SSC4 candidates in another population consisting of 174 pigs from 39 litters infected with *Ascaris*. The population used for the validation was produced by sows inseminated with mixed semen from three boars. The heritability ($\pm$ SE) of worm burden of non-larval *Ascaris* (>2 cm), adult *Ascaris* (>10 cm), and total number of *Ascaris* was 73.1% (± 0.17), 31.4% (± 0.15), and 36.9% (± 0.16), respectively. The two SSC4 SNPs were genotyped using the TaqMan SNP Genotyping Assay. One SNP was associated with both adult *Ascaris* burden ($P < 0.001$) and total *Ascaris* burden ($P < 0.01$). Future research will further investigate this QTL associated with *Ascaris* burden.

Survival and development of *Ascaris suum* and *Trichuris suis* eggs in deep-litter on an organic pig farm

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Background: Helminths are common in European organic pig production systems and the use of deep-litter has long been considered a serious risk factor contributing to this problem. However, until now hardly any data has existed to either support or reject this hypothesis, thus complicating formulation of farmer guidelines.

Methods: On an organic Danish pig farm, 3 different areas (latrine, resting area and the area in-between) of 2 indoor fattening pens with deep-litter (min. 2 months old) were examined for *Ascaris suum* and *Trichuris suis* eggs. The deep-litter was sampled vertically 10, 20, 30, 40, 50, and 60 cm from the surface, by pooling 4 sub-samples per level. In addition, eggs were isolated from the top, middle, and bottom of deep-litter (3-4 months old) of the same areas but in 3 other pens. Percentage embryonation was determined before and after incubation in H₂SO₄ at 25°C for 7 weeks. For comparison, control eggs isolated from fresh faeces were also incubated.

Results: Though less common in the resting areas, eggs of *A. suum* (0-481 eggs/g dry litter) and *T. suis* (0-58 eggs/g dry litter) were detected in all pen areas and vertically throughout the deep-litter, but the large majority of eggs was unembryonated and some were damaged. Hardly any eggs were partially or fully embryonated. Once isolated and given optimal conditions, eggs of both species from all 3 areas were able to develop to a larvated stage, but the ability to do so declined with the depth within the deep-litter. Compared to control eggs from faeces, *A. suum* eggs from deep-litter were less successful in embryonation while *T. suis* eggs from deep-litter had the same percentage embryonation as fresh eggs.

Conclusion: For the first time data has shown that deep-litter may not be a risk factor for *A. suum* and *T. suis* transmission within the pens as previously suspected. However, it does appear that a high number of eggs may survive and potentially embryonate if they are transferred to a more beneficial environment. The consequence being that manure should not be spread indiscriminately onto pastures, which may later be used for pigs. A solution may be to inactivate the eggs first.

Identifying Humane Endpoints in Chickens Artificially Infected with Coccidiosis

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This work follows previous studies to identify humane end points in turkeys artificially infected with coccidiosis. As in turkeys, coccidiosis remains one of the major concerns in chicken production. Clinical disease is associated with a variety of *Eimeria* species that vary in their pathogenicity, with *Eimeria tenella* infection characterised by blood in the faeces, anorexia, morbidity and mortality particularly severe. Studies of coccidiostats in artificially infected chicks are essential to determine the efficacy of novel products and relicensing of existing products. These studies are regulated in the United Kingdom (UK) by the Animals (Scientific Procedures) Act 1986, whilst guidance is provided by the World Association for the Advancement for Veterinary Parasitology (WAAVP) and the (European Medicines Authority) EMA 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 European Legislation (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 chicks. Preliminary data on clinical signs resulting from coccidiosis which preceded death was characterised and then used to identify birds that were ill. These birds were then euthanased and the diagnosis confirmed. The aim of this preliminary project was to assess the criteria previously identified in turkey poults, define any modifications necessary and then apply clear humane endpoints. The study demonstrated that with slight modification the techniques applied to turkey poults can be applied to chicks in order to identify and apply humane endpoints.

Evaluating the Effectiveness of an Insect Growth Regulator (Diflubenzuron 10%) to Control Flies (*Musca Domestica*) in High Posture Hens Manure Under Laboratory Conditions

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Background: The infestation with flies (*M. domestica*) is a severe problem in the intensive rearing of high posture hens because it is a source of discomfort and are cause of fine or closure by local authorities, and because they are vectors of diseases, too. Poultry manure is highly attractive so that flies oviposit growing until imagos. An assay was conducted to evaluate the effectiveness of an insect growth regulator, Ambiflud Benzuron (Diflubenzuron 10%), on larvae of *Musca domestica* taken from high posture hens manure. Method: It was collected high posture hens manure of a commercial warehouse without any antiparasitic treatment for a period of 21 days before its recollection. Once in the laboratory, they were placed 200 g. of manure in plastic trays in a pyramid form and 100 larvae of first stage. 4 treatments of six trays each one were performed at doses of: 25 ml/l Low dose (Db), 50ml/l Optimal dose (Do) and 100ml/l Double dose (Dd) and the control group (T). Then the count of pupae and imagos were made every seven days. For the statistical analysis the Proc Mixed procedure of SAS (2001) was used because the data are repeated measurements in the time or in the pace, and they are correlated. Results: Calculating the lines of dose-mortality-stages larvae-pupae and imagos was found 100% efficacy for both groups (Do) and (Dd) because the emergence of adults was 0% at 7 and 14 days. 73.3% of the larvae became adults at day 21 in the group (Dd) and 85.3% in the control group, 75.4% at day 28 in the group (Dd) and 92.4 % emerged in the control group. Conclusion: It concludes a high efficacy of the product at the doses mentioned for a period of 14 days.

Localization of *Ascaridia galli* larvae in chickens 3 days post infection

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The normal habitat of *Ascaridia galli* is in the small intestine of poultry. To achieve a better understanding of its life cycle, a histological study was conducted in order to localize the larvae during the early phase of the infection. Each of six Lohmann Brown chickens were infected orally with 20,000 embryonated *A. galli* eggs whereas four chickens were left as un-infected controls. At necropsy 3 days after infection the first half of jejunum/ileum was divided into two equally sized sections (J1 and J2) and examined for larvae. The junction of sections J1 and J2 was designated JX. Sections J1, J2 and JX were examined by standard histology, and the eosinophil response in the tissue was studied by special histochemical staining. A higher number of *A. galli* larvae was recovered from section J2 than J1. The majority of larvae was recovered from the most profound layers. Based on histology, 144 larvae were identified and their location noted. The highest number of larvae was observed in the JX sample as compared to J1 and J2 ($P < 0.001$), and most of them were located in the profound crypt zone of the mucosa as compared to the other zones ($P < 0.05$). The number of larvae was higher in the lumen compared to the epithelium and lamina propria ($P < 0.001$). A significantly higher number of eosinophils was found in lamina propria of the infected group when compared to the control group ($P < 0.001$). This experiment provided evidence of a true "histotrophic phase", as a few larvae were lodged in the lamina propria. However, only few larvae made a complete penetration of the epithelium indicating that perhaps penetration may be more of an accident. It was far more common that larvae were localized within the epithelium or in the lumen of the crypts. It is therefore suggested that "mucosal phase" is a more appropriate term to use for the early phase of *A. galli* infection as compared to "histotrophic phase" used in many textbooks.

***Strongyloides* spp. infections in domestic fowls in Chonburi, Thailand**

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Background: On July 2010, many domestic fowls died on 3 back yard system farms near our EvrdC, in Chonburi, Thailand. Special reference for *Strongyloides* spp. (ST) worm detection to the 8 carcasses, and the long term observation of ST larvae per gram feces (LPG) to the 3 survivors were carried out.

Methods: Farm-PT, -GS and -TN were raising the domestic fowls; those number of raised (number of carcasses autopsied) were 60 (5), 50 (2) and 60 (1), respectively. These 8 carcasses were detected of ST parasitic female in the intestine, and to detect the migrating larvae in 10g muscle of the limb, ankle and chest, respectively. Also EPG count with dilution technique and LPG count with the Polyethylene tube culture technique were performed. Three survivor fowls, Nos.700, 701 and 703 were provided from the Farms to the EvrdC, and LPG count has been examined for 8 months.

Results: Mortality of fowls on Farm-PT, -GS and -TN were 40/60 (66%), 30/50 (60%) and 8/60 (13%), respectively. The parasitic female in the intestine and the larvae in the feces were positive 7/8 carcasses, respectively. On the migrating larvae detection in the muscle, no larva was found in all carcasses. On the fecal examination, no pre-hatching egg was found, however, the larva was found of 7/8 carcasses. The maximum numbers of the parasitic female (LPG) found among 8 carcasses were 54 (40,100), 7 (7,400) and 37 (4,070), respectively. Degenerated parasitic females and the larvae were also found in the most of the carcasses. On the periodical LPG observation in 3 survivor fowls, the value was not constant, however, low number of LPG was noted contentiously for more than 8 months. In addition, the most of fowls in these farms, *Cestoda*, *Heterakis* and *Ascaridia* were found commonly.

Summary/Conclusions: ST infections in fowls were noted in the 3 farms at Chonburi, Thailand on July 2010. No pre-hatching egg in the feces and nor the migrating larvae in the muscle were found. Degenerated shape worms, such as the parasitic female in the intestine and the larvae in the feces, were found in many cases. These worms may degenerate and disappear within short time after the host died. Also, on the fecal examination, the larvae may died and disappear when provided feces is not fresh. Above all, the most of present ST data may be limited value than the real ST parasitism. This report shows the evidence that the massive infection of ST in fowls of Thailand. Further study of this *Strongyloides* spp. in fowls should be interesting to compare to worms of Family Strongyloididae.

Comparison of three faecal egg counting methods for recovery of *Ascaris suum* eggs

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In veterinary parasitology, faecal examination is used to monitor the prevalence and intensity of gastrointestinal parasites for subsequent planning of treatment and control strategies. An ideal diagnostic method should be fast and simple and at the same time give as accurate results as possible. In the present study, three faecal egg counting methods, namely simple McMaster, concentration McMaster, and FLOTAC, were compared and evaluated in terms of recovery, rapidity, and easiness to perform. In addition, influence of day of counting the sample, counting only one chamber of the slide, and order of filling and reading the sides of the slide on faecal egg count (FEC) were also investigated. A total of 25 subsamples of pig faeces spiked with 611 *Ascaris suum* eggs per gram (EPG) were analyzed for each method using the same flotation fluid (saturated NaCl with 500 g glucose per liter, specific gravity 1.27 g/ml). The mean (95% confidence interval) FEC's of the three methods were 312 (254-370), 493 (448-538) and 406 (385-426) corresponding to recoveries of 51.5%, 81.4%, and 67.0% for simple McMaster, concentrated McMaster, and FLOTAC, respectively. The recoveries of all 3 techniques were differing significantly ($P < 0.01$) from each other. The day of counting the sample and the order of counting the slide chambers did not influence the FEC. Of the three methods, the simple McMaster was easy and took least time but reading the chamber was difficult due to a lot of dirt. The concentration McMaster was also easy, quick and the chamber was clear making it easy to read. FLOTAC method was time consuming, somewhat complicated and presence of air bubbles made it difficult to read the chambers. Of the three methods, the concentration McMaster is the method of choice for FEC estimation of *A. suum* eggs. However, in cases of low FEC the FLOTAC method may be preferred due to the lower detection limit (1 EPG) where it is 20 EPG for the Concentration McMaster method.

Intestinal parasites in European organic pigs

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Background: Several national surveys have found high parasite infection levels in organic production systems, presumably due to risk factors such as traditional housing, less medication, and access to outdoor facilities. The present survey was conducted to obtain and compare parasite prevalences and infection levels from 6 European countries.

Methods: Thirteen-20 organic sow herds were visited per country in the spring and autumn 2008 in Austria, Denmark, France, Germany, Italy, and Sweden. At each visit, individual faecal samples were collected from 10 weaners, 10 large fatteners, and 10 sows. The samples were examined for parasite eggs/oocysts using the same concentration McMaster technique with a lower detection level of 20 eggs per gram. Before the survey, a ring test was performed to ensure that the implementation of the technique was comparable for the national laboratories.

Results: The dominating findings were eggs of strongylids (most probably *Oesophagostomum*, highest occurrence in sows), *Ascaris* (highest in fatteners), and *Trichuris* (highest in fatteners) and coccidian oocysts (highest in sows). The national prevalences of the most infected age groups of Austria were; 50%, 24%, 8%, and 42% for strongylids, *Ascaris*, *Trichuris*, and coccidia, respectively. The corresponding values for the other countries were 47%, 73%, 23%, and 79% in Denmark; 76%, 41%, 46%, and 96% in France; 46%, 22%, 10%, and 52% in Germany; 55%, 17%, 24%, and 90% in Italy; and 94%, 74%, 18%, and 81% in Sweden. A few herds were positive for *Metastrongylus* sp in Italy. Some of the observed differences between countries may be related to national housing systems. Most noticeable, Austrian and German outdoor facilities were mostly concrete runs, while the other countries had pastured pigs, which on some Italian farms were turned out on large forest areas.

Conclusions: Organic European pigs in general had very high infection levels with intestinal parasites and some sustainable control measures have to be implemented to limit the production losses due to parasites.

Swine mange in southeast Spain: a neglected and costly disease

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Swine mange (SM) caused by *Sarcoptes scabiei* is arguably the most important parasitic infection of intensively raised pigs worldwide. Murcia in Southeast Spain is one the main pig producing regions in the country. An abattoir study in the region in 1991 detected mites in the ears of 37% fatteners from 78% herds, suggesting infection was highly prevalent. Current prevalence and control of SM in Murcia was investigated in 49 sow herds, by analysing *S. scabiei* ELISA seroprevalence, investigating mites and papuloeritematous dermatitis in slaughtered animals and ascertaining acaricidal treatment use. Acaricidals, predominantly pouron organophosphates, were used by 98% of farmers, mostly in adult pigs only and none of the farmers had ever implemented a recognised protocol to eliminate SM from the herd. Seropositive pigs were found in 91% of the farms and seroprevalence was 26%, 9% and 4%, in sows and 75kg and 100kg feeder pigs, respectively. Seroprevalence varied significantly between herds, being <10% in 13 herds and =50% in 11 herds, and it was negatively associated to the frequency of acaricidal treatments used. The prevalence of pigs from 11 herds harbouring *S. scabiei* in their ears was 5%; in contrast, 76% of pigs had papuloeritematous dermatitis, the extent of which was significantly greater in pigs from herds with mites compared to other pigs. Moreover, in many non-infected pigs dermatitis was restricted to the perineal region and an immunohistochemical study of the skin of one of these animals failed to detect mite antigens and the presumptive diagnosis was irritant contact dermatitis. Study results suggest that SM is still widespread in southeast Spain, but prevalence appears to be lower than 20 years ago and this is encouraging. However, improved control has most probably been achieved by an increasing, repeated application of acaricidal compounds and such a strategy not only carries considerable drug toxicity and resistance risks, but it is costly and many farmers may not be able to maintain it in the economic recession presently affecting the pig industry in Murcia.

Long-term survival of *Ascaris suum* and *Trichuris suis* eggs in relation to pasture management

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Background: Organic pig production systems are commonly characterized by high helminth prevalences. This problem is partly associated with access to outdoor facilities such as pastures and more knowledge is needed on long-term pasture infectivity patterns to improve farmer advisory services and guidelines on pasture management.

Methods: Six identical paddocks were originally contaminated in the spring to autumn of 2001 by pigs infected with high levels of *Ascaris suum* and low levels of *Trichuris suis*. Since then, no further eggs have been deposited and 3 of the paddocks have been ploughed to a depth of 20 to 28 cm and re-sown once a year while 3 paddocks remained untouched apart from yearly cutting of the vegetation. In the late spring and late autumn of 2001, 2002, 2003, and 2004 as well as in the autumn of 2005, 2007, and 2010, survival of parasite eggs was measured by analysis of soil samples and by recovery of worms from short-term exposed helminth naïve tracer pigs.

Results: Following a high initial egg mortality in 2001-2002, the number of parasite eggs in the soil declined slowly over time for both species. In 2001, very few *T. suis* eggs developed to infectivity. The tracer data show that overall pasture infectivity for *T. suis* did not peak until 3-4 years after the initial contamination. Preliminary data from 2010 indicate that *T. suis* is still present on the paddocks, though at very low levels. Infective *A. suum* eggs were detected in 2001 and paddock infectivity levels peaked within 2 years after contamination, resulting in livers with very high numbers of white spots, irrespective of paddock treatment. Transmission of *T. suis* was more consistently reduced by ploughing compared to *A. suum*.

Conclusion: Development and maturation of *A. suum* eggs and especially *T. suis* eggs was overall slower than expected, indicating that pasture rotation schemes should ideally exceed 3 years. However, 9 years after initial contamination, both species were still detected and *A. suum* pasture infectivity was still too high for the paddocks to be suitable for pigs. Overall, transferring the eggs deeper into the soil by ploughing appeared to reduce parasite transmission.

Occurrence of gastrointestinal helminths in pigeons (*Zenaida auriculata*), captured in Londrina City, Parana State, Brazil
Gastrointestinal helminthes in pigeons (*Zenaida auriculata*)

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Zenaida auriculata is found throughout Brazil, both in rural as in urban centers. Studies on helminth parasites of the gastrointestinal tract of pigeons *Z. auriculata* may contribute to the knowledge of parasites biodiversity and the development of public health. There are, to date, few studies of the prevalence of gastrointestinal helminths in birds of this species in Brazil. The aim of the present study was to evaluate the occurrence of gastrointestinal helminths from pigeons (*Zenaida auriculata*) naturally infected, trap-captured in Londrina-PR. One hundred thirty five pigeons from both sexes were sampled. We carried out the euthanasia of the birds in a CO₂ chamber and the withdrawal of the gastrointestinal tract from the esophagus to the cloaca. The viscera were inspected and the gastrointestinal contents were placed in a Petri dish and observed by stereomicroscope for the presence of helminths. For identification, the helminths were placed between slide and coverslip, in acetic acid solution for clearing, and observed by an optical microscope, in objective of 5x and 10x. Sixty two (45.93%) from 135 birds were infected. *Dispharynx nasuta* was observed in 8 birds(5.93%), and *Tetrameres* sp. in ten birds (7.41%), all in proventriculus. In the small intestine, we identified *Ornithostrongylus quadri radiatus* in 26 (19.26%) birds, *Ascaridia columbae* in 27 (20%), cestodes in four (2.96%) and *Brachylaima mazzantii* in eight (5.93%). Forty-five animals (72.58%) were parasitized by only one species of parasite. Thirteen pigeons were seen to be parasitized by two species (20.97%) and four birds parasitized by three different species (6.45%). This study is expected to advance in knowledge on the epidemiology of gastrointestinal helminths of the species of *Z. auriculata*.

New methodology to evaluate chemical compounds in poultry hens infested by mites (Acari, Analgoidea)

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Chickens are frequently parasitized by several species of mites that cause dermatitis. In poultry farms this ectoparasites cause great economic losses to the farmers due to reduce in egg production. Parasitized birds are found under stress, scratching themselves most of the time, not eating or resting properly. The control of these arthropods, to minimize the losses of producers, is currently done applying chemicals. The standardization of laboratory methods, used in efficacy studies is from fundamental importance in evaluating and comparing the different products used. Thus, the present work has proposed a new evaluation methodology for laboratory "in vivo" assays to evaluate acaricides used to control mites. The evaluation method is based on three parameters: bird nuisance, degree of dermatitis and the presence of ectoparasites. The discomfort is measured counting the number of birds that scratch in one minute. The degree of dermatitis is evaluated by assigning scores from 0 to 5, where 0 = no dermatitis, 1 – low dermatitis, 2 - moderate dermatitis, 3 - median dermatitis, 4 - high-dermatitis and 5- very high dermatitis . The presence of live mites is verified by skin scrapings from the following regions: under the wing, flank and back, riding up between slide and coverslip with 0.1 N sodium hydroxide and immediately observed under an optical microscope for visualization of live mites . The proposed method proved to be practical, effective and represents a new alternative assessment in laboratory studies where necessary to assess damage caused by the ectoparasitic mite of poultry production.

Faecal egg counts from free range hens in the UK

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Background: The move to free range and organic egg production following the implementation of EU directive 99/74/EC was likely to subject chickens to increased levels of infection with nematodes and this has been shown in studies from Sweden. The present work looked at the prevalence of nematode infection in the UK and formed part of a larger study of welfare in free range hens.

Methods: Fresh faecal samples were collected either from the floor of sheds or from the outdoor range at set intervals throughout the laying cycle in 44 flocks. Egg counts were made using the FLOTAC method, sensitive to 2 e.p.g. of faecal material. The worming of flocks was under the control of the farmers. Standard procedures were used for the post mortem of birds or groups of ten birds.

Results: Ninety three per cent of flocks were infected with one or more species of nematodes. *Ascaridia galli* was present in 76% of flocks and *Heterakis gallinarum* in 56%. The prevalence of *Syngamus trachea* was 16% and was more common in faecal samples collected outside, whilst *Trichostrongylus tenuis* was present in 25% of flocks, and was more common in indoor samples. Chicken guts three weeks after treatment with flubendazole still contained mature *H. gallinarum* while six weeks after treatment they also had *A. galli* and *T. tenuis* present.

Summary/conclusion: Nematodes are common in free range commercial egg producing flocks in the UK, but the welfare implications and effects on egg production remain to be determined. As expected *A. galli* and *H. gallinarum* are prevalent. The finding of mature *H. gallinarum* worms three weeks after therapy with in diet flubendazole, emphasises the need for further investigations as it could not be determined if this was due to inadequate self medication or the development of anthelmintic resistance. Since flubendazole is the only anthelmintic licenced for use in laying hens, resistance could present a major problem for flock health. This work formed part of the Bristol Pecking Project which is funded by the Tubney Charitable Trust and separate work funded by the RSPCA.

Coccidian oocysts as type specimens: long-term storage in aqueous potassium dichromate solution preserves DNA

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Preservation of the exogenous oocyst stage of coccidian parasites (phylum Apicomplexa N. D. Levine, 1970) as type specimens of newly described species has long been problematical. Conventional fixatives have proved unsatisfactory, and compromises such as embedding oocysts in resin or photographing them are not entirely appropriate for various reasons. As an alternative, chilled potassium dichromate solution (normally used in the laboratory to prevent putrefaction of temporary preparations of live oocysts) has been tested as a long-term preservative of sporulated oocysts of *Eimeria brunetti* P. P. Levine, 1942, *E. maxima* Tyzzer, 1929, *E. mitis* Tyzzer, 1929, *E. necatrix* Johnson, 1930, *E. praecox* Johnson, 1930 and *E. tenella* (Railliet & Lucet, 1891) (family Eimeriidae Minchin, 1903: suborder Eimeriorina Léger, 1911). Oocysts from faeces of chickens, *Gallus gallus* (Linnaeus), were placed in 2.5% w/v aqueous potassium dichromate solution (PDS), and stored in the dark at $4 \pm 2^\circ\text{C}$. After 23 years in storage, oocysts of each species were administered orally to chickens and failed to initiate infections, indicating that the oocysts were dead. Nevertheless, after about 24 years, DNA was still recoverable from the oocysts, and the original species identifications made by classic parasitological methods were confirmed by polymerase chain reaction assays. Furthermore, after almost 25 years, microscopical examination revealed that the walls and internal structures remained well preserved in 83–98% of the oocysts of the six species investigated. Hence, PDS is potentially suitable for the long-term preservation of sporulated coccidian oocysts as type specimens for taxonomic purposes. The samples used in this study are now in the care of The Natural History Museum (London, UK). It is recommended that they be monitored in like manner, by suitably qualified scientists, at intervals of about 5 years to assess their state of preservation and the recoverability of DNA. Enough material is available to monitor it until it is at least 100 years old.

Control of *Ornithonyssus sylviarum* (Canestrini & Fanzago, 1877) (Acari: Macronyssidae) with vegetable oils

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Ornithonyssus sylviarum is one of the major haematophagous mite of poultry hens in Brazil. The control of this ectoparasite is done primarily through chemicals with acaricide action. Because of the importance of this mite in the Brazilian poultry industry, this paper presents a new alternative for control of ectoparasites using a natural product made from vegetable oils. Fifty white Leghorn hens naturally infested *O. sylviarum* were divided into two groups: treated and control (no treatment). The compound containing: 70% of Neem oil, 2.5% of *Melaleuca alternifolia* oil, 2.5% of *Melaleuca leucadendron*, 5.0% of Coconut oil, 10% of Sodium Lauryl Sulfate and 10.0% of vehicle. The compound was diluted in water, at a solution of 3%. To facilitate penetration of the product, detergent was added at a rate of 0.3%. The birds were treated by applying 200 mL of mixture per bird, carrying out two treatments at a 7 days gap. Evaluations were performed by counting the number of live mites before and after treatment. After 7 days of first spray, only one hen in the treated group had live mites and in all of the other hens, there was a 100% rate of mites mortality. After 14 days, there was a 100% rate of mites mortality in all the treated group, which has remained free of parasites until 21 days after the first application. Birds in the control group showed no change or decreased infestation of *O. sylviarum* in all days of observations. The results of this study indicate that the compound containing vegetable oils was effective in the control of *O. sylviarum* and may be a new alternative for the control of this ectoparasite.

A new porcine model to advance understanding of scabies immunopathology

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Background: Crusted scabies is a severe manifestation of *Sarcoptes scabiei* infection, akin to chronic mange in animals. Detailed understanding of scabies immunopathology is lacking, with research impeded by sporadic access to patients and the inability to undertake longitudinal study of infection in humans. We have responded to this by developing a tractable porcine model of human scabies. Pigs are a natural host of scabies, and show similar clinical and, immunological changes to humans, including susceptibility to hyperinfection that resembles human crusted scabies. In previous work, we established a porcine model of crusted scabies using the glucocorticoid dexamethasone. In this study, we report the temporal development of scabies immunopathology in different disease phenotypes.

Methods: We completed a 24-week trial involving four treatment groups. Pigs were scored for lesion development and parasite burden, and skin biopsies collected for parallel histologic studies. qRT-PCR was undertaken to assess transcriptional levels of key Th1, Th2, and Th17 cytokines across the trial.

Results: A range of different clinical phenotypes of scabies was observed. qRT-PCR analysis confirmed an association between disease severity and transcription levels of the Th2 cytokines IL-4 and IL-13, which were significantly increased at weeks 4, 8 and 12 post infection. Interestingly, we also saw significant and differential up-regulation of the Th17 cytokines IL-17 and IL-23 in pigs with crusted scabies. In contrast, no significant changes in IL-10 or IFN- γ transcription were observed.

Conclusions: We observed an association between crusted scabies and a dysregulated immune response involving increased Th2, IL-17 and IL-23 cytokine transcription. Development of an informative animal model represents a breakthrough in scabies research, establishing the foundation for prospective studies of the dermal immunopathology of scabies. Describing the skin pathogenesis of scabies in detail is essential for the future design of immunotherapeutics for this disease. This may lead to strategies to protect vulnerable subjects from contracting crusted scabies, and will ultimately result in improved skin health for disadvantaged communities and better management of scabies in veterinary settings.



SESSION F

Keynote Lecture

- **Basic research on helminths: platforms to support anthelmintic discovery and development** - T. Geary

Oral Communications

- **Comparative measurement of abamectin, ivermectin and moxidectin concentrations in resistant *Haemonchus contortus*** - A. Lifschitz
- **Interaction of macrocyclic lactone endectocides with *Haemonchus contortus* P-glycoproteins** - P. Godoy
- **Comparison of P-glycoproteins between *Parascaris equorum* populations to identify single-nucleotide polymorphisms correlating with macrocyclic lactone resistance** - I. J. I. Janssen
- **Triclabendazole: time-scale of action against *Fasciola hepatica* in the sheep host** - I. Fairweather
- **Investigation of benzimidazole drug metabolism in nematodes** - J. Gilleard
- **Evaluation of the effect of increasing albendazole dose rates against resistant *Haemonchus contortus*: Drug systemic availability, clinical efficacy and genetic polymorphism** - L. Alvarez
- ***Ascaris suum* SLO-1 receptor: is it the target for the anthelmintic emodepside?** - S. K. Buxton
- **Further pharmacological and molecular evidence for SLO-1-channel activity in emodepside mode of action** - G. von Samson-Himmelstjerna
- **How does monepantel kill nematodes?** - R. Kaminsky
- **How do antibiotics kill adult filariae – a novel immune-evasion strategy revealed?** - A. J. Trees
- **Multiple *Haemonchus contortus* GluCl subunits confer ivermectin sensitivity to a resistant strain of *C. elegans*** - A. J. Wolstenholme
- **A microfluidic platform for high-sensitivity, real-time anthelmintic screening on parasitic nematodes** - R. J. Martin

Posters

- **Pgp-expression in Ivermectin Selected GI Nematodes of Cattle** - A. Engström
- **Differences in Dynamics of Transcriptional Regulation of Selected Genes between Serum-Stimulated and Percutaneously Migrated Larvae of *Ancylostoma caninum*** - C. Welz
- **Identification of SLO-1 calcium- and voltage-activated potassium channels within different clades of parasitic nematodes: hints for a consistent mode of action of the anthelmintic Emodepside?** - D. Kulke
- **Characterization of non-specific resistance mechanisms against ivermectin in *Cooperia oncophora*** - J. Demeler
- **Differential exposure of *Haemonchus contortus* to albendazole according to the site of worm location in the abomasum of infected sheep** - G. Suárez
- **Emodepside potentially interacts with the γ -Aminobutyric acid (GABA) receptor and the acetylcholine receptor in *Caenorhabditis elegans*** - S. Miltsch
- **Effects of erythromycin on the maternal and fetal plasma concentrations of moxidectin after its intravenous administration in pregnant sheep** - R. Pérez
- **Inflammation and apoptosis in stomach wall of Mongolian gerbil (*Meriones unguiculatus*) infected with *Haemonchus contortus*** - Königová
- **Differences in nitrogen metabolism in *Haemonchus contortus* and *Teladorsagia circumcincta*** - S. Umair
- **New valerolactam anthelmintics for livestock production, molecular approach to the mechanism of action** - B. Munguía
- **Efficacy of nitroxynil and closantel against *Haemonchus contortus* -comparative parenteral treatments in lambs-** - G. Suárez
- **Development of alternative Albendazole pharmaceutical preparations** - S. G. Castro
- **Optimizing protease production from an isolate of the nematophagous fungus *Duddingtonia flagrans* using response surface methodology and its larvicidal activity on horse cyathostomin** - J.V. Araújo
- **Effect of the level of cellular immune response on *Haemonchus contortus* burden in sheep** - E. L. Ortolani
- **Ultrastructural reconstruction of *Taenia ovis* (Platyhelminthes: Cestoda) oncospheres from serial sections after high pressure freezing and freeze substitution** - A. Jabbar
- **Singularity in the burse venation of *Haemonchus contortus*** - C. Giudici
- **Progress on understanding the process of doramectin percutaneous absorption through ovine and bovine skin** - J. Sallovitz
- **Influence of the formulation on the pharmacokinetics of ivermectin 5% in cattle** - N. Mestorino,
- **Pharmacokinetics of a formulation containing ivermectin and levamisole in cattle** - J. O. Errecalde,
- **Combined administration of abamectin and albendazole as a controlled-release capsule in lambs: pharmaco-therapeutic evaluation** - G. Suarez
- **Assessment of the ex vivo interaction of antiparasitic drugs with efflux transporters proteins in rat intestine** - A. Lifschitz
- **Efficacy of Abamectin and Levamisole in combination against New Zealand cattle endoparasites** - J. Moffat

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Basic research on helminths: platforms to support anthelmintic discovery and development

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Background: Current efforts to discover and develop new anthelmintics for the veterinary market are spurred by reports of resistance in livestock animals in a global scale. The contraction of the animal health industry due to mergers and acquisitions limits to some extent our ability to do this work. Modern strategies for drug discovery revolve around mechanism-based approaches, which require knowledge of essential and vulnerable processes in the target organisms. Advances in genomic and proteomic technology reveal an unprecedented ability to quickly deduce gene and protein content in pathogens. The challenge is to develop experimental systems that can be used to validate predictions of function and essentiality in parasite biological systems. Exploitation of the modern technology platforms enables us to learn more about the basic biology of parasitic nematodes, which in turn provides new directions for drug discovery.

Methods: Identification of drug targets and anthelmintic mechanisms of action has been aided by new technologies. Examples of advances in these areas were obtained from the literature and the author's laboratory and will be used to illustrate the potential benefits of increased focus on basic research.

Results: A better understanding of the suitability of nematode nicotinic acetylcholine receptors as targets for novel drugs has been obtained through genomic-level studies on the receptor for monepantel. A clearer picture of how ivermectin acts against filariae has been developed through work in *Brugia malayi*. Finally, the potential of novel nematode neuropeptide G protein-coupled receptors for drug discovery has been illuminated through studies in *Caenorhabditis elegans*.

Summary/Conclusions: The ability of the pharmaceutical industry to employ modern methods for anthelmintic discovery depends on the availability of basic knowledge on key biochemical and physiological processes in parasitic nematodes. This knowledge can be translated into high-throughput screens through genomics/proteomics platforms. Information about parasite genomes must be interpreted in the context of parasite biology, a goal which remains challenging.

Comparative measurement of abamectin, ivermectin and moxidectin concentrations in resistant *Haemonchus contortus*

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Abamectin (ABM), ivermectin (IVM) (avermectins) and moxidectin (MXD) (milbemycin) are close related broad-spectrum endectocide compounds. However, differential efficacy patterns against resistant nematodes have been reported, which may be based on specific pharmacokinetic/pharmacodynamic features of each molecule. This work aimed to measure the comparative concentration profiles of ABM, IVM and MXD achieved in the bloodstream, the gastrointestinal contents/tissues and within a target abomasal parasite (*H. contortus*) in lambs naturally infected with resistant nematodes. Additionally, the pattern of the drug-transporter P-glycoprotein (P-gp) mRNA expression in *H. contortus* exposed to the three compounds was established. Thirty (30) naturally parasitized lambs were assigned into three experimental groups intraruminally treated with either ABM, IVM or MXD at 0.2 mg/kg. Blood samples were collected from six animals in each treated group over 14 days post-treatment. Four animals from each group were sacrificed at day 2 post-treatment. Mucosal and fluid content samples from the abomasum and small intestine were collected. Adult specimens of *H. contortus* were recovered from the abomasum. Drug concentrations were measured by HPLC. Specific P-gp mRNA levels in *H. contortus* were quantified by real time PCR. A similar plasma systemic exposure (AUC values) was obtained for the endectocide molecules. However, MXD persistence in the bloodstream was longer ($P<0.05$) compared to that of both ABM and IVM. Concentrations of IVM obtained in abomasal content (day 2 post-treatment) were higher ($P<0.05$) (125 ng/g) compared to that measured after MXD treatment (32 ng/g). There was a positive correlation between drug concentrations measured in abomasal content and those recovered in *H. contortus* collected from treated lambs. However, higher concentrations of ABM (146 ng/g), IVM (238 ng/g) and MXD (60 ng/g) were measured within *H. contortus* compared to those observed in plasma and abomasal content. No significant differences on P-gp expression were observed in *H. contortus* specimens *in vivo* exposed to the different drugs. Although MXD concentrations recovered at target tissues and within resistant *H. contortus* specimens tended to be lower than those of IVM and ABM, some differential pharmacodynamic features may have accounted for the advantageous efficacy pattern against the same resistant *H. contortus* strain observed in a parallel trial.

Interaction of macrocyclic lactone endectocides with *Haemonchus contortus* P-glycoproteins

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Haemonchus contortus is an important parasitic nematode of small ruminants that has developed anthelmintic resistance. This resistance represents a serious problem worldwide. Macrocyclic lactone (ML) anthelmintics, such as ivermectin (IVM) and moxidectin (MOX) have been extensively used against ecto and endoparasites in livestock. There is accumulating evidence that *H. contortus* has developed ML resistance due, in part, to the involvement of constitutive over expression of ABC transporters such as P-glycoproteins (P-gp), which may influencing the concentration of MLs that reaching the target inside the worm.

The goal of this work was to clone and express *H. contortus* P-glycoproteins in mammalian cells and establish the interaction of these transporters with IVM and MOX. After the characterization and stable expression of these *H. contortus* P-gps, the inhibition of transport function by the different MLs was assessed by measuring the accumulation of the fluorophore Rhodamine123 (Rho123). HcP-gps showed at least a 50% less fluorescence accumulation in comparison with untransfected parental cells, meaning that the nematode P-gp transfected mammalian cells actively transported Rho123. Following this, the effect of the MLs on the Rho123 transport was assessed. We found that IVM and MOX produced markedly different responses on the nematode transporters, with IVM being a much more effective inhibitor of nematode P-gp transport than was MOX. Furthermore, the extent of the transport inhibition produced by the different MLs differed between different *H. contortus* transporters. The results indicate that nematode P-gps may play an important role in regulating the concentration of IVM that reaches the mode of action receptors in the parasite, and thus the development of resistance, whereas the nematode P-gps appear to play much less of a role in regulating the availability of MOX at the receptors and possibly the development of resistance to MOX. These differences may account for differences in the expression of resistance between avermectins, such as IVM and MOX.

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Comparison of P-glycoproteins between *Parascaris equorum* populations to identify single-nucleotide polymorphisms correlating with macrocyclic lactone resistance

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Background: Anthelmintic resistance in parasitic nematodes is an increasing burden for animal husbandry and has therefore a high economic impact. As for other nematodes, macrocyclic lactone (ML) resistance has been reported for populations of *Parascaris equorum* in several parts of the world. This is caused by the excessive and/or exclusive use of ivermectin or moxidectin, but it can also be the consequence of an inappropriate administration or dosage. Drug resistance can be evoked by unspecific detoxification mechanisms often involving the activity of ABC-transporters such as P-glycoproteins (Pgps). These proteins are localised in the cell membrane and decrease the intracellular concentration of xenobiotics such as MLs by active extrusion.

Methods: *P. equorum* Pgp-gene sequences were amplified using a nested polymerase chain reaction (PCR) with degenerated primers followed by 5' and 3' rapid amplification of cDNA ends. Maximum likelihood analyses were performed to identify orthologs in other nematode species. In order to screen for single nucleotide polymorphisms (SNPs) potentially involved in ML-resistance, Pgp nucleotide sequences generated from eggs of several susceptible and resistant *P. equorum* populations were compared using the SeqDoC software.

Results: Full-length sequences of two *P. equorum* Pgps were obtained. After phylogenetic classification these Pgp sequences were designated as *PeqPgp*-11 and *PeqPgp*-16. Bioinformatic analyses revealed a typical Pgp-structure for both proteins with two similar halves, each containing six transmembrane helices and an intracellular ATP binding site in a nucleotide binding domain. Comparison of DNA sequences by SeqDoC showed several SNPs; some of these causing dramatic changes in biochemical properties of the deduced amino acids between susceptible and resistant *P. equorum* populations.

Summary/Conclusions: It is well known that Pgps play an important role in the development of parasite drug resistance. Knowledge of changes in Pgp sequences in populations under drug selection might give first hints for resistance associated biochemical changes of the protein. For further analyses, fragments of several additional putative *PeqPgps* have already been identified and will be investigated accordingly. Additionally, real-time RT-PCR is currently used to compare expression levels of *PeqPgp*-11 and -16 between ML-susceptible and resistant populations.

Triclabendazole: time-scale of action against *Fasciola hepatica* in the sheep host

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Sheep infected with the Cullompton isolate of *Fasciola hepatica* were treated with triclabendazole (10 mg/kg) at 12 weeks post-infection. Adult flukes were recovered from the liver and, where present, from the gall bladder at 48 h, 72 h and 96 h post-treatment (pt). The location and condition of the flukes at each time-point was recorded and morphological changes to the different tissue systems monitored by means of histology, together with scanning and transmission electron microscopy. Detachment and movement of flukes from the bile ducts to the gall bladder occurred between 48 and 72 h pt and, by 96 h pt, very few flukes remained (all of which were dead). This pattern fits in well with the known pharmacokinetics of the drug in sheep, as it follows the peak plasma concentrations of the two main metabolites, triclabendazole sulphoxide and triclabendazole sulphone, at 22 h and 36 h pt, respectively, following oral dosing. A severe necrosis of the posterior half of the body was observed at 72 h pt and, by 96 h pt, the tegument had been completely stripped off. The latter was probably due to the decrease in synthetic activity in the tegumental cells and the lack of production of the tegumental secretory bodies that are responsible for the maintenance of the surface tegumental membrane. Most of the major tissue changes took place between 48 h and 72 h pt. There was a significant loss of muscle fibres from the muscle blocks, which would impede the fluke's ability to remain attached to the bile duct. The loss of motility and the degeneration of the gastrodermal cells (seen by 72 h pt) would prevent the fluke from feeding and this would exacerbate the general disruption of the fluke. The testis was severely affected at 48 h pt. No eggs were present in the uterus at 48 h pt: this was the result of degenerative changes in the ovary, vitelline follicles and Mehlis' gland and disruption of egg formation in the ootype. So, drug action is relatively quick and the complete cessation of egg production has important implications, not only for pasture contamination by fluke eggs and the epidemiology of the disease, but also for the way in which drug efficacy is assessed.

Investigation of benzimidazole drug metabolism in nematodes

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Metabolism and excretion of drugs is known to have a profound effect on the activity of pharmaceutical compounds in humans and domestic animals. The major function of phase I and phase II biotransformation enzymes (eg cytochrome P450s and UGTs) is to increase drug polarity in order to enhance excretion by Phase III drug efflux systems (eg ABC transporters). Biotransformation can also directly increase drug activity (eg "pro-drugs") or reduce drug activity. We have an advanced understanding of these processes in mammals and the investigation compound biotransformation in the host is an intrinsic part of the anti-parasitic drug discovery and development process.

In contrast, little is known about drug metabolism and efflux in parasites and these processes are not generally considered during drug development. Nematode genomes contain all the enzyme systems necessary for biotransformation of pharmaceutical compounds. Hence it is likely that many pharmaceutical compounds are metabolized inside the parasite resulting in either direct inactivation (or activation) or enhanced excretion. Metabolism of anthelmintic compounds by parasites is also a possible mechanism of resistance that has received little research, despite there being precedence in the case of insecticide resistance. Consequently, we are investigating how nematodes metabolize and excrete the major classes of anthelmintic drugs. We have first used the model nematode *Caenorhabditis elegans*. HPLC-MS analysis has revealed the production of two albendazole-glucoside metabolites in the worm. These metabolites are distinct from any albendazole metabolites reported in mammals and may represent a nematode-specific biotransformation. In order to identify the enzymes that catalyze these biotransformation reactions, we used whole genome microarray experiments to identify a small subset of genes that are dramatically up-regulated following exposure of the nematode to albendazole, mebendazole and thiabendazole but not ivermectin and levamisole. These "benzimidazole-induced" genes include specific UDP-glucuronosyltransferases and cytochrome P450s (Cyp35C1, Cyp35A2, Cyp35A5, UGT-16 and UGT-63) families; enzyme families likely to be involved in the observed biotransformation. Expression of these genes is largely localized to the nematode gut, suggesting this to be the major site of drug metabolism and excretion. We are currently overexpressing these genes in transgenic *C. elegans* to look at the effects on both drug potency and glucoside-metabolite production.

Evaluation of the effect of increasing albendazole dose rates against resistant *Haemonchus contortus*: Drug systemic availability, clinical efficacy and genetic polymorphism

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The gastrointestinal absorption of most drugs follows a first-order kinetics, whereby a constant fraction of the total drug is absorbed in each equal interval of time. This is applicable to the most of the therapeutically used drugs, but remains unclear for the poorly water soluble benzimidazole (BZD) anthelmintics in ruminants. The main goals of the current trial were: a) to characterize the albendazole (ABZ) metabolites plasma disposition kinetics after ABZ administration at different dose rates to lambs, b) to compare the clinical efficacy of different ABZ dose levels in lambs infected with a BZD-resistant strain of *Haemonchus contortus*, and c) to analyze the frequency of mutations at three different single nucleotide polymorphisms (SNP) on the beta-tubulin isotype 1 encoding gene in worms surviving the treatments at different dosages. Twenty four (24) Corriedale lambs artificially infected with a resistant *H. contortus* strain were allocated into 4 groups (n=6) and intraruminally treated with ABZ at either 5 (ABZ₅), 15 (ABZ₁₅) or 45 (ABZ₄₅) mg/kg. Plasma samples were collected up to 120 h post-treatment and analyzed by HPLC. An untreated control Group was included to assess the comparative anthelmintic efficacy of the different treatments against *H. contortus*. Additionally, adult specimens of *H. contortus* were recovered directly from the abomasum for genetic analysis. Parametric and non-parametric tests were used to compare the statistical significance of the results (P<0.05). The area under the concentration vs time curve (AUC) of the active ABZ-sulphoxide metabolite increased from 21 (ABZ₅) up to 159 (ABZ₁₅) and 390 µg.h/mL (ABZ₄₅), which indicates some type of non-linearity in the relationship between dose level and systemic drug availability. The efficacies against resistant *H. contortus* were 20% (ABZ₅) 74% (ABZ₁₅) and 98% (ABZ₄₅). The enhanced systemic exposure achieved after treatments at the highest dose rates correlated with significant increment in drug efficacy against a resistant *H. contortus* strain. There was a strong relationship between the increase in drug selection pressure (treatments at the highest dosages) and a particular association between SNP200 and SNP167 in the population tested. The association Phe/Phe₁₆₇ – Tyr/Tyr₂₀₀ conferred high levels of resistance.

***Ascaris suum* SLO-1 receptor: is it the target for the anthelmintic emodepside?**

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Treatment and prophylaxis for parasitic nematode infestations of humans and animals relies on anthelmintic drugs because there are no effective vaccines. Emodepside, a cyclooctadepsipeptide, is a more recently introduced anthelmintic drug with a novel mode of action involving a calcium-activated potassium channel, SLO-1, and latrophilin (lat) receptor in *Caenorhabditis elegans*. Emodepside breaks resistance to the classical anthelmintics. The purpose of our experiments was to examine the mechanisms of action of emodepside in the parasitic nematode, *Ascaris suum*.

We used electrophysiological techniques to record emodepside effects in *A. suum* muscle and performed molecular biology experiments to investigate the expression of emodepside target genes.

Emodepside produced a slow, concentration (1-10 μ M) and time-dependent membrane hyperpolarization and increase in voltage-activated calcium-dependent SLO1-like potassium currents. The effect of emodepside was enhanced by stimulation of protein kinase C (PKC) and nitric oxide (NO). Sodium nitroprusside increased the emodepside hyperpolarization and reduced the V_{50} of the voltage-activation curve of the emodepside-activated K currents. N-Nitro-L-Arginine, an NO inhibitor, inhibited the emodepside hyperpolarization and the emodepside K currents. The PKC activator, 10 μ M phorbol-12-myristate-13 acetate increased the emodepside K currents. Conversely, staurosporine inhibited the emodepside hyperpolarization and the emodepside K currents. At the molecular level, *slo-1* and *lat-1* homologues in *A. suum* were found to be readily expressed in adult muscles by RT-PCR analysis. Sequence alignments showed that *Asu-slo-1* and *Asu-lat-1* genes are evolutionarily conserve. Altogether, these results are consistent with a model in which NO, PKC and emodepside signaling pathways are separate and converge on SLO-1 K channels, or in which emodepside activates NO and PKC signaling pathways to increase opening of the SLO-1 channels.

Our work improves the understanding of emodepside mode of action on economically important gastro-intestinal parasites and may help in finding other drugs that could potentiate emodepside's efficacy and stem development of resistance.

Further pharmacological and molecular evidence for SLO-1-channel activity in emodepside mode of action

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Background: The detailed understanding of anthelmintic mode of action and drug target characterization open opportunities for identification of new antiparasitocides and for investigation of possible target-related resistance. Emodepside represents the first commercialized anthelmintically active cyclooctadepsipeptide. Recently, the calcium-gated potassium channel SLO-1 was demonstrated to be involved in emodepside action. Here, a functional rescue approach in *Caenorhabditis elegans* SLO-1-mutants was used to further investigate the molecular biology and pharmacology of parasitic nematode SLO-1 proteins.

Methods: For functional characterization of parasite SLO-1, coding sequences and putative 5'-flanking promoters were cloned into expression vectors and transformed into *slo-1*-deficient *C. elegans*. Functional analysis was based on a body bend assay using young transgenic adults. Additionally the effect of penitrem A, an inhibitor of mammalian SLO-channels, on the locomotion was studied in *C. elegans* with the body bend assay and in *Ancylostoma caninum* in a larval migration inhibition assay.

Results: As expected, *slo-1* mutant worms were not affected by emodepside whereas Bristol N2 wild-type were concentration-dependently inhibited with EC₅₀ values around 135 nM and nearly complete paralysis or death at 10 µM. Expression of *A. caninum slo-1* from the *C. elegans slo-1* promoter completely restored susceptibility to emodepside. Worms expressing *A. caninum* or *C. oncophora slo-1* from the respective *A. caninum* or *C. oncophora slo-1* promoter showed intermediate responsiveness to emodepside with more than 50-fold higher EC₅₀ values than wild type worms or worms expressing the *slo-1* channel from the *C. elegans* promoter.

Concentrations of 1 µM penitrem A significantly antagonized the effect of emodepside on *C. elegans* worms in the body-bend assays and had very similar effects on *A. caninum* infective larvae in the larval migration test.

Summary/Conclusions: The present findings further support the current model of SLO-1 playing a key role in the emodepside mode of action on the nematode body wall musculature. Furthermore, it can be concluded that the function is conserved across the three nematode species employed herein.

How does monepantel kill nematodes?

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Monepantel from a recently developed class of AAD-derived anthelmintics offers an attractive solution to control all major gastro-intestinal nematodes in sheep including those which are resistant to classical anthelmintics. A genetic screen performed on the non-parasitic nematode *C. elegans*, identified the *acr-23* gene as a prominent target for monepantel. The search for parasitic nematodes with reduced sensitivity to the AADs has led to the identification of a similar gene, *mptl-1*, which is disrupted in AAD-mutant isolates of the parasitic nematode *Haemonchus contortus*. With the discovery of *acr-23* and *mptl-1*, it was now possible to investigate the mode of action of monepantel in nematodes up to the protein level. In the present study, we have found that a *C. elegans* mutant *acr-23* strain is fully rescued by expressing the wild type *acr-23* gene. In addition we have tested the potential of the wild type *H. contortus* *mptl-1* gene for restoring AAD sensitivity in the *C. elegans* *acr-23* mutant. Moreover, the tissues that are targeted by monepantel have been identified through the expression of a fusion protein between ACR-23 and the green fluorescent protein. Finally, we will present data on the successful expression of the ACR-23 receptor in *Xenopus* oocytes and for the first time, direct evidence of its modulation by monepantel. Together, these results reinforce the involvement of the ACR family of receptors in the AAD mode of action and our understanding of this new class of anthelmintic.

How do antibiotics kill adult filariae – a novel immune-evasion strategy revealed?

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Background: There is no safe macrofilaricidal drug available for the mass treatment of River Blindness (caused by *Onchocerca volvulus*) or lymphatic filariasis. Using *O. ochengi* in cattle, which is phylogenetically, biologically and immunologically closely related to *O. volvulus*, we have shown *in vivo* that tetracycline antibiotics kill adult worms, and trials with doxycycline in humans by others have recently shown macrofilaricidal action against *O. volvulus*.

In *O. ochengi* the macrofilaricidal action is related to the presence of endosymbiotic *Wolbachia* sp. bacteria, the prolonged suppression of which leads to eosinophil activation and death of worms. What has not been clear is whether eosinophils are a cause, or a result, of worm morbidity and death. We have previously shown that melarsomine is macrofilaricidal, but in *in vitro* studies, does not selectively act against *Wolbachia*. We hypothesised that if eosinophil activation was a result of worm death, cellular changes in the worm nodule would occur following melarsomine treatment and concurrently with oxytetracycline treatment.

Methods: Groups of cattle naturally infected with *O. ochengi* in Cameroon were treated either with oxytetracycline (OXY) or melarsomine.

Results: In sequential samples *ex vivo* quantitative analysis of granulocytes showed that only in the OXY group was there a significant expansion of eosinophil numbers and a significant early peak in degranulating eosinophils associated initially with viable worms. Eosinophil degranulation was associated with ultra-structural deformities in the OXY group and eosinophils were seen within worms. Quantitative PCR of RNA transcripts showed that the 16S r RNA : Oo GST1a ratio (a measure of anti – *Wolbachia* effect) was significant only in the OXY group, observations supported by immunohistochemical staining for *Wolbachia* surface protein. Bovine transcripts for key cytokines involved in neutrophil activation (GRO and IL-8) were significantly lowered in the OXY group.

Summary/conclusions: These results support the hypothesis that in this nodule-dwelling filarial worm, OXY-associated worm death is a result partly or wholly of eosinophil activity, and that in untreated worms, *Wolbachia* confound this by stimulating a neutrophil – rich cellular environment in which worms survive for many years.

Multiple *Haemonchus contortus* GluCl subunits confer ivermectin sensitivity to a resistant strain of *C. elegans*

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Background: Functional genetic studies on parasitic nematodes are virtually impossible, making research into serious problems such as anthelmintic resistance very difficult. The model organism *C. elegans* has been proposed as an expression system for parasite genes, but its use may be complicated by the different composition of the gene families that may be involved in resistance, such as the glutamate-gated chloride genes – the targets of the macrocyclic lactones.

Methods: We cloned GluCl subunits from *C. elegans* and *H. contortus* behind the *avr-14* promoter and introduced the transgenes into DA1316, a *C. elegans* strain with mutations in *avr-14*, *avr-15* and *glc-1*, which is highly resistant to ivermectin, by biolistics, along with a GFP marker. Worms expressing GFP were tested for ivermectin sensitivity in acute paralysis and thrashing assays and by measuring their ability to develop in the long-term presence of the drug.

Results: The *avr-14* promoter drove expression of GFP in a number of neurons, reproducing the pattern previously reported for this gene. Expression of the *C. elegans* AVR-14B and AVR-15 restored the ivermectin sensitivity of DA1316 to wild-type levels. AVR-14A gave a partial rescue. GLC-3 and GLC-1 rescued drug sensitivity but not as fully as the other subunits. When we tested the *H. contortus* subunits, Hco-AVR-14B also completely rescued drug sensitivity, and in a behavioral assay over-rescued the *avr-14* phenotype. The subunits Hco-GLC-5, which forms an ivermectin-sensitive channel in vitro, and Hco-GLC-6 also rescued drug sensitivity, though there were some quantitative differences between the results obtained with these subunits and AVR-14B. Hco-GLC-2, which does not form a drug-sensitive channel in vitro, had no effect on the response of DA1316 to ivermectin.

Conclusions: The results obtained by expressing GluCl subunit transgenes in a drug-resistant mutant mirrored those obtained by in vitro expression, suggesting that the proteins were expressed accurately and in a functional manner. Hco-GLC-6 has not so far been expressed in vitro and these data predict that it will also form an ivermectin-sensitive channel. Overall, these results suggest that expression in *C. elegans* accurately reflects the properties of the parasite subunits and is a useful way to study parasite genes in vivo.

A microfluidic platform for high-sensitivity, real-time anthelmintic screening on parasitic nematodes

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Background: Phenotyping of parasitic nematode larvae has relied on observations after fixed intervals of incubation in different concentrations of anthelmintics. We describe a microfluidic platform for screening drugs and their dose responses on locomotion behavior of parasitic and free living nematodes. The system offers reduced experimental time and higher sensitivity. The real-time video observation and computer tracking of drug effects at a single worm resolution is used, which allows identification of changes in locomotion parameters and the ability to follow effects as a function of time. Existing nematode motility and migration assays do not offer this level of resolution.

Methods: The microfluidic platform was manufactured using standard micromachining and soft lithographic procedures and composed of polydimethylsiloxane [1;2]. It had two components: (i) micro-channels to study nematode motility during the pre- and post-exposure periods of the experiment and (ii) a drug well for administering the dose and studying motility at different exposure times. The drug screening experiment can be described by three main phases: (i) 'pre-exposure study' - worms are inserted into the micro-channels and their locomotion is characterized, (ii) 'dose exposure' - worms are guided from the micro-channels into the drug well and held there for a predefined time, (iii) 'post-exposure study' - worms are guided back into the micro-channels and their locomotion is characterized and compared to pre-exposure motility.

Results: The direction of nematodes' movement was reliably controlled by the application of an electric field in a defined range. Control experiments (no drug) confirm that the applied electrotactic forces do not affect the worms' motility. We demonstrate the workability of the microfluidic platform on free living *Caenorhabditis elegans* (wild-type N2 and levamisole resistant *lev-8* ZZ15) and parasitic *Oesophagostomum dentatum* (levamisole sensitive, SENS and levamisole resistant, LEVR) using levamisole as the test drug. We measured: (i) % worms responsive; (ii) % worms leaving the drug well; (iii) mean post-exposure velocity; (iv) time to not responsive; and found significant differences, using two-tailed, nonparametric *t*-tests with Welch's correction between levamisole-sensitive and levamisole-resistant nematodes.

Summary/Conclusion: The proposed scheme of drug screening on a microfluidic device is expected to significantly improve the resolution, sensitivity, and throughput of *in vivo* testing, while offering new details on the real-time exposure effects of new and existing anthelmintics.

Pgp-expression in Ivermectin Selected GI Nematodes of Cattle

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Background: Anthelmintic resistance (AR) against the macrocyclic lactones (ML), has recently been reported as a widespread problem in gastrointestinal (GI) nematodes of cattle. In closely related GI-nematodes of sheep, ML-resistance has been suggested to be linked to an overexpression of P-glycoprotein (Pgp). The aim of this study was to investigate Pgp-expression in surviving *Cooperia oncophora* following treatment with ivermectin.

Methods: A conserved 202 bp Pgp sequence from *Cooperia oncophora* was identified by Clustalw2 with 83% similarity to Pgp9 of *C.elegans*. Primers specific to Pgp9 mRNA were then designed in Primer3 generating a 153 bp PCR-product. Sequencing of the PCR product was performed followed by a BLAST search in Wormbase (genome view). Total RNA was isolated, using NucleoSpin columns with DNase (Macherey-Nagel), from five single male and five female *Cooperia oncophora* each pre- and post (10 days) ivermectin treatment, from three different isolates, giving in total 60 worms. The three isolates represented one susceptible control group and two strains showing signs of phenotypic ML resilience according to FECRT results (81% and 86% reductions, respectively). The integrity of the RNA was checked visually following electrophoresis and quantification was performed according to the RiboGreen protocol (Invitrogen). One-tube RT-PCR QuantiTect with SYBR green (Qiagen) was used to measure Pgp9 gene expression by real time RT using known concentrations of Pgp9-cDNA as standards and non-template controls as blanks.

Results: The sequenced product matched with Pgp9 of *C.elegans*. Real Time PCR showed mean concentrations of 172 ($\pm$ SD of 123.6) ag/ μ l before, and 195 ($\pm$ 1.4) ag/ μ l after treatment in females and 27.4 ($\pm$ 25.4) ag/ μ l and 46.2 ($\pm$ 32.4) ag/ μ l respectively in male worms. No significant difference in gene expression was seen in comparisons between any group pre and post selection. Female worms showed a large variance ($p=0.747$), whereas the males showed slightly greater tendency ($p=0.091$) to increase Pgp9 gene expression after treatment.

Conclusions: Comparison of Pgp9 gene expression before and after treatment, showed no significant difference between the tested groups, but some tendency towards increasing expression in male as compared to female worms.

Differences in Dynamics of Transcriptional Regulation of Selected Genes between Serum-Stimulated and Percutaneously Migrated Larvae of *Ancylostoma caninum*

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Background: Serum-stimulation induces exsheathment, feeding, and transcriptional changes in infective larvae of the canine hookworm *Ancylostoma caninum* (Hawdon and Schad, 1990). Therefore, serum-stimulated larvae are commonly accepted as a model for early parasitic stages. *In vitro* percutaneously migrated larvae also show exsheathment and feeding, but the onset of feeding seems to be later than after serum-stimulation (Franke et al., 2011). The present study compares the dynamics of transcription of selected genes in serum-stimulated, percutaneously migrated, and infective larvae.

Methods: For serum-stimulation, larvae were incubated in 10% canine serum at 37°C and 5% CO₂. Percutaneously migrated larvae were collected using the PERL (percutaneous larval migration) chamber system (Franke et al., 2011). Larvae from both stimulation methods were collected at several time-points after the first contact with the stimulus. For analysis of the percutaneously migrating larvae at the early time points (30 min – 6h), not larvae but skin samples containing larvae were used. Transcriptional analysis was performed using real-time PCR. For normalization, the genes for the ribosomal protein RPL37A (60S) and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used. Genes of interest were coding for *Ancylostoma* secreted protein 1 (*asp-1*) and for metalloprotease 1 (*mtp-1*), both of them known to be downregulated in serum-stimulated larvae, furthermore so far unnamed *asp* and lysozyme genes, which are known to be upregulated in serum-stimulated (Datu et al., 2008) as well as in percutaneously migrated larvae (Strube et al., in preparation).

Results: The analysis confirmed the previously described downregulation of *mtp-1* and *asp-1* in serum-stimulated larvae and demonstrated it also in percutaneously migrating larvae. Transcription of the unnamed *asp* and lysozyme was also observed to be upregulated in both populations. The most interesting finding was a clear difference in regulation dynamics, especially for the unnamed *asp*. While in percutaneously migrating larvae the transcription was observed to increase during the first 6h and then to remain on a high level, in serum-stimulated larvae it showed a peak after 4-6h and then declined.

Summary/Conclusions: The results of the present study demonstrate that the transcriptional patterns in serum-stimulated and percutaneously migrated larvae are not identical. The function and the importance of the regulated genes remain to be investigated.

Identification of SLO-1 calcium- and voltage-activated potassium channels within different clades of parasitic nematodes: hints for a consistent mode of action of the anthelmintic Emodepside?

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Background: More than 2 billion people suffer from soil-transmitted helminthiasis and 140 million from lymphatic filariasis and onchocerciasis. The WHO list of essential drugs contains only a few drugs to treat human helminthiasis. However, veterinary medicine teaches us that resistance can spread rapidly in a multiplicity of nematode species. It is therefore necessary to meet the demand for broad spectrum anthelmintics with new modes of action. Such could be the cyclooctadepsipeptide Emodepside which has recently been characterized as a compound with a broad spectrum of antinematodal activity. As the first topical anthelmintic for felines and as an oral canine dewormer it was introduced to market (Profender®) a few years ago.

The mode of action of Emodepside is not completely understood yet. In *Caenorhabditis elegans* a functional large-conductance calcium- and voltage-gated potassium channel SLO-1 was described to be essential for Emodepside activity. SLO-1 channels of *Ancylostoma caninum* and *Cooperia oncophora* reconstitute Emodepside sensitivity in *Caenorhabditis elegans slo-1* loss-of-function mutants. Up to now, however, SLO-1 and its role in Emodepside action has only been investigated in nematodes belonging phylogenetically to clade V within the very heterogeneous phylum Nematoda. Therefore, it is of interest to examine if SLO-1 is a conserved channel within the other clades of nematodes.

Methods: For identification of SLO-1, adult *Onchocerca gutturosa* (clade III) and adult *Trichuris muris* (clade I) were collected and total RNAs isolated. PCRs using degenerated primers resulted in amplification of several cDNA fragments. Full length cDNA sequences were obtained by 5' and 3' RACE-PCR for *Onchocerca gutturosa slo-1* and *Trichuris muris slo-1*.

Results: *Onchocerca gutturosa slo-1* and *Trichuris muris slo-1* encoding proteins of 1119 and 1151 amino acids, respectively. Sequence homology searches using conserved domain database suggested that they belong to the superfamily cl04110 (Calcium-activated BK potassium channel alpha subunit).

Conclusion: High degrees of sequence identity and similarity to the SLO-1 proteins of *C. elegans* and strongylids suggest conserved function; however, further experimental evidence is required to unequivocally demonstrate Emodepside sensitivity of these channels.

Characterization of non-specific resistance mechanisms against ivermectin in *Cooperia oncophora*

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Background: While facing increasing anthelmintic resistance, knowledge about resistance mechanism becomes crucial. Despite its importance, for resistance to macrocyclic lactones this still remains unresolved. Non-specific mechanisms of resistance involving transporters, e.g. P-glycoproteins (Pgps), or inactivating enzymes, e.g. Cytochrome P450s (CyP450s), are believed to play an important role. Nematodes have a complex repertoire of both protein classes. It is currently unknown, which transporter or enzyme system is associated with resistance to certain anthelmintics. The cattle nematode *Cooperia oncophora* was used as a model to study contribution of both, CyP450s and two individual Pgps, to the development of resistance in more detail.

Methods: The response of susceptible and ML-resistant isolates of *C. oncophora* to ivermectin were evaluated *in vitro* in the presence of Pgp and CyP450-inhibitors verapamil hydrochloride (VPL) and piperonyl butoxide (PBO), respectively. Full-length Pgp cDNAs were cloned following amplification by RT-PCR and RACE-PCR. Sequence variation of two Pgps was compared between six *C. oncophora* isolates with different resistance status by SeqDoc-analysis. Inducibility of Pgp transcript level by ivermectin was analysed by real-time RT-PCR.

Results: In the presence of VPL and PBO the susceptibility of all tested isolates to ivermectin was significantly increased in the *in vitro* assays measuring development (100-fold) or motility (10-fold). Comparison of full length Pgp-2 and Pgp-3 (currently 65% of the sequence analysed) sequences between *C. oncophora* isolates revealed no major differences correlating with resistance though sequence variability was generally lower in resistant isolates. Preliminary results indicate only minor differences in Pgp-2 expression levels between *C. oncophora* isolates. Expression of Pgp-3 and inducibility of both Pgps by ivermectin are currently examined.

Summary/Conclusions: Though these data indicate that degradation and extrusion as non-specific xenobiotic detoxification mechanisms are both involved in ivermectin-resistance in *C. oncophora*, identification of the relevant genes still remains problematic. The characterised panel of *C. oncophora* isolates nevertheless provides a useful tool for further analyses of specific Pgp and CyP450 genes and their possible contribution to ivermectin efflux and metabolism.

Differential exposure of *Haemonchus contortus* to albendazole according to the site of worm location in the abomasum of infected shee

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The relationship between drug kinetic behavior and anthelmintic activity has been extensively studied. However, there is a need to further understand how the active drug reaches target parasites of different tissue location. The trial described here was designed to measure the accumulation (drug exposure) of albendazole (ABZ) and its active metabolite, ABZ-sulphoxide (ABZSO), in *Haemonchus contortus* recovered from two different locations within the abomasum. Drug measurement was carried out in worms attached to the abomasal mucosa and in those unattached “free” worms recovered from the luminal abomasal content. The data on drug accumulation within the target *H. contortus* was complemented with the assessment of ABZ/metabolites concentration profiles in plasma, abomasal fluid and mucosal tissue collected from the same infected lambs. Eight (8) lambs artificially infected with a benzimidazole-resistant *H. contortus* strain were divided into two experimental groups (n=4), and intraruminally treated with ABZ at either 5 mg/kg (ABZ₅ group) or 15 mg/kg (ABZ₁₅ group) dose rates. Animals were sacrificed at 12 h post-treatment and plasma, abomasal fluid and abomasal mucosa samples collected. Similarly, adult specimens of *H. contortus* from both abomasal locations (*attached to mucosal tissue* or *unattached worms*) were recovered from both experimental groups. ABZ/ABZSO concentrations were measured by HPLC. The highest ABZ concentration profiles were recovered from the abomasal mucosa, ranging from 4.7±3.2 (ABZ₅) and 7.9±1.2 (ABZ₁₅) µg/g. Conversely, the highest concentration of the more polar ABZSO metabolite was measured in the abomasal fluid content, reaching values of 8.0±2.4 (ABZ₅) and 14.5±3.9 (ABZ₁₅) µg/mL. Interestingly enough, significantly higher (P<0.05) ABZSO accumulation were observed in *H. contortus* recovered from the abomasal fluid content (2.6±0.5 and 3.7±0.7 µg/g for ABZ₅ and ABZ₁₅, respectively), compared to those measured in resistant worms attached to the abomasal mucosa (0.8±0.2 and 1.3±0.4 µg/g for ABZ₅ and ABZ₁₅, respectively). A favourable partitioning process between worm/fluid compared to that obtained for the worm/mucosa, may have facilitated the greater drug accumulation observed in unattached worms collected from the abomasal fluid. The observed differential pattern of drug exposure according to worm location within the abomasum, may have marked influence on the survival of resistant parasites after ABZ treatment.

Emodepside potentially interacts with the γ -Aminobutyric acid (GABA) receptor and the acetylcholine receptor in *Caenorhabditis elegans*

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Background: Emodepside belongs to a new class of anthelmintic drugs, the cyclooctadepsipetides, and shows good efficacy against resistant nematode populations. Although some putative receptors for emodepside have already been discovered, its mode of action is still not fully understood. The possibility of an involvement of the γ -Aminobutyric acid (GABA) receptors on the PF1022A mode of action was already postulated (Chen W. *et al.* 1996; Harder A. *et al.* 2003). Therefore, a possible involvement of the GABA receptor *unc-49* and the levamisole responsive acetylcholine receptor in the mode of action of emodepside was investigated using two different *Caenorhabditis elegans in vitro* assays.

Methods: The following *C. elegans* strains were used for the experiments: The N2 Bristol wild-type strain, two *unc-49* GABA receptor mutants, as well as five acetylcholine receptor (AChR) loss of function mutants.

For the development assay synchronised first stage *C. elegans* larvae were incubated for 54 hours with different concentrations of anthelmintics and development was compared between wild-type and mutants. In the body bend assay, the motility of *C. elegans* wild-type and mutants was compared after 24 h exposure to anthelmintics.

Results: In the development assay GABA receptor mutants showed significant ($p < 0.0006$) better development ($EC_{50} = 492.6$ nM/525.6 nM) following emodepside exposure than the wild-type ($EC_{50} = 180.4$ nM). This result was confirmed in the body bend assay, where motility of two GABA mutants ($EC_{50} = 738.1$ nM/963.6 nM) was significantly higher ($p < 0.002$) compared to the wild-type ($EC_{50} = 283.4$ nM).

In addition, the development rate of one of the acetylcholine receptor mutants, *unc-38*, in the presence of emodepside was 3.2-fold increased compared to the wild-type ($p < 0.003$). All other tested AChR mutants showed no altered susceptibility to emodepside.

Furthermore, the complete cDNA of the *Toxocara canis* GABA receptor *unc-49* was obtained and will be used for subsequent rescue experiments.

Summary/Conclusion: An involvement of the GABA receptor *unc-49* in the emodepside mode of action was demonstrated using two different *C. elegans in vitro* assay. Results of the development assay also indicate a possible involvement of the acetylcholine receptor subunit *unc-38*. Future studies will focus on *C. elegans* rescue experiments using parasite encoded receptors.

Effects of erythromycin on the maternal and fetal plasma concentrations of moxidectin after its intravenous administration in pregnant sheep

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Moxidectin (MXD) and erythromycin (ERM) are two highly lipophilic drugs that are substrates of P-glycoprotein (P-gp), an ATP-binding cassette (ABC) transporter that controls the disposition of both drugs in the animal's organism. Thus, co-administration of these drugs can lead to significant modifications in their pharmacokinetic behavior. P-gp also plays an important role in the regulation of the maternal-fetal transplacental exchange of xenobiotics between mother and fetus. In this study, we evaluated the effect of multiple doses of ERM on the maternal-fetal plasmatic disposition of MXD, after its intravenous administration in pregnant sheep. Ten Suffolk-Down pregnant sheep of 64.5 ± 4.0 kg body weight were surgically prepared at 120 days of gestational age, to insert polyvinyl catheters into the fetal femoral vein. Subsequently, the sheep were randomly assigned into two experimental groups. Group I (control), five pregnant sheep were treated with 0.2 mg/kg of MXD intravenously (IV). Group II (premedicated group), five pregnant sheep were pretreated with an IV dose of 5 mg/kg of ERM administered at 24 and 12 h before and at 12, 24 and 36 h after administration of 0.2 mg/kg of MXD. Blood samples were drawn before and at different times until 144 h post treatment. Plasma samples were analyzed by high performance liquid chromatography (HPLC), after solid phase extraction. A non-compartmental pharmacokinetic model was used to determine the pharmacokinetic variables. Results are expressed as mean $\pm$ standard deviation and compared by Student t-test for paired samples. Co-administration of ERM/MXD increased the MRT and the elimination half-life of MXD, from 45.6 ± 10.8 h to 109.2 ± 52.9 h ($P = 0.0129$), and from 48.7 ± 8.6 h to 120.4 ± 52.9 h ($P = 0.0228$), respectively. These changes were associated to an increase in the volume of distribution from 4758 ± 1844 mL/kg to 15417 ± 12094 mL/kg ($P = 0.093$). Our results suggest that the interaction of ERM with P-gp can reduce the rate of elimination of MXD. No significant differences ($P > 0.05$) were observed in the comparison of fetal pharmacokinetic variables of MXD, between both groups of fetuses, concluding that co-administration of ERM with MXD in pregnant sheep, does not modify the transplacental exchange of MXD between maternal and fetal circulation.

Inflammation and apoptosis in stomach wall of Mongolian gerbil (*Meriones unquiculatus*) infected with *Haemonchus contortus*

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Background: Apoptosis is a form of programmed cell death involved in a wide range of processes and parasites have evolved a number of mechanisms to induce or avoid host cell apoptosis in order to survive and complete their life cycle. In the present study we examined dynamics of nematode-induced mastocytosis, number of apoptotic mastocytes and eosinophils and distribution of apoptotic cells in the stomach wall of the laboratory model Mongolian gerbils.

Methods: Animals were infected with benzimidazole sensitive strain of nematode *Haemonchus contortus*. Apoptotic cells were visualised by TUNEL method, mast cells were stained with astra blue /safranin, eosinophils and neutrophils by Px-specific staining on the tissue sections on day 4, 7, 10 and 14 post infection (p.i.).

Results: The peak of connective tissue mast cells (CTMC) and mucosal mast cells (MMC) numbers was seen on day 4 p.i., and then their numbers gradually decreased. They were localised in the mucosal and submucosal layers of infected stomach, where the highest number was recorded in *tunica muscullaris*. Mast cells undergoing apoptosis appeared on day 4 p.i. and intensity of apoptosis peaked on day 7 p.i. In the infected stomach, apoptosis was also seen in the glandular cells in *submucosae* and muscle cells in *tunica muscularis*. No apoptotic eosinophils were observed during nematode infection.

Conclusions: These data suggest that infection triggered accumulation of mast cells and eosinophils in all stomach layers, being localised mainly in the sites of direct injury caused by the larvae. It seems that apoptosis is responsible for the regulation of mast cells numbers and that mast cells are the key cells in the initiation of larval expulsion.

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Differences in nitrogen metabolism in *Haemonchus contortus* and *Teladorsagia circumcincta*

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Nitrogen metabolism is essential for survival of all organisms and the enzymes involved present potential targets for development of vaccines or novel chemicals to control nematode parasites of domestic ruminants. The aim of the present study was to identify pathways involved in the metabolism of key amino acids (glutamate, arginine, and proline) in *Haemonchus contortus* and *Teladorsagia circumcincta* and particularly to identify enzymes not present in the host or with unusual properties. Biochemical assays showed that there are significant differences between parasite and host metabolism and between the two parasite species. Unlike mammals abomasal nematodes do not have a fully functional ornithine urea cycle (OUC), and arginase was the only OUC enzyme detected in either species. All enzymes of the ornithine-proline-glutamate pathway were detected in the two life cycle stages of both worm species making the parasites capable of interconversion of these amino acids. Polyamines are essential cell growth and differentiation regulators. In mammals arginine is metabolised into polyamines via ornithine (by arginase (E.C. 3.5.3.1) and ornithine decarboxylase (ODC) (E.C. 4.1.1.17)). In plants and bacteria polyamines are synthesised also via agmatine (by arginine decarboxylase (ADC) (E.C. 4.1.1.19) and agmatinase (E.C. 3.5.3.11)). ODC activity was detected in both parasite species but ADC activity was detected only in L3 and adult *H. contortus*. Parasitic nematodes may not be completely dependent on ingested amino acids, but be able to incorporate ammonia into glutamate and other amino acids. Ammonia can be incorporated into glutamate through the glutamine synthetase-glutamate synthase (GS-GOGAT) pathway. GOGAT (E.C. 1.4.1.14) activity was not detected in L3 *H. contortus*, but was present in L3 *T. circumcincta* and in adult worms of both species. Enzyme activity was inhibited by the specific inhibitor azaserine and was up-regulated by added ammonia. If proven important for worm survival, GOGAT can be an important target, as it is absent in the host. Because of similarity with the host pathway, ornithine-proline-glutamate might be a less useful target for the parasitic control. The flexibility in *H. contortus* of synthesis of putrescine may make this pathway less useful as a target for parasite control than in *T. circumcincta*, in which only the ODC pathway was detected.

New valerolactam anthelmintics for livestock production, molecular approach to the mechanism of action

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Livestock, one of the major markets for animal health drugs, urgently needs for anthelmintic drugs discovery, because the emergence of resistance against marketed drugs. In the search for new anthelmintics, we have communicated previously the synthesis and anthelmintic activity of a new series of valerolactam derivatives, using nematode parasite *Nippostrongylus brasiliensis* L4 in vitro model developed by us. Those compounds were more active than marketed anthelmintics as albendazole (standard drug), contrary to preliminary studies of activity of them in natural infected sheep. By mean of pharmacomodulation, a new series of mixed valerolactam-benzimidazole derivatives were synthesized, improving diffusion rate into parasites (patent application: DNPI n°32300 Uruguay, INPI n°P20100104489 Argentina) which are in development.

On the other hand, based on chemical considerations, it was explored the effects of a valerolactam derivative (product **1**) and α -Difluoromethylornithine (DFMO) used as a tool for comparison purpose, on the arginine cross-talk between polyamine (PA) and nitric oxide (NO) pathways of *N. brasiliensis*, as a molecular approach of mechanism discussed in this communication.

For the analysis of PA, dansylation procedure was performed. Parasites were collected from rats and cultured for the anthelmintic activity in vitro model. Independent experiments were carried out, with or without treatment with DFMO or product **1**. Parasite homogenates were dansylated and analyzed by HPLC. Anthelmintic activity studies of DFMO and product **1**, plus exogenous PA and precursors, were also performed. Finally, SDS-PAGE and Western Blotting of *N. brasiliensis* L4 parasites were carried out, and immunoreactivity with anti-NOS I (neuronal) or anti-NOS III (endothelial) was assayed.

Different behaviors were observed for both drugs. While DFMO depleted spermidine and spermine levels (2.5 and 3.3-fold decrease, respectively) paralleled with its anthelmintic activity, product **1** seemed not to affect, showing similar PA levels than untreated parasites. The results suggest DFMO is depleting PA via ADC (and not ODC) inhibition while valerolactam derivative is affecting arginine consumption probably by altering parasite NOS activity. The presence of nitric oxide synthase (NOS) in *N. brasiliensis* L4 was evidenced by immunoreactivity with anti-NOS I. Further studies will be carried out to confirm those observations.

Efficacy of nitroxynil and closantel against *Haemonchus contortus* -comparative parenteral treatments in lambs-

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Helminth infections are the most important cause of productivity losses in livestock. Drug-based treatments remain as the main tool to control parasitism in different animal production systems. Both nitroxynil (NTX) and closantel (CLS) are flukicidal compounds which have been shown to have good activity against the haematophagous sheep abomasal nematode *Haemonchus contortus*. However, the development of resistance to the available anthelmintic drugs is a seriously increasing problem. Resistance to anthelmintics from different chemical families (different mode of action) is widespread in sheep nematodes in Uruguay. The current trial describes the comparative clinical efficacy of nitroxynil (NTX) and closantel (CLS) against a CLS-resistant *H. contortus* strain in lambs. The CLS-resistant *H. contortus* strain was previously isolated from naturally infected lambs, where an efficacy failure was observed after oral CLS treatment. The isolated CLS-resistant strain was used to artificially infect (8000 L₃/lamb) fifteen (15) non-parasitized lambs. Twenty eight (28) days post-infection, the lambs were randomly allocated into three (3) experimental groups (each of n= 5), including an untreated control and those subcutaneously treated (10 mg/kg) with either NTX (NTX-treatment) or CLS (CLS-treatment). The efficacy estimation was performed by the faecal egg count reduction test (FECRT). Additionally, all animals were sacrificed at 13 days post-treatment to assess the comparative efficacy against adult *H. contortus* according to the WAAVP guidelines. Interestingly, while the FECRT reached a 39% for the NXT-treated lambs, a 100% reduction was observed in lambs treated with CLS at the same dose rate. Following a similar trend, the efficacy against CLS-resistant *H. contortus* was 99% after CLS treatment but only reached a 57% following NTX administration to infected lambs. An enhanced CLS systemic availability has been observed after parenteral compared to its oral administration to sheep. Thus, a subcutaneous injection at 10 mg/kg may have reached a CLS pharmacokinetic profile sufficient to kill the resistant strain, which has previously survived after the oral administration of the drug. Further work is required to understand the low efficacy observed for NTX, a drug usually active against susceptible *H. contortus*.

Development of alternative Albendazole pharmaceutical preparations

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The sustained therapeutic regimen (3-5 days) proposed in monogastrics to optimize the efficacy of methyl-carbamates benzimidazoles, may result impractical from the clinical view. Development of new formulations with prolonged permanence of the active moiety at the infection site is a challenge and may result in a shorter therapeutic regimen, with equivalent efficacy. Solids dispersions (SDs) have been proposed as new alternative to improve the dissolution rate of low solubility drugs. In previous in vitro studies, formulations of albendazole (ABZ) with SDs showed higher dissolution, when compared with ABZ conventional formulations. The dissolution profiles indicated that ABZ incorporated in SDs was rapidly released compared with free ABZ. The aims of the present study were: a) to validate a laboratory animal model (mice) to screen the anthelmintic drug absorption pattern and, b) to compare the plasma concentration profiles of two different SD containing ABZ in mice.

DSs with ABZ:P188 and ABZ:PEG6000 were prepared by the melting method. One hundred forty four BalbC mice (25 g b.w.) were divided in three groups (n=44) and orally treated with ABZ suspension (25 mg/kg) as follows: Group A, received a SD of ABZ (P188), the Group B was treated with the formulation ABZ:PEG6000 and, the Group C was dosed with ABZ conventional suspension. Blood samples were taken from the eye vein using 4 animals per sampling point, over 12 h post-treatment. Samples were analysed by High Performance Liquid Chromatography. ANOVA test was used for the statistical comparison of different points the concentration vs. time curves.

The mice model resulted useful to primary screen different ABZ formulations before using superior species (dogs). The concentration-time curve values obtained for the Group A described significant higher concentrations ($P<0.001$) for the active metabolite Albendazole Sulphoxide (ABZSO)(AUC values +50%), when compared with Groups B and C. Augmented Cmax (+130%) was also observed for the Group A. No statistical differences were found after the comparison of the ABZSO concentrations between the Groups B and C. In conclusion the formulation ABZ-based SD:P188, showed a markedly increase in ABZSO concentrations. The later, could be useful for the development of a potential new dosage form in monogastrics.

Optimizing protease production from an isolate of the nematophagous fungus *Duddingtonia flagrans* using response surface methodology and its larvicidal activity on horse cyathostomin

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Proteases are the most important group of commercial hydrolytic enzymes, constituting approximately 60% of the total enzymes produced worldwide. *Duddingtonia flagrans* is considered the most promising species of nematophagous fungi because of its large production of chlamydospores, resistant structures for survival. This species is also classified as predatory and can produce a range of enzymes, including proteases. Protease production from *D. flagrans* (isolate AC001) was optimized and the larvicidal activity of the enzymatic extract was evaluated on infective horse cyathostomin larvae (I₃). *D. flagrans* was grown in liquid medium with eight different variables: glucose; casein; dibasic potassium phosphate (K₂HPO₄), magnesium sulphate (MgSO₄), zinc sulfate (ZnSO₄), ferrous sulfate (FeSO₄) and copper sulfate (CuSO₄). The Placket-Burman analysis showed significant influence of MgSO₄, CuSO₄ and casein (p<0.05) on protease production by *D. flagrans* in liquid medium. The Central Composite Design indicated that the highest proteolytic activity was 39.56 U/ml as a function of the concentrations of casein (18.409g/l), MgSO₄ (0.10g/l) and CuSO₄ (0.50mg/l). Significant difference (p<0.01) was found for the larva number between the treated and control groups at the end of the experiment. It was found a reduction of 95.46% in the number of free-living larvae in the treated group compared with the control. The results of this study suggest that the protease production by *D. flagrans* (AC001) in liquid medium was optimized by MgSO₄, CuSO₄ and casein, showing that the optimized enzymatic extract exerted larvicidal activity on cyathostomin and therefore may contribute to a large-scale industrial production.

Effect of the level of cellular immune response on *Haemonchus contortus* burden in sheep

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Haemonchus contortus is the most important parasite to sheep raised the in tropical and subtropical areas producing major economical losses. One of the major host responses against the parasite infection occurs in the abomasum epithelium, throughout the action of the globule leukocyte (GL) and mucosal mast cells (MMC). Thus, the objective of this study was to evaluate the relationship between the level of the cellular immune response and the blood leukocyte counts and the worm burden. Thirty, nine-months-old, Suffolk x Greyface worm free sheep were used. Eighteen of those were orally infected with 500 *H. contortus* L3, daily, five times a week during six consecutive weeks. Twelve sheep were kept as controls. Blood samples were collected at day zero, 30 and 57 for leukocyte count. After 14 d from the last infestation (57th d), infected sheep were slaughtered and total worm counts were carried out in the abomasum. According to the worm burden, the sheep were divided into three groups. A < 1000 worms; B 1000 to 5000; C > 5000, with six animals each. Abomasal tissue samples were formalin fixed and colored with carbolcromatobe and toluidine blue for GL and MMC counts in 25 ocular fields, respectively. For statistical analysis, ANOVA with Duncan test were used. A linear regression was calculated between variables. Group A presented higher leukocyte counts compared to groups B (- 29%) and C (-39%) at 57th d, caused principally by the higher neutrophils counts in the group A. Compared to controls group A had a neutrophilia and eosinophilia, while the group C had a neutropenia and similar number of eosinophils. The mean values of GL and MMC were different (A>B>C) with 52, 33 and 14 counts for GL and 88, 69 and 35 counts for MMC, respectively. The higher the GL and MMC counts, the lower the worm burden (P < 0.0001). Sheep more resistant to *Haemonchus contortus* had a higher leukocyte, neutrophils and eosinophils counts, and higher GL and MMC counts in the abomasums epithelium showing the cellular immune response play an important role in the defense against *H. contortus* in sheep.

Ultrastructural reconstruction of *Taenia ovis* (Platyhelminthes: Cestoda) oncospheres from serial sections after high pressure freezing and freeze substitution

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Taenia ovis is a dog-sheep tapeworm and causes significant economic losses in the sheep industry due to the condemnation of infected meat. Sheep become infected by ingesting parasite eggs, each of which contains a larva termed an oncosphere. Following ingestion, the oncosphere hatches and becomes activated under the influence of the gastric and intestinal juices of the host. The activated oncosphere penetrates the intestinal wall of the sheep. Little information is available about the cellular components of the oncosphere. The present study was undertaken to interpret the cellular organisation of *T. ovis*. Oncospheres (non-activated) were snap frozen using a Leica High Pressure Freezer and freeze substituted in uranyl acetate in acetone before embedment in Lowicryl HM20 resin. For ultrathin serial sections, 90 nm thick sections were collected (at one micrometer intervals) onto pioloform-coated slot copper grids. The ultrastructure of the oncospheres was reconstructed from ultrathin serial sections using transmission electron microscopy. The surface of a hatched, non-activated *T. ovis* oncosphere is covered by an oncospherical membrane below which lies the tegument lined with microvilli. The basal matrix of the tegument is underlain by broad bands of peripheral somatic musculature. Three pairs of hooks and associated muscles are present in the somatophoric third of the oncosphere. Approximately 19 cells of seven different types were identified in oncospheres. These cells include: (a) penetration gland type 1 (PG1), a quadri-nucleated syncytium, consisting of two lateral pairs of cell bodies interconnected by narrow cytoplasmic bridges; (b) penetration gland type 2 (PG2), a second form of quadri-nucleated syncytium; (c) a uni-nucleated median mesophoric gland cell; (d) ten somatic cells; (e) two germinative cells; (f) two nerve cells; and (g) a pair of median somatophoric cells. This study provides a clear understanding of the morphology of *T. ovis* oncospheres and provides the basis for further investigations into the hatching and host-invasive biology of taeniid oncospheres.

Singularity in the burse venation of *Haemonchus contortus*

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Haemonchus contortus presents subcuticular structures in the burse, resembling a river delta (venations), which could be a biometric variable that would allow to identify individuals and/or related groups. The uniqueness of these structures could serve as markers of phenotype fitness attributable to environmental and/or anti-parasite drug resistance. This work analyzes venation differences between some strains of *Haemonchus contortus*. Adult parasite specimens were recovered from four sheep at slaughterhouses. Strains were named according to their geographical origin: a- Balcarce and Las Armas strains from Balcarce, Province of Buenos Aires, Argentina, and b- Casilda and Parnisari strains from Casilda, Province of Santa Fe, Argentina. Forty nematodes, ten for each strain were mounted on slides and clarified with lactophenol solution. Copulatrix venations of each specimen were photographed under light microscope at 400x, with a digital size of 100 pixels wide by 100 pixels long in the area between the 3rd and 4th ribs of the left lobe. To study the variability in venations, a numerical analysis of digital images was conducted by using an image editing software. The image was pixel-segmented in grayscale or black and white, with a cutoff ranging between 114 and 180 circular portion of the venations. Index values were subsequently obtained with the white/black pixel ratios (number of white pixels divided by the number of black pixels), called "Ib/n". A multivariate analysis of clusters was performed using the JMP 5.1 statistical software on the digital files with the full range of pixels in grayscale images of each individual. Analysis of variance showed significant differences among strains ($F = 2.9716$ - $P = 0.04$) of Ib/n index and the cluster analysis showed that the largest grouping occurred in Las Armas strain (50%), followed by Parnisari (25%), Balcarce (20%) and Casilda (5%) strains. These results suggest differences in the uniqueness of *Haemonchus contortus* venations. It can be concluded, at least in the strains studied, that by digitalizing images of *Haemonchus contortus* venations, unique differences can be identified and estimation of the white/black pixel ratios in gray scale images of these venations, would allow to determine differences of homogeneity, allowing the identification between strains.

Progress on understanding the process of doramectin percutaneous absorption through ovine and bovine skin

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Macrocyclic lactones are world-wide topically used to control endo and ectoparasites in cattle. A broad activity spectrum, easy administration and lack of residues in edible tissues are well established advantages for topical formulations. However, available knowledge on their pattern of transdermal absorption in ruminants is scarce. A practical *in vitro* method to assess dermal absorption through cattle skin was recently developed in our Lab. The objective of the work was to characterize *in vitro* the comparative pattern of doramectin (DRM) transdermal absorption (topical formulation) using both bovine and ovine epidermis. Hair/wool was clipped and the upper epidermal layers (500µm) split from dermis and subcutaneous tissue with a dermatome. Skin membranes (n=5) were mounted on Franz-type diffusion cells. DRM formulation (Dectomax® Pour-on for cattle, Pfizer, 0.5%) was applied on mounted skin membranes. Receptor medium was composed of buffer phosphate, bovine albumin (4.5%) and ethanol (20%) and samples collected up to 72 hours post-administration. Doramectin concentrations were determined by HPLC. DRM permeation through bovine upper epidermal layers stabilized after 38 ± 9.84 h post-administration. However, a plateau was not observed with ovine epidermal layers. Permeation fluxes were 39.92 ± 10.15 (bovine) and 9.21 ± 1.82 (ovine) $\text{ng} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$, differences being statistically significant ($P < 0.05$). Permeation coefficients (K_p) were significantly higher ($P < 0.05$) for bovine ($1.60 \times 10^{-04} \pm 0.41 \times 10^{-04}$ cm/h) compared to ovine ($0.37 \times 10^{-04} \pm 0.07 \times 10^{-04}$ cm/h) epidermal layers. The theoretical time required to DRM for reaching the perfusion medium (lag times) were similar between skin from both animal species (between 9.43 and 11.7 h). The differences observed in the dermal permeation *in vitro* kinetics may be due to quality-quantitative differences in skin composition. Ovine skin presents a higher lipid composition compare to bovine skin. This high lipid composition, along with the high DRM lipophilicity, would determine a depot effect from which DRM is slowly released. Results observed with bovine skin are in correlation with *in vivo* data obtained after topical treatment reported elsewhere. However, the data indicates that a limited absorption through skin may occur in sheep, which may be a serious impairment to reach an acceptable anti-parasite efficacy. Physiological species differences in skin physiology can determine marked differences in the topical absorption of highly lipophilic compounds.

Influence of the formulation on the pharmacokinetics of ivermectin 5% in cattle

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Introduction: Ivermectin (IVM) is a semi-synthetic avermectin broad-spectrum molecule active against endo and ectoparasites of clinical relevance in veterinary and human medicine. IVM is commercially available to use in livestock as injectable, oral and pour-on formulation. It has been shown that differences in drug formulation may affect the pharmacokinetics of endectocides in different animal species. The concept of antiparasitic persistence acquired great clinical significance after the discovery and commercial development of the endectocide compounds for use in ruminants. The vehicle in which these compounds are formulated may influence their absorption process and the resultant drug concentration profiles achieved in the different tissues. After the introduction of the first IVM 1% formulation, different pharmaceutical modifications have been assayed to extend the IVM persistent endectocide activity. More recently, highly concentrated (3.15%) long-acting IVM preparations were introduced into the veterinary market. In the present paper we compare the pharmacokinetic behaviour of two new experimental IVM 5% formulations after its subcutaneous administration to cattle.

Materials and Methods: Twelve castrated male healthy calves were the experimental animals. Animals were randomly divided into two groups and treated with either IVM 5% A (formulation predominantly saturated oil) or IVM 5% B (formulation predominantly unsaturated oil) by subcutaneous injection in the shoulder area at 1000 µg/kg body weight. Blood samples were obtained at different post-administration times until 90 days. Plasma concentrations of IVM were determined by HPLC with fluorescence detection using automated solid phase extraction. The concentrations versus time curves obtained for each treatment were fitted with WinNonlin Professional 5.2 (Pharsight Corp., Mountain View, Ca.). Pharmacokinetic parameters were determined using non-compartmental methodology.

Results: The pharmacokinetic behaviour of IVM was different between the two formulations. C_{max} was higher (104.33 vs 60.69 ng/mL), T_{max} (6.40 vs 10.50 days) was much shorter and the elimination was fast (T_{1/2λ} 11.12 vs 16.56 days) with the formulation B. This means that the formulation B showed less persistence than the formulation based on predominantly saturated oil.

Conclusion: The kinetic differences between these formulations resulted evident. The saturation of oils in the vehicle generated a higher affinity for ivermectin and consequent persistence of the drug at the injection site.

Pharmacokinetics of a formulation containing ivermectin and levamisol in cattle

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Introduction: Ivermectin (IVM) is a widely used long acting endectocide. Levamisol (LEV) is a short acting injectable anthelmintic.

A significant part of the investigation on antiparasitic treatments is directed towards synergistic associations. The association between IVM and LEV is a very rational one from a mechanistic point of view. The half lives of these drugs, however, are markedly different: Meanwhile IVM is a very persistent drug in the organism, LEV is an anthelmintic with short persistence. To balance this association, the levels of IVM in the animal should not be very prolonged meanwhile LEV levels should be more persistent than those obtained with ordinary formulations.

The procedure followed to reduce IVM persistence was based on the reduction of the dose to a minimum (200 µg/kg), and to formulate the drug more diluted than ordinarily. In the case of LEV, the vehicle used resulted of very high affinity for the drug, with the consequence of a slow liberation profile.

In the present paper the results of the pharmacokinetics of the combination IVM-LEV are described.

Method: Six Aberdeen Angus young and healthy bovines received 1 mL/30 kg of the formulation (IVM 0.6%-LEV 23%) (dose: IVM 200 µg/kg, LEV 7.7 mg/kg) by the subcutaneous route. Blood samples were obtained at different post-administration times. Plasma samples were stored at -20° C until assayed by HPLC with fluorescence detection in the case of IVM and UV detection in the case of LEV. Both techniques were validated at our lab.

Results: LEV presented a fast absorption profile with a Cmax of 1.71 µg/mL at 4.33 h. After that a very slow elimination profile for this kind of drugs (half life of elimination of 26.94 h) was obtained.

In the case of IVM, its absorption profile was fast in comparison with classical formulations. The Cmax (39.62 ng/mL) was determined at 2.17 days. Elimination half life was 7.55 days.

Conclusion: These kind of formulation could be useful in areas of mixed infections by ticks and endoparasites, especially when some degree of helminthic resistance to IVM is detected. The only possibility of usage of such a product is in the frame of programs which alternate IVM with agents with different mechanism of action.

Combined administration of abamectin and albendazole as a controlled-release capsule in lambs: pharmaco-therapeutic evaluation

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In an attempt to overcome the inconvenient of the development of resistance to the available anthelmintic chemical groups, pharmaceutical formulations combining benzimidazole and macrocyclic lactone compounds are available in the veterinary pharmaceutical market. The goal of the current work was to evaluate the anthelmintic efficacy and the pharmacokinetic profiles of both abamectin (ABM) and albendazole (ABZ) after their oral administration as a combined controlled-release capsule in lambs. Twenty (20) Corriedale lambs naturally infected with multiple anthelmintic resistant gastrointestinal nematodes, were randomly allocated into two experimental groups (n=10): an untreated control and a group treated with the ABM+ABZ controlled-release capsule (Bionic®, Ancare, New Zealand) by the oral route. Faecal and plasma samples were collected over 100 days post-treatment. ABM and ABZ sulphoxide (ABZSO) plasma concentrations were measured by HPLC. The efficacy of the treatment, estimated at different days post-treatment, was assessed by the faecal egg count reduction test (FECRT). Additionally, faecal samples were cultured to determine nematode genus and species. Both ABM and the ABZSO metabolite were measured in the bloodstream up to 92 days post treatment. The ABM peak plasma concentration (C_{max}) was 16.5 ± 7.7 ng/mL reached at 8 days post-treatment. The ABM area under the concentration vs. time curve (AUC_{0-t}) was 840 ± 377 ng.day/mL. While ABZ parent drug was not detected at any time post-treatment, its active ABZSO metabolite, reached a C_{max} value of 0.2 ± 0.1 µg/mL and AUC_{0-t} of 8.6 ± 3.4 µg.day/mL. Plasma concentrations of both ABM and ABZSO increased gradually to achieve the steady-state (10.3 ng/mL and 0.1 µg/mL, respectively) at approximately 8 days, which was maintained over 77 days post-treatment. The FECRT estimated at 13 days post-treatment was 73%. However, between days 29 to 92 post-treatment, an enhanced egg output reduction (87-98%) was observed. The faecal cultures showed *Haemonchus* spp. as the main nematode surviving the combined ABM+ABZ treatment. The sustained presence of the combined molecules delivered by the capsule for a long period, may have accounted for the observed efficacy against multiple resistant nematodes.

Assessment of the *ex vivo* interaction of antiparasitic drugs with efflux transporters proteins in rat intestine

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Transport proteins play an important role in the intestinal secretion of different drug molecules widely used in veterinary therapeutics. An active intestinal efflux of different antiparasitic compounds has been described. Although the main interaction of such drugs with efflux transporters seems to be mediated by P-gp, the tissue distribution of the breast cancer resistance protein (BCRP) overlaps extensively with that of P-gp, suggesting a similar role in substrate transport. The aim of the study was to characterize the *ex vivo* interaction of ivermectin and moxidectin with the efflux transporters P-gp and BCRP. The intestinal transport studies were carried out by the Ussing chamber technique. The ileal segment obtained from male Wistar rats was opened along the mesenteric border and the resulting flat sheets of gut mucosa were mounted into the Ussing-chambers. After a 20 min equilibrium period, rhodamine 123 (Rho 123) (5 μ M) as P-gp substrate, and danofloxacin (DFX) (50 μ M) as a BCRP substrate, were added to both mucosal (M) and serosal (S) sides. Ivermectin (IVM) and moxidectin (MXD), used as inhibitors, were added at equimolar concentrations (10 μ M). Additionally, PSC833 and pantoprazole (PTZ) were used as specific P-gp and BCRP inhibitors, respectively. The amount of permeated Rho 123 and DFX was determined by spectrofluorometric detection. The apparent permeability coefficients per unit of membrane surface area (P_{eff}) (cm/s) and the efflux ratio ($P_{eff_{S-M}}/P_{eff_{M-S}}$) for Rho 123 and DFX were calculated. The efflux transport of Rho 123 and DFX in rat intestine was demonstrated (Efflux ratio of 4.26 and 4.38 respectively). The $P_{eff_{S-M}}$ (secretion) of Rho 123 was significantly reduced ($P<0.05$) by IVM, whereas no differences were observed for MXD. On the other hand, the DFX efflux ratio was significantly reduced by both IVM (1.24) and MXD (1.67) compared to the control, suggesting inhibition of the active secretion process. The work permitted to demonstrate the ileal permeability of the efflux transporter substrates. The antiparasitic molecules under study showed a differential inhibitory effect on P-gp and BCRP-mediated intestinal transport. These preliminary results demonstrated the usefulness of this experimental approach for testing the mechanisms involved in the absorption/excretion processes for anthelmintic drugs, which may have therapeutic relevance.

Efficacy of Abamectin and Levamisole in combination against New Zealand cattle endoparasites

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Background: To combat widespread macrocyclic lactones (ML) resistance in N.Z. cattle parasites dual ML/levamisole anthelmintics have been developed. This paper reports, for the first time, the oral and topical efficacy of this combination in a controlled comparative slaughter trial and a multicentre longitudinal comparative faecal egg count reduction test (FECRT) field trial.

Methods: In the slaughter trial, 18 naturally-infected 7 month-old cattle were randomly allocated, within FEC ranked blocks, into treatment (Converge® 0.2g/L abamectin and 80g/L levamisole or Eclipse® Pour-on [Abamectin 10g/L and levamisole 200g/L]) or control groups. FECRTs were conducted 10 days after oral or topical administration. Four days later, following slaughter, abomasal and small intestinal trichostrongyloidea were counted using standard W.A.A.V.P. methodology.

In the field trial, 40 suitably naturally-infected 7-9 month-old beef or dairy cattle on 4 properties, identified as having ML resistant *Cooperia*, were randomly allocated into 3 treatment groups; Converge, Eprinex Pour-on (eprinomectin 5g/L), and Eclipse Pour-on or an untreated control group. FEC and coprocultures were conducted on the day of treatment and repeated on day 14, 21 and 28 post-treatment. FECRTs were calculated and FEC data were modelled with herd and treatment as fixed-effects in a zero-inflated negative binomial model that accounted for repeated-measures within cow.

In both trials stock handlers and laboratory staff were blinded to treatment.

Results: In the slaughter study, strongyle FEC 10days after treatment in the Converge (median 875epg: interquartile range 538-1625epg) and Eclipse (900epg: 525-1200epg) were 0epg. Total worm counts were dominated by adult *Ostertagia* (median 1900), *Trichostrongylus axei* (3700) and *Cooperia* (8800). Converge and Eclipse significantly reduced the total adult worm count by >99% and 100% respectively.

In the field trial, pre-treatment FEC ranged from 150 to 1100epg/farm. Converge and Eclipse reduced the total epg on days 14 and 21 on 3/4 farms by ≥99%; on the 4th farm the greatest efficacy was 93% and 97% respectively. The overall efficacy of Eprinex on day 14 was 92%, 61%, -30% and -31% in each of the 4 farms, and against *Cooperia* was 75%, 39%, -55% and -104%. On one farm *Ostertagia* eggs were also cultured on most days post-treatment in all groups.

Summary: These results indicate the inclusion of levamisole with an ML significantly increases the efficacy of an ML alone against ML resistant *Cooperia* in cattle.



SESSION G

Keynote Lecture

- **Livestock ectoparasites: integrated management in a changing climate** - R. Wall

Oral Communications

- **Emergent / re-emergent parasites and species migration: due to climatic changes and global warming or a consequence of human participation?** - G. M. Bulman
- **Adaptation of cattle ticks to new hosts, environment, and host location** - C. Almazan
- **Changes on distribution of the soft tick vector *Ornithodoros erraticus* in Portugal since the early 1990s: a comparison of two distribution surveys** - M. Figueiredo
- **New in vitro test to evaluate the resistance level of the cattle tick, *Rhipicephalus (Boophilus) microplus*, against acaricides** - L. Lovis
- **Transcriptomic analysis of the host response to infestation with the ectoparasitic mite *Psoroptes ovis* (*P. ovis*)** - S, T. G. Burgess
- **The role of scabies mite aspartic proteases in host selection and infestation processes** - D. C. Holt
- **Evolution of acaricide resistance: phenotypic and genotypic changes in field populations of *Rhipicephalus (Boophilus) microplus* in response to pyrethroid selection pressure** - A. J. Trees
- **Evaluation of different light or chemical traps for the control of *Culicoides* spp in sheep and cattle farms in Belgium** - B. Losson
- **Haematobia irritans: control through Essential Crude Oils (ECO) of Aguariabay (*Schinus molle* L.)** - M. O. Lapissonde
- **Population dynamics and control of the horn fly (*Haematobia irritans*) in a cattle farm from Tamaulipas, Mexico** - N. Soberanes
- **Functional genomics of the horn fly, *Haematobia irritans* (Linnaeus, 1758)** - L. Torres
- **Development of a diagnostic test for sheep scab based on biomarkers** - B. Wells

Posters

- **CLiKZiN, a new formulation of dicyclanil for the prevention of blowfly strike on sheep** - B. Hosking
- **Metabolic differences between pyrethroid sensitive and resistant horn flies, *Haematobia irritans*** - A. A. Marchiondo
- ***Rhipicephalus microplus*: Effect of the Essential Crude Oil (ECO) of Aguariabay (*Schinus molle* L.), *Lippia* (*Lippia alba* (Mill.) N.E. Brown) and *Carqueja* (*Baccharis articulata*)** - M. O. Lapissonde
- **Clinical findings and treatment of genital myiasis in ewes and rams in Greece** - ; E. Papadopoulos
- **Sarcoptic mange in guanacos: treatment with moxidectin and transmission of the disease to sheep and goats** - E. Papadopoulos
- **Influence of Herd Management on the Prevalence of Tick (Acari: Ixodidae) Infestation in Domestic Goats (*Capra hircus*)** - M. Sohail Sajid
- **Prevalence and associated risk factors of insects infesting domestic sheep (*Ovis aries*) of district Toba Tek Singh, Punjab, Pakistan** - M. S. Sajid
- **Evaluation of different light or chemical traps for the control of *Culicoides* spp in sheep and cattle farms in Belgium** - B. Losson
- **The Status Of Infestation In Small Ruminants (Sheep and Goats) With The Myiasis Causing Fly (*Oestrus ovis*) In Northern Nigeria** - B. D. J. George
- **Evaluation of tetrachlorvinphos as a feed -through insecticide for the control of horn fly (Diptera: Muscidae) developing in cattle manure** - O. S. Anziani
- **Epidemiology of *Oestrus ovis* larvae in goats in San Luis (Argentina)** - C. E. Rossanigo
- **Acaricidal activity of extracts from *Petiveria alliacea* against *Rhipicephalus microplus* resistant to acaricides** - J.A. Rosado-Aguilar
- **Sequence Analysis and Cloning of a Putative Serine Endopeptidase Inhibitor from the Cattle Tick *Rhipicephalus (Boophilus) microplus*** - L. Tirloni
- **Observed and predicted distribution of *Culicoides* and comparison with bluetongue outbreaks in Portugal (2005-2009)** - F. Boinas
- **Population dynamics of the sheep ked (*Melophagus ovinus*) in Patagonia Argentina** - M. Larroza
- ***Oestrus ovis* parasitism in Santa Ines and Ile de France young sheep** - A. F. T. Amarante
- **Seasonal evolution of pediculosis (*Linognathus stenopsis* and *Bovicola caprae*) in naturally infested goats in NW Patagonia** - F. Olaechea
- **Husbandry practices and acaricide control of *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) in Quindío - Colombia** - W. A. Cañón-Franco
- **First clinical trial for the biological control of psoroptic mange in cattle using entomopathogenic fungi** - B. Losson
- **Frequency and identification of Tabanidae (diptera) in relation to temperature and environment in an area subtropical Argentinian mount forest** - C. A. Luciani
- **Efficacy of fluzuron and flumethrin combination pour-on against a resistant field strain of *Bovicola limbatus*** - P. Qwalela
- **Efficacy of fluzuron and flumethrin combination pour-on against a resistant field strain of *Rhipicephalus (Boophilus) spp.* on a commercial cattle farm in South Africa** - P. Qwalela
- **First report of amitraz resistance in the cattle tick, *Rhipicephalus (Boophilus) microplus*, in Argentina** - C. Cutullé

- **Resistance Associated Factors To Acaricides In Rhipicephalus (Boophilus) Microplus In Brazil** - C. M. B. M. Rocha
- **Knowledge of Milk Producers on Cattle Ticks About Biology and Cattle Tick Control** - C. M. B. M. Rocha
- **The use of urea in a rotational grazing system and its effect on Rhipicephalus (Boophilus) microplus (Murrell and Barker, 2003) (Acari: Ixodidae)** - R. P. B. Wanderley Müller
- **Effect of urea on the tick Rhipicephalus (Boophilus) microplus mortality** - A. Pinto da Cunha
- **Characterization of workers from dairy cattle farms** - L. Nogueira Domingues
- **Integrated Control of Cattle Parasites** - L. Nogueira Domingues
- **Strategic Control of Rhipicephalus (Boophilus) microplus in dairy cattle** - P. Vieira Bossi Leite
- **Naturally infected cattle develop antibody responses against salivary proteins from H. irritans irritans** - M. Breijo
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Livestock ectoparasites: integrated management in a changing climate

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The prevalence of livestock ectoparasites is the result of a complex interaction of factors such as parasite and host abundance, host susceptibility, climate and, critically, farmer husbandry and intervention strategies, all of which change seasonally in space and time. Given the complexity of the interacting factors, the effects of any climate change on disease incidence are hard to predict, as accordingly are the optimal husbandry responses required to ameliorate any effects. Here cutaneous myiasis in sheep, by the blowfly *Lucilia sericata* in the United Kingdom, is used to highlight the impact of a range of such issues. Cutaneous myiasis would be expected to be highly sensitive to even small changes in climate and therefore provides a good model to illustrate the problems inherent in attempting to predict the effect of climate change on livestock disease incidence. Both simulation and spatial species distribution models, show that the range of elevated temperatures predicted by current climate change scenarios are likely to result in an elongated blowfly season with earlier spring emergence and a higher cumulative incidence of strike. Strike incidence would be expected to increase, particularly for ewes in early summer. However, under higher IPCC emissions scenarios (+3°C), parts of central and southern England may become too hot and dry for strike by *L. sericata* to persist in mid-summer. Under these conditions, it is possible that other, more pathogenic Mediterranean agents of myiasis, such as *Wohlfahrtia magnifica* could replace *L. sericata*. Nevertheless, the models suggest that simple changes in some husbandry practices, such as shearing or trap use, could have an important effect in reducing early season ewe strike incidences by *L. sericata*. The work reviewed here, suggests that climate warming is likely to increase the risk of fly strike incidence, with consequent animal welfare and economic problems. However, practical measures exist which, with modest changes in husbandry practices, should be able to manage expected increases in strike, under the range of climate changes currently predicted. The work demonstrates that attempts to predict the likely impact of climate change on disease incidence must take into account changes in farmer behaviour and animal management practices as well as parasite biology.

Emergent / re-emergent parasites and species migration: due to climatic changes and global warming or a consequence of human participation?

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Animal parasites possess amongst many others, two outstanding and distinctive qualities, they easily adapt to migration and advantage of climatic changes. Men are their irreplaceable allies, either by lax animal health controls, veterinarians who accept changes and migration patterns as inevitable, and cattlemen perhaps innocently or in their quest for short-term benefits, often conveniently overlook immediate and future damages. All three situations make them accessories after the fact. Losses and the cost of corrective sanitary measures are high.

Emergent Diseases (ED) are those discovered in the last 30 years and include known diseases whose agent has been revealed recently. Re-Emergent Diseases (RD) possess a history of previous discovery but with low incidence and have reappeared in the last two decades, with higher prevalence of cases or outbreaks, causing an impact in health or production in a geographical region.

Climatic changes from global heating exist and do not enter discussion, are commonly assigned to be the cause of species migration and a threat for further spreading of disease. In human diseases most papers foresee changes in the mode of transmission and in zoonosis. In veterinary science, however, case studies reveal a dominant system of unrestrictive migration and travel of both cattle and companion animals. This paper analyses 12 examples in cattle and sheep ectoparasites in the Argentine Mesopotamia and Patagonian regions.

These cover *Onchocerca cervicalis*, an ED in horses discovered in Formosa in 1984. *Dermatobia hominis*, the tropical warble fly known as *ura* in Argentina, Paraguay and Uruguay, an ED in new transitory habitats 850 km south. The horn-fly *Haematobia irritans*, entered Argentina from Brasil and Paraguay in late 1981. In less than 10 years it was taken on cattle and spread by man from the tropics and subtropics to the continental climate cattle farms in the centre of Argentina and even reached the north area of the cold Patagonia. *Dirofilaria immitis* or heartworm first described in large surveys in 1988 and 1989, has undergone a gradual migration and increase in prevalence rates along the large rivers and reached Buenos Aires. *Dracunculus insignis* is a rare dormant ED found in house dogs and a puma *Felis concolor concolor*, all in northern Formosa. *Raillietia auris* is an uncommon cattle ear-mite ED described in this same province in 1984. *Ornithodoros rostratus* an argasid tick in goat herds property of original people tribes moved over 400 km east from their first description in the arid west of Formosa near Salta. The ixodid tick *Amblyomma neumanni* found in the northwest of Argentina, but rarely in the northeast, was described during 2 consecutive years 600 km south, an ED way out of its known habitat. *Psoroptes cuniculi*, the goat ear-mite, follows a similar pattern. *Rhipicephalus microplus*, the common cattle ixodid tick and confined in an unstable balance to the north of Corrientes in 2000, has since reappeared as a RD in Entre Ríos and Buenos Aires, a direct result of cost restrictions in the official control campaign and drug resistance, a vivid example of migration and lack of control. In the Patagonia, *Melophagus ovinus*, the sheep ked, has migrated from the Atlantic coast to the Andes foothills and valleys and also registers a single case described in the south of Buenos Aires, a story of a ED in new geographical regions. Finally also in the vast Patagonia, eradicated *Psoroptes ovis* or common sheep mange mite, reappeared in two outbreaks, in Río Gallegos (Santa Cruz) in 1980 and on the island of Tierra del Fuego in 1997, in both cases parasite migrations through mismanagement and lack of control.

A strong sensation exists that the finding of new habitats for both known or relatively unknown parasites is far more frequent than informed, with a tendency to be accepted as a natural phenomena, at times predictable and in general considered unavoidable. In an attempt to better understand the growing list of ED and RD parasites and the diseases they cause in many new areas, the 12 chosen examples indicate that overall, the fundamental cause is animal migration, where man has helped and is responsible in many ways, thus diminishing the importance of easily blamed global warming and climatic changes. ED and RD will continue to appear, reappear and often become temporarily hidden. Man is a major player. Although mistakes can often be corrected, they must be avoided and not repeated.

Adaptation of cattle ticks to new hosts, environment, and host location

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Background: The cattle ticks, *Boophilus* spp. and *Amblyomma* spp. are the most economically important ectoparasites of cattle in Mexico and Latin America. *Boophilus* ticks were eradicated from the USA at the beginning of the last century, and after several efforts from the Mexican government, this tick remains under control and due to resistance to pesticides currently it is almost impossible to eradicate cattle ticks from Mexico and other Latin American countries. *Amblyomma cajennense* is native from the American Continent and although its bite is very aggressive and is known that it transmits several tick-borne pathogens, it has been underestimated. Herein we describe infestations of *B. microplus* ticks in hosts other than cattle from the Northeast of Mexico and Southern Texas, USA., as well as cases of *A. cajennense* infestations in newborn calves and oral cavity infestations.

Results: *B. microplus* tick infestations occurred from 2009-2010 in the Northeast of Mexico, and the area close to the border with Texas, USA; and infestations of dogs, horses, sheep, wild animals like the white tailed deer, the nilgai and the rat snake will be presented. Severe cases of infestations of newborn calves with *A. cajennense*, as well as adult tick infestations at the oral cavity in calves suffering from malnutrition will be discussed.

Conclusions: The cattle ticks are adapting to parasitize new hosts. The tremendous use of acaricides for the control of cattle ticks may be responsible of this adaptation. Cases of oral cavity infestations in calves will be presented. The role of the global warming, ecology and global change may be causing these changes.

Changes on distribution of the soft tick vector *Ornithodoros erraticus* in Portugal since the early 1990s: a comparison of two distribution surveys

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Ornithodoros erraticus is a soft tick normally found on pig holdings in Portugal and Spain which use traditional extensive production methods. It can transmit pathogens such as the spirochetes *Borrelia hispanica* and African swine fever virus (ASFV). During the 1980s, this tick was reported in six provinces in Spain and one province in Portugal (Alentejo). During the 1990s, a survey of its distribution was conducted as part of the ASF eradication programme in Portugal. Postal questionnaires were sent to veterinarians in 76 counties in four regions of Portugal along the Spanish border. Thirty-two veterinarians in 24 counties reported the presence of the parasite (a reply rate of 38%). A follow-up questionnaire was sent to these veterinarians to complement the information obtained previously; this had a response rate of 76%.

Follow-up field surveys of the 281 farms with suspected presence, using manual collection and/or an adapted CO₂ trapping system, confirmed the presence of ticks on 39 farms (14% of the total investigated) in 13 counties of the 24 investigated. The tick was detected for the first time in Algarve and in six counties of Alentejo.

A subsequent distribution study was conducted in 2009 via a telephone survey of 76 veterinarians working on farms with Alentejano-breed pig herds (which accounted for over 95% of the responses received in the first survey). Fifty-two farms were investigated in 12 counties in Alentejo and ticks were detected on nine, in three counties. No ticks were found in 11 farms surveyed in 2 counties in Algarve.

A comparison of the two studies showed that there was a decrease in the premises found with the parasite, and the majority of farms previously found to be infested were not used for pig production anymore. Nevertheless, new infestations were detected during the more recent survey. In both surveys the CO₂ trap was more efficient than manual collection.

An analysis of the influence of factors such as the type of premises, the production method and the size of the farm will also be presented.

New *in vitro* test to evaluate the resistance level of the cattle tick, *Rhipicephalus (Boophilus) microplus*, against acaricides

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Background: The cattle tick *Rhipicephalus (Boophilus) microplus* is a key vector of bovine babesiosis and causes high economic losses to farmers. The widespread use of acaricides has led to drug and multidrug resistance against nearly all commercially available acaricides. In this context, *in vitro* tests are used to determine the resistance against acaricides in order to evaluate its distribution and to choose the most efficient compounds for treatment.

Here we present a new *in vitro* test, the Larval Tarsal Test (LTT), and its comparison with the Larval Packet Test (LPT), recommended by the Food and Agriculture Organization (FAO).

Methods: Both tests are based on *in vitro* exposure of larvae to serial dilutions of acaricides. However, the type of contact with the active ingredient (AI) differs among the tests. The LTT is performed in microtiter plates and larvae have tarsal contacts with the AI coated at the bottom of the wells while in the LPT, larvae are enclosed in filters soaked with AI and forming a packet. A sensitive (Muñoz) and a resistant *R. microplus* reference strain (Ultimo) were selected and their susceptibility to 10 compounds from 6 classes (organophosphates, synthetic pyrethroids, formamidines, macrocyclic lactones, carbamates and pyrazols) was evaluated with the two tests.

Results: Both bioassays are equally sensitive at measuring resistance ratios and the LTT requires up to 150-fold lower doses to detect resistance than the LPT. Additionally, by avoiding the handling of larvae and using multi-well plates, the LTT is much more time-effective than the LPT.

Summary/Conclusions: The LTT is a sensitive, time-effective *in vitro* test which allows testing of a large number of compounds and doses in a single test. The LTT is therefore a suitable test for the assessment of the level of resistance of *R. microplus* and is currently used to test field strains originating from South America, South Africa and Australia.

Transcriptomic analysis of the host response to infestation with the ectoparasitic mite *Psoroptes ovis* (*P. ovis*)

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Background: 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 infestation is directed against mite secretory/excretory products and is typical of an immediate hypersensitivity reaction with a clear bias towards a Th2 type immune response. Sheep scab is currently controlled by chemical intervention however, there are concerns over chemical residues in meat, their effect on human health and the environment. There is also an emerging problem of resistance to the drugs used for treatment, which are also relied upon for the control of gastro-intestinal parasites. Vaccine candidates have been identified by fractionating mite protein extracts however, efficacy is low and the isolation of the individual protective proteins has proven difficult. To further improve development of a sheep scab vaccine we must first gain a better understanding of the host-parasite relationship. Sheep scab is an excellent model with which to achieve this as both host and parasite material is readily accessible from the host-parasite interface, the skin.

Methods: In order to further characterise the early events (first 24 hours) in the host response to infestation with *P. ovis* we utilised an ovine transcriptome microarray to interrogate the transcriptional events associated with the local skin response to infestation.

Results: Clustering and network analyses of this dataset have enabled the identification of key signalling events involved in the host response to infestation with *P. ovis*. This has provided unique insights into the pro-inflammatory response instigated by *P. ovis*, with a key role being identified for the transcription factor NF- κ B. It has also provided vital information regarding the nature of the mite factors that may trigger this response. Crucially these studies have elucidated a number of temporal patterns by which the host immune system is regulated following exposure to *P. ovis*.

Summary: The findings of this study have dramatically increased our knowledge and understanding of the host response to *P. ovis*, identifying potential strategies for novel methods of disease control, including vaccine development.

The role of scabies mite aspartic proteases in host selection and infestation processes

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Background: Scabies is a disease of worldwide significance, caused by infestation of the skin with the “itch mite” *Sarcoptes scabiei*. *S. scabiei* causes mange in many livestock and companion animals as well as in wild animal populations including foxes, camels, bears, wombats and koalas. In addition up to 300 million humans are estimated to suffer from scabies at any one time, primarily in disadvantaged communities worldwide. Aspartic proteases in many parasitic organisms facilitate the invasion and digestion of host tissue and are therefore considered to be potential targets for development of novel therapeutic agents. Emerging resistance of scabies mites to available treatments emphasises the need to develop effective anti-parasite therapies.

Methods and Results: We collected scabies mites from the skin of infested humans and animals. Aspartic protease activity in whole mite extract was measured using a fluorogenic substrate. Aspartic protease activity was detected which was inhibited by the aspartic protease inhibitor pepstatin A. A single aspartic protease like sequence identified in a library of scabies mite expressed sequence tags was cloned into the prokaryotic expression vector pQE-9 and expressed in *E. coli*. The recombinant protein was used to raise polyclonal antibodies in mice, which were used for immunolocalisation of the aspartic protease in sections of infested skin. The aspartic protease localised to structures within the mite corresponding to the mouth parts, anterior mid gut and the hind gut lumen, indicating a role in digestion. Enzymatically active recombinant protein was tested for the ability to digest various skin macromolecules. Digestion of fibrinogen and fibronectin was observed which could be inhibited with pepstatin A, however no digestion of laminin or collagen III was observed. Recombinant aspartic protease from human scabies mites was able to digest human haemoglobin but had little effect on canine haemoglobin, indicating a role of the enzyme in host specificity.

Conclusions: The scabies mite aspartic protease may play a key role in host specificity, digesting host skin macromolecules as part of the infestation process. The identification or design of inhibitors with specific activity against the scabies mite aspartic protease may be candidate molecules for effective chemotherapy for scabies.

Evolution of acaricide resistance: phenotypic and genotypic changes in field populations of *Rhipicephalus (Boophilus) microplus* in response to pyrethroid selection pressure

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There have been few, if any, studies of arthropod field populations quantifying the kinetics of evolution of phenotypic and genotypic resistance to chemicals in response to the presence or absence of selection pressure. A prospective intervention study was undertaken over two years in Mexico to measure changes in resistance phenotype and genotype in the presence or absence of pyrethroid selection pressure on field populations of *Rhipicephalus (Boophilus) microplus* ticks on 11 farms with varying degrees of pyrethroid resistance. The resistance phenotype was evaluated by bioassay in a larval packet test expressed as resistance factor (RF) derived from probit analysis of dose mortality regressions, and resistance genotype by an allele specific PCR (AS-PCR) to determine the frequency of a sodium channel mutation (F1550I) associated with pyrethroid resistance. To validate the AS-PCR, a Pyrosequencing™ method was developed to detect the F1550I mutation. There was good concordance with the genotypes identified by both Pyrosequencing™ and AS-PCR ($Kappa: 0.85$). On five farms cypermethrin (CY) was exclusively used at intervals and on six amitraz was used. On two of the five CY treated farms, the experiment was prematurely terminated due to unacceptably high levels of tick resistance. For all five farms, after 8-24 months of continued selection pressure with CY the RFs had increased 2-125 fold. The frequency of the resistance allele increased on all five farms from a starting range of 5-46 % to a range of 66-95 % after 8-24 months. On six farms treated with amitraz neither the RF nor the frequency of the resistance allele changed. A clear correlation between the phenotype and genotype was found in three of four treated farms confirming that the F1550I mutation is a major cause of synthetic pyrethroid resistance in Mexico. These results show that the pyrethroid resistance trait is stable (> 2 years) and that resistance is acquired much faster than it is lost. Hence, alternation of pyrethroid acaricide with other chemicals is likely to lead to the stepwise acquisition of synthetic pyrethroid resistance but no additional prolongation of its efficacious lifespan.

Evaluation of different light or chemical traps for the control of *Culicoides* spp in sheep and cattle farms in Belgium

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Background: Biting midges (*Culicoides* spp. Ceratopogonidae) are important vectors of animal diseases such as bluetongue and horse sickness. In Belgium, the first outbreak ever recorded of bluetongue was reported in 2006. In 2007-2008 the disease was responsible for huge economical losses through animal morbidity and mortality as well as movement restrictions. Several indigenous species of *Culicoides* were shown to act as competent vectors. Additionally to vaccination, vector control was proposed as a control measure. In the present study the trapping efficacy of several commercially available traps was compared to a reference light trap, the OVI (Onderstepoort Veterinary Institute) trap.

Methods: Seven cattle and sheep farms were selected. In each farm an OVI trap was operated outside the animal accommodation for 2 consecutive weeks. Inside the buildings and during the same period an OVI trap or the test material was operated 2 nights per week each. The trapped insects were collected on a daily basis and kept in 80° alcohol until identification. Thus each device was evaluated over a two week period. The evaluation started in early July and ended up at mid October 2010. Seven different traps were evaluated i.e. CDC™ light trap, white and yellow sticky tapes, UV trap for muscid flies (Belagri™), white light trap (EVS CO₂ Mosquito trap™) with or without solid CO₂ and cattle urine. The trapping efficacy of each device was compared to the OVI reference trap. Biting midges were identified according to morphological criteria.

Results: A total of 21,731 *Culicoides* were trapped with 82.5% belonging to the *C. obsoletus/scoticus* complex. Nineteen different species were identified. CDC™ and Belagri™ traps had a relative trapping efficiency of 10.9 and 33.1 % respectively. Other traps had negligible or no trapping efficacy for biting midges.

Conclusions: This study confirms the excellent efficacy of the OVI trap for trapping or monitoring biting midge populations in Western Europe at least. The available chemical or light traps which were evaluated seem unsuitable.

***Haematobia irritans*: control through Essential Crude Oils (ECO) of Aguaribay (*Schinus molle* L.)**

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Currently, the products used to treat bovines infested by *Haematobia irritans* are synthetic, what brings at least two inconvenients: the resistency presented by flies relatively fast and the negative impact on the environment and the users.

The ECO are substances obtained from vegetables that provide a solution to the above mentioned problems. They are totally biodegradable and are formed by more than two hundred components, that means that a priori the resistancy is imposible.

The present work belongs to a group of essays to study this and other ECO, what brings satisfactory results.

For this study were used six hundred forty eight steers crossbred of Braford, Brangus and Limangus, which weighted between three hundred and five hundred kilograms. Body Conditions Score from 3 to 4,5 (scale 1 – 5). They had approximately five hundred flies each. The product used was ECO of *Schinus molle* L. at 5 % in oil corn solution. 40 cc were spread over the animals' backs with a 10 cm³ automatic syringe with pour-on cannula. The animals were observed at the moment of the application, eight hours after that, and three days later.

During the treatment all flies that were reached by the solution fell immediately. Consequently, the ones that touched the animals' wet backs remained weak and fell later. Eight hours after the process it was observed that some flies were placed in the ventral part of the steers. At the third day the animals were again infested, but the amount they presented did not exceed eighty flies each.

To conclude, it could be stated that similarly to previous essays, the ECO of *Schinus molle* L. shows a strong insecticide and repellent power against *Haematobia irritans* what provides an alternative to the hard synthetic products. Nevertheless, its residual effect is short.

Population dynamics and control of the horn fly (*Haematobia irritans*) in a cattle farm from Tamaulipas, Mexico

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Background: The horn fly *H. irritans*, is one of the most economically important ectoparasites of cattle and currently became a huge problem of health of cattle from extensive areas of Mexico and Latin America. The aims of this research were: To determine the population dynamics of *H. irritans* in a farm located at Soto La Marina, Tam., Mexico and to evaluate treatment programs against this arthropod.

Results: Horn flies, from a group of 12 bovines without treatment during the time of the experiment, were counted monthly from one side of each bovine during one year (March 2007-March 2008) to obtain the population curve during a year. Also, several chemical treatments were evaluated against horn fly infestations. *H. irritans* was found over cows during the whole year with variations related to temperature, humidity, and water precipitation. Three peaks of flies were found during January, March and from July to August. Treatments based on phosphorades, combination of phosphorades and pyrethroids, and ivermectinas were applied.

Conclusions: Population dynamics curves were built. The results and the best treatment against *H. irritans* are discussed.

Functional genomics of the horn fly, *Haematobia irritans* (Linnaeus, 1758)

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Background: The horn fly, *Haematobia irritans* (Linnaeus, 1758) (Diptera: Muscidae) is one of the most important ectoparasites of pastured cattle. The aim of this study was to conduct a functional genomics study in female horn flies using expressed sequence tags (EST) analysis and RNAi.

Results: A cDNA library of adult female *H. irritans* was constructed. 2,160 high quality horn fly ESTs were sequenced and assembled into 992 unigenes (178 contigs and 814 singlets) representing molecular functions such as serine proteases, cell metabolism, vitellogenesis, cytoskeleton, DNA replication, cell response to stress and infection, cell proliferation and cell-cell interactions, and development. Functional analyses were conducted using RNAi for the first time in horn flies. Gene knockdown by RNAi resulted in higher horn fly mortality (for functional groups serine protease and protease inhibitor), reduced oviposition (VTG, FER and vATPase) or both (proteasome component, immune response and 5'-NUC) when compared to controls. Silencing of ESTs in the ubiquitination and vATPase functional groups did not affect horn fly mortality and oviposition or reduced mortality when compared to controls, respectively.

Conclusions: These results advance the molecular characterization of *H. irritans* and suggest candidate protective antigens for the development of vaccines against this important ectoparasite of cattle.

Development of a diagnostic test for sheep scab based on biomarkers

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Sheep scab is a highly contagious ectoparasitic disease caused by the mite, *Psoroptes ovis*. The disease causes intensely pruritic lesions with severe dermatitis and has become a major welfare and production issue in many countries. Control and treatment is complicated by various factors, including mite biology and resistance problems, indicating that early diagnostic tests are likely to be important for future disease control. In addition, sheep scab has recently become a notifiable disease in Scotland, further illustrating the usefulness of diagnostic test development.

Recent microarray studies have identified over 600 host genes differentially expressed in circulating leukocytes following *P. ovis* infestation. Many of these genes encode proteins known to be involved in inflammatory responses. These proteins were filtered, annotated and ranked using a combination of Ingenuity Pathway Analysis and Genespring GX software, to identify the most promising biomarker candidates. This resulted in the identification of a final list of 178 potential biomarker candidates, with the final selection being based on suitable antibody availability. To date, thirteen proteins from this list have been tested with heterologous anti-human antibodies against a range of sera from sheep scab infested and naïve sheep. Promising results have been obtained for three of these inflammatory proteins and further characterisation has demonstrated that they are rapidly up-regulated following infestation and that their serum levels correlate well with disease progression and severity. Further studies will investigate sera concentration trends post-treatment, as it is crucial that any potential biomarker falls off rapidly to base levels after treatment. Circulating levels of these proteins in healthy sheep are currently being established and the selected proteins will be further evaluated using positive and negative field samples, and samples from sheep with other common inflammatory conditions. Sequence analysis has been performed on one of the biomarker candidates and the expression of a novel ovine recombinant protein is currently underway. This will facilitate the production of an ELISA which will allow cost effective, extensive screening of serum samples from a range of diseased and healthy animals. The possibility of combining the biomarker diagnostic with a *P. ovis* specific antibody test currently being developed at Moredun will also be investigated, to provide a full profile of disease status of individual animals in a flock.

CLiKZiN, a new formulation of dicyclanil for the prevention of blowfly strike on sheep

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Background: Dicyclanil (CLiK[®]; 50 g/L) was first used as a preventative of blowfly strike in 1998. CLiK is now the biggest selling preventative worldwide and is characterized by its long protection period (e.g. 18-24 weeks in Australia). In some environments, sheep farmers require only 6-12 weeks protection (country-dependent), and a short meat withholding period. This led to CLiKZiN, a new formulation containing 12.5 g/L dicyclanil. Five efficacy studies were conducted. Two were for dose establishment and three were conducted on commercial farms in the target regions.

Methods: In an implant study three formulations (7.5 g/L dicyclanil, 12.5 g/L and 20 g/L) were applied to sheep. Sheep were challenged with larval implants (*Lucilia cuprina*) and inspected at 2-weekly intervals from 4-14 weeks post-treatment. In a fly release study, sheep were challenged with adult *L. cuprina*. Untreated controls were compared with sheep treated with either of three products (12.5 g/L dicyclanil [CLiKZiN], 60 g/L cyromazine, or an increased volume of 12.5 g/L dicyclanil (= 20 g/L formulation). Sheep were exposed to *L. cuprina* released into the rooms 4 days/week for 12 weeks post-treatment. Strikes were scored after treatment. In the field studies 18,410 sheep across 100 sites were treated with CLiKZiN and inspected at regular intervals after treatment.

Results: In the implant study inadequate efficacy occurred in the 7.5 g/L group. The 12.5 g/L group demonstrated 10-12 weeks efficacy while the 20 g/L group retained a small advantage in protection. In the fly release study only eggs on the controls developed into strikes. Treated groups displayed 100% control but larval death took longer near the end of the study. In the field studies, efficacy was determined from trial sites with blowfly exposure at critical times. CLiKZiN warranted up to 11, 6-9 and 8 weeks protection in Australia, New Zealand and Europe, respectively.

Summary: The controlled studies demonstrated that 12.5 g/L dicyclanil applied at the proposed dose, provided 10-12 weeks protection under experimental conditions. These findings were reinforced in the field although shorter protection periods occurred in New Zealand and Europe as the result of treating coarse-woolled sheep and the wetter climate.

Metabolic differences between pyrethroid sensitive and resistant horn flies, *Haematobia irritans*

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The objective of this study was to identify metabolic differences between pyrethroid sensitive and pyrethroid resistant adult horn flies systemically treated with doses of permethrin at 3 µg/mL and 30 µg/mL at 2, 12 and 24 hr feeding intervals. Treatments were carried out using sexed horn flies fed *in vitro* on permethrin-spiked citrated bovine blood at 25°C and then the flies were flash frozen for analysis. Samples of adult male and female horn flies were homogenized in bulk yielding 4 technical replicates. Each replicate was extracted and run on the Metabolon Platform Technology to identify biochemical pathway changes. A total of 468 biochemicals were classified, 295 named/identified and 173 unnamed. Welch's two-sample t-test was used to determine whether the means of two populations were different at P≤0.05 significance. Each sex was analyzed statistically for both a longitudinal analysis (time) and within concentrations of permethrin. The resistant and susceptible cohorts were compared for both males and females. Female resistant horn flies were less sensitive to permethrin than male resistant horn flies with an R/S ratio of 12X. Female susceptible horn flies were more sensitive to permethrin than male susceptible horn flies. Permethrin-resistant horn flies showed significantly different energy metabolism profiles in maltose/glucose metabolism, NAD metabolism, 2-methylcitrate metabolism and fatty acid/lipid metabolism – formation of carnitines, when compared to susceptible horn flies. Permethrin-resistant horn flies mobilized glycogen through the production of glucose-6-phosphate more readily than susceptible horn flies to harvest energy under stress-survival response induced by permethrin exposure. Permethrin-resistant female horn flies had higher maltose levels than permethrin-resistant male horn flies. Permethrin-sensitive horn flies had decreased levels of NAD precursors suggesting that these horn flies are more efficient in NAD metabolism to drive reactions more quickly as a survival mechanism. Permethrin-resistant horn flies had lower levels of 2-methylcitrate, methylfumarate and methylsuccinate. Carnitine species increased in the permethrin-resistant male horn flies. The largest differences in metabolites in this study were observed between permethrin-resistant male and female horn flies with the higher energy metabolism in permethrin-resistant horn flies possibly conferring an energetic advantage for survival.

***Rhipicephalus microplus*: Effect of the Essential Crude Oil (ECO) of Aguaribay (*Schinus molle* L.), Lipia (*Lippia alba* (Mill.) N.E. Brown) and Carqueja (*Baccharis articulata*)**

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The Essential Crude Oil has been demonstrated to be an efficient product for the control of ectoparasites, such as Horn flies (*Haematobia irritans*).

This work is based on studies that have been realised with the ECO obtained from Aguaribay which is a vegetable species native to America.

Until now, the efficacy shown on the control of the Horn flies meant that it could be used as an alternative to the hard synthetic products.

With the purpose of extending the spectrum utilization of Aguarubay it was tested on ticks. On the other side, different oils were tried for the same purpose.

Synthetic products have a negative impact on the workers' health and the environment. That is why the aim of this work is to probe the effectiveness of these three crude oils and evaluating their effect over the exoskeleton.

Each oil was utilised in 25 %, 50 % and 75 % concentration of water solution; and Cipermetrina concerning the lab prescriptions.

Firstly, engorged ticks were recollected from naturally infested bovines. The parasites were washed and the most alive ones were selected. Seven ticks per oil concentration were put in Petri dishes: six of them were treated and the other one acted as control. They were directly observed for 60 minutes. Finally, all the ticks were sent to the histology lab.

100 % of mortality was produced at 33 minutes and 5 seconds in all oils except for Carqueja which caused this effect at only 75 %.

Cipermetrina did not provoke mortality at all, and the control group stayed alive up to the end of the essay.

It was observed that treated ticks presented a thinner kitina in opposition to the control group.

To conclude, the tested ECO showed a parasiticide effect and a priori they might cause damage in the kitina of the exoskeleton. Furthermore, the advantage of these natural products is that they allow a healthier usage for farmers and the environment as they are biodegradable.

Clinical findings and treatment of genital myiasis in ewes and rams in Greece

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Background: Genital myiasis in sheep is important, because it causes discomfort of affected animals and impedes reproductive process. Here, we describe the clinical features and the treatment of genital myiasis in ewes and rams.

Methods: We examined 7 ewes and 9 rams with genital myiasis. External genitalia of the animals were examined clinically. Standard parasitological techniques were used for collection and identification of larvae from the affected sites.

Results: In ewes, symptoms of genital myiasis included valvular oedema, muco-haemorrhagic discharge, presence of hyperaemia and petechiae in vaginal wall and subcutaneous fistulae around the vulva. In rams, symptoms included difficult and partial exteriorising of penis and markedly thickened prepuce with fistulae along the tissue. Presence of dipteran larvae was consistently observed. *Wohlfahrtia magnifica* larvae were identified. For treatment, mechanical removal of the larvae was initially carried out by using medical forceps after massaging affected sites (peri-vaginal area or prepuce). This was followed by thorough cleansing of the area with 2% chlorhexidine solution (1:40 in water); removal of remaining larvae was then performed. Pharmaceutical treatment included administration of moxidectin 1% inj. sol. (0.3 mg per kg bodyweight, twice 10 days apart). In ewes, treatment also included systemic administration of a broad-spectrum antimicrobial agent. Mechanical removal of larvae was repeated 8 to 10 days after initial treatment; animals were monitored thereafter. In all cases, treatment was successful. However, 5 of the 16 animals (2 ewes, 3 rams) were culled, as the lesions would be causing difficulties in mating or lambing.

Conclusions: Genital myiasis in sheep can be a problem in extensive sheep-production systems and can lead occasionally to interfering with the reproductive process, hence leading to culling of affected animals. Moxidectin, although not licenced against cutaneous myiasis, was found to be of help as part of the therapeutic regime.

Sarcoptic mange in guanacos: treatment with moxidectin and transmission of the disease to sheep and goats

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Objective: We detail the therapeutic approach to sarcoptic mange in two guanacos and we present evidence of transmission of the disease to neighbouring sheep and goats.

Methods: Two guanacos were diagnosed with sarcoptic mange, confirmed on clinical evidence and recovery of mites from skin scrapings (by using standard parasitological techniques). Each animal was treated twice, 10 days apart, with moxidectin 1% inj. sol. given subcutaneously. Dose rate was 0.2 mg per kg bodyweight. After the injections, safety observations were made for local or general adverse reactions. Moreover, clinical examinations were performed and skin scrapings were collected and examined by using standard parasitological techniques.

Results: After treatment, neither changes were seen at the site of injection, nor were general reactions recorded in either guanaco. No mites were recovered 21 d post-treatment. Skin lesions had disappeared by the 42nd day post-treatment. Thereafter, sheep and goats neighbouring the guanacos developed sarcoptic mange, confirmed by clinical findings and recovery of mites from skin scrapings. In contrast, horses, donkeys and dogs, also neighbouring the guanacos, did not develop lesions characteristic of sarcoptic mange, nor were mites recovered from skin scrapings from these animals.

Conclusions: It is concluded that moxidectin can be used for the treatment of sarcoptic mange in guanacos. The findings support a hypothesis that mites may be transmitted easily among ruminants. The findings are in line with results of previous *in vitro* studies, indicating that mite strains from ruminants belong to the same cluster.

Influence of Herd Management on the Prevalence of Tick (Acari: Ixodidae) Infestation in Domestic Goats (*Capra hircus*)

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This paper describes the influence of herd management on the prevalence of goat tick infestation in lower Punjab, Pakistan. For this purpose, based on two stage cluster random sampling, a total of 136 groups of goats were surveyed for collecting information about following parameters: (a) herd size; (b) nature of herds; (c) farming pattern; (d) type of housing; (e) animal keeping; (f) floor pattern, and (g) hygienic measures. Within settled farms and nomadic herds, various parameters recorded were: (a) farmer's trend of therapy; (b) trend of consultancy; (c) therapeutic approach of farmers; and (d) choice of drugs. The selected herds were visited twice a month to collect relevant information on a pre-designed questionnaire containing open and closed type of questions. Of the total 136 groups of goats, the highest ($P<0.05$) proportion of herd was those having a size of 20-40 goats/herd followed in order by 10-20 goats/herd, 40-60 goats/herd, 60-80 goats/herd, and > 80 goats/herd (05/136; 3.6%). 109 of 136 (80.1%) were settled farms and 27 of 136 (19.8%) were nomadic herds. Of 109 farms, 81.6% (89/109) were associated with large ruminant farms while 20% (18.3%) were separate goat farms. There was significantly higher ($P<0.05$) proportion of open housing system, uncemented (kacha) floor and free animal keeping practice in the surveyed farms. The highest prevalence of tick infestation ($P<0.05$) was found in the herd size of 40-60 goats/herd and nomadic herds. Closed housing, uncemented floor pattern were having the highest ($P<0.05$) prevalence of ticks followed in order by semi-closed housing, partially cemented floor pattern and open housing, cemented floor pattern. Tethered animals were more likely infested ($P<0.05$) by ticks as compared to free housed. These observations provided a solid foundation that will aid in the planning of tick control program in lower Punjab.

Prevalence and associated risk factors of insects infesting domestic sheep (*Ovis aries*) of district Toba Tek Singh, Punjab, Pakistan

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An epidemiological survey was conducted from March 2009 to February 2010 in order to determine the prevalence and associated risk factors of insects infesting domestic sheep (*Ovis aries*) of district Toba Tek Singh, Punjab, Pakistan. A total of 5640 sheep were examined for the assessment of prevalence of ectoparasitic infestation. Out of 4020 animals examined, 684 were found positive (17.02%) for insects including lice (36.57%), fleas (3.23%) and flies (1.62%) were recorded. Amongst the identified species of fleas, the prevalence of *Ctenocephalides* (*Ct.*) *felis* was found higher (1.84%; 71/3864, $P < 0.05$; OR= 1.94) as compared to that of *Ct. canis* (0.96%; 37/3864). Among lice, *Haematopinus eurysternus* (13.56%; 524/3864 $P < 0.05$; OR= 3.97) was found predominant specie followed in order by *Damalinia bovis* (9.08%; 351/3864; OR= 2.53) and *Linognathus vituli* (3.80%; 147/3864). Only one species of flies i.e. *Stomoxys calcitrans* (3.42%; 132/3864) was found prevalent in the study area. Amongst various breeds of sheep, Cholistani (20.48% (85/415); OR= 1.46) indicated highest insect infestation followed in decreasing order by Kajli (17.48% (271/1550); OR= 1.75), Thalli (16.44% (226/1375); OR= 1.11) and Lohi (15.00% (102/680). In various seasons studied in the present survey, winter was having highest prevalence of fleas (7.87%; 76/966) and lice (47.72%; 461/966) while prevalence flies reached to their peak in spring season (5.80%; 56/966). It was observed that the prevalence of insects was higher in animals kept on non-cemented (uncemented) floor (19.19%; 1556/8108; OR=2.17) followed in order by partially cemented/bricked (12.19%; 767/6290; OR= 1.29) and cemented floor (9.85%; 485/4922) while feeding system, housing system and animal keeping methods were not found associated with the prevalence of insects ($P > 0.05$).

Evaluation of different light or chemical traps for the control of *Culicoides* spp in sheep and cattle farms in Belgium

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Background: Biting midges (*Culicoides* spp. Ceratopogonidae) are important vectors of animal diseases such as bluetongue and horse sickness. In Belgium, the first outbreak ever recorded of bluetongue was reported in 2006. In 2007-2008 the disease was responsible for huge economical losses through animal morbidity and mortality as well as movement restrictions. Several indigenous species of *Culicoides* were shown to act as competent vectors. Additionally to vaccination, vector control was proposed as a control measure. In the present study the trapping efficacy of several commercially available traps was compared to a reference light trap, the OVI (Onderstepoort Veterinary Institute) trap.

Methods: Seven cattle and sheep farms were selected. In each farm an OVI trap was operated outside the animal accommodation for 2 consecutive weeks. Inside the buildings and during the same period an OVI trap or the test material was operated 2 nights per week each. The trapped insects were collected on a daily basis and kept in 80° alcohol until identification. Thus each device was evaluated over a two week period. The evaluation started in early July and ended up at mid October 2010. Seven different traps were evaluated i.e. CDC™ light trap, white and yellow sticky tapes, UV trap for muscid flies (Belagri™), white light trap (EVS CO₂ Mosquito trap™) with or without solid CO₂ and cattle urine. The trapping efficacy of each device was compared to the OVI reference trap. Biting midges were identified according to morphological criteria.

Results: A total of 21,731 *Culicoides* were trapped with 82.5% belonging to the *C. obsoletus/scoticus* complex. Nineteen different species were identified. CDC™ and Belagri™ traps had a relative trapping efficiency of 10.9 and 33.1 % respectively. Other traps had negligible or no trapping efficacy for biting midges.

Conclusions: This study confirms the excellent efficacy of the OVI trap for trapping or monitoring biting midge populations in Western Europe at least. The available chemical or light traps which were evaluated seem unsuitable.

The Status Of Infestation In Small Ruminants (Sheep and Goats) With The Myiasis Causing Fly (*Oestrus ovis*) In Northern Nigeria

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Background: Small ruminants (sheep and goats) are a major source of income and security to the resource-poor livestock farmer/keeper in northern Nigeria. In addition to all other constraints and prevalent debilitating diseases both farmer and animals contend with, oestrosis appears to be of major concern in this group of animals. Infestations appear much higher in frequency during the rainy season (May to September). Pathophysiology of ovine and caprine oestrosis include strong inflammation presenting mucosal hypereosinophilia and mastocytosis, mucus hypersecretion and nasal discharge (Nguyen *et al.*, 1996; Dorchies *et al.*, 1998).

This is the background from which a prevalence study of oestrosis in sheep and goats was conducted during a 24month period (Nov. 2005 to Oct. 2007).

Methods Sampling: The sample size was obtained using the formula outlined by FAO, (1990).

The heads of slaughtered sheep and goats were purchased from butchers on weekly basis from abattoirs in five local government areas in Kaduna State. These were transported in polythene bags to the Entomology Laboratory of the Department of Veterinary Parasitology and Entomology where they were hand - sawn vertically through the nasal cavities, using forceps and pen knife to expose the nasal cavities, turbinate bones and frontal sinuses. With the aid of a hand lens and a pair of hand forceps, larvae were collected and examined as described by Horak (1977). The number of recovered larvae per head were recorded. Tissue sections of the turbinates were also collected and preserved in 10% formalin for histopathological studies.

Histopathological Studies: Sections of the nasal cavity and turbinate bones were processed using Technicon tissue processor. The tissues were sectioned and stained using Haematoxylin and Eosin (H & E) stain and mounted on glass slides. These were examined for histopathological lesions using the methods described by Kiernan (1990).

Results: In sheep, there was no significant difference ($P>0.05$) in infestation of *Oestrus ovis* according to seasons (dry 24.3% and wet 24.5%) seasons). In goats, infestation was higher ($P<0.05$) during wet (22.7%) than in dry season (14.7%). Histopathological lesions included irregularities of the venous sinuses, oedema, congestion and increase in fibrous tissue of the submucosa, which was infiltrated with polymorphonuclear leucocytes. To be discussed further.

Evaluation of tetrachlorvinphos as a feed –through insecticide for the control of horn fly (Diptera: Muscidae) developing in cattle manure

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Background: the efficacy of an insecticide used as a feed additive against the immature stages of a pyrethroid resistant population of *Haematobia irritans* was evaluated.

Methods: six Holstein steers 300 -350 kg were used as experimental animals. For three consecutive days, three of the steers were individually treated by adding tetrachlorvinphos (Rabon ® 97.3 % oral larvicide, KMG-Bernuth Inc, USA) to a corn ration at a feeding level of 70 mg per 45 kg body weigh daily. The rest of the steers remained as control animals. Bioassays of manure from each experimental animal were carried out before and after the three days treatment was finished. No manure bioassays were performed during treatment period. Adult horn flies, from a population with antecedents of pyrethroid resistance, were collected from naturally infested cows and the produced eggs were inoculated in manure of treated and untreated steers. After egg inoculations, manure samples were incubated at 27 °C and 50-60 RH for 7 days, the pupae were extracted by washing and then hold at 27 °C and 60-70 % RH until all the emerging adults died. The number of pupae and number of emerging adults were recorded and the percent of inhibition of development was calculated by comparing horn fly production in the manure from treated and control steers. The effectiveness of the treatment was determinate by comparing numbers of pupae and adult flies recovered with the numbers of eggs placed in the manure. Statistical differences in the distribution of pupae and adults emerged of *H. irritans* between both groups were measured using the “t” student test ($p < 0.05$).

Results: in the bioassays of manure carried out before treatment, the range of pupation observed were 60 % and 63 % and adult emergence of 56 % and 59 % for control and treated group respectively ($p > 0,606$). Meanwhile, horn fly did not survive in the manure of treated group collected on the first and second days after completion of treatment ($p < 0.0001$). On the third day, the activity of the insecticide still persisted and differences between the number of pupae of both groups remained significant ($p = 0,0275$). No significant differences were observed after ward between treated and control groups.

Epidemiology of *Oestrus ovis* larvae in goats in San Luis (Argentina)

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Introduction: Oestrosis is a myiasis caused by the larvae of the fly *Oestrus ovis* (Oo) (Linné 1761, Diptera: Oestridae). The larvae are obligatory parasites of nasal and sinus cavities of sheep and goats (cavitary myiasis). The development of larvae in the nasal-sinus cavities, can cause severe clinical signs including breathing difficulties, nasal discharge and emaciation, which together with the annoyance caused by the adult flies, may lead to significantly reduced animal production and economic losses. This parasite is found worldwide and in some regions of Argentina reached a 92% prevalence. The work aims to know the seasonal variation of Oo larvae in naturally infected adult goats in the area, to determine the most appropriate time for treatment delivery.

Methods: The trial was conducted in establishment goat herders in the area of Candelaria city (San Luis), in which the disease is a serious problem every year, especially in the summer. Thirty heads from goats were examined over a 14 month period, from February 2009 to March 2010 (2 to 3 per months). Heads were dissected and larvae found in nose and sinus cavities were recovered, counted and identified, under a stereoscopic microscope according to keys described by Cepeda-Palacios (1999). The developmental stage (L1, L2 or L3) of each larva was also recorded.

Results: A total of 26 goats (86.6%) were parasitized by larvae of Oo in different stages of development. The total number of parasites recovered was 227 larvae (97.4% alive and 6 dead), with a mean larval burden of 7.9 larvae per animal, with a minimum of 1 and maximum of 21 larvae. The highest number of larvae recovered was the third-stage larva (L3) (47.1%), then was the second stage (L2) (29.1%) and finally the first stage (L1) (23.8 %). As to the anatomical location within the head, 14.1% (19L1-7L2-6L3) were found in turbinate, 20.7% (18L1-11L2-18L3) in the ethmoid, 1.3% (2L1-1L2) in the maxillary sinus, 4.4% (4L1-1L2-5L3) in the lachrymal sinus, 0.9% (2 L1) in the palatine sinus, the 19.4 % (3L1-17L2-24L3) in the anterior frontal sinus and 39.2% (6L1-29L2-54 L3) in the posterior frontal sinus. The total recovery of larvae had two peaks, one in June-July (late fall-winter) and the other in January-February (summer). L1 larvae prevailed in autumn (April-May), decreased in June-July, disappeared in August-September to increase again in spring. The L3 instead, prevailed in late winter (August-September) and summer (February)

Conclusion: The observed seasonal patterns in larval abundance in the area are compatible with two annual peaks in adult fly activity, in spring and summer-autumn. Previous reports have also found oestrosis to be markedly seasonal, although the exact pattern varies between regions.

Acaricidal activity of extracts from *Petiveria alliacea* against *Rhipicephalus microplus* resistant to acaricides

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Chemical acaricides have played a pivotal role in the control of *Rhipicephalus microplus*. However, as a consequence of extensive use of chemicals this tick specie has developed resistance to the major classes of acaricides in different countries. In Mexico, tick resistance to acaricide is recognized in several states mainly in the Mexican tropics. Commercial acaricides are usually toxic to humans and leave residues in the environment. However, acaricide from plants are usually of low toxicity to mammals, water soluble, producing non-residual effects and have a low incidence of developing resistant strains. Therefore the aim of this study was to evaluate the acaricidal activity of crude extracts and fractions from stems and leaves of *Petiveria alliacea* on larvae and adults of *R. microplus* (resistant to organophosphates, pyrethroids and amitraz) using the larval immersion test (LIT) and adult immersion test (AIT), respectively. Methanolic extracts of stems and leaves of *P. alliacea* showed 100% mortality on the LIT bioassay. On the other hand, methanolic extracts of leaves and stem on the AIT test showed 26 and 86% of mortality, respectively, egg laying inhibition of 40 and 91%, respectively and hatchability inhibition of 26 and 17%, respectively. Purification of the active stem methanolic extract showed that the activity was present in the n-hexane non-polar fraction. Bioassay-guided purification of the n-hexane fraction produced 10 semi-purified fractions; fraction B had the highest activity against tick larvae (100% mortality). Gas chromatography-mass spectrometry demonstrated that the chemical composition of the active fraction B samples were mainly composed of benzyltrisulphide (BTS) and benzyldisulfide (BDS). These metabolites might be responsible for the acaricidal activity of stem extract of *P. alliacea*. However, further experiments to evaluate the acaricidal activity of BTS and BDS on larvae and adults of *R. (B.) microplus* are needed. Our results showed that *P. alliacea* is a promising biocontrol candidate as acaricide against *R. (B.) microplus* resistant strains.

Sequence Analysis and Cloning of a Putative Serine Endopeptidase Inhibitor from the Cattle Tick *Rhipicephalus (Boophilus) microplus*

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The cattle tick *Rhipicephalus (Boophilus) microplus* is a one-host tick and is considered the most harmful bovine parasite in several regions of the world, causing a severe impact on cattle industry. Until now, tick control depends on the use of acaricides. However, development of tick drug resistance, meat and milk chemical contamination restrains the use of chemical acaricides. Vaccines have been shown to be practical and sustainable. However it is necessary to identify and characterize potential antigens. An approach to select antigens is to identify tick proteins with key roles for tick survival, such as acquisition and digestion of the blood meal and modulation of host responses. In mammals, serine endopeptidases and their inhibitors are known to regulate important functions as blood clotting, fibrinolysis, pro-protein processing, releasing and inhibition of bioactive peptides, inflammatory and immune responses. Ticks produce endopeptidases and its inhibitors to disrupt the host homeostatic balance to their advantage. So, there is an increasing interest in testing these inhibitors as vaccine targets. This work aims to perform "in silico" analysis and cloning of a gene encoding a putative *R. microplus* serine endopeptidase inhibitor. Comparing *R. microplus* nucleotide sequences with serine endopeptidase inhibitor sequences of several species, a 2123-pb sequence with similarity for these inhibitors was found. The 1140-pb ORF starts from position 31 and encodes a 379-amino acid protein, with a theoretical isoelectric point of 6.56, and a molecular weight of 43,284 Da containing 22 cysteine residues. Also, the protein has four BPTI-like domains, suggesting that it belongs to Kunitz family. The ORF from gut and salivary gland cDNA was amplified and cloned. The identity of the amplicon was confirmed by PCR and enzyme restriction analysis. The expression of recombinant protein is in progress and will permit the characterization of inhibitory activity and test its immunogenicity properties.

Observed and predicted distribution of *Culicoides* and comparison with bluetongue outbreaks in Portugal (2005-2009)

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Bluetongue (BTV) is transmitted mainly by several species of the genus *Culicoides* and causes disease in domesticated ruminants. In November 2004 an outbreak of BTV-4 occurred in Portugal. This was followed in September 2007 by an outbreak of BTV-1, which continues to persist in some regions of the country.

To support policy decisions by veterinary authorities, a national entomological surveillance programme (ESP) was initiated in 2005 using a network of light traps. We describe the design of the ESP, report the observed distribution of species implicated as potential vectors of BTV, and use these data to extrapolate the probability of presence of *C. imicola* and *C. obsoletus* across the country.

Two sets of logistic regression models were developed using either bioclimatic variables or temporally Fourier-processed remote sensed variables and topographic data. For each set, "absence" was defined either as a location without any *C. imicola* or *C. obsoletus* or as a location with fewer than ten, resulting in four models.

Between 2005 and 2009, 5,045 valid catches were made. *C. imicola* was the most common species identified, accounting for 78% of the specimens collected. Its peak occurrence was in the month of September. Species in the *obsoletus* complex accounted for 6% of catches, peaking between May and July. The predominant geographical occurrence of *C. imicola* is in the East-Central and Southern Regions of Portugal while *obsoletus*-group species occurred throughout the country.

For the model using climatic variables, the most significant variables were the annual mean temperature and the mean temperature in the driest quarter, while for the model using remote sensed data, the most significant variables were the annual mean NDVI, and the mean, minimum and second term phase MIR. The models allowed the prediction of areas with a high probability of *C. imicola* and *C. obsoletus* activity. These predictions were tested against the results of the entomological surveillance. We discuss the model results and performance with reference to the distribution of bluetongue outbreaks in Portugal, as well as the use of the resulting data by national authorities to inform national risk management measures.

Population dynamics of the sheep ked (*Melophagus ovinus*) in Patagonia Argentina

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Background: *Melophagus ovinus* is common in sheep farms of Patagonia. Because of the limited local information available regarding the population dynamics of *Melophagus ovinus*, populations in sheep flocks were studied in two locations with different breeds and management conditions in the NW of the province of Río Negro.

Methods: One of the sites, henceforth Farm 1 (F1), had 21 six-month-old cross Corriedale sheep naturally parasitized, and the second site, Farm 2 (F2), had 11 artificially infested Merino sheep of the same age. The counting of keds and sheep body weight and condition score were undertaken monthly in all individuals. We also noted ambient temperatures from May to December 2009.

Results: In both farms a similar tendency was observed: the population of keds displayed a seasonal cycle, with a late summer low and highest numbers in winter and early spring, with few keds and pupae remaining after shearing. The increase in mean ked numbers was greater at F2, starting with an average of 7 keds per animal, and reaching a maximum of 157, a 22 fold increase in 7 months. In contrast for F1, the initial average count of 46 parasites reached a maximum mean of 174, increasing 4 times in a similar period of time. The body weight and the body condition score of sheep, and the ambient temperatures in both farms were within the normal parameters for each area.

Conclusions: The exponential growth observed in F2, was attributed to the inoculation of sheep that were not previously infested with keds, which meant that the parasites were in the establishment phase. On the other hand, in F1 the parasitic population was already established in the flock at the beginning of the experiment and their population growth may have been conditioned by management, mortality and other factors. These findings contribute to the knowledge of the population dynamics of *Melophagus ovinus* in the environmental and management conditions of NW of Patagonia and supports the importance of shearing and other factors like management and experience of previous infestation.

***Oestrus ovis* parasitism in Santa Ines and Ile de France young sheep**

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Previous studies showed that Santa Ines hair sheep was more resistant to gastrointestinal nematode infections (GIN) than Ile de France sheep. The present experiment aimed to evaluate if that reported difference in resistance against GIN also occurred against *Oestrus ovis* infestation. Santa Ines (n=12) and Ile de France (n=12) young male lambs were weaned at two months of age and moved to a paddock with *Brachiaria decumbens* grass, where they were kept together during the experimental period (September to December 2009). In December 2009 all lambs were sacrificed and *O. ovis* larvae were recovered, counted and identified according to the larval stage. All animals were infested by the different larval instars of *O. ovis* without statistics difference between breeds ($P > 0.05$). The Santa Ines lambs had an average of 24.8 larvae, and the intensity of infection ranged between 14 and 39 larvae, while the Ile de France lambs showed an average of 23.5 larvae with minimum and maximum of 11 and 36 larvae, respectively. The lambs of both breeds began to show clinic signs of oestrosis in early October coinciding with a significant increase ($P < 0.01$) in the serum levels of immunoglobulin G (IgG) against crude extract (CE) and against excretory and secretory products (ESP) of *O. ovis* larvae. Ile de France lambs showed overall mean serum IgG against CE and against ESP significantly higher than Santa Ines lambs ($P < 0.05$). Immunoglobulin A (IgA) level in nasal mucus and number of mast cells and eosinophils in the upper respiratory tract did not differ between breeds ($P > 0.05$). Ile de France lambs showed higher concentrations of IgG against *O. ovis*, however, no significant difference was observed between breeds regarding the infestation degree. Study supported by FAPESP.

Seasonal evolution of pediculosis (*Linognathus stenopsis* and *Bovicola caprae*) in naturally infested goats in NW Patagonia

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Lice infestations (pediculosis), produced by the biting louse (*Bovicola caprae*) and the sucking louse (*Linognathus stenopsis*), infest goats worldwide and are the most important parasitosis in goat herds in Patagonia. With the aim of describing the seasonal dynamics of pediculosis in goats naturally infested in this region, 3 groups of 12 untreated animals each were studied in Río Negro province. Group 1 (G1) had adult Angora goats; group 2 (G2), young Angora goats and group 3 (G3), adult Criollo goats, all kept under the usual extensive management system used in the area. During 8 months (from March to October 2010), monthly louse counts were made, recording goat body weight (kg) and condition score (1 to 5). Louse abundance was measured by counting adult lice and nymphs in twelve 10 cm partings: at the throat, chest, lower shoulder, upper shoulder, withers and hind-quarters on both sides of each animal. At the beginning of the trial (March), averages from 23 to 29 lice per group were recorded. The number of lice increased in a similar way in the three groups during the first half of the study, after which differences in the evolution of parasite populations were observed. In the case of G1 and G2, lice populations grew to a peak in spring, reached a maximum average of 174 lice in September, prior to shearing, and then decreased to averages of 85 (G1) and 95 (G2) by the end of the trial (October). In the G3, where goats were not sheared, the lice populations began to decline in July, bearing an average 94 lice per animal, until October. Regarding the body condition of the goats, it was low (1.5 to 2 in general), especially during the winter months, in accordance to the body weight that remained relatively stable, even in G2 (growing animals). While the evolution of lice populations in the different groups was similar, Criollo goats maintained lower levels of infestation throughout the study period. Although this preliminary data should be validated by further works, our findings suggest that, like noted for other ectoparasites, goat lice populations in Patagonia despite age and breed, increase through winter and spring and decline during summer.

Husbandry practices and acaricide control of *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) in Quindío - Colombia

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Introduction: Worldwide, the crisis in the production of new molecules and the rapid development of resistance to acaricides are factors associated with the control of the cattle tick *Rhipicephalus (Boophilus) microplus*. Colombia does not escape to this reality and few studies have characterized this problem in the country until now.

Methods: An epidemiological survey in 54 cattle farms distributed in 21 localities near to Filandia (Quindío-Colombia) was performed in order to measure the degree of farmers' perceptions related to biology and tick control. Simultaneously, field strains of *R. (B.) microplus* were collected aiming the diagnosis of acaricide sensitivity to commercial products commonly used in the region. The bioassay technique utilized was the "Shaw Larval Packet". The mortality for this test was evaluated by a probit analysis with GraphPad-Prism 5.01 to calculate the lethal concentration (LC) for commercial dilutions at 99%.

Results: Producers have low-level of knowledge on biological aspects of the tick, which may limit the performance of its control, although 57.4% of interviewed recognized its detrimental role. The acaricide control by using manual backpack sprayer has several shortcomings in aspects such as personal protective equipment (66.7% don't use), acaricides substances (92.6% unknown toxicological categories), equipment for the application of products (75.9% in good condition) and application techniques (72.2% inadequately).

Cypermethrin and diamidines support a stable market acaricides, however, it is noteworthy that Fipronil formulated for agricultural purpose, is the second molecule utilized for the tick control among farmers (16/54). The mixture of products, either among active molecules for veterinary use, or even between these and some agricultural products, are another handling practice that were registered.

The field strains demonstrated resistance to amitraz 0.02% in 14 of the 21 areas studied (LC₉₉ 0.042%), cypermethrin 0.39% (LC₉₉ 25%, 4/4) and chlorfenvinphos 0.25% (LC₉₉ 0.45%, 1/13). Additionally, the spatial distribution of acaricide resistance in Filandia established the presence of multidrug resistant strains of *R. (B.) microplus* in at least four regions.

Conclusions: The study concluded that producers show chemical dependence for acaricide control without technical support and, furthermore, improper practices, contributing for the emergence and spread of multidrug resistant strains under a great selection pressure of acaricide on the tick population.

First clinical trial for the biological control of psoroptic mange in cattle using entomopathogenic fungi

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Background: *Psoroptes ovis* is responsible for a severe dermatitis in sheep, cattle, goats and rabbits. Entomopathogenic fungi could represent an alternative to chemical acaricides. In sheep *in vivo* trials were so far unsuccessful despite a high *in vitro* efficacy. The low density of mites, the presence of toxic components in the fleece, high local temperatures and inappropriate formulation could explain these failures. In this study a field trial was conducted with two hydrophilic formulations of *Metarhizium anisopliae* in *P. ovis* naturally infected cattle.

Method: *M. anisopliae* IHEM 18027 was cultured on SDAY at 27°C. Mass production was performed according to Jenkins *et al.* (1998). Two hydrophilic excipients were used. Two groups of 8 naturally infected cattle were formed. Each group was sprayed with one litre of the excipient alone or the biopesticide (4 animals/formulation – 3 applications at 10 day intervals). Clinical and parasitological indices were monitored every 10 days from day 0 until day 40. Samples of hair were collected for fungal cultures. Body weights were evaluated on Days 10, 30 and 60 PT. Data were analysed with SAS.

Results: There was a rapid worsening of the clinical condition of the control animals and because of welfare concern two had to be treated during the trial with injectable ivermectin. At trial end 5 control animals were still positive for *P. ovis* ($P < 0.005$). In contrast, the condition of treated animals improved markedly and only one animal was still positive at trial end. Clinical and parasitological indices were significantly different ($P < 0.001$). Most of the control animals lost weight whereas treated ones took weight. Live conidia of *M. anisopliae* could be isolated from hair up to 60 days PT.

Conclusion: *M. anisopliae* was successfully used in two hydrophilic formulations for the control of naturally acquired psoroptic mange in cattle.

Frequency and identification of Tabanidae (diptera) in relation to temperature and environment in an area subtropical Argentinian mount forest

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Introduction: The tabanus are of great important when it comes to the development of illnesses in people as well as in wild animals which are of great economic importance suffer from these affections. The objective this work was to identify other species and gender within subfamilies Tabanidae, Pangoninae and Chrysopsinae and to relate them to the temporarily environmental distribution.

M and M: The research was carried out in the province of Chaco Argentina Lat. S. 27°20" Log. W. 58°56". The tabanus were captured and gathered by means of Malaise trap, between 10 a.m. and 3 p.m. The key Coscaron and Papavero (1993) were used for identification. The meteorological data is characteristic of the EEA INTA Colonia Benítez.

Results: 1.159 samples were captured; six Tabanidae with n: 1.141 (0,98%) samples of species in order of populations importance were *T. claripenis* n: 403 (0,35%); *T. occidentalis var dorsivitattus*, n:337 (0,29%); *T. nebuloso sp. ornativentris*, n: 176 (0,15%); *T. pseudonebulosos*, n: 104 (0,9%); *T. pungeons*, n: 70 (0,76%); *T. sorbillans*, n: 51 (0,4%); 2 Pangoninae, n: 2 (0,02%); *T. leucotabano*, n:1 (0,01%); *T. esenbeckia*, n:1(0,01%) and 3 Chrysopsinae, n:16 (0,14%); *Chrisops variegatum*, n:4 (0,035%); *Chrisops sp. I*, n:7 (0,06%); *Chrisops sp. II*, n:5 (0,043%). The monthly distribution of tabanus according to number were: Oct.n:279.; Nov.n:213.; Dic.n:131.; Ene.n:52.; Feb.n:12.; Mar.n:24.; Abr. n:16.; May.n:9.; Jun.n:9.; Jul.n:6.; Agu.n:76.; Set.n:139 and Oct.n:195. Frequency of individuals in relation to the seasons was higher in spring, n: 623 (0,54%). *T. claripenis* and *T. occidentalis var dorsivitattus*, constitutes 0.64 % of the tabanideos and their presence was significant all year round. The observation related to four species of tabanus in order of importance were n= 365 and or n=75 day per temperature, humidity, winds and precipitations respectively. The correlation relationship r and probabilities were found in the highest temperature (P<0.003) in the lowest humidity (p<0,05) and also between the lowest humidity with winds (p<0.04).

Conclusion: During spring, the highest number of species were captured, Tabanidae representing 0.98% within subfamilies. *T. claripenis* and *T. occidentalis var dorsivitattus* their temporary distributions were found all year round. The high temperature, the low humidity and the wind conditioned the capture of tabanus.

Efficacy of fluazuron and flumethrin combination pour-on against a resistant field strain of *Bovicola limbatus*

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Introduction: *Bovicola limbatus* is a biting louse parasitizing Angora goats. It is host specific with a life cycle of between 32 - 34 days from egg to adult. The louse feeds on epidermal debris causing skin irritation with resultant rubbing and scratching adversely affecting both quantity and quality of mohair produced in South Africa. The parasite is well established throughout the country with adults most numerous during the winter months. The louse strain used showed resistance to synthetic pyrethroids and insect growth regulators through field inefficacy observations and investigative trials in South Africa. .

Objective: To confirm lousicidal efficacy of a fluazuron 2.5%/ flumethrin 1% combination pour-on against a resistant strain of *Bovicola limbatus*.

Methods: Twelve Angora goats, with moderate off-shears infestation, were divided into a treatment and a control group containing six animals each. The fluazuron / flumethrin combination pour-on was administered to the treatment group at a dose rate of 0.1 ml /kg. The two groups were separated to prevent cross-contamination and were observed twice a day in their pens. Assessments were performed via lice counts at weekly intervals until day 56 post treatment.

Results: The fluazuron 2.5%/ flumethrin 1% combination pour-on controlled a lousicide-resistant *Bovicola limbatus* strain with 100% efficacy from day 21 to 56 post-treatment

Conclusion: A combination of fluazuron with flumethrin therapy controls lousicide-resistant *Bovicola limbatus* strains.

A combination of fluazuron and flumethrin treatment could be used to effectively control *Bovicola limbatus* strains showing resistance to currently registered lousicides.

Efficacy of fluazuron and flumethrin combination pour-on against a resistant field strain of *Rhipicephalus (Boophilus)* spp. on a commercial cattle farm in South Africa

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Introduction: *Rhipicephalus (Boophilus)* spp. are one host ticks with a 21 day life cycle. In South Africa they are well established in the warm, moist regions along the southern and eastern coasts and adjacent interior, as well as in the drier northern Highveld. This genus transmits *Babesia bovis*, *B. bigemina*, *Anaplasma marginale* and *A. centrale* parasites to bovines with resultant economic losses due to poor production, morbidity and mortality. Tick resistance to synthetic pyrethroid, organophosphate and amidine acaricides has been detected in various areas around the country using the Shaw Larval Packet Test method.

Objective: To confirm the field efficacy of a fluazuron 2.5%/ flumethrin 1% combination pour-on against a resistant strain of *Rhipicephalus (Boophilus)* spp. and to monitor efficacy via Shaw Larval Packet Testing.

Methods: Ten sentinel adult bovines were randomly selected on a commercial cattle farm in Grahamstown, South Africa. Samples of at least 50 engorged female *Rhipicephalus (Boophilus)* spp. were collected from their predilection sites at different times between May 2007 and April 2010. Ticks were collected prior to each application of a fluazuron 2.5%/ flumethrin 1% combination pour-on dip on the animals and were transported in plastic bottles with perforated lids containing tissue paper to independent laboratory. Results of the Shaw Larval Packet Tests over the period were compared.

Results: The fluazuron 2.5%/ flumethrin 1% combination pour-on controlled acaricide-resistant *Rhipicephalus (Boophilus)* spp. (>90% efficacy) as well as multi-host ticks on the treated bovines. The Shaw Larval Packet Test results showed an improved efficacy of amitraz (250 ppm) from 23% to 74%, cypermethrin (150 ppm) from 0.7% to 7.3% and chlorfenvinphos (500 ppm) from 10.9% to 14.1% over the study period.

Conclusion: A combination of fluazuron with flumethrin controls acaricide-resistant *Rhipicephalus (Boophilus)* spp. tick strains and may assist in improving their susceptibility profile, ultimately reversing the tolerance trend. The useful life span of existing single active acaricides may be extended by use of the combination.

First report of amitraz resistance in the cattle tick, *Rhipicephalus (Boophilus) microplus*, in Argentina

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The cattle tick, *Rhipicephalus (Boophilus) microplus*, has already developed resistance to amitraz in Australia, South Africa, Brazil, Colombia and Mexico. However, no cases of amitraz resistance in the cattle tick have been notified in Argentina yet. Amitraz was first introduced in Argentina in 1978. Nevertheless, its use was rapidly abandoned due to the concomitant appearance of less expensive chemicals such as the synthetic pyrethroids (SP). Following to the development of SP resistance, amitraz was reintroduced to the Argentinian market in 1997. More recently, in July 2010, a failure to control ticks using amitraz was recorded in a farm located in Santo Tomé, Corrientes province. Acaricide treatment was supervised by highly trained SENASA local staff. Engorged females ticks (EFT) collected from this farm were then submitted to both SENASA and INTA laboratories to determine not only the level of amitraz resistance but in vitro efficacy of deltamethrin, and ethion combined with cypermethrin. A modified Drummond adult immersion test was used, and two separate bioassays were run. EFT were randomised by weight to form as many groups as acaricides to be tested. The first bioassay compared the amitraz resistance level between the field isolate and a susceptible laboratory strain (INTA A26). The second bioassay assessed only the performance of the EFT field isolate immersed in 250 ppm amitraz, 50 ppm deltamethrin, and 400 ppm ethion combined with 100 ppm cypermethrin. Results from the first bioassay showed that oviposition was not inhibited in the EFT field isolate challenged with amitraz. In the second bioassay, amitraz, deltamethrin and the combination of ethion and cypermethrin showed 1.5%, 3%, and 90.6% efficacy, respectively. In the light of these results, samples were also sent to a laboratory in Switzerland to perform further analysis and new EFT were collected from neighbouring farms. Both *in vivo* and *in vitro* results account for the first evidence of amitraz resistance in *R. microplus* in Argentina. To further investigate this recent emergence of amitraz resistance, SENASA will conduct a local and regional sampling in the cattle tick area.

Resistance Associated Factors To Acaricides In *Rhipicephalus (Boophilus) Microplus* In Brazil

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Rhipicephalus (Boophilus) microplus is an important source of damage for the dairy farmers, and its control has become difficult due to the parasite resistance to acaricides. This research has aimed to assess the main factors which influence the resistance occurrence and prevalence of ticks in the farms. In order to do so, 105 randomly chosen dairy farms in southern of Minas Gerais were visited and were performed interviews which collected production, social, economics and cultural information as well as acaricides management data. The samples research corresponded to 45 farms where it was possible to collect ticks for acaricides efficiency test. Ticks' infestation level was evaluated by counting female over 4mm and engorged females were collected for efficiency test of the products. It was made a data bank on Epidata and analyzed with *software* PASW 18.0. Variables descriptive analyses were made. It were searched for the factors related to: 1. the tick resistance to the acaricides products and 2. the infestation levels. For this purpose the analysis were performed by the Chi-square test, for qualitative variables, and by Kruskal-wallis test for quantitative variables. The *odds ratio* and its confidence limits was calculated. The significance level required was 95%. It was noted great variation on dairy farmers' profile, on properties characteristics, on ticks handling and on ticks control. The test those farms showed differences between the products efficiency, even among those which had the same base. Many factors were related to the presence of tick resistance to different products, nevertheless, in general, all of them seems indicate that properties with a better technique level (mechanic milking, artificial insemination, animal density, among others) have shown more risk of installed resistance. Concerning some acaricides, control factors (frequency, equipments and concentration) were associated to resistance. In 77% of the properties were found resistance to more than four chemical bases, among which in 33% there were not efficient products. Multiple resistance is a frequent problem in the researched farms.

Knowledge of Milk Producers on Cattle Ticks About Biology and Cattle Tick Control

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This study has as its objective, the evaluation of milk producers knowledge regarding bovine ticks and their practices and control. 93 dairy farmers in the state of Minas Gerais were interviewed whom participated in various courses at EMBRAPA Gado de Leite. A formula was utilized which had already been tested in the study ROCHA *et al.*(2006). These producers had not received previous information in reference to acaricides and tick control efficiency tests. In order to analyze the information, the open answers were categorized through content and descriptive analysis extracting the profile highlighted by the highest frequencies. We tested the association between tick education, practice and knowledge by using Chi-square tests for trends. It was revealed that 92.3% of farmers had no knowledge of the non-parasitic period. For 96.4% what determined the moment of treatment was the degree of tick infestation and 77 of them (93.3%) used spray guns for the application of the acaricide. In seeking the intersection between biological variables and control with education, cooperatives, experience and herd size, it was found that there is a linear association between education and the practice of pre-dilution in acaricide preparation ($p=0,010$). The others showed no significant association. This data demonstrates that there is a need to instruct the farmers in relation to the biology and control of the *R.(B.) microplus*. It has been concluded that the majority of dairy farmers in MG are unaware of bovine tick biology and the factors that influence choosing a acaricide. This in turn hampers strategic control and furthermore that education has no bearing on more efficient tick control within the dairies.

The use of urea in a rotational grazing system and its effect on *Rhipicephalus (Boophilus) microplus* (Murrell and Barker, 2003) (Acari: Ixodidae)

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Parasitic infections are a recurring problem in cattle. The study was carried out from April/09 to April/10 and assessed the effect of nitrogen fertilizer (urea) in pastures on the infestation of *Rhipicephalus (Boophilus) microplus*. We used twenty crossbred cows kept under rotational grazing in the Experimental Field of Embrapa Dairy Cattle - MG, Brazil. The counts of ticks were performed every 14 or 21 days after the tick-bath. The baths were determined by the presence of engorged tick females. The animals were divided into treated group (T1), animals in paddocks fertilized with urea and control group (T0), animals in the paddocks without fertilization. In total we used 25 paddocks of 400m² for each group and these were grazed for 24 hours. The treated group after the departure of the animals received 4.6 kg of urea, equivalent to 2.116 kg of nitrogen. The paddocks were fertilized control maintenance of pasture with ammonium sulfate. In broadcast fertilization with urea, a loss of nitrogen (NH₃) that has a toxic effect on the engorged female. To analyze the results were pre-defined four groups: group 1, animals treated with urea pickets during the rainy season (T1S1), group 2, animals in paddocks not fertilized, in the rainy season (T0S1), group 3, animals in paddocks with urea in the dry season (T1S0), group 4, animals in paddocks not fertilized, in the dry season (T0S0). The data were compiled by the Kruskal-Wallis (1952), and significant difference ($p < 0.05$) between counts of ticks in groups 1, 2 and 3. The result of group 4 was equal to that of groups 1 and 2. The best outcome observed in the rainy season indicates a greater effect on ticks, allowing to infer a seasonal influence. The less moisture in the dry season acted directly result in reducing the occurrence of hydrolysis of urea and reducing the toxicity of ammonia on the engorged female.

Effect of urea on the tick *Rhipicephalus (Boophilus) microplus* mortality

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This study assessed the effect of urea on *Rhipicephalus (Boophilus) microplus*. The experiment was divided into two stages. In Phase one was used 30 plastic buckets, with an area of 18 cm² each, containing *Brachiaria brizantha* grass where were dropped three engorged female. These containers were equally divided in three groups: G1 control group, G2 treated with 15g of urea and G3 treated with 15g of urea plus 10% ammonium sulfate. 1.8 liters of water was added *per* bucket after fertilization. In Phase two was used 20 plots of *Panicum maximum* cv. Mombasa grass with 1 m² area each, divided in two groups of ten: a group without the addition of urea and one urea-treated group. Twelve engorged females were placed at each plot on day zero with 60g of urea in the treated group. On the 27th day grass of plots 1-5 of both groups was trimmed, and urea group was fertilized. On the 40th day a white flannel 1.60 m x 1.00 m was placed on the grass in order to verify larvae presence. During Phase I, 24 h after contact with urea, all engorged females had died in G2 and only one survived in G3. In Phase II, tick counts showed 85.97% ($P < 0.0001$) less larvae in the treated group. Results showed an increase mortality rate in urea exposed groups under the experiment conditions. Keywords: *Rhipicephalus (Boophilus) microplus*, tick mortality, pasture fertilization, urea.

Characterization of workers from dairy cattle farms

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Four workers at two farms that apply an integrated strategic control of endo and ectoparasites on cattle and horses (GE), and 10 workers at eight properties with a conventional parasite control (GC) were interviewed to characterize the profile of them. Farms were located at Minas Gerais state and Rio de Janeiro state, Brasil. All respondents had an incomplete elementary education. Milk production was the main source of income for all individuals at GE farms and at GC it was pointed out by 90% of respondents. Regarding the composition of the herd, the average values of number of cows and other animal categories in the GE were 47.96% and 52.04% respectively and at GC farms it was 43.28% and 56.72%. All respondents of GE farms said that veterinarians were the source of information, at GC this were present at 30% of the farms only. On the GE farms zootechnical, economic and health bookkeeping were performed as a routine while at GC farms zootechnical recording were made only at 70%, economic at 40% and health at 60% of the farms. GE farms received technical assistance monthly while at GC this assistance was present only at 50% of farms. For GE respondents the major problems of dairy cattle were ticks infestations (75%) and tick fever (75%), while GC respondents said that ticks (70%), *Dermatobia hominis* larvae (30%) and worms (30%) were the main problem. Regarding cattle worm control individuals at GE said that performed drug application's at beginning and end of the dry season and didn't perform this treatment at lactating cows, the CG respondents said that this practice was made randomly and lactating cows' treatment were performed at 62.50% of the farms. All GE respondents said that change the antiparasitic drugs sporadically while at GC farms this was made often at 50% of farms and the same amount said that never do this. There is a better organization in GE farms what shows technical support importance in planning and implementing health programs, aiming to the rational use of pesticides.

Integrated Control of Cattle Parasites

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An integrated strategic control of endo and ectoparasites of cattle was evaluated from April/06 to March/09 on two farms of dairy cattle, in the states of Minas Gerais (Farm 1) and Rio de Janeiro (Farm 2), Brazil. Farms 1 and 2 had 250 and 150 animals on average respectively. It was proposed strategic treatments with doramectin to helminth control in females from weaning to first calving in May, July, October and January, and for the cows, treatments only in the pre-partum. To *Haematobia irritans* control, it was proposed treatments in October and April according to infestation score of 200 flies per animal. To *Dermatobia hominis* and *Cochliomyia hominivorax* control treatments were proposed in October and in newborn animals as prevention of umbilical myiasis, respectively. In this case treatments were performed according to observation of larvae in animals. Samples of feces were collected on days zero, 14 and 60 after the first treatment each year (2006-2008) to OPG counting. Livestock production data were compared with data from October/03 and March/06 when the program of integrated control of parasites was not performed at farms. Strategic treatments to *H. irritans* and *D. hominis* were not necessary, showing that anthelmintics and acaricides treatments certainly interfered on these populations. The treatment strategy for prevention of umbilical myiasis also have been 100% effective. The results of OPG counts showed 100% efficacy of doramectin 14 days after treatments. During the implementation of integrated control program the mean weight gain of heifers from birth to first calving was 500 and 515 g per day, at Farms 1 and 2, while before the program implementation these rates were 303 and 333 g per day, respectively. Consequently, the age at first birth evolves from 56 to 34 months on Farm 1 and from 51 to 33 months at Farm 2. Results that clearly reflect the economic efficiency and sustainability of the program of integrated control of parasites in cattle. Key words: dairy cattle, horses, parasites, integrated control strategy.

Strategic Control of *Rhipicephalus (Boophilus) microplus* in dairy cattle

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The cattle tick, *Rhipicephalus (Boophilus) microplus*, is highly adapted to tropical and subtropical climates conditions and is spread almost all over the country. The objective of this study was evaluate the *Rhipicephalus (Boophilus) microplus* strategic control in dairy cattle, with a proposed treatment by the semi-engorged female with length ≥ 3 mm presence, without fixed interval. The study was conducted on a farm in Minas Gerais (F1) and another in Rio de Janeiro (F2), with 250 and 150 animals respectively. Both herds were divided into dairy cows (VL), heifers and dry cows (NVS), calves (BD) and calves infants (BM). A period of 120 days for strategic control was established - from April to July, with the range of treatments determined by the presence of semi-engorged female ≥ 3 mm. The treatments were performed by spray bath through a motorized system and chute containment. There were significant reductions in *R. (B.) microplus* populations in both farms and gradual reductions in the number of treatments and acaricide used. *R. (B.) microplus* parasitic load evaluations showed a reduction at F1 of 38.55, and 24.58% respectively in the second and fourth years of the experiment compared to the first, while the third increased by 19.55%. Counts conducted at F2, showed a reduction of 31.99, 30.52 and 51.10% respectively in the second, third and fourth years, when compared to the first one. Was used an average emulsion insecticide volume of 3.5 liters at VL and NVS categories, at category BD and BL, 2.5 and 2 liters, respectively. The acaricide product spraying cost was: US\$0.10 for VL and NVS and US\$0.07 and US\$ 0.04 for BD and BL, respectively, considering the period in which the trial was conducted. The spraying operation had an average duration of 25 seconds per animal, when performed by two operators. Strategic control of *R. (B.) microplus* in dairy cattle demonstrated significant results in reducing the parasite population. Key words: dairy cattle, *Rhipicephalus (Boophilus) microplus*, strategic control.

Naturally infected cattle develop antibody responses against salivary proteins from *H. irritans irritans*

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The horn fly (*H. irritans irritans*) is a blood-sucking insect parasitizing cattle of worldwide distribution. Parasite burdens in excess of 200 flies may cause significant stress, leading to decreases in weight gain and milk production. In Uruguay, losses in the leather industry are considerable (USD 3,5 million per million of processed leathers) and in USA, annual losses for USD 1 billion are attributed to this parasite (Cupp M et al., 2004).

The saliva of blood-sucking parasites ensures that they get a proper blood meal, by interfering with host physiological processes. In this context, it is generally proposed that blocking salivary components is a useful strategy to compromise parasite development and, therefore, that it may contribute to parasite control.

With the purpose of identifying components from the saliva of *H. irritans*, we carried out a small scale analysis of the salivary gland transcriptome of adult flies. We thus identified several proteins with high similarity to those described in the saliva of *Stomoxys calcitrans* by proteomic studies (Wang et al., 2009). The cDNAs coding for two identified proteins - the highly abundant Ag5 and a putative member of the HYP16 family- were expressed in *E. coli* and the purified recombinants used to assess the humoral immune response of naturally infected cattle. Parasite burden and antibody titers against recombinant Ag5 and HYP16 were estimated weekly in 30 infected bovines (24 - 36 months old).

Our results indicate that, upon appearance of *H. irritans* in the herd (which coincides with the beginning of warm days), all animals developed a humoral response against the assayed antigens. The IgG levels were higher at the end of spring, remained stable during the summer and in the early autumn, and fell to undetectable levels in the winter. No relationship was observed between parasite burden and antibody titers. We conclude that antibody responses against the assayed salivary proteins do not interfere with the progress of infection. In other words, no experimental evidence was obtained regarding the potential of Ag5 and HYP16 as candidates for the preparation of a vaccine to control horn fly infections.

Real-time PCR to assess the *Leishmania* load in *Lutzomyia longipalpis* sand flies: screening of target genes and assessment of quantitative methods

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Visceral leishmaniasis is an endemic disease in Brazil. Its etiological agent is *Leishmania infantum chagasi*, and its main vector species is the sand fly *Lutzomyia longipalpis*. Epidemiological studies have used conventional PCR to measure the rate of infection of sand flies collected in the field. However, real-time PCR can detect lower parasitic loads, reducing the number of false negatives and improving the quantification of *Leishmania* in the sand fly. This paper aims to compare genes with various numbers of copies to detect and quantify *L. infantum chagasi* in pools constituted by distinct numbers of *L. longipalpis* specimens by real-time PCR. We mixed pools of 1, 10 and 30 male sand flies with various amounts of *L. infantum chagasi*, forming groups with 50, 500, 5000 and 50000 *Leishmania* parasites. For the amplification of *L. infantum chagasi* DNA, primers targeting kDNA and the genes encoding the polymerase α and the 18S subunit of the ribosome were employed. Parasites were measured by absolute and relative quantification. The PCR analysis using kDNA amplifications presented the greatest sensitivity among the genes tested and showed the capacity to detect the DNA equivalent of 0.004 parasites. Additionally, the relative quantification using these primers was more accurate and precise. In general, the number of sand flies used to DNA extraction did not influence the *Leishmania* quantification. On the other hand, only for low-copy targets, as polymerase α gene, the less number of parasites in the sample produced inaccurate quantifications. Thus, qPCR measurement of *L. infantum chagasi* in *L. longipalpis* is improved by amplification of molecular targets with multi-copies per parasite, especially by increasing the sensitivity, accuracy and precision.

The cattle tick *Rhipicephalus (Boophilus) microplus*: Fluctuation and chemical control in the Northern Mexico

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Background: The cattle tick *Rhipicephalus (Boophilus) microplus* is the most important ectoparasite of livestock in Mexico. *R. (B.) microplus* transmits diseases such as babesiosis and anaplasmosis, the most important diseases in Mexican tropics. Tick control is difficult because of resistance to pesticides. In order to have an effective control, the biological fluctuation of this tick as well as evaluation of different treatments is required. The aims of this work were to determine the fluctuation of cattle ticks and to evaluate the efficacy of different chemical based on commercial acaricides.

Results: The fluctuation of cattle ticks was determined in a farm at the municipality of Soto la Marina, Tamaulipas, Mexico from March 2007 to March 2008. A group of 12 adult European pasturing cattle was used. Total number of ticks from the right side of each animal was obtained and ticks identified. Most of the ticks were identified as *B. microplus* but also *Amblyomma cajennense* was found. Six groups of 12 animals were used to evaluate acaricide treatments as follows; G1 Garraban®, G2 Garraban® and Dectiver® Premium, G3 Garraban®, Dectiver® Premium and Ectoline®, G4 Garraban®, Acatak® and Dectiver® Premium, G5 Neocidol® and Dectiver®, Premium G6 without treatment was used as control. Ticks numbers were statistically significant ($p>0.05$) during the year in all treatments, excepting for group 1, where no significance was found on April and December and groups 4 and 5, without significance on December.

Conclusions: The biological fluctuation of *R. (B.) microplus* during a year was constructed. Tick control with the different treatments demonstrated the efficacy of tick control without significant difference when different combinations were used, thus demonstrating the feasibility of tick control by using inexpensive treatments.

Comparative evolution of *Melophagus ovinus* populations in ked-naive Merino sheep and Angora y Criollo goats

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Although sheep are generally considered to be the only definitive host of the sheep ked, *Melophagus ovinus* (L.), the parasite has been reported also from a range of domesticated and wild animals, including goats. When populations of adult sheep keds infesting goats were diagnosed on a mixed sheep-goat farm in Patagonia, it was decided to carry out a controlled trial to confirm these findings and compare the evolution of the infestation in ked-naive sheep and goats. Three groups of 4 animals each were established: Group 1 (G1) adult Angora goats, Group 2 (G2) adult Criollo goats, and group 3 (G3), Merino lambs.

An experimental infestation was induced in all animals with 6 *M. ovinus* on each animal. Body surface of each lamb and goat of the three groups was inspected monthly and the number of *M. ovinus* and pupae were tallied.

Each group of animals was kept in separate, covered pens, which provided a suitable environment for the spread and survival of keds. Since the beginning of the trial, averages of 1.3 to 7 keds per group were recorded in G1 and G2, and a mean of 2.8 keds per group at the end of the trial (half of the initially inoculated keds). In these groups, some goats were negative to ked counts and most of them remained with countings below the number of keds transferred. In contrast, G3 showed that 2 months after infection, 91.6% of *M. ovinus* used in the original infestation became adapted and continued to live on their new hosts, with individual variation ranging from 1 to 12 keds. In this group, lambs showed a marked increase in their ked populations throughout the experiment (7 months), reaching a maximum mean of 131.3 keds at the end of the trial. This represents a 22 fold increase from the original population. Pupae counts followed a similar pattern to that of adult keds in the three groups of animals throughout the study period. These results confirm a significant adaptation of the parasite to sheep conditions, and the status of reservoir of goats. Despite the establishment of keds on goats, this was considered less relevant than that of sheep. Still keds on goats may affect control plans, as these animals are not part of routine treatments against keds.

Social representation of parasite control in dairy farms

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The social representations studies are recognizing way to constituted social imaginary based on shared particular group experiences. In attempt to elaborate the characteristic representations regarding parasite control in herds of horses and cattle were conducted semi-structured interviews with four workers on two dairy farms, which used the integrated program of parasitic strategic control of and received monthly assistance of veterinarians (GE) located in Minas Gerais e Rio de Janeiro states and 10 employees on eight dairy farms in Rio de Janeiro state, which did not use strategic program to control parasites (GC). Even after receiving epidemiology and parasite control lectures, only one person from GE showed better cattle tick program assimilation. But none was able to associate the strategic control program implementation with the parasites epidemiology and with the production system goal, so it was felt that the program was no sufficient apprehended. However, was observed an acaricide amount reduction. One GE respondent described correctly the treatment program proposed against cattle worm, and none described the program control used for horses nematode parasites. The GC actions reports express parasite control empiric and products misuse, mainly ivermectins bases. The GC antiparasitic compounds choice was made according to the lowest price, neighbors advice or by the shops veterinary products clerk. The tick annual treatments numbers varied between one and two times, according to reports, on entire herd. Six CG individuals reported satisfaction when using homeopathic product for parasite control, however, reported use of conventional acaricide treatment when the situation got worse. All GC expressed little about the importance of equine parasite control. Despite all have horses, baths with acaricides were random, related to the ticks presence and unsuitable products. From the GC reports, there were no parameters regarding the acaricid products used in the dairy herd, demonstrating the technical monitoring importance of strategic program of control implementing.

Social representation of ticks biology knowledge in dairy farms

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The summary results in question originate from qualitative study with milk property workers. The term Qualitative Research understands a diverse interpretative techniques set that aim describe and decode meanings complex system components.

While quantitative studies generally seek to follow with severity a predetermined plan (based on clearly indicated hypotheses and variables that is operational definition object) the qualitative research does not seek to enumerate or measure events and, generally does not employ elastic instrumental data analysis, its interest focus is ample and emerge from a differentiated perspective of the quantitative methods adopted. The qualitative and quantitative researches are not incompatible opposites that should not be combined.

The analysis methodological approach for data collected through semi-structured interviews was the Social Representations Theory, a qualitative approach, originated by the Social Psychology. The representations set form the existing imagery about a subject in study. A social representation conceiving way consists of understanding them as the thinks expression of certain population about a certain topic.



SESSION H

Keynote Lecture

- **The biology and control of equine parasites: is there anything new** - T. R. Klei

Oral Communications

- **Strategic Parasite Control for Working Donkeys Worldwide; Responsible, Effective and Sustainable** - A. F. Trawford
- **Helminths of horses in the federal state of Brandenburg (Germany): Prevalence, risk factors and anthelmintic efficacy** - B. Hinney
- **Gastrointestinal nematode effect on the reproductive performance of mares and stallions under an intensive equine embryo transfer program** - M. Mejia
- **Control of Parascaris equorum Infections of Foals on a Swedish Stud** - E. Osterman Lind
- **In Vitro Selection Demonstrates Variation in Ivermectin Susceptibility in Cyathostomin Larvae** - F. Kooyman
- **Strategic control Anocentor nitens (Neumann, 1897) (Acari: Ixodidae) on horses** - A. Pinto da Cunha
- **Strategic control of Amblyomma cajennense (Fabricius, 1787) (Acari: Ixodidae) on horses** - P. V. Bossi Leite
- **Population Genetics of Parascaris equorum Based on AFLP Marker Technique** - E. Tydén
- **Towards diagnosing migrating Strongylus vulgaris: Preliminary data** - U. V. Andersen
- **Selective anthelmintic treatment of horses - The practical implementation is a chance for veterinarians** - A. Becher
- **Targeted selective treatment in horses - future approach to encounter anthelmintic resistance?** - D. Hamel
- **It's Time For a Change: The Rationale For Evidence-Based Parasite Control In Horses** - R. Kaplan -

Posters

- **Sustainable Management of a Group of Donkeys Harbours Anthelmintic Resistant Cyathostomins** - A. Trawford
- **Efficacy of a bovine formulation of moxidectin on the control of equine strongyles** - H. Garcia
- **Anthelmintic resistance in horses in Uruguay** - A. Drocco
- **Diagnosis of helminthiasis in horses. Comparison coproparasitologic techniques** - G. Arduoso
- **Helminth in horses of Rio Grande do Sul Brazil** - M. J. T. Mattos
- **Factors associated to IgG antibodies against Gasterophilus SP in horses from an oceanic climate area** - J. Sanchís

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The biology and control of equine parasites: is there anything new

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We will attempt to highlight areas that bring new thoughts to the field of equine helminth parasitology, confirm the status quo, or repudiate accepted dogma.

Identification of drug resistance populations of worms has been a focus of research in recent years. It is clear that macrocyclic lactone (ML) resistant populations of *Parascaris equorum* have a worldwide distribution. The long held prediction of emerging, wide spread ML resistance of cyathostomins has yet to be clearly documented. This is likely related to the methods used and complexity of the worm and host populations involved. Limited studies have reported decreased egg reappearance periods and follow up critical trials indicate that this may be due to a reduced larval efficacy (Lyons et al. 2010). Other recent studies have demonstrated the *in vitro* selection of ivermectin resistant in larval migration inhibition assays coupled with species identification using the reverse line blot method (RLB) (van Door et al.2010).

The development of molecular methods such as the RLB to identify individual species of cyathostomins (Traversa et al. 2007,2009) have increased in use in a wide range of studies. Other investigations have identified a cyathostomin larval protein that maybe important in anti-mortem diagnosis of mucosal larval burdens (McWilliam et al. 2010). There are also reports on the significance of the appropriate statistical analysis of FECRT data that are important but rarely considered (Denwood et al. 2010, Vidyoshankar, et al 2007). Similarly, the necessity (Chapman et al.2003) of the identification of a large portioned of the adult cyathostomin population to produce an accurate population estimates have largely been ignored.

While not new the focus on selected treatments and preservation of the cyathostomin refugia have become more accepted. The establishment of these approaches and their widespread use will depend on education. Open discussions of the use of the new class of anthelmintic monapantel in horses are not published but if ML resistance becomes a documented reality this would likely occur. Little has been published recently to investigate alternative control strategies.

With recent advances in cell biology, molecular biology and immunology it is disappointing that more has not been done to use these tools to advance out understanding of equine helminth parasitism. This is especially true in the area of host response where investigations in other host systems have clearly demonstrated that helminth infections regulate the outcome of vaccination and other infections. Much remains to be learned and applied.

Strategic Parasite Control for Working Donkeys Worldwide; Responsible, Effective and Sustainable

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Background; Throughout the world working equids remain an essential part of life for resource-limited communities. By providing essential draught, pack and ridden transport these animals contribute significantly to the livelihoods and welfare of their owners. Many organisations work with such equids by providing veterinary care and owner education. Treatment for parasites has been an important component of this work, however, little work has been undertaken to determine when, if and how treatment should take place. The Donkey Sanctuary (DS) is taking an active role in determining strategic de-worming protocols for all developing country projects using scientific methods.

Methods: Studies to determine gastrointestinal parasite burdens of donkeys were undertaken in Kenya and Ethiopia during 2007-2010. Faecal egg counts (FEC) were carried out in a cohort of donkeys during the rainy season and at the end of the dry season to determine seasonal fluctuations. Assessment of the health status of the donkey, including body condition (BCS), was made at the time of sample collection.

Results: Studies in Ethiopia showed that there was no significant benefit of de-worming twice yearly as opposed to once yearly if treatment is carried out at the end of the rainy season ($P < 0.05$) and that FEC is not significantly correlated with BCS. Studies in Kenya also showed no correlation between BCS and FEC with the majority of donkeys (>95%) being in good BCS. Mean FEC in the study population was 516epg in the rainy season and 220epg during the dry season.

Conclusions: These studies in Kenya and Ethiopia have provided evidence for changes in treatment practices undertaken by DS veterinary teams. Based upon these studies treatments will be performed once yearly as no significant benefit is derived from multiple treatments. The practice of blanket de-worming will eventually be discontinued in all project countries, only those animals which are immuno-compromised due to illness or age will be selected for treatment. These studies have shown that when in good health donkeys are able to cope with moderate levels of parasite infection and de-worming may be neither cost effective, ethical nor responsible.

Further studies of this nature are currently being carried out in other countries where the DS works and each project area will be individually assessed to design protocols for the future.

Helminths of horses in the federal state of Brandenburg (Germany): Prevalence, risk factors and anthelmintic efficacy

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Background: To optimize control strategies of horse helminths, it was aimed to get up-to-date data of relevant parasites in the federal state of Brandenburg, to detect risk factors for a high endoparasitic burden and to monitor efficacy of Pyrantel (PYR) and Ivermectin (IVM).

Methods: The number of horse farms in Brandenburg was estimated. A questionnaire was sent to these farms (700) in June/July 2006. 235 of those were returned. A sample of 126 study farms was selected at random from this subgroup. 1407 randomly selected horses were examined coproscopically. Risk factors for a high burden with cyathostomes were identified using multivariate logistic regression.

For the efficacy study for IVM and PYR in the years 2007/2008, 23 Horse farms with a high endoparasitic burden were selected. In these farms horses (min. 9 animals, max 40) with at least 100 strongyle eggs per gram faeces were randomly allocated to either a treatment or a control group.

In the IVM study, 213 horses were treated orally with 0.2 mg IVM/kg BW (Eqvalan duo[®], Merial). In the PYR study, 220 animals were treated with 6.6mg PYR/kg BW (Banminth[®], Pfizer). The untreated control group consisted of 193 and 198 animals respectively. Two weeks after treatment, faeces were examined coproscopically to detect the egg reduction.

Results: The prevalence on farm level was: cyathostomes 98.4%, ascarids 16.7%, tapeworms 14.3%, pin worms 8.7%. Large strongyles (*Strongylus vulgaris*) were detected in one farm only. Rare deworming and infrequent cleaning of stables were the most important risk factors for a high burden of cyathostomes.

Animals treated with IVM showed a mean egg reduction of 100%, whereas for PYR, egg reduction was less than 90% in two farms.

Conclusion: The high intensity of cyathostome-infections demonstrates that their epidemiological control is insufficient. Horse farms should be better advised about stable hygiene. IVM is still fully efficient. Sporadic resistance against PYR is assumed.

Gastrointestinal nematode effect on the reproductive performance of mares and stallions under an intensive equine embryo transfer program

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Nematode strongyles are usually present in grazing horses. A control parasite program was carried out in a horse reproductive center in Argentina (Doña Pilar Embriones, Lincoln, Buenos Aires). The objective of the present work was to evaluate the effect of nematode infection on reproductive performance of mares (donor and recipient) and stallions. Individual EPG were performed to 616 mares (73 donors and 543 recipients) and 38 stallions at least 2 times during the breeding season. Data from 471 flushings and 426 embryo transferes were analyzed. In fecal samples, small strongyle eggs were mostly prevalent, with sporadic appearance of big strongyles and tapeworms. Mares were classified in two groups accordingly to EPG counts: group with low parasite burden (L): EPG < 100 in all samples and group with high parasite burden (H) EPG ≥ 300 in at least one sample. Donor L mares had higher rate of positive flushings ($P = 0.013$) and more embryos per flushing ($P = 0.032$) than donor H mares. Transferred embryo from L donor mares resulted in similar pregnancy rates than embryo from H donor mares. Pregnancy rate was unaffected by parasite egg presence in recipient mares. Stallions were classified in three groups accordingly to EPG levels: low (L) EPG < 100, medium (M) $100 \leq \text{EPG} < 500$ and high $\text{EPG} \geq 500$. Positive flushing rates were negatively affected by increasing stallion EPG level ($P = 0.016$); whereas number of embryos and pregnancy rates remained unaffected. In conclusion small strongyle infection impaired reproductive performance in horses under embryo transfer program in early stages (until embryo recovery), whereas subsequent phases of transference and pregnancy rates were not modified by parasite burden.

Control of *Parascaris equorum* Infections of Foals on a Swedish Stud

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Introduction: A study was performed during two consecutive years on a large stud where two foals had died due to heavy *Parascaris equorum* burdens, although a preventive deworming program had been operated for several years. The aim of the study was to formulate a control strategy based on targeted treatment following faecal egg counts (FEC).

Methods: In the first year, 14 foals were included in the study. Individual faecal samples were collected weekly between 10-23 weeks of age. The samples were analysed by a McMaster method with a detection level of 50 eggs per gram (epg). Whenever the *P. equorum* FEC > 200 epg, the foal was drenched with 7.5 mg fenbendazole per kg bodyweight. In the second year, 28 foals were dewormed with fenbendazole at 13 weeks of age without being diagnosed. Individual faecal samples were then collected and analysed every second week. When the FEC > 200 epg, the foal was dewormed just like in previous year. In addition, instead of permanent paddocks used in the spring of the first year, foals of the second year were offered new paddocks.

Results: In the first year, all the foals became infected with *P. equorum*. Egg excretion started at 12-18 weeks of age with a large variation between 50-9500 epg. The first generation of ascarid worms resulted in higher epg values than the second generation; mean epg at the first deworming occasion was 2139, whereas it was 540 at the second occasion. Among foals that were sampled for at least 16 weeks, the average number of anthelmintic treatments was 1.6. In the second year, the egg shedding was considerably lower than in the previous year. A maximum of 900 epg was measured during the study period. The average number of anthelmintic treatments during 16 weeks was 1.9.

Conclusions: *Parascaris equorum* control including fenbendazole treatment at 13 weeks of age followed by targeted selective treatment based on FEC for the rest of the season appeared to be a successful control strategy on this stud.

In Vitro Selection Demonstrates Variation in Ivermectin Susceptibility in Cyathostomin Larvae

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Background: The study of macrocyclic lactone (ML) resistant cyathostomins in the field is difficult. Therefore a reiterative Larval Migration Inhibition Assay (rLMIA) is used, based on migration of exsheathed L3 (xL3) in the presence of ivermectin. The xL3 were differentiated by reverse line blot (RLB), a technique based on the hybridisation of the amplified intergenic spacer sequence with species specific probes for 13 common species. When larvae migrate through consecutive sieves, the migration percentage was found to increase. This indicates selection of the least susceptible larvae in a cyathostomin population that is heterogenic for ivermectin susceptibility. The selection resulted in a change in the cyathostomin population composition; a 5 fold increase of *Cyathostomum catinatum* and a 70-100% reduction of *Cylicocyclus nassatus* larvae.

Method: In the present study we investigated if selection for ivermectin resistance of larvae also occurred within cyathostomin species. rLMIA with 2 consecutive sieves was used and xL3 before selection and after the first and second migration were counted and differentiated by RLB. An increased migration percentage through the second sieve was considered to indicate heterogeneity for ivermectin susceptibility for larvae of that species. Five populations of cyathostomins from horses with different ML treatment history were assayed.

Results: The dominant species before selection were *Cyathostomum pateratum*, *C. nassatus* and *C. catinatum* and after selection *C. catinatum*. The percentage migration through the first sieve of *C. catinatum* in the presence of 30 µg/ml ivermectin was high in every population (20-30%). In populations with a history of ML treatments the percentage migration of *C. catinatum* was higher in the second migration (60-75%) compared to the first. This was not observed in a population that was never treated with MLs. Larvae of *C. nassatus* were the most ivermectin-sensitive in every population.

Conclusions: rLMIA was able to detect inter- and intraspecific differences in ivermectin susceptibility of xL3 from different cyathostomin species. In particular *C. catinatum* larvae showed an increased variation in ivermectin susceptibility in populations that had been exposed to MLs before. When this can be confirmed in in vivo resistant population(s), the current results provide a starting point for further molecular research on the mechanism of ML susceptibility in larvae as well as adult cyathostomes.

Strategic control *Anocentor nitens* (Neumann, 1897) (Acari: Ixodidae) on horses

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This study evaluated a control strategy of *Anocentor nitens* on horses based on spraying and acaricide paste formulation application in the auricular pavilion and nasal diverticulum. The study was conducted from October 2003 to March 2009 and the evaluations had been divided into the following phases: Phase 1 - Oct/03 to Mar/04 and the Phases 2, 3, 4, 5 and 6, respectively, corresponding to the same periods subsequent until Mar/09. The infestation levels in the auricular pavilion and nasal diverticulum were classified with scores 0 to 3. Phase 1 observations referred to the control period. From Apr/04 to Mar/06 was implemented a scheme acaricide sprayings carried out every seven days and divided in two series: the first one beginning in April 2004 and the second beginning on July, six spraying treatments regimen programmed for each, using cypermethrin at a concentration of 0.015%, and topical treatments applied monthly in the ears with an insecticide powder product. From April/06 to March/09 the same previous period spraying treatments regimen was performed and added a acaricide paste application, at each two months, applied in the auricular pavilion and nasal diverticulum. Phases 2 and 3 there was no reduction in parasitic loads of *A. nitens* in relation to Phase 1. While in Phases 4, 5 and 6 were recorded significant reductions in relation to the control period and also in comparison with the evaluations of Phases 2 and 3, characterizing the effectiveness of treatments using the acaricide paste. Results show that populations of *A. nitens* present in the nasal diverticulum are important in the maintenance of the infections in horses, being necessary control measures against the ticks that parasitize this anatomical structure. Keywords: Equine, *Anocentor nitens* diverticulum nasal, auricular control, acaricide tick paste.

Strategic control of *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae) on horses

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Amblyomma cajennense is a trioxen tick and has low host specificity, but the horses are their preferred host. It causes serious economic losses as a result productivity decreased, acaricides spending and is responsible for pathogens transmission. This study verified the efficacy of a strategic control program of *A. cajennense* in horses under field conditions in a proposed Integrated and Strategic Control of Parasites in Cattle and Equines. This study was conducted from October 2003 to March 2009. Were used 11 males and 5 females adults horses of mixed breed kept in property standard management. The initial scheme proposed for acaricide spraying was six treatments with a seven days interval and divided into two modules: the first one beginning in April of each year and the second beginning in July, seeking to reach ticks larvae and nymphs. The pyrethroid - cypermethrin 0.015% was used to horses spraying. However, there were initial regimen variations due animals and pastures larvae presence, climatic interference and operational matters at property (eight treatments April 2004 and five treatments July 2004). Adult tick counts were performed on horses from October to March of each year. After five years using the strategic control program the adult tick parasitism rate was reduced by 91.54%. The strategic control program results in a product rationalization use and manpower involved, determining a lower risk of animals, man and environment contamination. Results show the strategic control effectiveness. However, it is important consider some management and operational factors that can interfere with the practical application of this technology. Keywords: *Amblyomma cajennense*, strategic control, equines. Acknowledgment: CAPES, CNPq e INCT de Informação Genético-sanitária da Pecuária Brasileira.

Population Genetics of *Parascaris equorum* Based on AFLP Marker Technique

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Background: *Parascaris equorum* is common and important parasite of foals, where moderate to high infection levels may result in pulmonary and gastrointestinal dysfunction, poor growth, general illness and possibly death. Several reports from different European countries and North and South America show that macrocyclic lactones have reduced efficacy to *P. equorum*. The aim of the present study was to examine genetic variation of *P. equorum* from various stud farms in Sweden using amplified fragment length polymorphism (AFLP).

Methods: Approximately 100 adult parasites were collected from foals after they had been expelled with fenbendazole. *P. equorum* isolates in this study were collected from stud farms (n=10) with different geographical locations in Sweden. A control isolate from another population was from a stud in Germany. Total DNA was extracted and the AFLP method was basically performed as described by Applied Biosystems (ABI, CA, USA).

Results: Peak patterns of individual worms were determined, and data were analysed for various population parameters. With a peak amplitude threshold of 80, an average of 44 peaks were obtained in each worm. The AFLP analysis of all worms thus provided >350 markers with most being variable. Preliminary results from parasites collected from different foals on the same stud indicate they were more closely related than parasites from different studs. Based on results from worms analysed so far, the population structure suggests relatively high genetic relatedness among worms, low mutational rate, low gene flow between studs, and absence of strong genetic selection even from worms collected on different studs.

Conclusions: Our results show that AFLP is an effective method to demonstrate intra-specific genetic variation among geographical isolates of *P. equorum*. Thus, this technique has high potential for screening a maximum number of loci, and is therefore useful for analysis of population diversity.

Factors associated to IgG antibodies against *Gasterophilus* SP in horses from an oceanic climate area

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Background: Gasterophilosis is a equine myiasis caused by *Gasterophilus* sp larvae (Linne 1761, Diptera: Oestridae) mainly detected in areas under mediterranean climate, where elevated prevalences (>95%) have been reported. Although most horses can support substantial populations of these parasites without apparent disease, their presence can cause disease too subtle for current detection methods. This myiasis is commonly found at the slaughter of the horses, or even when L3 instars are passed by faeces.

Methods: A serological survey to assess the risk factors associated to the presence of IgG antibodies against *Gasterophilus* sp in horses from an oceanic climate area was conducted. 672 blood samples were analyzed by using an enzyme-linked immunosorbent assay (ELISA) and excretory/secretory antigens from *G. intestinalis* and *G. nasalis* second-stage larvae. The results were analyzed according to the breed, age (<3, 3-10 and >10 years), gender and housing of the equines (box, pasture and forest).

Results: The percentage of positive horses was 67% (CI 95% 63, 71). The autochthonous Pura Raza Galega (PRG) horses reached the lowest seroprevalence and the Arabian Pure Blood the highest ($\chi^2= 40.758$, $p= 0.001$). No differences in the percentages of seropositivity regarding the age were achieved ($\chi^2= 5.211$, $p= 0.074$). Geldings showed the highest values of seroprevalence, while the lowest were observed in mares ($\chi^2= 12.596$, $p= 0.002$). Horses maintained in the pastures exhibited significantly higher seroprevalences than the equines kept in boxes or those maintained in forests ($\chi^2= 53.238$, $p= 0.001$).

Conclusions: Our results demonstrated that sensitization in horses against *Gasterophilus* depends on their breed, gender and housing. Ocean climatic areas offer also suitable conditions for the development of this myiasis.

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Selective anthelmintic treatment of horses

The practical implementation is a chance for veterinarians

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Growing anthelmintic resistance has led to the first studies using selective anthelmintic therapy (SAT) in horses in the early 1990th. For the same reasons SAT for horses was introduced 2008 in the area of Southern Germany and Austria. The main aim was to evaluate this method under field conditions.

Fecal samples of 99 horses on 15 farms in an area of 40 km around Salzburg (Germany and Austria) were collected monthly from March until November 2008 and 2009, resp. The samples were analysed using a modified McMaster method. Every horse shedding ≥ 250 strongyle eggs per gram feces (EPG) was treated with anthelmintic according to following schedule: 1st fecal egg count (FEC) ≥ 250 EPG: pyrantel. 2nd FEC ≥ 250 EPG: Ivermectin. 3rd FEC ≥ 250 EPG: moxidectin. At the end of the grazing season, every horse shedding ≥ 250 EPG in October and every other horse was treated independent of the FEC in november by administering moxidectin plus praziquantel. According to the fecal egg count reduction tests, pyrantel was only used in 2009 when efficacious.

In 2008 no eggs were detected in the fecal samples of 38 horses and 2009 in the fecal samples of 40 horses. Furthermore 66 and 76 resp. horses in 2008 / 2009 did not have to be treated due to FEC ≥ 250 EPG. The mean EPG in March 2008 was 147 EPG and was reduced to 105 EPG in march 2009.

FEC monitoring as part of worm control can be used to reduce the pasture contamination with strongyle eggs.

According to a questionnaire horse owners favour worm control methods which (i) prevent anthelmintic resistance, (ii) analyse the farm specific worm population and (iii) are specific to their own individual horse. However costs and effort of worm control are less important to them.

In conclusion the results of these two studies suggest that FEC - monitoring can support a diagnostic based, medical approach to equine worm control which –according to a large scale questionnaire – is widely preferred by horse owners. Equine practitioners can thus clearly benefit from such an approach in terms of veterinary medical expertise and economic success, resp.

Targeted selective treatment in horses – future approach to encounter anthelmintic resistance?

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Background: Resistance in small strongyles to anthelmintics, especially to ivermectin, pyrantel and fenbendazol, is a huge problem for the future management of worm infections in horses. Since several years efforts have been undertaken to introduce control programs to reduce the development of anthelmintic resistance in small strongyles. A promising attempt is the use of refugia, which aims in treating high egg shedders while low shedders are not treated and therefore a considerable proportion of nematode population is excluded from impact of treatment. An essential technique of this measure is the use of fecal egg counts for quantifying of egg excretion. Considering recent publications on targeted selective treatment, cut-off for treatment is set around 250-300 epg. Such regimes have successfully been introduced in Denmark.

Methods: In the following study we present preliminary results from 104 horses examined with McMaster-technique and combined sedimentation-flotation. Additional information on treatment regime was provided by the owner.

Results: A total of 104 animals (91 horses of 12 breeds, 13 ponies/3 breeds) were enrolled in this study. Mean age of 93 animals with age provided was 12.8 years, gender could be attributed to 41 mares, 51 geldings and one stallion, gender was not provided for 10 animals. 83 (79.8%) horses had egg counts of 0 - <30 epg, 14 (13.5%) of 30 – 240 epg and 7 (6.7%) of > 240 epg. Maximum epg was 1650. Eggs of *Anoplocephala* spp. were detected in two, *Parascaris equorum* eggs in none of the fecal samples. Treatment was given in an average of 120 d prior to fecal examination. Ivermectin was given in 38 cases, pyrantel in 11, moxidectin in 5, others in 3 cases. There was no information on treatment in 47 cases.

Conclusion: Although treatment dated back more 4 month in average, more than 80% of all animals based on a cut-off of 250 epg would not need to be treated. The period of 4 month by far exceeds the possible egg reappearance of small strongyles in horses.

The use of targeted selective treatment can considerably reduce the use of anthelmintics in a huge proportion of the horses. Future follow-up examinations will identify possible changes in individual epgs and efficacy of anthelmintics.

It's Time For a Change: The Rationale For Evidence-Based Parasite Control In Horses

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In 1966, a groundbreaking publication by Drudge and Lyons led to the interval dose program, where horses are treated with anthelmintic every two months throughout the year. This program was designed primarily to control the highly pathogenic *Strongylus vulgaris*, and was extremely successful in reducing parasite-related morbidity and mortality. This strategy rapidly became adopted worldwide, and in the 1970's and 1980's with the introduction of new classes of anthelmintics, the practice of rotating drugs at each treatment also became commonplace. Also during this time, anthelmintics became available as easy to administer pastes, eliminating the need for veterinary administration via nasogastric tube. As a result, veterinary involvement in parasite control declined drastically. Without veterinary involvement, parasite control became removed from the medical scrutiny that other medical treatments receive. However, much has changed over the past 45 years, and current 'traditional' approaches routinely fail to provide optimal or even adequate levels of parasite control. It is now becoming obvious that this approach is no longer viable, and an evidence-based approach is needed. Evidence based veterinary medicine (EBVM) is defined as the conscientious, explicit and judicious use of current best evidence in making decisions about the care of individuals and populations. EBVM is gaining wide acceptance across the spectrum of equine veterinary practice with the exception of parasite control. But this must change given the new biological realities. *Strongylus vulgaris* now is very uncommon. In contrast, cyathostomins were historically of little pathogenic concern, but now are considered the primary nematode pathogen of horses. *Strongylus* and cyathostomin parasites have very different life cycles and host-parasite dynamics, and therefore require very different approaches for successful control. Furthermore, years of intensive anthelmintic use have led to high levels of anthelmintic resistance in both cyathostomins and *Parascaris equorum*. In addition, the host-parasite relationship is characterized by an overdispersion among hosts, such that a small percent of animals harbor the majority of the total worm burden in a herd. Given these biological realities, there no longer is any reasonable medical justification for treating all horses at frequent intervals, often with drugs that are ineffective due to resistance. Rather, an evidence-based approach is required that takes into account the numerous medical, biological and environmental factors that should be considered when making anthelmintic treatment decisions.

Sustainable Management of a Group of Donkeys Harbouring Anthelmintic Resistant Cyathostomins

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Background: The Donkey Sanctuary has 2500 donkeys resident on its farms in the UK. Parasite control in these herds focuses treatment on the basis of a positive faecal egg count (FEC). Monitoring of the herds has identified ongoing problems at one site which houses 293 donkeys (Farm P); a number of animals are known to harbour cyathostomins with suspected ivermectin and moxidectin resistance. This study reports the findings of reduced efficacy of pyrantel embonate (PYR) at Farm P and the management practices in place to manage the resultant population.

Methods: A proportion of donkeys resident (n=47) at Farm P were selected for a faecal egg count reduction test (FECRT) on the basis of an FEC indicating the need for treatment (FEC $\geq$ 300 epg), donkeys were treated with equine paste PYR (19 mg/kg) and dung samples were taken for FECRT 14 days post-treatment.

Results: FECRT showed a mean reduction of 85% (range 14 – 100%). 66% (n=31) of donkeys showed an FECR of less than 95% at 14 days post treatment when treated with PYR. Under field conditions this may indicate a lack of efficacy of PYR.

Conclusions: Detection of lack of efficacy to PYR at Farm P may cause concern for those managing this group of donkeys. This population have been previously reported to harbour cyathostomins which appear to be resistant to both moxidectin and ivermectin; unpublished data also suggests lack of efficacy of benzimidazoles. Management of these animals is challenging with limited effective anthelmintics alongside day to day management of a geriatric donkey population. On the basis of these and previous results Farm P is now closed with no movements into or out of the population and undertakes 4 weekly FEC monitoring with individual treatment plans. Pilot studies have shown the safety of increasing the treatment threshold to 1000epg with no significant clinical effects, leading to adoption at Farm P. Donkeys are removed from pasture for at least 5 months over winter; pasture larval counts have shown no viable larval recovery after the winter of 2009/10. Management of this group will involve limited, targeted treatment and management of pasture to prevent further resistance. Ongoing research continues to determine the reasons for the lack of efficacy to all classes of anthelmintic and to determine best management practices.

Efficacy of a bovine formulation of moxidectin on the control of equine strongyles

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Anthelmintics for horses are almost exclusively available in oral formulation. Venezuelan creole horses are usually difficult to treat with these oral formulations because they are not used to human contact, and besides, these formulations are usually expensive and time-consuming for horse owner on cattle farms. The aim of this study was to determine the efficacy of a bovine formulation of Moxidectin (Cydectin NF[®]) for controlling strongyles infections in wild creole horses from a cattle farm in Guarico (Venezuela) based on percentage of epg reduction (EPGR). The study was performed between June and July, 2009 at Research Station La IGUANA, (Universidad Simon Rodriguez, Guarico, Venezuela). Twenty adult animals over 3 years-old age, 10 males and 10 females were treated once with Cydectin NF[®] with the dose of 400 µg/kg (subcutaneous way at the neck). Coprological samples were taken from each horse before treatment (day 0) and 24 hours (h), 48 h and 15 days after treatment (DPT) and analyzed using the Mc master method. Prevalence before treatment was 80% (16/20) with an epg mean of 792. Amazingly epg mean rose to 1015 epg at 24 hours post treatment, then declined to 596 epg at 48 h post treatment. At 15 DPT, efficacy was 96,6% and the epg mean was reduced to 27 epg.

Anthelmintic resistance in horses in Uruguay

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Introduction: Small strongyles or cyathostomins are the most prevalent parasites in horses of all ages and can cause severe disease like diarrhea, colic, dehydration and even death. Resistance of these parasites to benzimidazoles is well known all around the world. In Uruguay this situation was described in only one farm, in a study published in 1995. Anthelmintic resistance of cyathostomins to ivermectin has been observed in many countries and recently, resistance to ivermectin was reported in one farm in Brazil. The objectives of this work were to evaluate the presence of anthelmintic resistance in nematodes of horses and to identify the most prevalent parasite genus in horses of different farms in Uruguay.

Methods: The presence of anthelmintic resistance was evaluated by the fecal egg count reduction test (FECRT). Feces of 306 horses of different breeds, sexes and ages from 8 farms of Uruguay were studied. In each farm horses with more than 100 eggs per gram (epg) were selected and randomly assigned to 4 equal groups of 6 to 10 animals. Three of this groups were treated orally with fenbendazole 10% (Panacur®, Intervet), Ivermectin 1,87% (Eqvalan®, Merial) and pyrantel pamoate 24% (Totalject P®, Santa Elena Laboratories), one control group was not medicated. Individual fecal samples were collected pre-treatment and 14 days after treatment and processed by a modified Mc Master technique. Additionally larval cultures were performed before and after treatment.

Results: Only cyathostomins were identified in pre and post treatment larval cultures. Resistance of small strongyles to fenbendazole was present in all farms, with 0 to 37% of reduction of the fecal egg count. Ivermectin and pyrantel pamoate showed 99-100% and 92-100% efficacy respectively, in all farms surveyed.

Conclusions: This is the first wide survey of anthelmintic resistance in horses reported in Uruguay. Results indicate that resistance of cyathostomins to benzimidazole is present and widespread in Uruguay and corroborate that small strongyles is the most prevalent group in the country.

Diagnosis of helminthiasis in horses. Comparison coproparasitologic techniques

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Helminthiasis of horses have a negative impact on production. Routine deworming every eight weeks, was used throughout the world. Currently more attention is placed on epidemiology, and the egg reappearance period to implement the treatment, reducing the number of doses, trying to delay the emergence of resistance. Several authors question the McMaster technique, suggesting that in horses is not sensitive or accurate. Techniques were compared: Mc Master Modified, Robert and O'Sullivan 1949 (MMM), Mc Master Original Gordon and Whitlok 1939 (MMO) and method of Stoll. Two operators performed the tests. The MMM compared Operator N° 1 vs. MMO and Operator N° 2 with the method of Stoll. 54 samples were processed. There were two replicates for each sample with each technique. The variable analyzed was the epg. Each sample was divided into two subsamples. First analyzed the two subsamples using the same technique and then between techniques. The results were analyzed by nonparametric Wilcoxon test for paired data. Operator N° 1 **A**-Analysis of subsamples using the same technique. There were no significant differences between samples with no technique (MMO: $P = 0.9685$; MMM: $P = 0.2757$). **B**-Analysis between techniques for each of the two subsamples, MMO vs. MMM. In both there was a highly significant difference ($P < 0.0001$). MMM detecting more epg than MMO. Both have approximately the same coefficient of variation. Operator N° 2 **A**-Analysis of subsamples using the same technique. There were no significant differences between samples with no technique (MMM: $P = 0.07$, Method Stoll: $P = 0.11$). **B**-Analysis between techniques for each of the two subsamples, MMM vs. Method Stoll. In both there was a highly significant difference ($P < 0.0001$). MMM detecting more epg than the method of Stoll. Both have approximately the same coefficient of variation while in the case of the sub-sample 1 Method Stoll also presented greater relative variability. We conclude that, even taking into account the diversity of uncontrolled variables, can affect the egg count, the evidence indicates that the method of Stoll would be less desirable, since not only detects the lowest median number of eggs but, also does so with the highest coefficient of relative variation, while the MMM technique detected a greater number of eggs present, with less relative variability.

Helminth in horses of Rio Grande do Sul Brazil

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The state of Rio Grande do Sul has an area of 281.749 Km² and the population of horses is estimated to be 468. 447 head, equivalent to 8.15% of the national herd. The region has a subtropical weather, precipitation between 400 and 1200 mm per year. The climate is characterized by a distinct dry season (winter) and a wet season (summer). Helminth infections in horses can cause emaciation and diarrhea, which account for significant losses in breeding. Horses are kept in native field and are treated with ivermectin and moxidectin every 45 days. This study was carried out to determine the occurrence of helminth infections among horses in two cities of Rio Grande do Sul Brazil. Fecal samples of 136 animals, were analyzed for the identification and quantification of eggs per gram and infective larvae. Results showed that 82,35% of the samples were infected by Strongyloidea/cyathostominae eggs/larvae. We concluded that the infections by helminths, especially the cyathostominae, are significantly common in the region, so that greater importance should be given to this situation.

Towards diagnosing migrating *Strongylus vulgaris*: Preliminary data

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Increased awareness of anthelmintic resistance among cyathostomins and *Parascaris equorum* has initiated a change towards less intensive treatments. Faecal egg count techniques only detect egg-laying parasites, but in the case of *Strongylus vulgaris* it is migrating stages that can be highly pathogenic. Currently there are no reliable ways to detect migrating *S. vulgaris* antemortem. The aim of this study was to identify molecules that potentially can be used as diagnostic markers. The approach involved generation of antibodies against adult *S. vulgaris* excretory-secretory (ES) fraction and immunoscreening of a larval cDNA library in order to identify secreted, immunogenic proteins. ES fractions were collected from adult worms after 13, 24, and 48 hours and from larvae after 13, 18, and 27 days. The adult ES fraction with highest protein concentration was dialysed (3.5 kDa molecular weight cut-off) and used to immunise a rat. The antiserum's reactivity was assessed on western blots (WBs) with adult and larval ES as antigen. The cDNA-library was constructed from mRNA extracted from migrating *S. vulgaris* larvae. Immunoscreenings were performed with hyperimmune rat serum and insert sizes assessed by PCR and gel electrophoresis. In WBs, the antiserum recognised approximately 22 molecules from the adult worm ES fraction with one (20 kDa) that appeared to be immunodominant. When probed against larval ES, the antiserum identified approximately 12 molecules, with highest reactivity against a 60 kDa protein. Silver-staining indicated that both adults and larvae produce a highly diverse ES-fraction. When comparing the silver stained gel and the WBs, the molecule with highest antibody reactivity in larval ES was not the most abundant, whereas the strongest reacting molecule in adult ES was abundant. So far, immunoscreening of 10,000 plaques from the cDNA library yielded 32 reactive clones. PCR performed on secondarily screened positive clones revealed cDNA of similar size with some slight variation (744, 755, and 776 bp). Further immunoscreening will be performed, and the immuno-reacting clones will be sequenced. Sequence data obtained from this library will be searched against non-redundant protein sequences using BLASTx, and full-length cDNAs will be obtained. An identified ES protein may prove useful for detection of migrating *S. vulgaris* stages in horses.



SESSION I

Keynote Lecture

- **Developing vaccines to control protozoan parasites in ruminants** - E. A. Innes

Oral Communications

- **What's New Is Old: Novel Approaches For The Control Of Parasitic Worms** - D. Piedrafita
- **Molecular Variability of H11 Antigen in Brazilian Isolates of *Haemonchus contortus*** - M. B. Molento
- **H11 – a host-protective intestinal aminopeptidase from *Haemonchus contortus* and a vaccine target with anomalies** - D. Knox
- **Immunomodulatory effects of a nematode carbohydrate antigen** - I. Sutherland
- **Use of phage display libraries to identify conformational epitopes of *Teladorsagia circumcincta* antigens** - S. Ellis
- **Prenatal exposure of *T. colubriformis* affects postnatal response to *D. pteronyssinus* in lamb** - R. McFarlane
- **The role of the ovine MHC in resistance to nematode infection** - M. J. Stear
- **Evaluation of low dose immunization with gut membrane glycoproteins of *Haemonchus contortus* against nematode infection in grazing lambs** - C. J.H. Souza
- **Biological differences among *Neospora caninum* isolates obtained from oocysts and from clinical cases of naturally infected dogs** - A. Dellarupe
- **Candidate genes for the improvement of the DNA vaccine pCI-neo-LACK/MVA-LACK against canine leishmaniasis** - V. Larraga
- **Antigenic variation of the *Plasmodium chabaudi* cir multigene family and its implication in host tissue localization** - P. Ebbinghaus
- **Novel approaches for the development of vaccines against the blood fluke, *Schistosoma japonicum*** - E. N. T. Meeusen

Posters

- **Effect of host immunosuppression on response to infection with *Haemonchus contortus*** - S. Walkden-Brown
- **Vaccination against *Haemonchus contortus* on boer goats grazing contaminated pasture** - A. Spickett
- **Domestication of the *Trypanosoma congolense* transmission cycle selects for strains of reduced virulence** - S. Chitanga
- **Towards a Commercial Vaccine for Haemonchosis in Ruminants** - W.D.Smith
- **Field evaluation of a *Rhipicephalus (Boophilus) microplus* multiantigenic vaccine in cattle** - I. da Silva Vaz Junior
- **Vaccination with a recombinant chimeric antigen confers protection against *Neospora caninum* cerebral infection in experimentally infected mice** - T. Monney
- **Comparison of gene expression profiles between resistant and susceptible ovine breeds to *Haemonchus contortus*** - E. Liénard
- **Potential of lipid-core peptides as an effective vaccine delivery system for parasitic helminths** - A. M. Dougall
- **Evaluation of an experimental vaccine against *Haemonchus contortus* and *Haemonchus placei* in calves** - C. C. Bassetto
- **Desensitising sheep to *Trichostrongylus colubriformis*** - A. W. Greer
- **Identifying transcriptional changes of key molecules of *Teladorsagia circumcincta* upon encountering the ovine host** - A. M. Halliday
- **What Cytokines are Expressed on Abomasum Lymph Nodes when Somalis Crossbreed Sheep are Infected by *Haemonchus contortus* in Northeast of Brazil?** - L. Giotto Zaros
- **Novel Control Strategy for Cattle Tick: Development of a recombinant vaccine Performance optimization and Efficacy** - T. Poggio
- **Cytokines Expressed in Crossbreed Sheep from Northeast of Brazil naturally infected by *Trichostrongylus colubriformis*** - Silva Vieira
- **The Th2 immunomodulative effect of somatic extract of *Trichinella spiralis*** - E. Dvorožňáková
- **Production and Immunological Characterization of a Recombinant *Rhipicephalus microplus* Cysteine Endopeptidase** - D. P. Oldiges

Developing vaccines to control protozoan parasites in ruminants

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Protozoan parasites are among some of the most successful organisms worldwide, being able to live and multiply within a very wide range of hosts. The diseases caused by these parasites cause significant production losses in the livestock sector involving reproductive failure, impaired weight gain, contaminated meat, reduced milk yields and in severe cases, loss of the animal. In addition, some protozoan parasites affecting livestock such as *Toxoplasma gondii* and *Cryptosporidium parvum* may also be transmitted to humans where they can cause serious disease.

Our approach at Moredun is to establish experimental models of infection in ruminant species to enable us to study the interactions between parasite and host. We are interested in how the parasite initiates infection become established and multiplies within the host and the critical pathways that may lead to a disease outcome. Once the parasites invade the hosts they induce both innate and adaptive immune responses and the induction and function of these immune responses are critical in determining the outcome of the infection.

Using different protozoan parasitic diseases of livestock as examples this paper will discuss how the parasites may cause disease and how the host can defend itself against the parasite. By understanding this critical relationship between parasite and host and determining which immune responses are critical for host protection and survival, as opposed to those immune responses that may in fact protect the parasite or cause disease in the host, will enable us to develop effective vaccines to help control disease.

This paper will discuss different vaccination targets and strategies to help protect against: acute disease, congenital infection and abortion, persistence of zoonotic pathogens in tissues of food animals and passive transfer of immunity to neonates.

What's New Is Old: Novel Approaches For The Control Of Parasitic Worms

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Background: Most successful vaccines currently available for viral, bacterial or protozoal infections accelerate the development of immunity naturally acquired by the host after infection. Natural immunity to gastrointestinal nematode (GIN) parasites develops in ruminants after repeated infections, and most animals are refractive to infection after 1-2 grazing seasons. This indicates that the development of GIN vaccines based on 'natural' antigens, is an achievable goal. The major objective of the work presented here was to determine (i) the protective potential of a surface larval molecule of *Haemonchus contortus* in vaccination protocols using commercially acceptable adjuvants and immunization schedules and (ii) the identification of mimotopes to allow large scale commercial vaccine production.

Methods: Large scale vaccination (twice, 4 weeks apart) in commercial adjuvants was carried out in groups of 50 sheep per trial and various immunological (antibody isotypes, hypersensitivity reactions, quantification of immune cells) and parasitological parameters (worm burdens, worm length, egg production) were measured. Mimotope identification and functionality studies were carried out using phage display libraries and in vitro functional assays, respectively.

Results: The larval antigen was validated as a vaccine antigen based on protection in sheep against experimental *H. contortus* infection, with significant and consistent protection levels of greater than 60% for egg counts and worm recoveries in antigen vaccinated animals. We have also identified several mimotopes to allow commercialisation of this vaccine.

Conclusions: We are now looking to commercialise this vaccine with our partners, Pfizer VMRD Asia Pacific.

Molecular Variability of H11 Antigen in Brazilian Isolates of *Haemonchus contortus*

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Background: *Haemonchus contortus* is an intestinal nematode of ruminants that is found worldwide. The parasite causes significant economic and sanitary problems to food producing sub/tropical countries. As an alternative control method, researchers are trying to produce a recombinant vaccine, with special attention to H11 aminopeptidase complex. Due to a post-translational process of glycosylation, this antigen requires complex expression systems to have the recombinant form.

Methods: The aim of this work was to investigate the molecular variability of two regions of H11 to verify whether the presence of nucleotide mutation in the whole gene extension could result in different post-translational modifications. We used larvae and adults (n=35) isolates of *Haemonchus contortus*, from São Paulo and Paraná State farms. **Results:** The amplified regions were sequenced on Applied Biosystems SOLiD™ Sequencer, in a total of 881.255 reads of 50 bp fragments. It was observed 33 nucleotide substitutions on the 549 bp 'smaller' fragment (29 transition type and 4 transversion type) and 17 substitutions on the 713 bp 'bigger' fragment (16 from transition type and 1 of transversion type). Inside the exonic regions, the single nucleotide polymorphism identified resulted in 10 amino acid substitutions (2 synonymous, 7 non-synonymous, 1 stop codon).

Conclusion: This is the first molecular variability analysis to the H11 gene, whose results demonstrate the minimum nucleotide mutational rate existing on Brazilian isolates. Deeper studies about post-translational alteration from these nucleotide mutations are necessary to define the most adequate expression vector to maintain the immunogenic capacity of an epitope recombinant protein against the majority field isolates of *Haemonchus contortus* in Brazil.

H11 – a host-protective intestinal aminopeptidase from *Haemonchus contortus* and a vaccine target with anomalies

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Background: An intestinal aminopeptidase-enriched protein fraction from the microvillar surface of the intestine in the blood-feeding adult stage of *H. contortus* stimulates high levels of protective immunity when used as a vaccine against homologous challenge. Although referred to as H11, it is a mixture of several intestinal proteins with the aminopeptidase being dominant and this has a 5 isoforms (H11 -1 to -5). Attempts to reproduce protection with recombinant protein versions (bacteria, yeast, insect cell) of H11 have, by and large failed although some recent reports indicate some protective efficacy when H11 is delivered as a DNA vaccine. The failure of recombinant vaccines could be because a combination of proteins is required to achieve full efficacy or the nature of the recombinant (folding, post-translational modifications) may be vital.

Methods: We have examined the *in vivo* effects of RNAi on the H11 -1 gene in infective larvae prior to infection. Subsequently, treated larvae were used to infect lambs and infection parameters compared to larvae exposed to control dsRNA. We have expressed H11 -1 and H11 -4 isoforms in *C. elegans* and characterized the protein products.

Results: H11 -1 RNAi resulted in a 57% reduction in faecal egg count (FEC), 40% reduction in worm burden and 64% decrease in aminopeptidase activity compared L3s pre-soaked in control dsRNA when the larvae were used to infect lambs. The H11 -1 and H11 -4 isoforms were both expressed in *C. elegans* insufficient quantity to establish a protection trial and the outcome of this will be reported. The *C. elegans*-expressed H11 isoforms were enzymically active indicative of correct folding and early analyses indicate that they are glycosylated in a manner similar to the native H11 protein.

Summary/Conclusions: The RNAi data do indicate the H11 is essential for normal worm development. The successful expression of H11 isoforms in *C. elegans* underlines the utility of this system for nematode vaccine production.

Immunomodulatory effects of a nematode carbohydrate antigen

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A carbohydrate larval antigen (CarLA), released from the surface of infective-stage trichostrongylid larvae following ingestion and exsheathment, induces a protective antibody response in sheep over a period of months. However, little is known as to how CarLA is recognised or processed by the immune system. A combination of genotypic and phenotypic techniques were used to describe changes in human monocyte-derived dendritic cells (DC), key antigen presenting cells, following exposure to CarLA *in vitro*. The DC internalise CarLA and responded to exposure, as measured by fluorescence and altered gene and protein expression. However, these changes were relatively limited compared to the response of DC to two model antigens (OVA and LPS). There was no significant alteration of the expression of genes encoding the major families of pattern recognition molecules by CarLA, while a few cytokine genes were up-regulated (IL1b, IL-6, IL-8TNFa). However, when DC were exposed to a combination of CarLA with either OVA or LPS, several hundred genes were uniquely modified. Of these genes, 89 were common to CarLA+OVA and CarLA+LPS. These genes included many with known immune function, implying significant modulation of the immune response to unrelated antigens following co-incubation with CarLA. Phenotypic studies using flow cytometry confirmed that DC exposed to CarLA undergo only minor changes in the expression of surface antigens. Furthermore, DC exposed to the combinations of CarLA-OVA and CarLA+LPS tended to have down-regulated expression of the co-stimulatory molecules CD80, 83, 86 and MHCII, but increased expression of CD40. This implies a possible function of CarLA in down-regulating signalling to immune-effector cells such as T-lymphocytes.

The results obtained demonstrate that CarLA is a powerful modulator of immune responses, and current work is focused on a more complete understanding of the interactions between DC exposed to CarLA and components of the acquired immune response.

Use of phage display libraries to identify conformational epitopes of *Teladorsagia circumcincta* antigens

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Background: *Teladorsagia circumcincta*, an abomasal nematode of small ruminants, is a major welfare and economic issue in temperate regions. Currently, control is based on anthelmintic use and management; however, anthelmintic resistance has been identified. An alternative control method is vaccination, as natural immunity can be acquired through trickle-infection. Vaccination trials testing lead candidate protein antigens, expressed by recombinant systems often fail to induce the same level of protection as the native antigen, possibly because of incorrect protein folding or lack of other appropriate post-translational modifications associated with conformational epitopes. We have therefore probed random peptide phage-display libraries (PDL) with antibodies from immune sheep to identify peptides that mimic epitopes of the infective larval stage (L3) of *T. circumcincta*.

Methods: A fraction highly enriched for IgA was prepared from the abomasal mucus of *T. circumcincta*-infected sheep by affinity chromatography, using an L3 somatic extract as the affinity ligand. The enriched IgA was subjected to three rounds of panning against a 7-mer peptide PDL to obtain peptide sequences that were bound by the target antibody. Individual phage plaques were sequenced, and their target-binding affinity assessed by ELISA.

Results: After three rounds of panning, fifty clones were selected for high binding affinity to the target IgA. Five peptides dominated the dataset: these contained a high proportion of aromatic amino acids and a number of these sequences showed high target-binding affinity and low affinity for the plastic substrate, highlighting the specificity of the technique. Preliminary dot-blot analysis showed that several of these peptide sequences bound IgA from the abomasal mucus of animals rendered immune to *T. circumcincta*.

Conclusion: Here, we report the use of PDL in the identification of conformational epitopes of *T. circumcincta* L3. The high prevalence of aromatic amino acids within the clones selected indicates that such residues could potentially mimic conformational epitopes. Further investigation highlighted the selectivity of bio-panning, as selected clones had high affinity for the antibody target. Preliminary immunoreactivity assays indicated that a panel of phage clones are recognised by abomasal IgA from trickle-infected animals. This study has potentially identified a number of peptide sequences that may be mimicking the conformation of L3 antigenic epitopes.

Prenatal exposure of *T. colubriformis* affects postnatal response to *D. pteronyssinus* in lamb

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Background: There is a worldwide consensus that asthma and allergies are witnessing a rising trend in western societies. In New Zealand, protection against asthma is conferred on individuals conceived and raised in a rural environment. The reasons for this are unknown, although an inverse relationship exists between internal parasite prevalence and sensitivity to house dust mites (asthma-causing allergin). The aims of this study were to a) set up a sheep model for the study of asthma and, b) study the effect of intrauterine inoculation of the asthma-causing allergen [*Dermatophyoides pteronyssinus*- (HDM)] or parasite antigen [*Trichostrongylus colubriformis* L₃ (TCL₃)] on the development of 'asthma' in sheep.

Methods: Foeti of twin bearing ewes (n=23) were dosed orally with HDM, TCL₃ or PBS as a sensitizer followed by an identical booster at birth. The animals were grazed on ryegrass/white clover pasture and at nine months of age, the lambs were injected subcutaneously with HDM (sensitizer followed by a identical booster). All animals were subsequently sampled and challenged (with HDM) intra-bronchially using a bronchoscope.

Results: Neonatal lambs that had been treated *in-utero* with HDM antigen (and their contra-lateral controls), had higher levels of HDM-specific IgE (but not IgG₁) compared to lambs treated with TCL₃ or PBS. Following challenge, there were significant differences due to treatment in the cell populations of broncho-alveolar lavage fluid (p<0.05), but not in blood. Serum HDM-specific IgE, but not HDM-specific IgG₁, levels of the different treatment groups were significantly different. Following necropsy, there was less hepatitis in the TCL₃-treated animals. Upon histological examination, inflammation due to HDM infusion was diminished significantly in those animals that had been exposed to TCL either *in utero* or post-natally.

Conclusions: The oral exposure of sheep foeti to parasite antigen *in-utero* affects the degree of local inflammation in the lung following exposure to HDM, later in life.

The role of the ovine MHC in resistance to nematode infection

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The primary manifestations of resistance to *Teladorsagia circumcincta* are reduced parasite growth and fecundity as well as reduced numbers of adult nematodes. IgA and eosinophils are associated with reduced growth and fecundity while IgE and mast cells are associated with reduced worm burden. There is considerable genetic variation in resistance to nematode infection in sheep. We tested 1000 lambs for genetic variation in class II loci of the major histocompatibility complex. There were several associations with specific alleles but these appeared secondary to heterozygote advantage. Heterozygotes have lower faecal egg counts and heterozygosity appears to act through the parasite-specific IgE response rather than the IgA response. One consequence is that resistance is likely to involve the recognition of multiple parasite molecules and there will be reduced selection pressure on parasite molecules to evolve and evade the immune response. Another consequence is that selective breeding for nematode resistance is unlikely to fix particular MHC alleles.

Evaluation of low dose immunization with gut membrane glycoproteins of *Haemonchus contortus* against nematode infection in grazing lambs

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A vaccine containing integral membrane proteins from *Haemonchus contortus* intestinal cells has been shown to be effective for lamb immunisation at a 50ug dose. However, in order to develop a viable commercial product, lower doses of vaccine were tested in two-month old grazing Corriedale lambs. The animals were kept in a single paddock and allocated to five groups according to their liveweight, sex and faecal egg counts (FEC). Vaccinates received 50, 10, 5, 2 or 0 ug of the antigen diluted in QuilA adjuvant. Vaccine shots were given at days 0, 21, 49 and 91 and all lambs were weaned at day 56. Lambs were weekly sampled for FEC, packed cell volume (PCV), and plasma was stored for subsequent serological analyses. Logarithmic transformation was used for FEC (logFEC+25). Lambs with PCV equal to or less than 15% were drenched with levamisole as a salvage treatment. After the second vaccine injection there was a significant reduction in FEC in the higher dose group. Natural infection levels were low due to drought, therefore 21,000 L3 mainly *H. contortus* field isolate, originated from contemporary lambs, was orally administered during a five-week period, starting at day 70. Post-artificial challenge responses (from day 91-112), showed that vaccinates had lower FEC counts and higher PCV than controls ($p<0.0001$) and salvage treatment was needed in 9/17, 1/16, 1/18 lambs for controls, 2 and 5ug, respectively, while none of the animals treated with 10 and 50ug reached $PCV\leq 15\%$. Animals treated with 50ug had the lowest FEC and the highest PCV value and the results suggested a dose-dependent effect for both FEC and PCV responses in the vaccinates. If the salvage criteria is taken into consideration, a minimum suitable vaccine dose for field application would be 10ug, but 5 and 2 ug doses were also effective in the great majority of lambs. Serological analysis may reveal whether these exceptions were "non-responders".

Biological differences among *Neospora caninum* isolates obtained from oocysts and from clinical cases of naturally infected dogs

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A pregnant BALB/c mouse model is suitable for examining the virulence of different *Neospora caninum* isolates. The aim of this work was to examine biological differences among 4 *N. caninum* isolates obtained from oocysts shed by naturally infected dogs (Nc-GER2, Nc-GER3, Nc-GER-6 and Nc-6 Arg), one isolate obtained from a clinical case (Nc-Bahia, kindly supplied by Dr. L. Gondim) and a well characterized *N. caninum* isolate (Nc-Liv, kindly supplied by Dr. D. Williams). Pregnant BALB/c mice were randomly assigned to one of 7 experimental groups. At day 7 of pregnancy, mice were subcutaneously inoculated with tachyzoites of Nc-GER2, Nc-GER3, Nc-GER6 (German isolates), NC-6 Arg (Argentinean isolate), Nc-Bahia (Brazilian isolate), Nc-Liv (UK isolate used as infection control) or PBS (negative control). Pregnant mice and neonates were examined daily for detection of clinical signs compatible with neosporosis. Neonatal morbidity and mortality were compared using Chi-square tests and survival curves and log-rank tests. Blood samples were collected from dams at 30 days post partum. Sera were analysed by ELISA for *N. caninum*-specific IgG1 and IgG2a. Differences among groups were determined by ANOVA. Mortality was not observed in neonates from groups inoculated with German or Argentinean isolates obtained from oocysts. Morbidity from German isolates and Nc-6 Arg was lower than 32%. On the contrary, groups inoculated with Nc-Liv or Nc-Bahia showed 100% morbidity and mortality. Differences were not found for IgG1 and IgG2a levels between groups inoculated with Nc-6 Arg and Nc-GER6, which were different from those inoculated with Nc-Bahia or Nc-Liv. Nevertheless, OD values of NC-6 Arg and Nc-GER6 were higher than those of Nc-GER2 and NC-GER3. A predominant IgG2a response (IgG2/IgG1 ratio > 2) was observed with Nc-GER6, NC-6 Arg and NC-Bahía as well as the infection control. In conclusion, low virulence isolates (Nc-GER6 and NC-6 Arg) could elicit a Th1-biased immune response which may potentially be related with a protective effect.

Candidate genes for the improvement of the DNA vaccine pCI-neo-LACK/MVA-LACK against canine leishmaniasis

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Background: *Leishmania infantum* is the etiological agent of canine leishmaniasis in the mediterranean basin. The DNA vaccine based on the activated protein kinase C receptor analogue gene (LACK) has been developed in our laboratory as the recombinant plasmid pCI-neo-LACK. It is partially protective as homologous plasmid/plasmid vaccination. An heterologous vaccination regime with pCI-neo-LACK (prime) and recombinant modified vaccinia virus Ankara (MVA-LACK, boost) increases the level of protection according to the evaluation of symptoms, parasite load, humoral response, lymphoproliferation and cytokine mRNA profiles.

We have also analyzed the expression profiles of the parasite at the non-differentiated and differentiated stages of *L. infantum* by custom shotgun genomic DNA microarrays. Metacyclic PNA- promastigotes up-regulate genes related with infectivity.

Methods: The experience has been performed again for further confirmation and this time cytokine production against crude Leishmania antigen (CLA) and LACK has been evaluated by ELISA in supernatants of the lymphoproliferation assays. The parasite load was determined by the efficiency corrected ΔC_t RT-PCR quantitative method. Normalization was performed with the 18S reference gene. We have performed a selection of vaccine candidates in the basis of i) the stage (metacyclic promastigotes and amastigotes), ii) function and iii) predicted antigenicity. As for function, it has been described that low copy number genes involved in lipid metabolism and intracellular signalling are generally immunogenic.

Results: The parasite load (normalized quantities) is considerably reduced in the vaccinated group G3: 0.01 (days 21 and 120 post-infection), 0.02 (day 180 p.i.) and 0.22 (day 300 p.i) for negative control group G1; 20.30 (day 21 p.i.), 5.05 (day 120 p.i.), 6.12 (day 180 p.i.) and 3.50 (day 300 p.i.) for positive control group G2; and 0.39 (day 21 p.i.), 0.01 (day 120 p.i.), 0.00 (day 180 p.i.) and 0.00 (day 300 p.i.) for G3. IFN- γ production is much higher in G3 (100-1500 pg/ml for CLA and 100-450 pg/ml for LACK depending on the day p.i.) than in G2 (10-25 pg/ml for CLA and 2-4 pg/ml for LACK).

Some of the selected vaccine candidates that are up-regulated in amastigotes are: a lipase, a MAP kinase, and the phosphatidylglycerophosphate synthesis protein. Some of the selected candidates that are up-regulated in metacyclic promastigotes are: the enoyl-CoA hydratase-isomerase, the 3-hydroxyisobutyryl-CoA hydratase and a protein kinase A regulatory subunit.

Conclusions: The vaccine pCI-neo-LACK/MVA-LACK induces partial protection in dogs against canine leishmaniasis according to an additional parameter: IFN γ production in lymph proliferation supernatants. The vaccine candidate genes previously mentioned are suitable to be tested in mice for the selection of protective genes. Finally, epitopes of the selected genes are going to be included to develop a multi-gene vaccine as an improvement of pCI-neo-LACK.

Antigenic variation of the *Plasmodium chabaudi* *cir* multigene family and its implication in host tissue localization

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Background: In *Plasmodium* parasites variant proteins expressed on the parasitized erythrocyte surface play a crucial role in immune evasion. Surface antigens are also involved in sequestration of late trophozoites and schizonts at endothelia of postcapillary venules of the host. The *Plasmodium chabaudi* CIR antigens belong to the largest multigene family in *Plasmodium* genomes formed by the *Plasmodium interspersed repeat* (*pir*) genes present in the human pathogen *P. vivax* (*vir*), in monkey parasite *P. knowlesi* (*kir*) and in the rodent malaria species *P. yoelii* (*yir*), *P. berghei* (*bir*) and *P. chabaudi* (*cir*). In this study, host/parasite interactions were analyzed *in vivo* correlating antigenic variation and localization in different tissues.

Methods: Bioinformatic analyses of all annotated sequence data of putative *cir* genes in the *Plasmodium* Genome Database Resource (plasmodb.org) were performed for identification of subfamilies. In order to examine tissue-specific differences in the transcription of *cir* genes, RT-PCR with subfamily-specific primers was used. In total, 432 cDNAs derived from 6 different tissues were sequenced to characterize the transcribed repertoire of *cir* genes. To confirm differences in transcription profiles of *cir* genes between parasites in different host tissues, restriction fragment length polymorphism (RFLP) of RT-PCR products were analyzed with the Agilent 2100 bioanalyzer for accurate and reproducible separation and size determination.

Results: The preliminary phylogenetic analysis of the annotated *P. chabaudi* *cir* genes identified distinct subfamilies that probably differ in their function and localization. Further phylogenetic analysis of amplified transcribed *cir* genes from six different organs of an infected mouse showed significant differences in the frequency between these tissues. Comparing the RFLP profiles, clear differences in the banding patterns obtained from individual organs were also observed. Changes in the transcription pattern of *cir* genes during the course of infection could also be detected by RFLP.

Summary/Conclusions: In comparison to the widely used *P. yoelii* model, analysis of *cir* gene antigenic variation in *P. chabaudi* has the advantage that antigenic variation and sequestration can be directly correlated, as *P. chabaudi* shows much stronger sequestration of schizonts than *P. yoelii*. The differences in *cir* gene transcription profiles between tissues are important hints for a correlation of surface antigen expression with sequestration in the *P. chabaudi* model.

Novel approaches for the development of vaccines against the blood fluke, *Schistosoma japonicum*

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Background: *Schistosoma japonicum* is a zoonotic disease with serious health impacts for humans and agricultural animals of the developing world. The only available treatment, praziquantel, has a short efficacy period and requires repeated treatment, making this control method impractical. An effective control program of this parasite could be achieved through vaccination. Vaccination of animals used for draught power such as buffaloes, a significant reservoir for human infection, would be an important target to prevent zoonosis. We have employed a novel strategy for the discovery of larvae-specific *Schistosoma japonicum* vaccine antigens.

Methods: In this study, we focused on the migrating stages (schistosomula) of the parasite, which are important targets in protective immunity. Isolation of tissue-specific antibody responses induced by the migrating parasite has been pioneered by our laboratory [1]. Rats, our experimental infection model, were given primary and secondary *S. japonicum* infections and killed at various times post-infection. Lymph nodes draining the different migration sites were removed and isolated cells subsequently cultured and the supernatants harvested. Supernatants containing secreted antibodies unique to the life-stage infecting the corresponding tissue(s), termed antibody secreting cell (ASC) probes, were used to reveal novel, stage-specific antigens. ASC probes isolated from rats were generated from the skin-, lung- and liver-draining lymph nodes, as well as from the spleen.

Results: ASC probes had unique tissue-restricted recognition patterns and identified several stage-specific antigens using a number of approaches including proteomic analysis, a schistosome protein chip [2] and a glycan array.

Conclusions: A number of interesting vaccine candidates will be cloned and tested for vaccine efficacy. In the long term, this may aid in the development of an effective anti-zoonotic schistosome vaccine.

References:

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Effect of host immunosuppression on response to infection with *Haemonchus contortus*

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Background: The host immune response is strongly implicated in the pathogenesis of infection of sheep with the “scour worms” *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* as determined by an experimental model enabling separation of the effects of parasitism and immunosuppression. We aimed to clarify the role of the immune response in the pathogenesis of infection by the more damaging haematophagous nematode *Haemonchus contortus* (Hc) using the same experimental model.

Methods: The experiment used 2x2 factorial design with 6-month-old non-naïve merino wether lambs. The factors were infection (IF) and immunosuppression (IS) resulting in 4 treatment combinations, each with 10 sheep: Control (C, non-IF no-IS); Immunosuppressed (IS, non-IF IS); Infected (IF, IF non-IS) and Immunosuppressed and Infected (ISIF, IS and IF). IF comprised 150 Hc L3/kg on day 0 followed by 250 L3 three times per week. IS comprised 1.3 mg/kg methylprednisolone acetate IM administered weekly. IS and IF treatments commenced on day 0 and the experiment ended on day 56. Anthelmintic treatment was given at pasture 6 weeks prior to, and at individual housing at day -21. Sheep were offered food (50:50 lucerne and oaten chaff) and water *ad libitum* and weighed 23.6±0.86kg on day 0. Feed intake, live weight, faecal worm egg count (WEC), dipstick assay for faecal blood and haematology were monitored weekly while abomasal worm counts, organ weights and body composition were determined on day 56.

Results: Immunosuppression was successful as evidenced by effects on the haematogram, liver weight and adrenal weight. Immunosuppression had no significant effect on either WEC^{1/3} d21-56 (IF 21.9±0.6, ISIF 21.0±0.6; P=0.33) nor Log₁₀ total worm burdens at d56 (IF 3.78±0.03, ISIF 3.80±0.03; P=0.32). Infection significantly reduced (P<0.001) and immunosuppression increased (P=0.003) feed intake but neither had a significant overall effect on liveweight or growth. Efficiency of growth was significantly reduced by IS (P=0.03) with the effect greatest in non-infected sheep. Haematocrit declined in IF sheep from d14 coincident with an increase in faecal blood but neither were affected by immunosuppression.

Summary/Conclusions: Contrary to findings in sheep infected with scour worms, immunosuppression for 56 days had no major influence on WEC, worm burdens or the pathological consequences of infection in non-naïve sheep infected with *Haemonchus contortus*.

Vaccination against *Haemonchus contortus* on boer goats grazing contaminated pasture

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Globally, *Haemonchus contortus* is considered to be the most economically important gastrointestinal nematode of small ruminants. As to its control is complicated by a high prevalence of anthelmintic resistance worldwide, alternative control strategies are needed.

Boer goats grazing contaminated pastures were successfully protected against *H. contortus* using an antigen vaccine. Two groups of 6-months-old male Boer goats were inoculated subcutaneously with either 5 or 50 µg of the vaccine containing Quil A as adjuvant. A third, control group was inoculated with adjuvant alone. After two vaccinations (V1; V2), 4 weeks apart, the goats were put to pasture seeded with *H. contortus*. Subsequent booster doses of vaccine were given after 11 (V3) and 17 (V4) weeks. Packed cell volume (PCV), antibody titre measurements and faecal egg count analyses (FECs) were done weekly. Anti-vaccine antibody titres increased in both vaccinated groups after V2, V3 and V4, but none were evident in the controls. FECs of the control goats increased to a mean of 2 900 by day 74, similar to those of the 5 µg group. Mean FECs in the 50 µg vaccine group rose more slowly, reaching a mean of 1 418. A week after V3 there was a marked rise in the anti-vaccine antibody titres of both vaccinated groups, associated with a marked decline in mean FECs (means of 266 and 230) two weeks later, while FECs of the control group had risen to a mean of 10 777. After administration of the final booster dose of vaccine (V4) mean FECs in both vaccinated groups remained well below those of the control group for the rest of the trial.

It is concluded that repeated immunisation by administration this vaccine could substantially reduce *H. contortus* infestation in grazing goats. Had the third immunisation (V3) been administered two weeks earlier, the protective effect would probably have been stronger. A vaccine of this type may be produced cost effectively and in quantity, and we believe that it would be useful in the control of haemonchosis in small ruminants in Africa and beyond.

Domestication of the *Trypanosoma congolense* transmission cycle selects for strains of reduced virulence

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Background: Animal African trypanosomiasis, mainly caused by *Trypanosoma congolense* (*T. congolense*), is a major constraint to animal health and production in sub-Saharan Africa. There exists a domestic and a sylvatic transmission cycle. A large number of wildlife species have evolved to become tolerant to the disease thus not developing clinical disease, whilst livestock develop clinical disease which impacts their productivity. It is hypothesized that in trypanotolerant wildlife, parasite strains which have the ability to replicate quickly have a higher selective advantage and when these highly virulent strains spill over into susceptible hosts, they will likely cause more damages to their hosts. In susceptible livestock, those quickly replicating strains will cause a high morbidity resulting in selection against them either through host death or treatment with consequent reduction in proportion of such strains. The objective of this paper is to compare the virulence of sylvatic, mixed and domestic *T. congolense* strains

Methodology: The virulence of 23 *T. congolense* strains (6 domestic, 5 mixed & 12 sylvatic) was tested in OF1 mice using the method described by Masumu *et al.* (2006). Animal ethics approval was obtained from Ethics Commission of the Institute of Tropical Medicine, Antwerp, Belgium (Ref DG 008). Mice survival was summarized in a Kaplan-Meier survival curve and further analyzed in a parametric survival model in Stata 10. Median survival time was used to categorize the strains as either lowly or highly virulent.

Results: Survival of mice varied considerably between *T. congolense* isolates. Mice infected with strains from domestic cycle survived significantly longer than those infected with strains from the sylvatic cycle ($P < 0.001$). Mice infected with strains from interface cycle also survived longer than those infected with strains from sylvatic cycle ($P = 0.03$). All strains isolated from domestic cycle were of low virulence while 20% (1/5) of the mixed cycle strains and 83.3% (10/12) of sylvatic strains were highly virulent

Conclusion: There is a higher proportion of highly virulent strains within the sylvatic cycle whilst most of the strains in domestic cycle are of low virulence. Domestication of the transmission cycle thus seems to select for strains of low virulence. Hence, in areas where increased animal production can be sacrificed, it should be possible to achieve endemic stability.

Towards a Commercial Vaccine for Haemonchosis in Ruminants

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Globally *Haemonchus contortus* is arguably the most important nematode parasite of sheep and goats, but isolates resistant to anthelmintics are very common and increasing. Non chemical ways of controlling this parasite are needed, vaccination being one option. Although highly protective antigens for *Haemonchus* were discovered some 20 years ago, numerous attempts to synthesise protective recombinant versions of these have not been successful.

Recently it has been discovered that the dose of native antigen required to induce effective protection is substantially lower than originally thought. Several dose-response vaccine trials will be described where calves, lambs or goats were immunised successfully against a *Haemonchus* challenge with a vaccine containing low doses of native antigen. Several adjuvants were evaluated during the course of these trials and it was found that simultaneous administration of a multivalent *clostridial* vaccine had no adverse effect.

A machine has been designed, built and patented which can rapidly harvest clean adult *Haemonchus* from the abomasa of infected sheep. Hundreds of grams of worms, sufficient for commercial scale vaccine production, have been obtained after deliberately infecting donor lambs with *Haemonchus* larvae. These lambs were rapidly processed through a commercial abattoir in the normal way, except their abomasa, which are normally discarded, were retained and the parasites harvested.

Methods have been devised for simplifying, streamlining and increasing the efficiency and yield of the antigen purification process. A Good Manufacturing Practice licence has been obtained for the whole vaccine production procedure from sourcing the donor sheep right the way through to formulating and bottling the antigen. Large scale field trials are planned for the next *Haemonchus* "season" in Australia. If these are successful, the plan is to submit the data for registration approval in Australia in two years time.

Field evaluation of a *Rhipicephalus (Boophilus) microplus* multiantigenic vaccine in cattle

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Background: The tick *Rhipicephalus (Boophilus) microplus* is a blood sucking ectoparasite of bovines, causing serious damages to the livestock production. Efforts to develop new vaccines for the parasite control have been attempted. In this work we tested the effects of simultaneous immunization of bovines with three recombinant proteins (Glutathione S-transferase (rGST-HI), Vitelin-Degrading Cysteine Endopeptidase (rVTDCE), and *Boophilus* yolk pro-cathepsin (rBYC)) under field conditions.

Methods: Two groups of twenty animals each received four inoculations of the multiantigenic vaccine (vaccine group) or adjuvant alone (control group). In the vaccine group, the inoculations with 21-day intervals contained 600 µg of recombinant proteins (200 µg of each antigen rBYC, rGST-HI and rVTDCE) mixed with 0.5 mL of adjuvant (Montanide 888 and Marcol 52). During all experiment (126 days), the bovines were continuously exposed to ticks under natural conditions in tick-infested pastures without use of chemicals for tick control. Adult female ticks were counted to follow the tick infestation rate and to determine the control of tick infestation obtained after vaccination. Cattle humoral immune response was determined by dot blot and western techniques.

Results: Immunological analyses showed sera from vaccinated bovines reacted with the three recombinant proteins. Along the experiment, the bovines vaccinated with recombinant proteins showed a statistically significant reduction that ranged of 36% to 62% in number of engorged ticks, as compared with the control group. Also, the percentages of weight gain of cattle during the experiment were statistically different in vaccinated and control groups.

Conclusions: Results show that simultaneous immunization could induce an immunity against the antigens which confers a partial protection against *R. microplus* natural infestation. We propose these proteins can be incorporated as antigens in a multiantigenic vaccine against tick infestations.

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Vaccination with a recombinant chimeric antigen confers protection against *Neospora caninum* cerebral infection in experimentally infected mice

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Neospora caninum (Apicomplexa: Eimeriina: Sarcocystidae) is reported as the leading cause of bovine abortion, thus the disease represents an important veterinary health problem and is of high economical significance. Currently, only one vaccine against bovine neosporosis is available on the market (Bovilis Neoguard®, Intervet). This vaccine is based on tachyzoite protein extract but confers only partial protection against the disease. There is therefore an urgent need for an efficient vaccine against bovine neosporosis.

The overall goal of our investigations on *N. caninum* is to develop a vaccine that limits both the cerebral infection and the transplacental transmission. It is very unlikely that this could be achieved by a single antigen, but most likely by a mixture of parasite antigens or a vaccine that contains a number of relevant antigenic domains of different proteins. Since promising results were obtained with a combination of the recombinant forms of three secreted proteins, NcMIC1, NcMIC3 and NcROP2 in the reduction of cerebral infection and vertical transmission in infected mice, we focused on the use of these proteins for further vaccination strategies. In order to increase the immunogenic potential of these antigens, the production of four different chimeric proteins based on their putative antigenic domains was investigated. Balb/C mice were vaccinated with the different proteins and challenged with *N. caninum* tachyzoites. The survival rate and health of the mice was monitored and one of the chimeric proteins conferred complete protection against clinical symptoms in the vaccinated mice. The parasite burden in the brain was assessed by conventional quantitative real time PCR. In order to compare the immune responses of the different groups, total IgG as well as IgG1 and IgG2a titers were assessed by ELISA, and selected cytokines (IL-4 and IFN- γ) levels in mice sera and supernatants of stimulated splenocytes were quantified. An additional trial in the pregnant mouse model to assess the protective efficacy of the chimeric antigen to block the vertical transmission of the parasite during acute infection is currently in progress. The results achieved so far will be presented.

Comparison of gene expression profiles between resistant and susceptible ovine breeds to *Haemonchus contortus*

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Background: *Haemonchus contortus* causes strong economic losses for the small ruminant industry. Moreover, the increase of anthelmintic resistance leads to look for complementary alternative solutions to control infection by gastrointestinal worms. Genetic selection may help inasmuch genes and associated mechanisms would be known. Phenotypically, some ovine breeds are more resistant to primary infection by this blood-feeding nematode. Indeed, Martinik Black Belly (MBB) sheep have lower infection rate and are considered resistant, compared to Romane (ROM) sheep that are susceptible. However, causal genes and differences of immune mechanisms remain poorly characterized. We used gene expression profiling with an ovine-specific microarray to identify and compare gene expression responses in draining lymph nodes and abomasal tissue between these two breeds after a primary infection by *H. contortus*.

Methods: Six-month old MBB (N=8) and ROM (N=8) lambs, reared indoors, were orally infected with 12 000 *H. contortus* L3 and sacrificed at 8 days post-infection (d.p.i.). Eight other lambs (4 MBB and 4 ROM) were uninfected controls. Differences of gene expression in abomasal lymph node and mucosa within and between each breed (before and after infection) were assessed using Agilent's ovine-specific oligoarrays. Candidate gene identified as differentially expressed were also assessed by RTqPCR. Analysis of functions and pathways of differentially-expressed genes with a fold change higher than 1.5 were performed through Innate DB software.

Results: At 8 d.p.i., over 250 transcripts differed between breeds for each tissue and between infected and controls. Among them, *itln2*, *gal11*, *tff3* and inflammatory mediator genes were significantly over-expressed in the abomasal mucosa of MBB after infection. In the lymph node of infected lambs, the *ccl26* cytokine which is chemotactic for eosinophils was over-expressed in MBB, whereas the *cxcl14* chemokine that is chemotactic for monocytes was up-regulated in ROM lambs.

Conclusions: Gene functions and mechanisms mobilized during a primary *H. contortus* infection indicate that resistant MBB sheep have a higher reactivity with a stronger inflammatory response and an earlier Th-2 immune response associated with the expression of two lectin genes. Moreover, those lectins could play a role in the innate immune response to *H. contortus*.

Potential of lipid-core peptides as an effective vaccine delivery system for parasitic helminths

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In sub-Saharan Africa and Latin America, co-infections with the hookworm *Necator americanus* and the intestinal schistosome *Schistosoma mansoni* are common. The aspartic protease (APR) of these blood-feeding organisms initiate the proteolytic cascade of haemoglobin within the gut. Subsequently they have undergone major characterisation as vaccine candidates. Certain epitopes found within the structure of *N. americanus* aspartic protease 1 (APR1) are binding sites for neutralising antibodies which can inhibit the activity of the enzyme with the parasite gut. One epitope, an exposed α -helix peptide (A₂₉₁Y) from *N. americanus*, the homologue in *N. americanus* APR2 and the orthologue *S. mansoni* APR1 have been engineered into lipid-core peptides (LCPs). The LCPs are novel, synthetic, self-adjuvanting vaccines proven as an effective delivery system for Group A streptococci. This study aimed to assess the effectiveness the LCP delivery system to induce antibodies against APR. Constructs NaAPR1(A₂₉₁Y), NaAPR2(N₃₀₄R) and SmAPR1(A₂₆₂K) alone, these peptides again attached to a LCP, the NaAPR1(A₂₉₁Y), NaAPR2(N₃₀₄R) and SmAPR1(A₂₆₂K) peptides including a DNA binding GCN4 peptide from yeast and finally these GCN4 peptides attached to a LCP were immunised into BALB/c mice (4 mice per group). Peptides without a LCP were co-immunised with Freund's complete adjuvant, along with three negative control groups without adjuvant for the GCN4-epitope peptides. The IgG1 and IgG2a response were measured by ELISA. Splenic cells from the SmAPR1(A₂₆₂K) epitope groups were stimulated with anti-CD3, NaAPR1(A₂₉₁Y), SmAPR1(A₂₆₂K) and *Schisto* soluble worm adult protein. The supernatant was collected after 3 days. We used Cytokine Beta Array to measure IL-10, IL-13, IL-17a, IFN-g, IL-5, IL-9, MCP-1 and TNF levels in each of the stimulated groups.

Three immunised groups SmAPR1(A₂₆₂K)+GCN4, SmAPR1(A₂₆₂K)+GCN4+LCP and NaAPR1(A₂₉₁Y)+GCN4+LCP had a significant IgG1 response to their immunised peptide construct. The mice showing the highest antibody responses to SmAPR1(A₂₆₂K)+GCN4 also gave elevated IL-10, IFN-g and TNF after the spleen stimulations. The antibodies induced in this study will be further analysed for neutralising activity against their corresponding APR1 from *S. mansoni* or *N. americanus*. Our results warrant a further assessment of the SmAPR1(A₂₆₂K)+GCN4 constructs and NaAPR1(A₂₉₁Y)+GCN4+LCP in animal vaccine trials.

Evaluation of an experimental vaccine against *Haemonchus contortus* and *Haemonchus placei* in calves

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A vaccine containing integral membrane glycoproteins from the intestine of *Haemonchus contortus* was evaluated in 4 groups of 9 worm free calves, either vaccinated or not and challenged with either 8,000 *H. contortus* or *Haemonchus placei* infective larvae. Vaccinates received 50 µg of the antigen diluted in QuilA adjuvant, while the controls got adjuvant alone. The vaccine was administered three times 21 days apart. The calves were challenged 7 days after the last immunization and killed for worm counts 43 days later. Antibody titres close to zero were detected in all calves at the start of the trial and remained low in both control groups throughout. The vaccinated calves first showed an obvious rise in titre 7 days after the second dose of vaccine. A second increase in titre occurred after the third dose so that peak levels were attained at about the time of challenge. Thereafter values started to decline, but remained well above background until the end of the trial. Vaccination significantly reduced faecal egg counts (FEC) and worm burdens ($P < 0.01$). Calves vaccinated and challenged with *H. contortus* did not shed eggs in faecal samples, while the controls showed a maximum mean ($\pm$ standard error) FEC of 61.1 (± 42.3) at 31 days post infection. Then, FEC progressively declined in this group with only one animal shedding eggs in faeces 42 days post infection. With *H. placei* the controls FEC peaked at 35 days post infection (61.1 ± 21.7) and remained relatively constant until the end of the study, while in the vaccinated group only two animals shed eggs and only on the last collection date. With *H. placei* the controls contained a mean of 551.1 (± 93.7) parasites, while the vaccinates had 174.4 (± 56.3) worms. The establishment rate of *H. contortus* was lower than that of *H. placei* ($P < 0.01$) with an average of 163.9 (± 39.4) and 74.4 (± 20.4) specimens in the control and vaccinated groups, respectively. It was concluded that vaccination of calves with antigens obtained from *H. contortus* conferred protection against both *H. placei* and *H. contortus*.

Desensitising sheep to *Trichostrongylus colubriformis*

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Background: Recent studies have provided evidence the majority of the production penalty that is incurred in sheep during *Trichostrongylus colubriformis* infections are a consequence of the hosts' own immune response, rather than the parasite *per se*. With this in mind, it is possible that regimes intended to desensitise animals to parasite antigens may provide a possible method through which the impacts of such infections can be mitigated.

Methods: Eighteen helminth-naïve 5 month old Romney sheep that had been selected for resistance against gastro-intestinal nematodes were allocated to one of two treatments (n=9). One treatment (group Tol) was exposed to a desensitising regime designed to induce mucosal tolerance to *T. colubriformis* antigens. The remaining group (group Con) acted as a control and were not subjected to the desensitising regime. Following the desensitising regime all animals were trickle infected with the equivalent of 2000 *T. colubriformis* L3 larvae per d for nine weeks. Parasitological and production parameters were monitored.

Results: Compared with the Con group, Tol animals, on average, consumed more total feed, viz, 158 ± 7.8 v 178 ± 10.2 kg dry matter (DM) ($P < 0.01$) and had a greater liveweight gain during weeks 3-6 of infection, viz, 140 ± 21.9 v 271 ± 47.6 g per d ($P < 0.01$) which resulted in a 4kg advantage in live weight and 2kg advantage in carcass weight by day 63. Tol animals also had less faecal scouring at day 63, viz, 2.15 v 0.91 (scale 0-4, where 0=no scouring) ($P = 0.03$), similar FEC throughout ($P > 0.05$) and tended to have greater worm burdens at slaughter on day 63, viz, 14050 v 6130 ($P > 0.05$) for Tol and Con animals, respectively.

Summary: Overall, the desensitising regime appeared to at least delay, if not abrogate, the components of the immune response associated with reduced animal performance, feed intake and faecal scouring. Desensitising animals to otherwise harmless parasite antigens, such as those for *T. colubriformis*, may provide a method through which the production and welfare costs of such infections can be mitigated. Investigations into the mechanisms, practicality and the suitability for other temperate parasite species are ongoing.

Identifying transcriptional changes of key molecules of *Teladorsagia circumcincta* upon encountering the ovine host

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Background: *Teladorsagia circumcincta* is an important abomasal nematode of sheep in temperate regions, and impacts both animal welfare and productivity. Exclusion, or expulsion, of parasites from immune sheep occurs within the first 48 hours following challenge. Here we identify parasite genes which are up- or down- regulated during this initial host-parasite interaction in hosts of differing immune status.

Materials and methods: Abomasal extracts were created from either naïve or immune sheep, and exsheathed infectious *T. circumcincta* larvae exposed to these. Next generation sequencing using Roche-454 technology was carried out on RNA extracted from the larvae. The resulting read data was assembled and subjected to bioinformatic analysis to elucidate sequences unique to, or with transcriptional changes between, larvae exposed to each environment. LC-MS/MS proteomic analysis was carried out on the abomasal environment samples.

Results: The more abundant transcripts in larvae exposed to the immune abomasal environment included, NADH dehydrogenase subunit 4, late-embryogenesis abundant protein and a peptidyl-glycine alpha-amidating monooxygenase homologue. A C-type lectin family member, metallocarboxypeptidase and cytochrome b were among the transcripts more abundant in larvae exposed to the naïve environment. Proteomic analysis of the abomasal environments revealed proteins unique to each environment. Proteins present exclusively in the immune environment included intelectin and galectin.

Conclusions: These results provide valuable insight into host immunity and the immediate parasite response to the host, assist in further understanding the complex host-parasite interaction and could potentially inform new drug or vaccine development.

What Cytokines are Expressed on Abomasum Lymph Nodes when Somalis Crossbreed Sheep are Infected by *Haemonchus contortus* in Northeast of Brazil?

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Cytokines play an important role in response to gastrointestinal nematodes infections and the knowledge about its expression is one way to infer how the host is responding to parasite. The present study evaluated the expression of cytokines IL-4 (interleukin-4), IL-13 (interleukin-13), TNF- α (tumoral necrosis factor) and IFN- γ (interferon) in Somalis crossbreed sheep resistant and susceptible to *Haemonchus contortus* infection in northeast of Brazil. From a Somalis sheep herd, 75 young animals were kept together on pasture without anthelmintic treatment for 4 months. The eight most resistant and the eight most susceptible animals were chosen based on the mean of fecal egg counts and slaughtered to recover the parasites and abomasum lymph nodes tissue samples collection. 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 contortus* than susceptible ($P < 0.05$). IL-13 was up-regulated in resistant animals (3.6 fold; $P < 0.05$) and IFN- γ was up-regulated in susceptible one (2.7 fold; $P < 0.02$). The other two genes analyzed had the same expression pattern in both groups ($P > 0.05$). IL-13 is a cytokine that stimulates the T_H2 response, leading the host to quickly and efficiently respond to the infection, contributing to parasite expulsion. On the other hand, IFN- γ activates a T_H1-type response that has been observed in susceptible animals. It can be inferred that in resistant animals a T_H2-type response was activated and in susceptible animals this response was characterized by T_H1-type response. Study funded by FUNCAP, CNPq and Embrapa Caprinos e Ovinos.

Novel Control Strategy for Cattle Tick: Development of a recombinant vaccine Performance optimization and Efficacy

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Introduction: In Argentina the cattle tick *Boophilus microplus* is considered the most important endemic ectoparasite and causes significant economic losses.

We propose an approach to reduce the incidence of infestation and tick borne diseases by means of cattle immunization with recombinant concealed gut antigens of ticks.

Because of sequence variations in the Bm86 locus, among other factors, could affect the effectiveness of Bm86-containing vaccines, in the present study larval isolates from endemic areas of our country were selected for sequencing and alignment of the Bm86 coding sequence.

A gene coding for autochthonous tick gut protein was cloned and the recombinant BmTVP protein expressed in bacteria was evaluate as an antigen in a single optimized oil formulation, called Providean Garrattack, in target specie.

Methods: A nucleotide sequence of the cDNA containing a 933 base-pair open reading frame was cloned and a N terminal truncated fusion protein BmTVP consisting of 311 amino acids was expressed as inclusion bodies.

Providean Garrattack contained 100ug/dose of recombinant BmTVP antigen combined with Montanide ISA 70 plus saponin as emulsifying agent.

Two groups of five bovines were immunized intramuscularly on at day 0 – 28 – 49. One group received Providean Garrattack and a Control Group received only adjuvant plus saponin.

Three weeks after the last immunization, cattle were infested individually with 1000 larvae per day for 3 weeks. After 21 days, teleogines dropped from the cattle were daily collected, counted, weighted and assessed for oviposition and egg fertility.

Efficacy of Providean Garrattack was proved by monitoring the seroresponse by ELISA, and the protection rate after challenge as described in Canales et al (2009).

Results and Conclusions: The recombinant truncated BmTVP protein showed a sequence identical to those of *Boophilus microplus* from Mexican origin (Canales et al 2009) and Brazilian Campo Grande strain (Andreotti et al 2008). Our sequence differed from Argentinean strain A (Garcia-Garcia et al, 1999) in a single position and shared 97% identity with Cuban Camcord strain (Gavac Vaccine).

Providean Garrattack vaccine was secure, harmless and highly immunogenic. Specific-IgG against BmTVP elicited in bovine immunized were 100-1000-fold higher than those obtained in the control group ($p < 0.05$ t-test) at all time points sampled. The protection level in vaccinated cows is being evaluated. These experiments support the development and testing of a anti-tick vaccine and how it might be introduced as a part of an integrated pest management control system.

Cytokines Expressed in Crossbreed Sheep from Northeast of Brazil naturally infected by *Trichostrongylus colubriformis*

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The present study used the real-time PCR methodology to quantify the expression of cytokines (IL-4, IL-13, TNF- α and IFN- γ) in small intestine of Santa Inês crossbreed sheep and Dorper crossbreed sheep resistant to *Trichostrongylus colubriformis* in Northeast of Brazil. From a sheep herd, 45 young animals were kept together on pasture without anthelmintic treatment for 4 months. The eight most resistant animals from both breeds were chosen based on the mean of fecal egg counts and slaughtered for recover the parasites and small intestine tissue samples collection. RT-PCR was performed using the LightCycler PCR and SYBR Green I dye. SDHA (succinate dehydrogenase complex subunit A) was used for normalization and the relative quantification of genes was calculated by REST software. Santa Inês crossbreed sheep had 5 fold less *Trichostrongylus colubriformis* than Dorper crossbreed sheep. Among the cytokines analyzed, only TNF- α was up-regulated in Dorper crossbreed sheep (3.2 fold; $P < 0.01$). The other genes analyze had the same expression pattern in both groups ($P > 0.05$). TNF- α is a cytokine is associated with both T_H1 and T_H2 response and stimulates the effects of IL-4 and IL-13, fact not observed in this study, where both cytokines remained the same. It was expected that Santa Inês crossbreed sheep, as more resistant than Dorper crossbreed sheep, presented higher levels of IL-4 and IL-13, which was not observed in this study. It can be concluded that TNF- α was up-regulated in Dorper crossbreed sheep, not activating the polarization of immune response in T_H1 and T_H2 -type response, although these animals presented the highest number of parasites. Study funded by FUNCAP, CNPq and Embrapa Caprinos e Ovinos.

The Th2 immunomodulative effect of somatic extract of *Trichinella spiralis*

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Background: Epidemiological data support the assumption that parasitic helminths may provide some protection against autoimmune and idiopathic bowel diseases. Helminths are able to evade the host immune response and suppress the Th1 response of acquired immunity. The cytokine environment is essential, Th cells secrete amounts of IL-4, IL-5 and IL-13 to direct the Th polarisation, but primarily, they have an anti-inflammatory effect on macrophages and dendritic cells. The strong Th2 immune response is an accompanying sign. On the contrary, autoimmune and inflammatory diseases induce Th1 response, that could be suppressed by Th2 response caused by the helminth presence. The aim of this study was to modulate the immune system but to avoid the parasite infection therefore there were examined effects of somatic antigen of *Trichinella spiralis* larvae on the T cell immune response, the production of Th1 and Th2 cytokines and the metabolic activity of macrophages.

Methods: The experiments were carried out using 105 mice of C57BL/6 strain. The somatic extract of *T. spiralis* larvae (1 mg/kg body weight) in adjuvant monophosphoryl lipid A was administered from one to five doses of antigen on experimental days 0, 5, 10, 15, and 20. Immunological parameters (proliferative activity of T cells, CD4 and CD8 T subpopulations, IL-5, IL-10, IFN- γ and TNF- α cytokine productions and metabolic activity of macrophages) were examined.

Results: The administration of three doses of *T. spiralis* antigens seems to be an optimum, that did not disturb the functional status of the cellular immunity (proliferation of lymphocytes, macrophage's metabolism), but increased the numbers of CD4 T cell subpopulation. Th cells determine the Th immune profile by cytokine secretion. Somatic antigen of *T. spiralis* induced an increasing in the production of Th2 cytokines (IL-5 and IL-10) from day 15 of the experiment. The inflammatory Th1 cytokine TNF- α showed only a moderate inhibition, but the production of IFN- γ was suppressed from day 10 at very low concentrations. The results suggest a possibility of successful therapeutic effect of *Trichinella* antigen in autoimmune inflammatory diseases.

Production and Immunological Characterization of a Recombinant *Rhipicephalus microplus* Cysteine Endopeptidase

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Background: The cattle tick *Rhipicephalus microplus* is a hematophagous arthropod responsible for severe losses in livestock industry. Control of this parasite is based on the application of chemical acaricides, which brings numerous setbacks to milk and meat production, in addition to environmental contamination and selection of drug resistant populations. Immunological control with vaccines is a promising alternative. However, it is necessary to find and to characterize molecules which serve as target for the immune response. *The Vitellin-degrading Cysteine Endopeptidase (VTDCE), an enzyme involved in degradation of storage protein during embryogenesis, is an essential molecule in tick embryogenesis. Due to its importance, VTDCE was tested as a vaccine target. As the production of native VTDCE is a complex and lengthy process, in this study we performed the cloning of VTDCE gene, expression and immunological characterization of the recombinant VTDCE (rVTDCE).*

Methods: Native VTDCE amino acid sequence was obtained by Edman degradation and mass spectrometry using LCMS/MS. By RT-PCR, the coding region of VTDCE was amplified from engorged *R. microplus* ovary RNA. Amplicon was ligated into the cloning vector pGEM-T. *Escherichia coli* TOP 10 strain was transformed and correct cloning was confirmed by DNA sequencing. A 285bp fragment was subcloned in pET32a expression vector, and *E. coli* strain BL21Star(DE3) was transformed. Recombinant enzyme was purified by immobilized metal ion affinity chromatography. rVTDCE activity was determined using fluorogenic substrate in presence or absence of inhibitors. Purified enzyme was also used to immunize cattle and rabbit.

Results: VTDCE amino-terminal and three internal tryptic peptides amino acid sequences were determined. *Based on these sequences we clone the VTDCE gene. The recombinant protein was expressed and purified from bacterial extract. Immunological analyses showed that VTDCE is immunogenic for cattle and rabbit and sera against recombinant form recognized the native form.* The recombinant enzyme shows activity upon the synthetic substrate and is totally inhibited by specific inhibitor of cysteine endopeptidase (E-64).

Conclusions: Results demonstrate that recombinant protein maintains activity and immunogenic properties of native proteins. The recognition of recombinant protein using sera from animals immunized with the native protein shows rVTDCE potential as a vaccinal antigen.



SESSION J

Keynote Lecture

- **Trichinellosis: epidemiology, risk factors, prevention and control** - M. Ribicich
- **Food-borne trematodiasis: drug discovery, global burden and integrated control** - J. Keiser

Oral Communications

- **Dynamic of excretion and molecular characterization of Cryptosporidium isolates in preweaned French beef cattle** - C. Paraud
- **Zoonoses of public health interest in horses from slaughterhouses Zoonoses in horses** - F. Evers

Posters

- **Toxoplasma gondii infection in bovine meat consumed in France** - J. Guillot
- **Effect of activated charcoal containing wood vinegar liquid (Obionekk ®) against Cryptosporidium parvum infection in experimentally infected calves** - C. Paraud
- **Analysis of Water Used for Human Consumption in Four Water Treatment Plans from Metropolitan Region of Curitiba, Parana, Brazil** - S. C. Osaki
- **Persistence of Trichinella spiralis in mice corpses exposed to natural conditions** - E. Riva
- **The potentially of necrophagous insects as paratenic host in the transmission of Trichinella spiralis in nature** - E. Riva
- **Monitoring of Trichinella sp. infection in wildlife of North-Eastern Italy: where has Trichinella britovi gone?** - G. Capelli
- **PCR confirmation of Trichinella spiralis isolated from small mammals trapped in rural areas of Buenos Aires province** - G. Morici
- **Molecular identification of Anisakis type I larvae from hairtail fish in Taiwan and Japan** - H. Sugiyama
- **Preliminary evaluation of 2-(2'-hydroxyphenyl) benzazole isothiocyanate for histological studie in Sarcocystis spp** - S. M. Tietz Marques
- **Effects of ultrasound on the survival and characteristics of Cryptosporidium oocysts and Giardia cysts** - M. Al-Sabi

Trichinellosis: epidemiology, risk factors, prevention and control

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In South America and Central America trichinellosis is endemic in several countries (Argentina, Chile, Mexico). In Canada, U.S.A. and several European countries, where pigs are raised in an industrialized manner, domestic trichinellosis has practically disappeared and outbreaks are more frequently due to the consumption of hunted meat or that of pigs raised in small farms. The domestic cycle of *Trichinella* occur in those farms where pigs are fed with garbage, inappropriate disposal of carcasses or raised under poor hygienic conditions. In the sylvatic cycle, the ability of L₁ within the nurse cell to retain its infective capacity for weeks/months and/or resist freezing temperatures for years plays a key role. These two cycles are in a close and dynamic interchange in the nature and may be influenced by management practices. *T. spiralis*, *T. nativa*, *T. T6*, *T. murrelli* and *T. pseudospiralis* have been identified in America. Predominant species in Argentina are *T. spiralis* with an important infective capacity for pigs, wild boars and rodents and low thermal tolerance and *T. T12* which was isolated from muscle tissues samples of *Puma concolor* in southern Patagonia. Risk factors for pigs include feeding them with meat waste, accidental consumption of rodents or dead animals and cannibalism within the herd. In humans, risk factors vary depending on the habits and customs of each region: religious customs, consumption of pig meat, equine meat, hunting animals' meat, handmade sausages and cold cuts, all of them without a previous trichinellosis diagnosis. The prevention and control of trichinellosis entail several aspects that vary according to the geographic area, animal species and predominant *Trichinella* species. The direct method for the parasitosis detection in meat packing factories by means of manual or automatic artificial digestion is the most widely known, but the high costs entailed results in the non-use of this method in those countries with low prevalence. Within the indirect methods, the ELISA test is efficient for the detection of trichinellosis in pig herds and to establish epidemiologic vigilance systems in endemic and non-endemic zones. Human prevention is carried out via: consumer education, education of hunters, pig breeding in confined farms with good management conditions, diagnosis of all susceptible animals in meat packing factories. Thermal treatment of meat, such as cooking, irradiation and freezing, allows the control of the disease, but, taking into account that some of the *Trichinella* species are resistant to freezing conditions, i.e., *T. nativa*, *T. britovi*, *T. T6*.

Food-borne trematodiasis: drug discovery, global burden and integrated control

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Background: While precise estimates are still lacking, up to 1 billion people might be at risk of food-borne trematodiasis, which comprise the liver flukes (*Clonorchis sinensis*, *Fasciola gigantica*, *Fasciola hepatica*, *Opisthorchis felinus* and *Opisthorchis viverrini*), the lung flukes (*Paragonimus* spp.) and the intestinal flukes (e.g., *Echinostoma* spp., *Fasciolopsis buski* and the heterophyids). Food-borne trematode infections are of considerable public-health, veterinary and economic relevance. Treatment is unsatisfactory; there are only two drugs currently available for treatment and control, praziquantel and triclabendazole. This presentation provides an update on recent developments in the treatment of food-borne trematodiasis. In addition, the latest estimates on the number of infections, deaths and for the first time ever, the global burden of food-borne trematodiasis, expressed in disability-adjusted life years (DALYs) lost are presented.

Recent findings: The fasciocidal properties of the artemisinins have been characterised, including studies in naturally infected sheep, pharmacokinetics and a proof-of-concept study in Egypt. Synergistic interactions of combinations of triclabendazole plus artesunate or artemether were observed in rats harbouring chronic *F. hepatica* infections. We also found that tribendimidine possesses promising opisthorchicidal properties; high cure and egg reduction rates were observed in an exploratory open-label trial in *O. viverrini*-infected patients in Lao PDR. For the year 2005, we estimate that 56 million people were infected with food-borne trematodes, more than 9 million people suffer from symptomatic sequelae and approximately 11,000 people die each year due to these infections.

Conclusion: Food-borne trematodiasis are culturally deeply rooted, emerging diseases due to inadequate sanitary conditions, changing food habits and rapid expansion of aquaculture. Chemotherapy-based morbidity control should continue to serve as the backbone of control programmes in highly endemic areas. Nonetheless, integrated control approaches and intersectoral collaboration between public health and veterinary medicine warrant more attention.

Effects of ultrasound on the survival and characteristics of *Cryptosporidium* oocysts and *Giardia* cysts

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Background: Sonication of fluids has been widely used for cell lyses and decontamination of water and food due to its three major physical and chemical effects: cavitation, implosion of water bubbles with subsequent increase in substrate temperature and liberation of free radicals such as O⁻. Additionally ultrasound has been used for cleaning filters used for water sampling and purification. Other studies have shown that backwash sampling of filtrates, and thereby collection of microorganisms, can be facilitated by sonication.

Methods: We studied the effects of ultrasound with different sonication power and time durations on two of the most common waterborne protozoa *Cryptosporidium* and *Giardia*, and examined its effects on parasite characteristics and survival rate using immunofluorescence dyes; DAPI (4',6-diamidino-2-phenylindol) staining/PI (propidium iodide), and analyzed by flow cytometry.

Results: The results showed that exposure of *Cryptosporidium* oocysts and *Giardia* cysts to ultrasound could reduce their survival rate even if the exposure time was limited to a few seconds. When sonication time was extended, changes in parasite characteristics became clearly visible. Several other factors determine the effects of ultrasound on the parasite, including the sonication power of ultrasound as well as substrate temperature.

Conclusions: Ultrasound is harmful for waterborne protozoa even when momentarily applied. However, a mode of operation may exist in which ultrasound can be used for collection of parasites for further study. The specific operational conditions may be tailored to control survival rates and collection efficiency.

Dynamic of excretion and molecular characterization of *Cryptosporidium* isolates in preweaned French beef cattle

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Background: Studies about excretion and molecular characterization of *Cryptosporidium* have been mostly conducted in dairy calves diarrheic or not. Little is known about *Cryptosporidium* in beef calves, especially in non-diarrheic calves.

Methods: This study was conducted in a flock of Parthenais beef cattle in Deux-Sèvres department (France) with no history of cryptosporidiosis. Twenty-five calves were sampled once a week from birth to one month of age. At each sampling date, presence of clinical signs of cryptosporidiosis was recorded. Oocyst excretion was assessed using two methods: the Heine staining method and a direct immunofluorescence method (Merifluor® C/G) which allowed quantification. Positive samples were submitted to molecular characterization of the 18S rRNA gene.

Results: None of the calves presented diarrhoea. Twenty-three of them excreted oocysts at, at least, one sampling date. Prevalence of excretion was maximal when calves were aged of 30 days with a percentage of excretion of 90% while mean excretion was maximal when calves were aged of 15 days with a mean excretion of 649 657 opg. 33 isolates were successfully characterised: 28 isolates were identified as *C. bovis*, 4 as *C. ryanae* and 1 as *C. parvum*. *C. bovis* was isolated from samples of calves aged of 10 to 34 days. *C. ryanae* was isolated from samples of calves aged of 17 to 34 days. *C. parvum* was isolated from one calf aged of 13 days. *C. bovis* was identified at several sampling dates from 6 calves, *C. ryanae* from one calf. Two calves excreted different species between 2 sampling dates (*C. parvum* – *C. bovis* and *C. bovis* – *C. ryanae*).

Conclusions: This survey demonstrated the high prevalence of excretion of *Cryptosporidium* in non-diarrheic beef calves. Our results demonstrated the predominance of species other than *C. parvum* in non diarrheic beef calves, which is similar to the results reported by other authors. Complementary studies should be conducted in other French beef flocks without cryptosporidiosis to know if these results can be generalized to other flocks.

Zoonoses of public health interest in horses from slaughterhouses

Zoonoses in horses

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The aim of this work was investigate the presence of *Trichinella* spp in musculature and antibody anti-*T.gondii*, anti-*Leishmania* spp and anti-*Leptospira* spp in equine from slaughterhouses with inspection service. It was collected 398 samples of masseter and blood from horses of both genders and different ages, from six Brazilian states. The sera samples were subjected to indirect fluorescence antibody test (IFAT) for *T. gondii* (IFAT $\geq$ 64) and *Leishmania* spp (IFAT $\geq$ 40), resulting in 46 (11.6%) and 183 (46%) of positives, respectively. For *Leptospira* spp, the sera were tested through microscopic agglutination ($\geq$ 100), with an outcome of 123 (30.9%) of positive results. It was possible the identification of the most likely serovar of infection in 95 (77.2%) of them. The serovars Hardjo (26.3%) and Autumnalis (12.6%) were the most present. All the 398 masseter samples analysed did not reveal the presence of *Trichinella* spp larva. The results reveal that the horses researched and destined to slaughtering have been exposed to the toxoplasmosis, leishmaniasis and leptospirosis agents, but presented no epidemiological importance to trichinellosis. The implementation of quality measures to breeding horses destined to slaughtering would decrease the exposition of these animals to risk factors, improving food safety for the consumers of this meat. The results reveal that the horses destined to slaughtering have been exposed to the toxoplasmosis, leishmaniasis and leptospirosis agents and demonstrate the spread of these diseases in the environment of animal and human. Trichinellosis not presented epidemiological importance in these horses. The implementation of quality measures to breeding horses destined to slaughtering would decrease the exposition of these animals to risk factors, improving food safety for the consumers of this meat.

***Toxoplasma gondii* infection in bovine meat consumed in France**

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Background: *Toxoplasma gondii* is generally transmitted by ingesting tissue cysts from undercooked or raw meat or consuming food or drink water contaminated with oocysts. France is the most important consumer of bovine meat within the EU, with a traditional raw meat consumption. The objectives of the present survey were to evaluate the seroprevalence of *T. gondii* in the bovine population of France; to identify the risk factors of *T. gondii* infection of an animal; to genotype bovine-related isolates of *T. gondii*.

Methods: 2349 hearts and diaphragms of bovines originating from 46 selected slaughterhouses all over France were analyzed in correlation with their age, sex and geographical origin; 570 muscles samples (diaphragms) originating from imported bovine carcasses (from EU and Brazil) were also included. Serology was performed on muscular fluids using ELISA (I.POURQUIER®, France) and on cardiac fluids using MAT (Modified Agglutination Test). Direct detection of parasites was performed by mouse bio-assay and real time-PCR. The concordance of the various results was evaluated according to the Kappa test and X2 test of Mac Nemar.

Results: The seroprevalence for *T.gondii* infection was comprised between 11 and 14% (mean value of 13%) within the bovine population slaughtered in France. For calves (< 8 months) the seroprevalence was comprised between 2 and 5% (depending on the geographical origin) (mean value 3 %). For adult bovines, the seroprevalence was comprised between 16 and 20% (mean value of 18%). *Toxoplasma gondii* was isolated from two samples by means of xeno-diagnostic and the PCR characterization identified them as genotype II.

Conclusion: This study represents the first large-scale analysis of *Toxoplasma* infection in bovine population slaughtered in France. The seroprevalence was as high as 13 % with a strong predilection for old female animals. Further analyses are required to better understand the origin of the contamination and its link to the type of farming. Two isolates were identified indicating that the risk of human *Toxoplasma* contamination following the consumption of bovine raw meat should not be neglected.

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Effect of activated charcoal containing wood vinegar liquid (Obionekk®) against *Cryptosporidium parvum* infection in experimentally infected calves

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Background: Many compounds have been screened for their potential anti-cryptosporidial activity in ruminants but none of them has been totally efficient in controlling the disease. The efficacy of Obionekk®, containing Nekka-Rich (activated charcoal and wood vinegar liquid), was evaluated against cryptosporidiosis in experimentally infected calves.

Methods: Fourteen dairy calves separated from their dams at 24 hours were orally inoculated at between 48 and 72 h of age with 3×10^7 oocysts of *Cryptosporidium parvum*/calf. At the onset of diarrhoea, calves were assigned to 2 groups according to the level of excretion of oocysts evaluated by an immunochromatography dipstick assay (BioX Diagnostics). Seven calves did not receive any treatment (control group) while calves in the treated group received 60 ml of Obionekk® (corresponding to 20 grams of Nekka-Rich) twice a day individually for 4 days. Clinical signs were monitored daily. Oocyst excretion was assessed daily using two methods: the Heine staining method and a direct immunofluorescence method (Merifluor® C/G).

Results: All the calves began to excrete oocysts five days post-inoculation. No difference in clinical signs was seen between control and treated groups. Agents of neonatal diarrhoea other than *Cryptosporidium parvum* were identified (rotavirus and coronavirus). No significant difference in the faecal oocyst excretion was seen between the 2 groups when using the Heine staining method. The immunofluorescence method was applied on the samples from day 6 (beginning of administration) to day 12 (2 days after the end of treatment) and allowed quantification. Before treatment, individual oocyst excretions varied from 8×10^3 oocysts per gram (opg) to 1×10^6 opg and were not different between control and treated calves. During treatment (day 7 to day 10), calves receiving Obionekk® excreted significantly less oocysts than control calves ($p < 0.05$), with a mean reduction in oocyst excretion over this period of 52 % in the treated group when compared to the control group.

Conclusions: No efficacy against diarrhoea was observed in our conditions. However, administration of Obionekk® resulted in a strong reduction of oocyst excretion which is similar to previous results. This reduction will reduce the environmental contamination and may lead to an expected reduction of clinical signs.

Analysis of Water Used for Human Consumption in Four Water Treatment Plans from Metropolitan Region of Curitiba, Parana, Brazil

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Introduction: *Cryptosporidium* spp. is a protozoan responsible for gastroenteritis outbreaks in humans both in developed and developing countries. Its resistance to disinfectants commonly used in water treatment plants (WTP) makes their detection extremely important.

Methods: In order to investigate the occurrence of *Cryptosporidium* spp. oocysts in water and in sludge resulting from the water treatment process, three sample collections were performed in four WTP in the metropolitan area of Curitiba-Brazil. The material collected consisted of water at the entrance of the water treatment plant and sludge resulting from the treatment process. The 12 samples tested were collected from December 2008 to January 2009. The water was treated at laboratory scale, followed by filtration. The cellulose membranes were washed with PBST and sent for DNA extraction and Direct Immunofluorescence. The sediment resulting from flocculation was submitted to oocyst recovery processes. For this, techniques of concentration by sucrose were used and then DNA extraction was performed, and slides were prepared in order to evaluate its presence by DIF. The sludge from the WTP was processed by the Yanko-1987 method, and the resulting sediment was divided into two aliquots: one aliquot was used to evaluate the presence of protozoa and helminths, and another aliquot was processed for DNA extraction and DIF. **Results:** By the Yanko and Direct Immunofluorescence techniques, in none of the 12 samples analyzed was it possible to detect *Cryptosporidium* spp. When the polymerase chain reaction (PCR) technique was used, the presence of oocysts in sludge water was detected in two (50%) WTP, corresponding 16, 7% of water samples and 25% of sludge analyzed.

Conclusions: These results reveal the occurrence of this protozoan in the water supply system of the metropolitan area of Curitiba-Brazil indicating a public health risk.. The detection of the protozoan using the PCR technique shows that this technique can be an important tool for detecting *Cryptosporidium* spp in the environment.

Persistence of *Trichinella spiralis* in mice corpses exposed to natural conditions

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T. spiralis infective larvae (L1) in carrion ensure the infection in nature. The aim of the present study was to evaluate the influence of different natural factors on viability and reproductive capacity of *T. spiralis* L1 present in corpses. Corpses of experimentally infected mice were exposed by 6 weeks to three different conditions: controlled refrigerator, summer and winter environment in Tandil, Argentina (37° 32' S; 59° 15' W) during a period of 0, 1, 2, 4 and 6 weeks and then, corpses were recovered for digestion and recovering of L1. Viability (%) and reproduction capacity index (RCI: recovered larvae/ inoculated larvae x 100) were calculated. Muscle tissue samples were used for morphologic and glycogen content analysis by hematoxylin - eosin and Periodic Acid Schiff's (PAS) stains. Temperature (T) and relative humidity (RH) were recorded daily. In summer (T average: 23.4° C; RH average: 54.7%), L1 were recovered from corpses exposed by one week only. The viability was 54.2% and the RCI (13.1) was significantly lower than control (174.4) ($P < 0.05$). From the first week onwards a coagulative necrosis was observed. The finding of cysts with irregular contours and PAS colour reduction by week 2, indicates a morphologic and metabolic deterioration of the parasite, which may be correlated with the null survival obtained. In winter (T average: 6,1° C; RH average: 75.1%) as in refrigerator, L1 alive were always recovered through the exposition period. The RCI were all high but significantly lower than control 0 ($P < 0.05$). Cyst and L1 morphological structure and the high levels of glycogen observed were equivalent to control. An inflammatory response was observed around muscle cyst which was distinguishable until week 2 of exposition. The present study has confirmed that *T. spiralis* L1 is able to survive in nature and keep infective by 1 week in summer and at least 6 weeks in winter. Probably, the persistence of L1 as source of infection in winter exceed the time measured in this study. Although exposed alive larvae presented a lower reproductive capacity than non exposed larvae, the former could also became an important source of infection for animals and perpetuate the environmental presence of the parasite.

The potentially of necrophagous insects as paratenic host in the transmission of *Trichinella spiralis* in nature

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T. spiralis affects a great diversity of mammals. Predation, scavenger and cannibalism behaviour on infected animals perpetuate trichinellosis in the domestic, sinantropic and selvatic cycle. It has also been demonstrated that certain arthropods -dipterous, crustaceans, beetles- with necrophagous habits may act as paratenic hosts of the infective larvae (L1). However, these experiments did not pay attention to the natural environment influence on arthropods populations. The aim of the present study was to evaluate the viability and infective capability of *T. spiralis* L1 ingested by necrophagous arthropods from cadaveric infected muscle tissue exposed to natural environment conditions. Corpses of CF1 mice harbouring L1 of *T. spiralis* were deposited in a pitfall trap and exposed by 25 days to natural summer conditions in Tandil, Argentina (37° 32' S; 59° 15' W). The trap was inspected daily searching for maggots of arthropods. The number and viability of *T. spiralis* L1 in maggots and from adult stages obtained by culture were determined and inoculated to CF1 mice. Five weeks post inoculation, mice were necropsied, digested and the recovered L1 were used to determine the reproductive capability index (RCI: recovered larvae / inoculated larvae x 100). On day 3 of exposition, 70 maggots of the dipterous *Phaenicia sericata* were studied and 29 alive L1 were recovered. The RCI for these larvae was 133.6. By day 5, four maggots of *Sarcophaga sp.* were isolated and 17 alive L1 were recovered from them. In this instance, the RCI was 43.4. From day 5 of exposition onwards L1 of *T. spiralis* were not recovered from maggots as well as not from adult stages obtained by culture. The present study demonstrates that *P. sericata* and *Sarcophaga sp.* necrophagous maggots are able to ingest and support *T. spiralis* L1 viable at least during the first stages of the deathly pale decomposition. The high RCI registered in the L1 recovered from maggots together with their dispersion behaviour to pupate on the soil are important observations that reinforce the potentially of arthropods in the transmission of *T. spiralis* in nature. Because the annual variation may influence the diversity and abundance of arthropods population it is possible that other organisms may also act as paratenic host of *T. spiralis*.

Monitoring of *Trichinella* sp. infection in wildlife of North-Eastern Italy: where has *Trichinella britovi* gone?

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The Regulation (EC) No. 2075/2005, reporting specific rules on official controls for *Trichinella* in meat, requires the onset of a risk-based wildlife monitoring programme in those areas where wildlife and holdings applying for *Trichinella*-free status coexist. For this reason, the wildlife monitoring of target animals (mainly foxes, wild boars and mustelids) has been implemented since 2006. We report the results of the wildlife monitoring for *Trichinella* sp. in three regions of north-eastern Italy during 2006-2010, in comparison with those of 1958-1960 and 1988-2005 periods in the same regions.

Muscles of wild animals were tested for *Trichinella* sp. larvae using a pepsin digestion method according to the Commission Regulation EC 2075/2005. Recovered larvae were identified by biochemical or molecular tests since 1988. In a survey performed in 1958-60 in the same area, 86 foxes (*Vulpes vulpes*) out of 275 (31.3%) were found positive for *Trichinella* spp.; prevalence ranged from 15% to 44.4% among provinces.

From 1988 to 2005, a total of 2,746 wild animals were tested including 1,639 foxes, 72 mustelids (62 martens, *Martes martes*; 10 beech martens, *Martes foina*) and 1,035 wild boars (*Sus scrofa*). Nine foxes (0.55%) were positive for *Trichinella britovi*. In the period 2006-2010 a total of 17,881 wild animals were tested including 1,904 red foxes, 618 mustelids (287 european badgers, *Meles meles*; 225 martens; 106 beech martens; 1 weasel, *Mustela nivalis*; 1 polecat, *Mustela putorius*), 1,322 birds (828 corvids, 16 falconiformes, 478 other families) and 14,037 wild boars. *T. britovi* was identified in one fox (0.05%) and in one beech marten (0.94%; 0.16% on overall mustelids).

Conclusion – In north-eastern Italy, the decrease of *T. britovi* prevalence in red foxes from the sixties to nowadays is impressive. Comparing the period 2006-2010 with 1988-2005 there has been a further 10-fold decrease of *Trichinella* prevalence in foxes ($p < 0.01$). In the last 20 years, foxes have expanded their distribution areas from mountain to lowland habitats. The reduction of the *Trichinella* prevalence witnesses the probable shift of the feeding behaviour of foxes from hunting and scavenging to food sources provided by humans settling. Changing ecosystems are expected to affect wildlife populations and the pathogens they carry.

PCR confirmation of *Trichinella spiralis* isolated from small mammals trapped in rural areas of Buenos Aires province

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To date, there are few published reports in Argentina about the existence of *Trichinella spiralis* in rodents and marsupials. Knowledge of its presence in this kind of animals is important, especially in those endemic areas where pigs are raised outdoors and potentially in contact with them. These animals infected with *Trichinella* represent a risk not only for being a reservoir but also for helping the potential introduction of this parasite into a domestic swine population. Therefore, to know the occurrence of *Trichinella* in this population, we collected 220 samples in rural areas of Buenos Aires province from six different species of small rodents (*Rattus rattus*, *Rattus norvegicus*, *Mus musculus*, *Akodon azarae*, *Calomys laucha*, *Oligoryzomys flavescens*) and a marsupial (*Didelphis albiventris*). Muscle samples from the trapped animals were then artificially digested to potentially isolate *Trichinella*. As a result, a low burden of *Trichinella* spp could be isolated from 33 % of *R. rattus*, 1 % of *R. norvegicus* and 6 % of *D. albiventris*. However, no larva was isolated from the other species. Larvae obtained from the positive samples were then identified as *T. spiralis* after performing a nested-multiplex PCR. Three of these larvae could further be identified to the genotype level through DNA sequencing. Although the burden of *Trichinella spiralis* found in the wildlife population was low (it varied from 0.6-3 larvae per gram among the different species), and *Trichinella* was not present in all the trapped wild animals, this finding confirms the presence of this parasite among feral animals and thus, this may represent a risk to the swine and human populations under the current pig management practices held in some areas of Argentina.

Molecular identification of *Anisakis* type I larvae from hairtail fish in Taiwan and Japan

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It is imperative to identify *Anisakis* larvae detected in food fish in order to improve food safety. Hairtail fish (= cutlass fish, *Trichiurus spp.*) is one of the most common and economically important food fish in the East and South China Seas. In Japan, Taiwan, and Korea, hairtail is often sold in markets and is favored for raw consumption as sashimi or sushi. However, few studies have investigated the identification of anisakid larvae isolated from hairtail. The parasites most frequently associated with the human anisakiosis are categorized as *Anisakis* type I, which comprise several species of the genus *Anisakis*. The anisakid larvae show primitive forms and lack the detailed morphological characteristics required for precise species identification. Thus, molecular characterization is necessary for determining the species and then acquiring important epidemiological information. In this study, we detected *Anisakis* type I larvae in hairtail caught off the coasts of Taiwan and Japan, and applied the molecular methods for species identification. The ribosomal DNA internal transcribed spacer (ITS) region was amplified by PCR and restriction fragment length polymorphism analysis (PCR-RFLP) was carried out for species identification. Some amplicons were also sequenced to validate the identification by PCR-RFLP. Most larvae isolated from hairtail caught off the coast of Taiwan were *A. typica* (84%), while those isolated from hairtail caught in Japan were identified as *A. simplex* sensu stricto (65%), *A. pegreffii* (33%) or *A. typica* (2%). This is the first report of *A. typica* in fish obtained from Taiwan and the first report of *A. typica* in hairtail obtained from Japan. Our results shed the light on the epidemiology of *Anisakis* type I larvae, which is a potential cause of human anisakiosis in Taiwan, Japan and countries where the presence of *A. typica* and other species comprising the *Anisakis* type I has been indicated.

Preliminary evaluation of 2-(2'-hydroxyphenyl) benzazole isothiocyanate for histological studie in *Sarcocystis* spp

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The parasites of genus *Sarcocystis* is one of the most commonly found parasite in domestic animals worldwide. *Sarcocystis* species are intracellular protozoan, are obligate two host life cycles, alternating between hosts that serve as each other's predator and prey. After ingestion of *Sarcocystis*, merozoites subsequently released and enter muscle cells and neural tissue where develop to mature *Sarcocystis*. Heart, diaphragm and skeletal muscles are the preferred organs of *Sarcocystis* spp. location in the intermediate host and can persist through life in the hosts but many start to disappear after three months of inoculation. Is highly prevalent in livestock animals and some species cause important economic loss when causing clinical and subclinical disease. The aim of this study was to evaluate a fluorescent dye 2-(2'-hydroxyphenyl) benzazole isothiocyanate (BzICT - 3×10^{-3} molar) how new dye to stain and to identify *Sarcocystis* spp. cysts in muscle biopsy specimens for epifluorescence microscopy, in comparison of light microscopy pieces of samples processed by standard histological technique stained with Hematoxylin and Eosin (H-E). Bovine heart samples were fixed in 10% neutral buffered formalin solution. The tissues were then processed to paraffin wax blocks by the acetone benzene method and 5mm thick sections were obtained using a rotatory microtome; stained with H-E and BzICT and examined at 400-1000 magnification for *Sarcocystis* cysts. The technique with BzICT in epifluorescence microscopy showed easy visualization to cysts in both magnifications. The result suggests that BzICT is a good tool to sarcocystosis diagnostic in bovine heart samples.



SESSION K

Keynote Lecture

- **Pet-associated helminthic zoonoses** - P. Deplazes

Oral Communications

- **Current situation about canine hemoparasites in Southern Greater Buenos Aires, Argentina** - D. F. Eiras
- **Encephalitozoon cuniculi infections in cats and dogs** - J. Csokai
- **Research on Canine Vector- Borne Diseases: the multidisciplinary approach as a resource** - D. Otranto
- **Endemic Toxoplasma gondii genotype II causes fatal infections in animal hosts in Finland** - P. Jokelainen
- **Serological Survey of Babesia canis in Dogs from Rural and Urban Areas of Seropédica, Rio de Janeiro State, Brazil** - J. Aparecida Rezende Vilela
- **Results of parasitological examinations of fecal samples from cats and dogs in Germany between 2003 and 2010** - D. Barutzki

Posters

- **Seroprevalence of Toxoplasma gondii in pigs in La Pampa – Argentina** - M. Fort
- **Canine dirofilariasis in Marajó Archipelago, Pará State, Brasil: an epidemiological, morphologic and molecular study** - A. P. Furtado
- **Morphological aspects of Dirofilaria immitis by Scanning Electron Microscopy** - A. P. Furtado
- **Intestinal parasites in different cat populations in northern Belgium** - E. Claerebout
- **Canine leishmaniasis: use of serological tests in three non-endemic areas from Northern Argentina** - D. F. Eiras
- **The epidemiology of Cryptosporidium spp in domestic animals (cats and dogs) in Thohoyandou, South Africa** - A. Samie
- **Zoonotic transmission of Microsporidia in patients with AIDS** - J. N. Velásquez
- **Gastrointestinal Parasites in an Urban Feline (Felis catus L 1758) Population Presented to a Spay & Neuter Program in Rio de Janeiro, Brazil** - N. Labarthe
- **Rickettsia spp. identification and differentiation in ticks collected in the city of Hannover (Germany) by quantitative real time PCR and pyrosequencing technology** - C. Strube
- **Efficacy of a spot on combination containing imidacloprid 10 % and moxidectin 1 % (Advocate® / Advantage® Multi, Bayer Animal Health) against Ancylostoma ceylanicum in cats** - P. Taweethavonsawat
- **Canine hepatozoonosis: a case in San Luis (Argentina)** - S. R. Aubert
- **Hippobosca longipennis – a potential intermediate host of newly proposed Acanthocheilonema species in northern India** - P. A. Megat Abd Rani
- **Stain techniques for detection of Encephalitozoon cuniculi in tissue sections** - M. A. Lallo
- **Occurrence of microsporidia in fecal samples of Nasua nasua (South American coati) from Ecological Park, São Paulo, Brazil** - M. A. Lallo
- **Limited risk of canine babesiosis in the Czech Republic** - V. Svobodova
- **Diagnosis of Spirometra spp. in cat (Felis catus)** - M. F. Bono Battistoni1;
- **Case report of canine visceral leishmaniasis in Concordia city, Argentina** - R. O. Sánchez
- **New Insights into Morphological and Biological Features of Capillaria aerophila (Trichocephalida, Trichuridae)** - D. Traversa
- **First Description of Gurltia Paralyans in a Domestic Cat in Argentina** - A. Paludi
- **Canine Visceral Leishmaniosis: An evaluation of Laboratory Diagnosis five years from the first autochthonous case in Argentina** - C. Nevot
- **Canine and Feline Infections by Cardiopulmonary Nematodes in Central and Southern Italy** - A. Di Cesare
- **Helminths infections in kennel dogs from southern Italy** - L. Rinaldi1,
- **Phylogenesis of Capillaria aerophila (Trichocephalida, Trichuridae) from Different Hosts and Countries** - G. Castagna
- **Retrospective study of 302 cases of canine dirofilariosis treated in Virreyes Veterinary Hospital province of Buenos Aires, Argentina** - G. Pérez Tort
- **Molecular characterization of Blastocystis isolated from humans and various animal hosts in Flanders (Belgium)** - J. Vercruysse
- **Retrospective study of 302 cases of canine dirofilariosis treated in Virreyes Veterinary Hospital province of Buenos Aires, Argentina** - G. Pérez Tort
- **Update on the Distribution of Dirofilaria immitis in dogs from the State of Rio de Janeiro, Brazil** - N. Labarthe
- **Prevalence of antibodies anti-Leishmania (L.) infantum chagasi in dogs from the Tapirapé indigenous community in Brazilian Amazon region: Preliminary results** - J. T. R. Lima
- **Feline intestinal parasites in Finland: focus on prevalences, risk factors and anthelmintic treatment** - A. Näreaho
- **Control of Giardia duodenalis in a dog kennel** - M. Schnyder
- **Zoonotic hookworm transmission in a highly endemic region of Southeast Asia** - R.C. A. Thompson
- **Survey of Endo- and Ectoparasites of Cats from Tirana, Albania** - M. Knaus
- **A canine Cercophithifilaria species (Spirurida, Onchocercidae) with dermal microfilariae, Italy** - D. Otranto

- **Vector-borne infections in dogs from Hungary and Romania** - D. Hamel
- **Bacterial and protozoan pathogens of veterinary and medical importance in Romanian ticks** - C. Silaghi
- **Natural infections of *Aelurostrongylus abstrusus* in cats in Germany** - D. Barutzki
- **First Report of *Trypanosoma cruzi* Infection in Naturally Infected Dogs from Southern Bahia, Brazil** - G. Albuquerque
- **Serological and Molecular Survey for Detection of *Leishmania Braziliensis* in Naturally Infected Dogs in Southern Bahia, Brazil** - G. Albuquerque
- **Prevalence of gastrointestinal helminths in cats admitted at the Veterinary Policlinic of the University of Zulia, Venezuela** - R. A. Ramírez-Barrios
- **Efficacy of emodepside plus toltrazuril suspension (Procox® oral suspension) against prepatent and patent *Isospora* spp. infection in dogs** - G. Altreuther
- **Efficacy of emodepside 0,1 % / toltrazuril 2 % suspension (Procox®) against mixed experimental *Isospora felis/Isospora rivolta* infection in cats** - K. Krieger
- **Stress response in *Giardia* cysts** - K. Troell
- **Evaluation of zoonotic parasites in dogs in a rural area of Portugal** - M. A. P. Conceição
- **Cutaneous leishmaniasis in a guinea pig (*Cavia porcellus*) diagnosed in Passo Fundo, state of Rio Grande do Sul, Brazil** - M. I. Vieira
- **Incidence of Compatible Injuries with Hidatidosis in Plants of Task in the Province of Corrientes** - S. Romero
- **Epidemiology of gestational toxoplasmosis in five municipalities in Parana State, Brazil** - R. Mitsuka-Breganó
- **Histopathology and parasite burden of golden hamsters experimentally infected by *Leishmania chagasi* and treated with resin-oil *Copaifera reticulata* and ethyl acetate fraction of *Ricinus communis*** - C. M. L. Bevilaqua
- **Validation of a rapid immunochromatographic test Dual Path Platform (DPP®) for serodiagnosis of Canine Visceral Leishmaniasis** - C. M. L. Bevilaqua
- **First record of *Angiostrongylus cantonensis* (Chen, 1935) in *Rattus rattus* and *R. norvegicus* in Belém, Pará, Northern Brazil** - J. N. dos Santos
- **First Record of *Calodium hepaticum* (Bancroft 1893) (sin. *Capillaria hepatica*) of *Rattus rattus* and *R. norvegicus* in Belém-Pará, North of Brazil** - J. N. dos Santos
- **DLA-DRB1 Gene Polymorphisms and Susceptibility of Dogs to Visceral Leishmaniasis** - C. M. Nunes
- ***Leishmania* (Viannia) *braziliensis* in popliteal lymph node of a dog in a *Leishmania* (*Leishmania*) *infantum chagasi* vulnerable area in São Paulo State, Brazil** - A. A. Cutolo
- **Chemical composition of *Eucalyptus staigeriana* and *Lippia sidoides* essential oils and insecticidal effects on *Lutzomyia longipalpis*** - I. T. Freitas Macedo
- **Prevalence of antibodies anti-*Rickettsia rickettsii* in dogs from the Tapirapé indigenous community in Brazilian Amazon region: Preliminary report** - A. H. H. Minervino
- **Occurrence of parasites of zoonotic importance in stray dogs collected by the Center for Zoonosis Control (CCZ) of Lages, Santa Catarina** - R. M. de Quadros
- ***Ancylostoma* spp. in sand of beaches of central zone of Sinaloa, México** - M. C. R. Robles
- **Diagnosis of *Echinostoma paraensis* Lie and Basch, 1967 (Trematoda, Echinostomatidae) in hamsters *Cricetulus griseus*** - M. Lurdes de A. Rodrigues
- ***Trypanoxyuris* (*Trypanoxyuris*) *minutus* (Schneider, 1866) (Nematoda, Oxyuridae) in *Alouatta guariba clamitans* Cabrera, 1940 (Primates, Ateleidae) in Rio de Janeiro, Brazil** - P. Breves
- **Helminthes parasites in dogs of fishing villages of central zone of, Sinaloa, México** - M. C. R. Robles
- **Diversity of helminthes eggs in *Cercocyon thous* Linnaeus, 1766 feces: a comparison among two different Brazilian Biomas** - S. Oliveira
- **Spotted Fever Group (SFG) *rickettsia* antibodies on horses in the State of Santa Catarina, South region, Brazil** - A. Barbosa de Moura
- **Comparison of indirect immunofluorescence assay, enzyme-linked immunosorbent assay and Dual Path Platform (DPP®) immunoassay for the diagnostic of canine visceral leishmaniasis** - S. S. Albano Amóra
- **Anti-*Trypanosoma cruzi* antibodies detection in dogs of Mossoró, Rio Grande do Norte, Brazil** - S. S. Albano Amóra
- **The Repellent Effect of Permethrin (70 Mg/Kg) in a Tick and Flea Spot-On Formulation for Dogs against Haematophagous Mosquitoes** - N. J. Scherling
- **Repellent Effect against Haematophagous Diptera of a 70 mg/kg dose Permethrin Spot-on formulation** - C. R. Francia
- **Diagnosis of Imported Canine Filarial Infections in Germany 2008–2010** - A. Dauschies
- **Studies on the prevalence of haematozoa in dogs** - L. D. Singla
- **A comparison of 2-(2'-hydroxyphenyl) benzazole isothiocyanate and fuchsin staining for determining *Cryptosporidium* spp. oocysts** - S. M. Tietz Marques
- **Seasonal dynamics of asymptomatic *Leishmania* infection in dogs in Spain** - E. Berriatua

Pet-associated helminthic zoonoses

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The close emotional tie between people and companion animals is recognized as a fruitful relation known as the human-animal bond. However, pet dogs and cats can play an important role in the transmission of helminthic zoonotic agents. Some of them, e.g. *Echinococcus* and *Toxocara*, are directly transmitted from pets to the human environment without the involvement of vectors or intermediate hosts. A number of canine parasites have extended their distribution ranges in Europe. Among the reasons for this trend are the displacement of dogs (mainly from the South and the East of the continent to Central Europe by animal protection organisations), the reduction of border controls within the European Union but also political and socioeconomic transitions especially in the former states of the Soviet Union. Ecological changes also contributed to parasite dispersal: Fox populations have grown in many areas of Europe, and the raccoon dog, an invasive carnivore species, has colonized large parts of the continent. These species are highly susceptible to most canine helminths. Furthermore, high populations of freely roaming stray dogs and cats maintain a permanent infection pressure of many parasites towards the domestic pet populations. In fact, human alveolar echinococcosis emerged in Europe in the context of the fox populations invading urban areas. The trend to design urban recreational environments closer to natural ecological systems also boosted vole populations that represent urban reservoirs for zoonotic parasites as was shown for *Echinococcus*, *Toxoplasma* and *Toxocara* spp. A good understanding of the parasites' epidemiology is required for planning and implementing effective prevention strategies. The continuous education of veterinarians and the information of the pet owners by providing uniform recommendations is of priority importance. Guidelines for the control of parasites of dogs and cats have been developed for the USA (www.capcvet.org) and for Europe (www.ESCCAP.org). A close collaboration between veterinary and public health professionals in a 'One Health' concept is required.

Current situation about canine hemoparasites in Southern Greater Buenos Aires, Argentina

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The prevalences of canine dirofilariasis, hepatozoonosis and babesiosis have been studied in temperate Argentina during the last decade. The parasite species of these infections have been identified by PCR or serological methods as *Dirofilaria immitis*, *Hepatozoon canis* and *Babesia vogeli*. The aim of this report was to assess their prevalence during the last year in Southern Greater Buenos Aires, and to compare with previous reference values available for each parasite species.

Between March 2010 and February 2011 a total of 14364 blood samples of owned dogs were submitted by veterinary practitioners to DIAP laboratory. Whole blood samples collected with EDTA were stored at 4 °C for parasite examination within 48 h. Samples were examined to detect circulating microfilariae in the buffy coat interface of the microhematocrit tube. Blood stained smears were observed under light microscope at 1000x to look for *Hepatozoon* gamonts or *Babesia* merozoites. Prevalence of each hemoparasite was calculated as the percentage of positive blood samples. These values were compared with those published in previous years for the same study area and methodology as follows: *H. canis* 1.34%=377/28051 samples (period 2002-2008), *Babesia* 0.2%=34/16767 (2003-2007), and *D. immitis* 1.63%=312/19136 (2001-2006). Statistical comparisons were performed using the X2 test for two independent proportions.

The three hemoparasites involved in the study were found in relatively low prevalence; *Hepatozoon* 2.26% (325 positive samples), *Babesia* 0.19% (27), and *Dirofilaria* 0.31% (45). However, each parasite showed a different pattern compared with the previous findings. *H. canis* and *D. immitis* prevalences were significantly higher ($X^2=49.2$, $P<0.001$) and lower ($X^2=134.7$, $P<0.001$) respectively, than the historic records. On the contrary, *Babesia* prevalence did not differ ($X^2=0.087$, $P=0.768$) with the previous reference value.

Our results strongly suggest that the infection by *H. canis* increased almost twice and that by *D. immitis* decreased around 5 times in the recent period. A gradual but constant decrease of *D. immitis* prevalence was previously demonstrated during the period 2001-2006, and the most reasonable hypothesis is the extended use of ivermectin. Ecological factors driving the different patterns observed for the 2 tick-transmitted parasites (*Hepatozoon* and *Babesia*) will be matter of future studies.

***Encephalitozoon cuniculi* infections in cats and dogs**

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The microsporidium *Encephalitozoon cuniculi* (*E. cuniculi*), infects rabbits as the main host with high prevalences and worldwide distribution. However, little is known about the relevance and abundance of infection in other hosts. While seroprevalence studies have been conducted in non-rabbit species clinical outcomes of infection are only described infrequently. We here describe cases of feline and canine encephalitozoonosis associated with different clinical pictures to highlight the importance of *E. cuniculi* in differential diagnosis.

Cases in cats include 16 animals with focal anterior cortical cataract or mature cataract and secondary uveitis in one or both eyes (totally 26 eyes) for which *E. cuniculi* could be detected as the causative agent. Serological tests as well as PCR and histological examination of the eyes were performed after phacoemulsification or enucleation. All cats had a positive antibody titre for *E. cuniculi*. *E. cuniculi* DNA could be detected in 25/26 lenses and 10/19 aqueous samples. The sequencing of samples yielded strain II (mouse) and a previously unknown strain. Histologically, spores could be detected in 17/26 lenses by acid-fast trichrome staining.

After descriptions of canine encephalitozoonosis from South Africa and the USA we could confirm the first cases in two dogs in Europe. Two puppies of one litter showed reduced weight gain, apathy, inappetence and distended abdomen. One puppy died, the other was euthanized. The histological examinations showed disseminated granulomatous encephalitis and some oligofocal granulomas in other organs. *E. cuniculi* antigen was detected by immunohistochemistry in many organs (e.g. brain, kidney, liver, spleen), which indicates a systemic infection. PCR of one dog (brain and kidney positive) was performed and sequencing showed strain III (dog).

Thus, *E. cuniculi* should be considered as a cause of ocular and neurological disease not only in rabbits but also in dogs and cats. Mice may be the source of infection in cats. Manifestation in the lens results from in utero infection in latent infected dams. The neonatal disease in dogs also suggests in utero infection.

Research on Canine Vector- Borne Diseases: the multidisciplinary approach as a resource

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National Governments have been drastically reducing funds to the majority of academic institutions working in the field of Research and Development, worldwide. This has impacted on the way of approaching research in veterinary parasitology, with a prioritizing of a multidisciplinary approach in sample collection, data analysis and discussion. A key example for this is represented by a series of recent studies on canine vector-borne diseases (CVBDs) which primarily had the aim to determine the protection of imidacloprid 10% and permethrin 50% (ImPer) against *Anaplasma platys*, *Babesia vogeli*, *Bartonella* spp., *Ehrlichia canis*, *Hepatozoon canis* and *Leishmania infantum* in autochthonous young dogs and in naïve beagles. Indeed, for the complex interactions CVBD- causing pathogens have among themselves, as well as with their vectors (e.g., ticks, mosquitoes, and sand flies) and the vertebrate host immune system (at both individual and population levels), a multidisciplinary study allowed researchers to gain information on diverse aspects of these infections. A longitudinal field survey (March 2008 to April 2009) was conducted in Southern Italy, an area highly endemic for many CVBD causing pathogens. Autochthonous young dogs and naïve beagles living in a municipal dog shelter were treated with ImPer, and biological samples (i.e., blood, bone marrow and skin samples) were collected from all enrolled animals four times. Serological, cytological and molecular tests were performed simultaneously on different tissue samples of all animals to detect the above pathogens. Ectoparasites (i.e., fleas, ticks and sand flies) were also monitored throughout the study period and aspects of the biology and ecology of *Rhipicephalus sanguineus* and of phlebotomine sand flies were investigated. In addition, clinical signs induced by single or multiple vector-borne pathogens were recorded and the interdependencies between the presence/absence of clinical signs were compared with trends in laboratory parameters. The results of this study provided the following evidence: 1) ImPer treatment protects young dogs (both autochthonous and beagles) against CVBDs, in a highly infested environment; 2) Sand flies are well adapted to the urban environment of southern Italy, thus representing a public health concern; 3) The presence of *R. sanguineus* ticks infesting dogs the year around indicates that control measures should be carried out throughout the year in southern Italy; 4) The diagnosis of CVBDs remains a challenging task due to the limitations of individual tests, which supports the use of multiple diagnostic techniques when attempting to confirm a CVBD in endemic areas; 5) In endemic areas, it is difficult to assign a specific clinical sign or hematological abnormality to a particular CVBD.

Endemic *Toxoplasma gondii* genotype II causes fatal infections in animal hosts in Finland

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Background: *Toxoplasma gondii* is a zoonotic parasite capable of infecting and causing disease in a wide variety of host species. To further understand its epidemiology, genotyping the parasite strains from different hosts is needed. *T. gondii* genotype II is considered endemic in Europe, but the situation in Finland has been unknown until recently. Fatal toxoplasmosis is diagnosed sporadically among domestic and wild animals examined postmortem in Finland, but the cases had not been further investigated. At present, we have genotyping results from *T. gondii* parasites causing fatal infections in selected animal host species, and these results are summarized here.

Methods: Retrospectively, we have searched the records of European brown hares (*Lepus europaeus*), mountain hares (*Lepus timidus*), and Eurasian red squirrels (*Sciurus vulgaris*) examined postmortem in 2006-2009 at the only wildlife pathology laboratory in Finland, Evira, for cases of fatal toxoplasmosis. In addition, diagnosed cases of fatal toxoplasmosis in cats necropsied at the University of Helsinki in 2008-2010 have been thoroughly investigated. We confirmed the cases immunohistochemically and performed direct genetic characterization of the parasite strains causing the deaths, using a multiplex-PCR-method with seven microsatellite markers.

Results: Naturally acquired toxoplasmosis was the cause of death of 14 (8.1%) of 173 European brown hares, 4 (2.7%) of 148 mountain hares, 3 (14.3%) of 21 Eurasian red squirrels, and 5 (3.2%) of 157 cats. All the cases were caused by *T. gondii* parasites belonging to the genotype II.

Conclusions: Thus far, we have genotyping results from fatal toxoplasmosis cases in four host animal species from Finland, and they are all consistent with *T. gondii* genotype II. Although the material available for these investigations cannot be regarded as representative of the selected host animal populations, these fatal infections were naturally acquired, suggesting this genotype is endemic in Finland. It remains to be seen whether other genotypes are circulating in this country as well.

Serological Survey of *Babesia canis* in Dogs from Rural and Urban Areas of Seropédica, Rio de Janeiro State, Brazil

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Canine babesiosis is a tick-borne disease with great clinical importance for dogs caused by the protozoa *Babesia* spp. The species that affect dogs are *Babesia gibsoni* and *Babesia canis*, being the latter more widespread, pathogenic and divided into three subspecies: *Babesia canis canis* (Europe), *Babesia canis rossi* (South Africa) and *Babesia canis vogeli* (North Africa, North America and Brazil). The disease is characterized by fever, pale mucous membranes, anorexia, depression and hemolytic anemia may occur. Diagnosis is usually made by clinical signs and peripheral blood smears. However, as parasitaemias are usually very low or the parasite is not detectable in blood smears, serodiagnostic methods are preferable for epidemiological studies. The aim of the present study was to determine the seroprevalence of *Babesia canis* in dogs from rural and urban areas within the district of Seropédica, Rio de Janeiro State, Brazil, using an indirect immunosorbent assay (ELISA). Blood samples were collected from 311 dogs, being 168 (54%) and 143 (46%) from rural and urban areas, respectively. Crude antigenic preparation of *Babesia canis* was used in ELISA test at the concentration of 10 µg/mL, sera diluted at 1:50 and alkaline phosphatase conjugated anti-dog IgG 1:2000. The immunological activity of each serum tested was calculated by determining the positive serum ratio (S/P), considering positive and negative sera as reference. Antibodies anti-*Babesia canis* were detected in 21.4% (n=36) of the dogs from rural area, whereas only 2.1% (n=3) dogs from the urban area were seropositive. In this study, 35.9% (n=14) of the dogs serologically positive had ages between 6 months and 2 years, 28.2% (n=11) between 2 and 5 years and 35.9% (n=14) between 5 and 10 years old. In relation to ectoparasitism, 84.6% (n=33) of seropositive dogs had ticks at the time of blood collection, and 15.4% (n=6) didn't. The results from the present study indicate that babesiosis should be considered as an important agent in rural areas of Rio de Janeiro State and points out the importance of tick control.

Results of parasitological examinations of fecal samples from cats and dogs in Germany between 2003 and 2010

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Background: Dogs and cats are frequently infected with various helminth and protozoan endoparasites. They pose a threat to the health of animals and some of them also to humans. It is therefore important to have information about parasites in individual animals as well as in pet populations. Data analyses of results from diagnostic laboratories provide such information and allow guidance for control strategies.

Methods: In a retrospective study the results of parasitological examinations of fecal samples from 8,560 cats and 24,677 dogs between January 2003 and December 2010 in Germany were analysed.

Results: 30.4% of the examined dogs and 22.8% of the cats were infected with endoparasites. The examination of fecal samples from dogs revealed stages of *Giardia* spp. (18.6%), *Toxocara canis* (6.1%), *Toxascaris leonina* (0.6%), Ancylostomatidae (2.2%), *Trichuris vulpis* (1.2%), *Capillaria* spp. (1.3%), *Crenosoma vulpis* (0.4%), *Angiostrongylus vasorum* (0.5%), Taeniidae (0.4%), Dipylidiidae (<0.1%), *Mesocostoides* spp. (<0.1%), *Isospora* spp. (5.6%), *I. ohioensis*-complex (3.9%), *I. canis* (2.4%), *Sarcocystis* spp. (2.2%) and *Hammondia heydorni*/*Neospora caninum* (0.3%). Dogs in the age groups up to 3 months and >3 up to 6 months of age showed significantly higher infection rates with *Toxocara canis* (12.0% and 12.4%, respectively), *Toxascaris leonina* (1.1% and 1.6%, respectively), *Giardia* spp. (37.5% and 38.2%, respectively), *Isospora* spp. (23.4% and 11.8%, respectively), *I. ohioensis*-complex (15.6% and 7.2%, respectively) and *I. canis* (11.8% and 5.2%, respectively) compared to older dogs. In fecal samples from cats stages of *Giardia* spp. (12.6%), *Toxocara cati* (4.7%), *Toxascaris leonina* (0.1%), *Ancylostoma tubaeforme* (0.2%), *Aelurostrongylus abstrusus* (0.5%), *Capillaria* spp. (1.0%), Taeniidae (0.6%), *Dipylidium caninum* (<0.1%), *Mesocostoides* spp. (<0.1%), *Isospora* spp. (6.0%), *I. felis* (4.4%), *I. rivolta* (2.2%), *Toxoplasma gondii*/*Hammondia hammondi* (0.8%) and *Sarcocystis* spp. (0.3%) were detected. Cats in the age groups up to 3 months and >3 up to 6 months of age showed significantly higher infection rates with *Giardia* spp. (19.5% and 24.0%, respectively), *T. cati* (8.1% and 6.9%, respectively), *Isospora* spp. (12.8% and 8.6%, respectively), *I. felis* (10.0% and 5.9%, respectively) and *I. rivolta* (4.6% and 2.9%, respectively) compared to older cats.

Conclusions: Despite continuous efforts of the veterinary profession to reduce parasitism in dogs and cats, infection rates with endoparasites remain on a high level. Further parasite control is warranted to reduce infections with parasites, which may be controlled by future long-term studies.

Seroprevalence of *Toxoplasma gondii* in pigs in La Pampa – Argentina

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The seroprevalence of toxoplasmosis in pigs was investigated in La Pampa –Argentina. A total of 415 serum samples from 82 farms were collected from 3 regions (North, Central and South) and assayed for *Toxoplasma gondii* antibodies by latex agglutination (LA) as screening test and indirect haemagglutination (IHA) as confirmatory test. An antibody titer $\geq 1:8$ in IHA test was considered positive. Antibodies to *T. gondii* by LA were detected in 237 pigs (57.4%). However, the prevalence of anti-*T. gondii* antibodies by IHA test was 30.6% (95% CI: 26.2-35.3%). No statistically significant differences were observed among regions: 31%, 26% and 29% of the pigs from North, Central and South region had anti-*T. gondii* antibodies. The herd prevalence was 59.5% (95% CI: 47.1-69.2). The results of the present survey indicate that *T. gondii* infection is a significant health problem in pigs and represents a public health concern in La Pampa-Argentina.

Canine dirofilariasis in Marajó Archipelago, Pará State, Brasil: an epidemiological, morphologic and molecular study

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Background: Marajó Archipelago is an interesting area for research in veterinary medicine, since domestic and wild animals share same ecological niche, facilitating the establishment of more complexes parasitic cycles than observed in urban areas. In this context, the observation of microfilariae in peripheral blood of domestic dogs can not be directly associated with *Dirofilaria immitis* infection, because co-infections with unidentified filarids may occur.

Methods: This study was conducted in two municipalities of Marajó Archipelago (Salvaterra and São Sebastião da Boa Vista), to obtain epidemiological data (presence of microfilariae in blood stream of 188 domestic dogs), morphological (taxonomic analysis of adult worms obtained from pulmonary artery and right ventricle of domestic dogs) and molecular (genetic comparison between ribosomal regions of circulating microfilariae in canine blood and adult worms).

Results: We observed 37.34% of microfilaremic dogs in Salvaterra and 6.67% in São Sebastião da Boa Vista, resulting in a total prevalence of 32.45%. Adult heartworms in pulmonary artery and right ventricle of dogs from both municipalities showed morphological characteristics of *D. immitis*. Molecular analysis showed that parasited dogs did not present mixed infection by filarids and the circulating microfilariae had similar sequences to *D. immitis* adult worms obtained in the same area and sequences of the same species previously published in the other areas.

Conclusions: The prevalence of microfilaremic domestic dogs in Marajo Archipelago is high. The dogs studied were parasitized by *D. immitis* in the pulmonary artery and right ventricle. The adult heartworms presented molecular similarity with circulating microfilariae and filarids mixed infection were not observed.

Morphological aspects of *Dirofilaria immitis* by Scanning Electron Microscopy

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Background: Data about morphology of helminths is very important to classical taxonomy (alpha taxonomy), and scanning electron microscopy (SEM) has become an important tool to add new taxonomic characters to previous described species made by light microscopy. For this reason, we carried out morphological studies in *Dirofilaria immitis* using this technique, since data about this filarid by SEM is scarce.

Methods: The filarids were collected from the right ventricle and pulmonary artery of domestic dogs in Salvaterra municipality, Marajo Island, Pará State, Brazil. The worms were fixed in AFA (2% acetic acid, 3% formaldehyde, and 95% ethanol 70°GL) at 60°C. Two centimeters of anterior and posterior ends of 10 males and females were processed according for SEM analysis previously as described.

Results: Morphological data of filarids were compatible with previous descriptions of *D. immitis*, but several anatomical structures are described by SEM for the first time: body surface with delicate transversal striation randomly in cephalic extremity and in posterior extremity of females and absent surrounding deirids, vulvar opening and anus; amphids presenting basal opening directed to posterior region of body; small lateral deirids are located in center of cuticular depression, next to lateral line, with a filamentous structure, base wider than apex that are trifurcated, and longitudinal concavity; inconspicuous lateral lines constituted by discrete discontinuous ridges in anterior and posterior portion of filarids, and by parallel continuous lines throughout body; vulvar opening elliptical and transverse next to anterior end, delimited by fine, but not prominent lips, without papillae or fringes; anus sub-terminal with incurved opening, toward posterior extremity.

Conclusions: We have added several new features for *D. immitis* using SEM, providing a much more robust characterization of the species.

Intestinal parasites in different cat populations in northern Belgium

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Background: World-wide only a limited number of recent studies have been performed to investigate the prevalence of intestinal parasites in cats. Moreover, in most studies only one particular cat population was targeted (e.g. feral cats or cats with clinical signs of gastrointestinal disease).

Methods: A cross-sectional survey was conducted to estimate the prevalence of intestinal parasite infections in household cats, feral cats and cats with gastrointestinal disorders in Flanders, Belgium. Samples were examined for the presence of worm eggs and protozoal oocysts using a sedimentation-flotation technique. The perianal region of the feral cats was examined for cestode proglottids. Fecal swabs were taken from the feral cats for detection of *Tritrichomonas foetus* using the InPouch™ TF - Feline test. For the detection of *Giardia* a direct immunofluorescence assay was used. PCR protocols targeting the β -giardin gene and the triose phosphate isomerase gene were used for the molecular identification of *Giardia* isolates.

Results: Between November 2008 and October 2010 faecal samples were collected from 47 household cats, 189 feral cats and 128 cats with diarrhea. In household cats the prevalence of intestinal parasites was relatively low. *Toxocara cati* was the most commonly found parasite (8.2%), followed by *Giardia* (4.1%). Much higher infection rates were observed in feral cats, especially for *T. cati* (66.7%), *Giardia* (16.7%) and *Cystoisospora* spp. (5.6%). Also in cats with gastrointestinal problems, *T. cati* (8.6%), *Giardia* (7.8%) and *Cystoisospora* spp. (10.9%) were the most frequently detected parasites. The cat-specific *Giardia duodenalis* assemblage F, as well as potentially zoonotic assemblages were present in different cat populations. *T. foetus* was not detected in the feral cats.

Conclusions: The prevalence of intestinal parasites in cats in northern Belgium is high, especially in feral cats. *Toxocara* and *Giardia* are commonly found parasites, and present a zoonotic risk.

Canine leishmaniasis: use of serological tests in three non-endemic areas from Northern Argentina

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Canine infection with *Leishmania chagasi* (syn. *Leishmania infantum*) can cause serious disease (CanL: canine visceral leishmaniasis) and infected dogs are important for human disease epidemiology. Accurate diagnosis of canine infections is essential to veterinarians and leishmaniasis surveillance programs. Diagnosis in asymptomatic dogs is usually difficult due to the low level of parasites in tissues and detectable antibodies in serum. Moreover, cross reactions in serological tests among *Leishmania* and *Trypanosoma* species has been reported. During the last years the northeast area of Argentina became an endemic area of CanL.

A total of 131 serum samples from CanL-asymptomatic dogs were taken during 2010 in Taco Pozo (n: 37), Concordia (n: 50) and Salta suburbs (n: 44) from Chaco, Entre Rios and Salta provinces respectively. The sampled area is currently considered non-endemic for CanL, and Salta and Chaco provinces are endemic for chagas disease. All samples were evaluated for anti-*Leishmania* antibodies by IFAT and RK39 Immunochromatographic strip test and for anti-*Trypanosoma* antibodies by IFAT. Serum dilutions 1/40 or greater were considered positive in both IFATs. Positive results for IFAT-*Leishmania*, RK39 and IFAT-*Trypanosoma* were respectively as follows: 2.3%, 11.3% and 7% in Salta; 11.4%, 16% and 22.2% in Chaco and 26%, 18% and 32% in Entre Rios. Regarding IFAT results, positive animals had titer 40, except one with titer 80 to *Leishmania* and another with titer 160 to *Trypanosoma*. Agreement between test for all samples was estimated and Kappa values were obtained: IFAT-*Leishmania* and RK39: 0.200; IFAT-*Trypanosoma* and RK39: 0.303; IFAT-*Leishmania* and IFAT-*Trypanosoma*: 0.388.

The three locations showed similar seropositivity for each serological test. Surprisingly seropositivity by IFAT was always higher for *Trypanosoma* than *Leishmania* antigen. This fact is especially interesting in Entre Ríos province, considered chagas vector transmission-free since 2004. The agreement between tests was low and both *Leishmania* tests show the lowest value. Considering all these facts, the accuracy of these tests to diagnose CanL and/or chagas disease in Argentinean asymptomatic dogs can be questioned. Additional studies are needed in order to determine if the protozoans are present in areas considered non-endemic or in asymptomatic animals.

The epidemiology of *Cryptosporidium* spp in domestic animals (cats and dogs) in Thohoyandou, South Africa

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Background: *Cryptosporidium* spp have emerged over the past decade as important diarrheal causing agents and responsible of diarrheal outbreaks around the world. Zoonotic transmission has been reported and their occurrence in domestic animals is of potential significance from both clinical and public health perspectives yet, the occurrence of these organisms among animals in South Africa, particularly the Vhembe district has not been described. The objective of this study was to determine the prevalence of *Cryptosporidium* spp in domestic animals, particularly cats and dogs in the Thohoyandou region.

Methods: Fresh stool samples were collected from cats (25 samples) and dogs (25 samples) including stray and home based animals in different areas of the Thohoyandou region. The stool samples were homogenized and genomic DNA was isolated from the stool using the Qiagen Kits. A Polymerase Chain Reaction (PCR) protocol based on the amplification of a specific sequence of the 18S rRNA gene was used to detect *Cryptosporidium* spp from the stool samples.

Results: From the 50 samples tested, 8 (32.0%) of the cats samples had *Cryptosporidium* and 11 (44%) of the dogs samples had *Cryptosporidium* with no significant difference ($\chi^2=0.764$, $p=0.280$). *Cryptosporidium* was more common in diarrheal samples in cats and dogs, 3/4 (42.9%) and 7/14 (50%) respectively. The infection rate was higher in stray animals particularly in the stray cats [3/4 (75%)] compared to home based cats [5 (23.8%)] ($\chi^2=4.046$, $p=0.044$). Stray dogs were also more infected [6 (46.2%)] than home based dogs [5 (41.7%)], but the difference was not statistically significant ($p=0.821$). Animals from the more rural parts of the region were more infected.

Summary/Conclusions: The results of this study have demonstrated the high prevalence of *Cryptosporidium* infections among cats and dogs in the Thohoyandou region and its potential implications in causing diarrhea among these animals. Stray animals were more infected and pose a threat to the community and other animals.

Zoonotic transmission of Microsporidia in patients with AIDS

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Background: Microsporidiosis is an emerging and opportunistic infection in AIDS patients associated with a wide range of clinical syndromes. Among the 14 species of Microsporidia currently known to infect humans, *Enterocytozoon bienersi* and *Encephalitozoon intestinalis*, are the most common causes of human infections associated with diarrhea and systemic disease, respectively. Species of Microsporidia infecting humans have been identified in water sources as well as in wild, domestic and farm animals.

The aim of this study was to describe the association between Microsporidia infection in AIDS patients and infection in their respective companion animals.

Methods: This study included 7 adult patients (6 male, 1 female) with AIDS and chronic diarrhea and 13 domestic animals, 3 cats and 10 dogs, with intradomiciliary habits. Feces samples from human and animals were obtained, concentrated by ethyl-ether centrifugation and stained with Weber trichrome and Gram-chromotrope stains. Biopsy specimens from patients were obtained by fiberglass endoscopy, embedded in polybedaraldite and stained with Azur II.

Results: We identified Microsporidia spores in fecal samples of the patients and at least one companion animal of each case. Biopsy specimens showed intracellular organisms compatible with microsporidia. We identified spores in 11 samples from animals, including 3 cats and 7 dogs. For one case, species identification was carried out with biopsy and fecal specimens from the patient and stools from his animals corresponding to a dog and a cat, by PCR amplification employing Eb.gc/Eb.gt primers for *E. bienersi* and SINT F/R primers for *E. intestinalis*. In this case, we detected a coinfection with both species for the patient and his dog and *E. Intestinalis* infection for his cat.

Conclusions: Cats must be added to the list of reservoirs of microsporidiosis caused by *E. Intestinalis*. Zoonotic transmission of Microsporidia should be taken in account for the management of AIDS patients.

Gastrointestinal Parasites in an Urban Feline (*Felis catus* L 1758) Population Presented to a Spay & Neuter Program in Rio de Janeiro, Brazil

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Global climate changes will determine, and probably are already determining, changes in epidemiological patterns of parasitic infections. In this forthcoming scenario it is possible that feline gastrointestinal parasitic infections become more frequent in urban environments, facilitating the spillover among mammalian hosts, including humans. The knowledge of the current frequency of these infections in urban cats along with constant monitoring will facilitate previewing outbreaks and therefore making it possible to draw preventive and therapeutic measures to the new scenario.

A total of 54 fecal samples from clinically healthy cats regardless of age, sex or breed presented to a free spay-neuter program were included. Samples were preserved in acetic-formalin solution, until processing. Parasitological exams were performed by three different techniques: i) centrifugal flotation using sugar solution ($d = 1.20 \text{ g/cm}^3$); ii) centrifugal flotation using saturated zinc sulphate ($d = 1.18 \text{ g/cm}^3$) and; iii) spontaneous sedimentation.

Approximately half of the samples (53.7% - 29/54) harbored parasitic forms and in 62.1% (18/29) of these samples at least one species capable of infecting humans was detected: *Toxocara* sp (27.6% - 8 / 29), *Ancylostoma* sp (27.6% - 8 / 29), *Giardia duodenalis* (20.7% - 6 / 29), *Cryptosporidium parvum* (10.3% - 3 / 29) and *Dipylidium caninum* (3.4% - 1 / 29). Other species found were: *Platynosomum fastosum* (20.7% - 6 / 29); *Isospora rivolta* (20.7% - 6 / 29), *Isospora felis* (13.8% - 4 / 29) and *Sarcocystis* sp (6.9% - 3 / 29). Ten out of the 29 positive samples had multiple parasitisms (34.5%) with different associations that included trematodes, nematodes and protozoans. Interesting to highlight that no oocyst of *Toxoplasma-Hammondia* was detected. Parasites were eliminated by 50% of kittens (4/8); 44% of young adults (15/34) (6months to 2 years) and 83% of adults (10/12) (>2 years).

These results show that the most frequently found gastrointestinal parasites probably circulate within the urban environment unnoticed once none of the animals showed clinical signs. Therefore, monitoring of these parasites over time must be enforced to allow early detection of spreading and spillovers and to make the development of preventive measures possible.

***Rickettsia* spp. identification and differentiation in ticks collected in the city of Hannover (Germany) by quantitative real time PCR and pyrosequencing technology**

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Background: Rickettsiosis is an emerging disease which often remains unnoticed or misdiagnosed due to the lack of appropriate diagnostic tools. To detect *Rickettsia* in the arthropod vector, mainly ticks, quantitative real time PCR (qPCR) is a fast and reliable method. However, a genus specific qPCR is not able to differentiate between species. To date, species identification is mainly achieved by amplification of the positive samples via conventional PCR and subsequent Sanger sequencing.

In the present study, the *Rickettsia* infection rate of ticks of the city of Hannover, the capital of the federal state Lower Saxony, Germany, was examined via qPCR. For subsequent species identification the pyrosequencing technology was evaluated as a potential sensitive diagnostic tool to identify different *Rickettsia* species.

Methods: 1089 *Ixodes ricinus* ticks (328 adult females, 341 adult males, 332 nymphs and 88 larvae) were analysed via qPCR for the presence of *Rickettsia*. Positive samples were amplified through PCR and sent for Sanger sequencing for species differentiation. 263 of the total of 363 positive ticks were available for evaluation of species differentiation through pyrosequencing by targeting the citrate synthase gene (*gltA*).

Results: 33.3% of the ticks (363 individuals) were found *Rickettsia*-positive by qPCR, consisting of 36.5% positive adults (41.8% females and 31.4% males), 28.6% positive nymphs and 27.3% positive larvae. Sanger sequencing identified 178 ticks (49.6%) for their species, of which 176 were *R. helvetica* and 2 *R. monacensis*. The remaining 50.4% ticks could not be differentiated. The 263 tick samples sequenced by pyrosequencing were accurately identified for their species if more than 10 *gltA* copies were present in the template. Besides confirmation of two *R. monacensis* infected ticks, *Rickettsia helvetica* was found in 188 samples of which 35 were previously undifferentiated.

Summary/Conclusions: The overall prevalence of 33.3% *Rickettsia* infected ticks in the city of Hannover is the highest found in Germany so far. Consistent with other German studies, *R. helvetica* was the most prominent species identified by Sanger sequencing. The pyrosequencing technology proved to be more sensitive than Sanger sequencing towards identifying *Rickettsia* species. Thus, the evaluated pyrosequencing method represents a reliable tool for *Rickettsia* species differentiation in diagnostic tick material and prevalence studies.

Efficacy of a spot on combination containing imidacloprid 10 % and moxidectin 1 % (Advocate[®] / Advantage[®] Multi, Bayer Animal Health) against *Ancylostoma ceylanicum* in cats

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Ancylostoma ceylanicum is common zoonotic hookworm of dogs and, cats in Asia and Australia. The objective of this study was to determine the efficacy of a spot on combination product containing imidacloprid 10 % and moxidectin 1 % (Advocate[®] / Advantage[®] Multi, Bayer Animal Health) against *A. ceylanicum* in experimentally infected cats. Sixteen kittens were each subcutaneously injected with 100 infective third-stage larvae of *A. ceylanicum*. Kittens were stratified by egg count and randomly allocated equally into control and treatment groups. The kittens in the treatment group were each treated with a spot on combination of 10 % (w/v) imidacloprid and 1% (w/v) moxidectin, administered topically at the skin surface between the shoulder blades. The kittens in the control group were not treated. Egg counts were performed daily until the end of the study period and compared for the treated and control groups. No eggs were detected in the treated group of kitten within 3 days of treatment and faecal samples from this group remained negative throughout the rest of the study, resulting in a treatment efficacy (egg reduction) of 100% (P<0.0001). The egg counts remained high (993 ± 666 epg) in the untreated control group for the rest of the study period. This study demonstrated that the spot on combination containing imidacloprid 10 % and moxidectin 1 % (Advocate[®] / Advantage[®] Multi, Bayer Animal Health) given at the recommended dose is highly effective against infection with *A. ceylanicum* in cats.

Canine hepatozoonosis: a case in San Luis (Argentina)

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Introduction: The canine hepatozoonosis is a systemic parasitic disease caused by a cosmopolitan protozoan (Phylum *Apicomplexa*) named *Hepatozoon canis* (HC). The disease is transmitted with the ingestion by dogs of contaminated ticks of the genus *Rhipicephalus sanguineus* (RS). The cycle continues in the canine and the first parasites appear in peripheral blood 3 months after ingestion. Vertical transmission it has been also reported in puppies. In the canine the parasite is found in gametocyte stage within the monocyte and neutrophil. The cycle is completed when an uninfected tick sucks blood from the infected host. Infected patients usually have no clinical signs, with the exception of current infected or immunosuppressed dogs, or puppies under 4 months old. The first symptoms are eye and nose discharge, locomotion disorders, anorexia, anemia, weight loss, emaciation, fever, weakness and death. The first description in Argentina was reported in 1999 in the city of Buenos Aires.

Methods: In the summer of 2009, a crossbred male dog two years old, large size and very thin, with clear signs of weakness, was submitted to consultation. The animal was born in the San Luis city, and had no travel history. Had frequent contacts with other neighborhood dogs. At the first examination, very pale mucous membranes were observed, and also anorexia, 42 ° C, dehydration, cachexia, and the presence of a large number of ticks. On palpation, a significant splenomegaly was detected. After primary treatment with antibiotics and anti-inflammatory the animal had a slight improvement, but after three days the animal started vomiting, and presented high fever and signs of jaundice, which guided to the presumptive diagnosis of a hemoparasitosis. A complete blood count (CBC), urinalysis and blood smears were performed to confirm the diagnosis.

Results: The ticks were classified as *Rhipicephalus sanguineus*. The CBC revealed a severe anemia, leucocytosis, and hypo proteinemia. Urine analysis identified a proteinuria of 4 crosses. Direct observation of blood smear stained with Wright, showed the presence of cigar shaped bright blue with a size between 6 to 11µ in neutrophils and / or monocytes. These structures are compatible with gamont of *Hepatozoon sp.* Facing the parasitological diagnosis of hepatozoonosis, treatment was started with Toltrazuril using a dose of 10 mg / kg every 12 hours for six days.

Conclusion: The clinical and laboratory results were consistent with a generalized hepatozoonosis infection. The finding of gamonts of *Hepatozoon sp.* in blood smears confirmed the infection. The treatment with toltrazuril was effective for symptom improvement despite frequent relapses. A year after the treatment applied the animal shows improvements but not a true recovery in its general condition. Renal failure (glomerulonephritis) following of the parasitosis causes a loss of protein in urine, which results in ascites and decreased body weight. The appearance of this case of Hepatozoonosis diagnosis in the province of San Luis alert the clinician regarding the need to consider in the differential diagnosis, along with other protozoal diseases such as toxoplasmosis and neosporosis in patients with advanced cachexia.

***Hippobosca longipennis* – a potential intermediate host of newly proposed *Acanthocheilonema* species in northern India**

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Canine infections with four species of filariae, namely *Dirofilaria immitis*, *Dirofilaria repens*, *Acanthocheilonema reconditum* and *Brugia* spp. have been reported in India. In previous studies, diagnosis was made by morphological identification only. The present study utilised a combination of conventional and molecular diagnostic techniques to gain further insight into the species and distribution of canine filarial species in India. Two filarial species were reported in dogs in India for the first time in this study, namely *Microfilaria auquieri* Foley, 1921 and a novel species of canine *Acanthocheilonema*, discovered in the Himalayan city of Ladakh, which is currently designated as *Acanthocheilonema ladakhii* until further and more detailed studies are undertaken. Almost all dogs in Ladakh, and a small proportion in Delhi, were found infested with a species of blood-sucking fly, identified as *Hippobosca longipennis* Fabricius, 1805, which is known to be an intermediate host and vector of *Acanthocheilonema dracunculoides*. Flies were examined using a stereomicroscope and identified morphologically. The flies were then individually dissected in phosphate buffered saline (PBS). A total of 11 infective larvae were found in 9 out of 40 flies dissected; 8 from the head, 2 from the thorax and 1 from the abdomen. The larvae averaged 2,900 (± 60) μm in length and 34 (± 5) μm in width and possessed characteristic features of the family Onchocercidae. Genetic analysis confirmed the identity of the larvae as the newly proposed *A. ladakhii*. This study is the first to illustrate a potential intermediate host-parasite relationship between *H. longipennis* and the newly proposed *A. ladakhii* in northern India.

Stain techniques for detection of *Encephalitozoon cuniculi* in tissue sections

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Background: The diagnosis of encephalitozoonosis employing histological sections is difficult because of the small size of the parasite and its staining characteristics. In this study, we investigated different staining techniques applied to histological sections for identification of *Encephalitozoon cuniculi*.

Methods: It was used liver fragments from Balb-c mice immunosuppressed with cyclophosphamide and inoculated with spores of *Encephalitozoon cuniculi*. The histological paraffin embedded sections were stained with hematoxylin-eosin (H-E), modified trichrome, Gram-Chromotrope, Giemsa, Brown-Hopps, PAS, Ziehl-Neelsen, and Grocott and also by the technique of calcofluor. Toluidine blue-fuchsin and toluidine blue stainings were used for the cuts embedded in plastic resin.

Results and Conclusion: Gram-Chromotrope, Brown-Hopps and Ziehl-Neelsen staining techniques permitted the best visualization of the spores for the diagnosis of *E. cuniculi* in histological paraffin embedded sections, since they allowed the clear differentiation of the spores among other tissue structures. In tissues embedded in plastic resin, toluidine blue-fuchsin and toluidine blue stainings also facilitate the finding of the agent. On the other hand, the trichrome technique showed great variability on results, therefore, being enable to identify the parasite in tissue. The other techniques, H-E, calcofluor, Grocott, Giemsa and PAS did not permitted the easy identification of the *E. cuniculi* spores.

Occurrence of microsporidia in fecal samples of *Nasua nasua* (South American coati) from Ecological Park, São Paulo, Brazil

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Background: Microsporidia are obligate intracellular parasites that are emerging as opportunistic pathogens that infect immunocompromised and immunocompetent individuals. The phylum Microsporidia contains 144 genera and 1,200 species that infect vertebrate and invertebrate hosts. Many questions about microsporidial transmission remain unanswered and the zoonotic potential of human-related microsporidia is the focus of discussion. As these parasites have been reported in wild animal as potential infection sources, the aim of the present study was to investigate the presence of microsporidia in fecal samples of South American coati (*Nasua nasua*) in the city of São Paulo (Brazil).

Methods: A total of 30 coatis were captured from ecological park and placed in individual cages in order to obtain fecal specimens. Thin smears were made from the fecal samples and submitted to Weber's Chromotrope and Gram-Chromotrope stains. The positive samples were submitted to PCR investigation for species characterization using the primers EBIEF1/EBIER1 for *E. bieneusi*, ECUNF/ECUNR for *E. cuniculi* and SINTF/SINTR for *E. intestinalis*. PCR amplification was done with GenAmpkit (Perkin Elmer Cetus) according to manufacturer's procedures.

Results: Microsporidia spores were found in 10 coatis (33.4%). The stool samples showed a variable number of spores stained in pinkish red with the use of Weber's Chromotrope and Gram-Chromotrope stains. The spores showed an ovoid shape and measured 0.9 to 1.6 micrometer, some presenting the typical belt-like stripe of these protozoans. Six (20%) coatis were positive by both techniques, 3 (10%) for *E. bieneusi*, 2 (6.7%) for *E. intestinalis*, and 5 (16.7%) for *E. cuniculi*. Coinfections were not detected in this study.

Conclusion: These results show that environmental contamination with microsporidia from coatis feces can be an important source of human infection with different species of *Encephalitozoon*.

Limited risk of canine babesiosis in the Czech Republic

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Large *Babesia canis* namely *B. canis canis* transmitted by *Dermacentor reticulatus* is spread in Europe. The incidence of *Dermacentor reticulatus* in the Czech Republic is limited to the Moravia and Thaya basins along the border with Slovakia. No autochthonous case of canine babesiosis was reported so far in the Czech Republic, even if babesiosis is present in neighboring countries. The aim of our study was to examine group of dogs coming from the *Dermacentor* locality.

Materials and methods: From March to November 2010, a total of 41 dogs of 11 breeds without travel history were examined. All animals were clinically healthy. Blood samples of some of them were collected repeatedly. Out of a total of 41 dogs, blood samples of 21 animals were collected once, 7 dogs were sampled twice and 13 animals three times. A total of 68 blood samples were collected. Samples were taken monthly. Full blood samples were examined by PCR method. DNA was extracted using the commercial kit QIAGEN NucleoSpin Blood (producer Machery-Nagel, Germany) as prescribed by the manufacturer. To amplify a diagnostic fragment of the 91 piroplasmid SSU rRNA gene, we have designed the forward primer TB-F (5'-CTTCAGCACCTTGAGAGAAAT-3') and the reverse primer TB-R (5'-CTTCAGCACCTTGAGAGAAAT-3') and the reverse primer TB-R (5'-TCDATCCCCRWACGATGCRBAC-3'). The dog with imported *B. canis* infection was used as a positive control. Specific IgG antibodies against *Babesia canis* were detected by indirect immunofluorescence using the commercial Babesia canis IFA IgG Antibody Kit (Fuller Laboratories Fullerton, California, USA).

Results: A total of 68 blood samples taken from 41 dogs were examined by PCR. No sample contained DNA of *B. canis*. Specific antibodies were detected in 5 dogs (12.2%). Serological examination detected 6 positive serum samples. One dog was positive in two samplings within 3 months. Titres ranged from 50 to 200.

Discussion: The Czech Republic has today a unique position as compared with adjacent countries where vector's area is expanding as well as babesiosis. Long-term incidence of *Dermacentor reticulatus* in the Czech Republic was confirmed only in a small area. On the other hand we detected the antibodies against babesiosis in 5 dogs (12.2 %). Although babesiae were not detected directly by PCR, the results of our study indicate that the presence of *B. canis* in the Czech Republic cannot be excluded.

Diagnosis of *Spirometra* spp. in cat (*Felis catus*)

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"Parasites in the *Spirometra* genus are Pseudophyllidea that are differentiated from *Diphyllbothrium* in that the uterus of *Spirometra* is spiral.

The adult worm is found in the small intestine and it can measure up to 60 cm in length, the scolex has elongated bothria, eggs are elliptical, yellowish, operculated and they measure 60 x 30 µm. Definite hosts are felids, but they can also parasitize dogs, swine, wild carnivores and other mammals.

The life cycle needs two intermediate hosts: copepods intake the coracidium that hatched from the eggs that were in the feces shed on water, and a proceroid develops inside them. When the infested crustacean is eaten by a second host, the proceroid transforms into a plerocercoid or sparganum. The second intermediary hosts may be reptiles, birds, small mammals, pigs, and primates (including humans). The cycle is completed when cats feed on the infested second intermediary hosts.

Sparganum measure between 4 and 10 cm in length, they are found in second intermediary hosts tissues. Humans may also become infested. The goal of the present report is to communicate the finding of *Spirometra* spp. in a one-year-old female cat, which was found in a dump as a kitten in Nuevo Torino (31° 21' latitude y 61° 14' longitude), Las Colonias Department, Santa Fe province. The cat was taken to the animal hospital due to a recurrent diarrhea and weight loss. According to its owner, no fish is included in its diet. Feces were analyzed using Teuscher technique and eggs compatible with *Spirometra* were found. Measures ranged from 58 – 72 x 30 – 36,5 µm.

Taking into account the anamnesis, clinical signs, the fact that plerocercoids of *S. mansonioides* develop in any vertebrate except in fish, we conclude that our finding corresponds to a Pseudophyllidea of the *Spirometra* genus. The cat was treated with Praziquantel, 35 mg/kg, following which the diarrhea ceased and the patient recovered its body weight. Unfortunately, the adult worm could not be recovered.

Case report of canine visceral leishmaniasis in Concordia city, Argentina

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Visceral leishmaniasis is a worldwide zoonotic disease in which wild and domestic mammals act as important hosts. In South America it is caused by the protozoan *Leishmania chagasi* (syn. *L. infantum*), being the sand fly *Lutzomyia longipalpis* the arthropod vector responsible for transmission. Dogs are considered the main domestic reservoir of the parasite in urban or suburban areas, representing an important part of the epidemiology of the disease. Clinical signs in dogs may vary from no clinical manifestation to a severe life threatening disease. During the last years, the northeast of Argentina (especially Misiones province) became an endemic area with severe cases of canine visceral leishmaniasis (CanL). Canine infection has been presented in some localities near Concordia (Northern Entre Ríos) and vector presence has been reported in this province recently.

A natural case of CanL in a 4 years old mixed-breed female dog raised in Concordia, Entre Rios, Argentina is presented. The dog was adopted from a suburban area of the city when it was a puppy and no history of travel to other cities was recorded. The animal presented clinical signs compatible with CanL: weight lost, forelimb skin ulceration, epistaxis, alopecia, exfoliative dermatitis, conjunctivitis, uveitis and onychogryphosis. Blood serum analyses revealed increased total protein concentration, hypoalbuminemia and hyperglobulinemia.

Serology using an rK39 immunochromatographic strip was positive. Cytology of the lymph nodes and bone marrow smears revealed low amount of *Leishmania* amastigotes. Following natural death, the dog underwent necropsy. No important lesion was observed except for mild hepatomegaly and cachexia.

The ITS1-PCR from bone marrow and a mix of spleen, bone marrow and liver DNA samples were positive for *Leishmania* spp. Sequencing and BLAST analysis showed that infection was caused by *L. chagasi*.

This is the first report of a severe CanL case in a dog raised in Concordia. Considering the present report of natural *L. chagasi* infection from an area where suitable conditions could exist, CanL should be considered a potential emerging infectious disease in the zone. It's important the surveillance programs in order to reduce the spread of visceral leishmaniasis in Argentina.

New Insights into Morphological and Biological Features of *Capillaria aerophila* (Trichocephalida, Trichuridae)

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Background: *Capillaria aerophila* is a trichuroid nematode affecting carnivores (e.g., cats and dogs) and humans. Animals become infected by ingesting larvated eggs or earthworms, which act as facultative intermediate hosts. Adult stages live underneath respiratory epithelia and cause different degrees of respiratory distress and symptoms. Indeed, this nematode is underestimated and several aspects remain to be elucidated and better clarified, including routes of transmission and diagnostic aspects. The aim of this study is to present new insights into morphological and biological features of this neglected lungworm.

Methods: Eggs of *C. aerophila* were collected from four naturally infected dogs and one cat with monospecific infection. Eggs were used to examination and measurements by light microscopy (LM; Leica DM LB2 microscope) and to scanning electron microscopy (SEM; FEI Quanta 200 SEM), respectively, and compared with eggs of the intestinal trichuroid *Trichuris vulpis*.

Eggs were also split in different coprocultures for their *in vitro* developing over a period of two months at a temperature of 20 ± 2 °C and relative humidity from 80 % to 85 %.

Results: LM showed typical features of *C. aerophila* eggs: size, asymmetry of bipolar plugs and a wall with a network of anastomosing ridges. These features were all confirmed upon SEM, in that all examined eggs showed an outer densely striated and net-like shell. Eggs of *T. vulpis* were bigger than those of *C. aerophila* and showed a thick and smooth wall at both LM and SEM. Eggs of *C. aerophila* started to develop after 35 days, and at day +60 all eggs contained a mobile larva.

Conclusions: This study reports a thorough LM and SEM morphological and morphometric analysis of eggs of the neglected lungworm *C. aerophila* and provides key information for their identification, which in turn represent a diagnostic dilemma, especially in comparison with whipworm eggs. Furthermore, this is the first information on the *in vitro* developing of *C. aerophila* eggs, providing important information on the biological cycle of this nematode, to be still fully elucidated.

First Description of *Gurltia Paralysans* in a Domestic Cat in Argentina

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Background: *Gurltia paralysans* is a nematode found in some countries of South America. Its location is in the blood vessels of the central nervous system (CNS) that causes progressive nervous disorders of the hindquarter in domestic cats. The authors describe one case of flaccid paralysis of the hindlimbs occurring in a cat in Argentina.

Method: A female, 2 year old domestic cat with a previous history of chronic paraparesis was brought to Dr. Paludi's clinic with paraplegia. Radiographic studies were performed to rule out possible spinal cord trauma, neoplasia, vertebral and intervertebral disk diseases. The clinical, neurological and blood exams, serology and microbiological studies did not express an etiology. Because of this and the epidemiological characteristics of the case (7 cats affected in a population of 12), the decision was taken to sacrifice the cat (The animal slaughtering followed the animal procedures and management protocols approved by the Ethics Committee according to the Animal Welfare Policy (act 087/02) of Faculty of Veterinary Sciences, UNCPBA, Tandil, Argentina [<http://www.vet.unicen.edu.ar>]) and perform the necropsy as a diagnostic test. Samples were taken from various organs for histopathology, toxicology; spinal cord samples for bacteriological diagnosis. The tissues were paraffin-embedded and stained with haematoxylin and eosin.

Results: Macroscopically both lungs had spongy consistency and multiple hemorrhagic foci were observed in the spinal cord from T11 to S5 segment.

Histopathological examination of the spinal cord revealed swelling in meninges and nerve tissue characterized by marked dilatation of blood vessels and perivascular cuffing and the presence of parasitic nematodes cuts in the light of several blood vessels. In the brain was observed inflammation of the meninges and in same sectors parasite eggs. In the lungs, perivascular inflammatory infiltrates were observed.

Conclusion: The final diagnosis was parasitic nonsuppurative meningomyelitis. The lesions observed were similar to those reported in other cases in which the agent was identified as *Gurltia paralysans*.

We are currently working on new cases and testing potential treatments.

Canine Visceral Leishmaniosis: An evaluation of Laboratory Diagnosis five years from the first autochthonous case in Argentina

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Introduction: Canine Visceral Leishmaniosis (CVL) is a severe zoonotic and systemic disease caused in Latin America by an intracellular protozoon, *Leishmania infantum/chagasi*. In urban environments, the domestic dog is the main animal reservoir. The parasite is transmitted by a night-biting sandfly, *Lutzomyia longipalpis*, an insect well adapted to peri-domestic environments and distributed throughout South America in tropical and temperate areas.

The urbanization of CVL has been observed due to changes in demographic and ecological factors. In April 2006, the first focus of CVL in Argentina was detected in the city of Posadas (Misiones).

The aim of this study is to communicate the experience obtained in Veterinaria del Oeste of clinical findings and the effectiveness of laboratory tools such as cytology and serology. Statistic register of sex and age of 3.006 symptomatic dogs tested were compared and studied in the same laboratory.

Methods: Dogs mainly from Posadas but also from other cities of the region underwent clinical examination. Symptomatology related to canine leishmaniosis was recorded, while cytologic samples from lymph node, bone marrow and skin were collected. In some of these animals, Anti-Leishmania antibodies were detected using rK39 immunocromatographic tests (Kalazar Detect Test).

A retrospective study based on the laboratory data was performed. The research was focused to evaluate the utility of these techniques for clinical purposes.

Results: A total of 3006 dogs, were tested by cytologic samples between 2006 and 2010; 48 % were females, 52% were males. Their average age was 4,6 years old.

2521 hyperplastic lymph nodes samples (84%) were positive to Leishmania. All 261 bone marrow samples taken were positive. From a group of 1017 symptomatic dogs, 341 had skin lesions. The parasite was found in 78% of these cases. In 25% of these, a *Malassezia* co-infection was found.

From 252 sera of symptomatic patients, all positive to Leishmania in cytologic samples, only 64,7% was found positive, while 35,3 % was negative, to Kalazar Detect Test.

Conclusions: Cytological diagnosis was proved to be accurate and proved to be a very useful tool in clinical practice, also providing additional clinical data. Further research is needed to support the use of the Kalazar Detect Test as a unique diagnosis tool for this emergent disease in Argentina.

Canine and Feline Infections by Cardiopulmonary Nematodes in Central and Southern Italy

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Background: Canine and feline cardiopulmonary nematodes are emerging in several countries, thus reviving the attention of the Scientific Community. *Dirofilaria immitis* and *Capillaria aerophila* affecting dogs and cats, *Aelurostrongylus abstrusus* and *Angiostrongylus vasorum* affecting cats and dogs, respectively, have major pathogenicity. With the aim to enhance knowledge on distribution and spread of these nematodes, this study investigated their occurrence in selected regions of Central and southern Italy.

Methods: From January 2009 to December 2010, a total of 970 individual faecal samples were collected from dogs (n = 534) and cats (n = 436) living in central (Marche and Abruzzo regions – Sites A and B respectively) and southern (Apulia – Site C) Italy. Overall 471 and 34 faecal environmental samples were also taken from kennels and catteries, respectively, from the three sites. Individual blood samples (n = 192) were collected from dogs from Sites B and C always in early morning. Faeces were examined using flotations and Baermann techniques, whereas blood was tested by Knott's method.

Results: *Capillaria aerophila*: 1.48 % and 20 % (Site A), 8.67 % and 2.71 % (Site B) and 16.67 % and 0 % (Site C) individual and environmental, respectively, canine samples were positive. With regard to cats, individual samples scored positive with percentages of 2.90 % (Site A), 3.03 % (Site B) and 14.29 % (Site C), whereas those collected from the environment were negative in Sites B and C. *Angiostrongylus vasorum*: larvae were found in 0.96 % and 2.48 % of individual and environmental samples from Site B, respectively. *Aelurostrongylus abstrusus*: 1.82 % (Site A) and 9.96 % (Site B) individual samples were positive. *Dirofilaria immitis*: 2.56 % of samples collected from Site B scored positive upon Knott's method.

Conclusions: Though the small sample size, these results indicate that cardiopulmonary nematodes are present in Central and southern Italy. Thus it is advisable to routinely include these parasites in the differential diagnosis of pet cardiorespiratory diseases. Larger surveys should be carried out to gain more information on the diffusion of the above pathogens, especially for those species, e.g., *C. aerophila* and *A. vasorum*, for which the actual distribution is poorly known.

Helminths infections in kennel dogs from southern Italy

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Background: Dogs are associated with many zoonotic infections among which helminthosis can pose serious public-health concerns worldwide. Kennel dogs can be considered as sentinels and reservoirs of zoonotic parasites and therefore the guidelines from the European Scientific Counsel Companion Animal Parasite recommend that careful consideration should be given to helminth control programmes for dogs in kennels and that faecal monitoring should be conducted regularly to identify helminth species present and the effectiveness of control programmes (<http://www.esccap.org/>).

The aim of the present cross-sectional copromicroscopic survey was to evaluate the extent of helminth infections (with particular regard to the zoonotic agents) in kennel dogs from Campania region of southern Italy.

Methods: The survey was conducted in 47 kennels distributed on the whole territory of the Campania region. A minimum of 20 boxes were examined in each kennel; fresh faecal samples were collected from the ground of each box (composite sample) and preserved in formalin 5%. Each faecal sample was then examined using the FLOTAC dual technique, using two flotation solutions on the same faecal composite, namely a sodium chloride based solution (specific gravity = 1.200), and a zinc sulphate based solution (s.g. = 1.350).

Results: Helminth infections were found in the 100% of the 47 studied kennels. Intestinal and pulmonary species were found as follows (no. of positive kennels; prevalence): *Trichuris vulpis* (46; 97.8%), *Ancylostoma caninum* (34; 72.3%), *Toxocara canis* (33; 70.2%), *Dipylidium caninum* (31; 65.9%), *Toxascaris leonina* (12; 25.5%), *Crenosoma vulpis* (9; 19.1%), *Oslerus osleri* (2; 4.2%) and *Angiostrongylus vasorum* (1; 2.1%).

Summary/Conclusion: The findings of the present survey show a high prevalence of helminths (including many zoonotic agents) in kennel dogs from southern Italy despite the regular use of anthelmintic treatments. This situation has important consequences on different issues concerning animal welfare, treatment and control, and public health. Because of failures in individual (use of anthelmintics) and collective (reduction of environmental contamination) preventive measures currently in place for kennel dogs, regular parasitological surveillance, appropriate treatment strategies and high quality *standard of hygiene* are strongly needed to guarantee the health and welfare of pets, and to enhance the safety of the public.

Phylogenesis of *Capillaria aerophila* (Trichocephalida, Trichuridae) from Different Hosts and Countries

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Background: The globally distributed trichuroid parasitic nematode *Capillaria aerophila* affects the respiratory tract of different carnivore species and humans. Because the infection causes respiratory conditions of varying severity several aspects on the biology of this parasite need to be elucidated. In addition, given the absence of molecular information, the aim of this work was to characterise a genetic region of *C. aerophila* isolates from different hosts and countries towards further diagnostic and phylogenetic studies.

Methods: Single adult nematodes from 9 red foxes (4 from Portugal, 3 from Romania and 1 from Serbia and UK, respectively), from 3 beech marten (from Portugal), and 5 egg batches from 3 dogs and 2 cats (from Italy) were subjected to a PCR-coupled sequencing protocol to amplify a ~ 344 bp informative region within the mitochondrial *cox1* gene. The 17 *C. aerophila* sequences were undertaken to a phylogenetic analysis which was conducted by MEGA4 using the neighbour-joining tree-building method. Sequences were compared with *cox1* sequences available in GenBankTM for the Nematoda belonging to the Capillarinae (n = 7) subfamily. *Thelazia callipaeda* was used as the outgroup.

Results: No substantial differences were found in the *C. aerophila* sequences from single adult nematodes collected from wild animals and egg batches from domestic species. Furthermore, a very low rate of variation was found in the sequences obtained from different countries. These results were confirmed by phylogenetic analysis in that all sequences of *C. aerophila* were grouped into a single clade clustering with ones including those of *Capillaria* spp. available in GenBankTM.

Conclusions: These preliminary results represent a foundation for further approaches to the study of lung capillariosis, e.g., validation of molecular tools instrumental to bio-epidemiological and diagnostic studies, and open avenues to address the phylogeography and population-genetic make-up of this neglected lungworm and to investigate its taxonomic position within the phylum Nematoda.

Molecular characterization of *Blastocystis* isolated from humans and various animal hosts in Flanders (Belgium)

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Background: *Blastocystis* is found in stool of humans and a wide variety of animal hosts, including insects, birds, reptiles, amphibians and mammals. Recent molecular analyses revealed that *Blastocystis* exhibits a remarkable genetic diversity, resulting in the description of 13 different subtypes (ST). ST1 to ST9 have been identified both in humans and in animal hosts, whereas ST10 to ST13 are exclusively found in animals. Yet, there are little data supporting the host specificity of these STs. Therefore, the objectives of the present study were (1) to assess the occurrence of different *Blastocystis* subtypes in a wide range of hosts in Flanders (Belgium) and (2) to evaluate the role of animals as potential zoonotic reservoirs for this parasite.

Methods: A total of 199 faecal samples from humans, horses, cattle, sheep, goats, lamas, dogs and cats were screened for the presence of *Blastocystis* spp. using a formol-ether extraction or sedimentation flotation technique. Samples containing cysts of *Blastocystis* were further examined using a nested PCR protocol targeting the SSU rDNA. Subsequently, *Blastocystis* isolates were cloned and sequenced to identify the different subtypes.

Results: A high percentage of samples were positive for *Blastocystis* (76%). Yet, there was a large variation in occurrence across the different host species examined, ranging from 54.5% in pigs to 93.6% in horses. The ongoing molecular characterization revealed the presence of both zoonotic (e.g. ST1 and ST5) and animal specific subtypes (e.g. ST10).

Summary/Conclusions: This is the first study that assessed the presence of *Blastocystis* in Belgium. The results suggest that *Blastocystis* is highly prevalent in different host species. Moreover, the preliminary results of the molecular analysis confirm that animals are a potential reservoir for zoonotic *Blastocystis* STs. Finally, the present study provides novel insights in the epidemiology of *Blastocystis* in animal hosts for which molecular data are scarce.

Retrospective study of 302 cases of canine dirofilariosis treated in Virreyes Veterinary Hospital province of Buenos Aires, Argentina

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The authors report a retrospective study of 302 dogs naturally infected with *Dirofilaria immitis* treated in Virreyes Veterinary Hospital from 1997 to 2011. The diagnosis was made using Knott test and Antigen test (Witness, Snap test or Speed diro). A complete blood count, creatinine, ALT, urinalysis, coproparasitological exam, thoracic radiography, electrocardiography and abdominal and cardiac ultrasound were performed to all of them(these findings are described). All dogs were classified using the classification system used in clinical trials: Class I, (30%of the dogs), Class II(60%) , Class III (10%), and IV or caval syndrome, only one dog that died without treatment .The medical treatment sequence recommended by the American Heartworm Society was followed. Complications of heartworm disease or other diseases were managed before adulticide therapy was begun. During all these years the adulticide treatment has been made with melarsomine dihydrochloride, 2,5mg/kg deep in the epiaxial muscles. All dogs (but one who was phobic) , were kept at the hospital for a fortnight . In order to avoid adverse reactions from 2002 on, we have treated all dogs but Class I over 35 kg, with the alternate dose regimen (one injection and two other injections a month after) Only one treatment failed and was repeated six months after. As our first 10 patients, 10 minutes after melarsomine injection had pain that lasted for 4 to 12 hours , anorexia and lethargy, we developed an analgesic protocol from one hour previous to the injection, diminishing the pain, and avoiding anorexia. 40% developed myositis in the site of injection. All the dogs were fed cardiac diets (HD (hills) or Early cardiac (Royal Canin) Supportive therapy included aspirin , sucralfate, glucocorticosteroids, diuretics, angiotensin-converting enzyme inhibitors, cough suppressants, anthelmintic and fleas drugs, antibiotics, iron, vitamins, etc. During the last two years our patients have also received doxycycline orally 10 mg /kg once a day , two weeks before the first injection and during the following 15 days, (due to *Wolbachia*) (with caution to avoid oesophagitis) . All of these dogs had no fever, less cough and lung lesions during treatment. Microfilaricide treatment is also reviewed.

Update on the Distribution of *Dirofilaria immitis* in dogs from the State of Rio de Janeiro, Brazil

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The national prevalence in Brazil has declined from 7.9% in 1988 to 2% in 2001. Rio de Janeiro state coastal sub-urban lowlands are the most extensively surveyed areas and at those sites prevalence has decreased from 43.4% (1988) to 0.6% (2003). After 2001 most small animal practitioners reported they were finding no heartworm infected dogs throughout the state until 2005 when a young adult dog was found infected in an urbanized area of the city of Rio de Janeiro. The dog was found to spend weekends in remote areas where heartworm infections were frequent in 1980 decade. To update information on the occurrence of heartworm infections and to pose an alarm to veterinarians in the case of a rising trend in occurrence, dogs from 2 coastal sub-urban lowlands where prevalence was high in 1988 were examined

Half the canine population (28) from the remote area where the dog was supposedly infected (area A) and 168 dogs from the suburbs of a large city (area B) were examined in 2005. In 2009 a new survey was conducted in area B (147 dogs). A blood sample was drawn from dogs, after their owners consent, and examined by Knott's modified test for microfilariae.

In 2005 microfilariae were detected in 9/28 area A dogs (32.1%) and in 0/168 area B dogs. In 2009, 18/147 (12.2%) dogs from area B (2009) harbored microfilariae.

The downward trend in canine heartworm prevalence observed from 1988 to 2003 was shown to have turned into an upward trend after 2005. No difference in the use of macrocyclic lactones or tetracyclines, once thought to push heartworm prevalence down, has been reported in Brazil suggesting that transmission pressure has built up in the remote area (area A) and from there the parasite spread back to areas where environment is favorable for transmission, although being close to an urbanized center (area B). These results suggest that the parasite has become less sensitive to the chemoprophylactics in use or that non-compliance is yet to be reported in Brazil.

Prevalence of antibodies anti-*Leishmania (L.) infantum chagasi* in dogs from the Tapirapé indigenous community in Brazilian Amazon region: Preliminary results

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Background: Dogs have an important role on epidemiology *L. (L.) infantum chagasi*, acting as domestic reservoir of the parasite, where even the asymptomatic dogs can transmit the infection to human, throughout the arthropod vector. Recent surveys, conducted in Brazilian Amazon, showed a high occurrence of visceral leishmaniosis in humans and 23% occurrence in dogs, especially among the animal living in urban area. There is no data regarding the prevalence of this specie of *Leishmania* among dogs from indigenous population from Amazon region.

Methods: A cross-sectional study was conducted to determine the prevalence of anti-*Leishmania (L.) infantum chagasi* antibodies in dogs from 7 villages from the Tapirapé indigenous community, located in the state of Mato Grosso, Brazilian Amazon. For this purpose, during September 2010, 110 blood samples were collected from all dogs, of different ages and gender, residents in the villages. The sera obtained were stored at -20°C until analysis. The detection of anti-*Leishmania spp.* was performed by indirect ELISA with total antigen, using promastigotes of *Leishmania (L.) infantum chagasi* strain MHOM/BR/72/cepa46 from chronically infected hamster spleen. The effects of gender, age and hunting activities were analyzed using Fisher's exact test and $P \leq 0.05$ were significant.

Results: Of the 110 samples analyzed, only three were positive, with an overall prevalence of 3.3%. No association with the risk-factor evaluated was obtained. From the three positive dogs, two were males and one female, two were adults and one juvenile (<1-yrs-old) and two dogs were used for hunting.

Conclusions: These results could indicate a low prevalence of the disease in the region. Hemoculture and isolation of the agent from the positive animals will provide a better understanding of the epidemiology of this disease in the area and the importance of the dog to human transmission.

Feline intestinal parasites in Finland: focus on prevalences, risk factors and anthelmintic treatment

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Background: The main aim of the study was to estimate the prevalences of the intestinal parasites of cats in Finland, and to identify possible risk factors for the infections.

Methods: Altogether 411 faecal samples of household cats representatively from Finland were inspected for cestode proglottids, and analyzed with a flotation method to detect worm eggs and protozoan oocysts. In case of *Toxocara cati*, a resampling kit was sent to the owner along with pyranthel embonate oral paste (Mirrix vet., Pfizer Animal Health) to treat the infection. Samples taken at the day of the medication, and two weeks after it, were quantitatively analyzed in McMaster chamber, to evaluate the efficacy of the treatment. Of the original faecal samples, 403 were also screened for *Giardia* antigens with a commercial ELISA kit (ProSpecT *Giardia* 96 test). In addition, the cat owners completed a questionnaire with questions about the cat's habitat, feed, travelling history and anthelmintic treatments.

Results: *Toxocara cati* prevalence was 5.4% and *Toxascaris leonina* 0.2%. *Taenia* eggs were found in 1.5% of the samples and isospora (*Cystoisospora* spp.) in 0.7%. We defined 3.2% of the samples as clearly *Giardia* spp. positive. Risk factors for *Toxocara* infection were being a non-pedigree cat, having access outdoors, and living outside of the cities. *Giardia* risk factor identified, was being a pedigree cat. No signs of pyranthel embonate resistance were noted in *Toxocara cati* (n=9, only the samples taken according to instructions were included).

Conclusions: The prevalence of cat GI-parasites was in low to moderate level in Finland. Risk factors for *Toxocara cati* and *Giardia* infections were predictable. However, the expected age related risk was not seen in this material.

Control of *Giardia duodenalis* in a dog kennel

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Diarrhoea in dogs and cats is often attributed to infections with *Giardia duodenalis*. The control of this potentially zoonotic parasite is frequently a frustrating issue for animal owners and veterinarians. Drugs with antiprotozoal activity such as fenbendazole and metronidazole are recommended, however, they do not show 100% efficacy and reinfections regularly occur. Ronidazole is currently the drug of choice for the treatment of *Tritrichomonas foetus* in cats and there is limited information available about its efficacy against *Giardia* spp. Animals of the dog kennel of the veterinary faculty of Zurich regularly showed loose faeces and the presence of *Giardia duodenalis*, genotype C. An elimination strategy comprising strict hygiene management and disinfection of the enclosures with 4-chlorine-M-cresol, oral treatment with ronidazole (30-50 mg/kg BW bid for 7 days) and two dog shampoos (containing chlorhexidine) at the beginning and the end of the treatments was implemented for a group of 6 dogs. Control dogs (n=7) were also transferred into clean enclosures and shampooed, but left untreated. Dog faeces were tested for the presence of *Giardia* cysts (SAF concentration technique) or *Giardia* antigen with a commercial ELISA (NOVITEC®) and a quick immunochromatography-based test (SensPERT®) before and 5, 8, 12, 19, 26, 33 and 40 days after the last treatment day. All treated dogs were negative for both *Giardia* cysts and antigen up to 26 days after the last treatment, while 1-5 samples per testing day of the control animals were positive. However, all dogs of the treatment group turned positive in at least one of the three applied diagnostic techniques within 33-61 days after treatment. Furthermore, all treated dogs had episodes of diarrhoea (for 1-4 days) within 14-31 days after treatment and unfurled faeces during the whole experiment. No further side effects were observed. Ronidazole confirmed in this study its good in vivo antiprotozoal effect provoking few side effects in the dogs. In particular, the combination of ronidazole treatment combined with the disinfection of the environment and shampooing of the dogs had an impact on *Giardia* cyst elimination and may represent a good control strategy of this dog parasitosis of top priority.

Zoonotic hookworm transmission in a highly endemic region of Southeast Asia

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Background: Southeast Asia accounts for one quarter of the global burden of hookworm disease. In an era of mass drug administration (MDA) for the control of human hookworm disease, the emergence of zoonotic hookworm presents as a problem for public health officials. *Ancylostoma ceylanicum* is prevalent in Southeast Asia and has been implicated in human hookworm disease. We present results of a survey investigating hookworm infections in people and dogs from communities living in northern Lao People's Democratic Republic (PDR).

Methods: We conducted a cross-sectional survey of 1,358 people and 105 dogs in four provinces in northern Laos. Samples were stored in two separate tubes containing preservation media, 10% formalin and 80% ethanol. Human samples were tested by formalin-ether concentration technique (FECT) and dog samples by saturated sodium nitrate floatation. A subset of 23 dog and 46 human samples with hookworm eggs were selected and tested by PCR and DNA sequencing to identify the hookworm species present.

Results: Hookworm eggs were detected in 46% and 85% of the human and dog populations, respectively. PCR amplification and sequencing was successful for 17 (37.0%) human samples. All 17 were identified as a single infection with either *Necator americanus* (14/17; 82.4%) or *A. ceylanicum* (3/17; 17.6%). The PCR-sequencing protocol was able to successfully amplify and characterise 18 (78.3%) samples from dogs. Single DNA amplifications of *A. ceylanicum*, *A. caninum*, *A. braziliense* and *N. americanus* were detected in 7 (38.9%), 2 (11.1%), 1 (5.6%) and 1 (5.6%) dogs, respectively. Dual DNA amplifications of *A. ceylanicum* and *A. caninum* were detected in 4 (22.2%) dogs and dual DNA amplifications of *A. ceylanicum* and *N. americanus* were detected in 3 (16.7%) dogs. *A. ceylanicum* was the most prevalent hookworm species infecting village dogs.

Conclusions: In regions of Southeast Asia where *A. ceylanicum* is highly endemic in the dog population, human *A. ceylanicum* infections are also present. Further work is needed to characterise the clinical significance of these infections. Furthermore, our evidence supports the notion that dogs are a potential transport host for *N. americanus* in areas where this hookworm species is highly endemic in the human population.

Survey of Endo- and Ectoparasites of Cats from Tirana, Albania

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Background: Recent studies showed a high prevalence of both external and internal parasites in dogs from suburban areas of Tirana, Albania with some of the parasites representing potential risks for both animal and public health. The aim of the study was to investigate the current prevalence of endo- and ectoparasites in cats and thus to provide information on the epidemiological significance of the parasites identified.

Methods: During 2008 to 2011, fecal samples, ear swabs, and ectoparasites obtained by body searches and total body combs were collected from 192 cats (82 males, 110 females; 75 $\geq$ 9 months, 117 > 9 months) from the greater Tirana area. Fecal samples were macroscopically and/or microscopically (modified McMaster and Baermann techniques) examined for helminth and protozoan infections; ear swabs were microscopically examined for the presence of ear mites.

Results: Overall, 91.7% of cats harboured one or more parasite species; 87.0% and 55.2% of the cats were tested positive for endo- or ectoparasites, respectively; concomitant presence of endo- and ectoparasites was found in 44.3% of the cats. The most prevalent endoparasite was *Toxocara cati* (56.3%), followed by *Aelurostrongylus abstrusus* (37.0%), dilepidid cestodes (34.9%), *Capillaria* spp. (29.2%), hookworm (26.6%), *Cystoisospora* spp. (26.0%), and taeniid cestodes (2.1%). Prevalences for ectoparasites were 49.0% for fleas (*Ctenocephalides felis* 49.0%, *C. canis* 2.1%), 8.3% for *Otodectes cynotis*, 7.8% for *Felicola subrostratus*, and 2.1% for *Rhipicephalus sanguineus*.

There were no differences between sexes for the prevalence of any parasites. Prevalence of infestation with ectoparasites did not differ between cats of different ages; however, cats $\geq$ 9 months of age harboured *Toxocara* and *Cystoisospora* infections more frequently ($p < 0.05$) than older cats, while *A. abstrusus* infection was detected in significantly ($p < 0.05$) more older cats than in younger cats.

Mixed infections with up to six endoparasites or three ectoparasites were recorded in 74.3% and 22.6% of the positive cats, respectively.

Conclusions: This study indicated a high prevalence of parasites in cats from Tirana. Given the impact that some of those parasites may have upon animal health and their zoonotic potential, measures should be taken to minimize the transmission of these parasites including raising awareness, effective treatment, and improvement of animal care.

A canine *Cercopithifilaria* species (Spirurida, Onchocercidae) with dermal microfilariae, Italy

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Background: Differently from the most widespread filarioids of dogs presenting blood microfilariae (e.g., *Dirofilaria immitis*, *Dirofilaria repens*, *Acanthocheilonema reconditum*), those with skin microfilariae belonging to the *Cercopithifilaria* genus have been little studied. In order to investigate the occurrence of *Cercopithifilaria grassii*, originally described from Italy, dermal microfilariae were detected in a dog in Sicily, which also presented *A. reconditum* blood circulating larvae.

Methods: The dermal microfilariae were collected from six skin sites by biopsy. Retrieved larvae were characterized morphologically and differentiated from those of all the other blood microfilariae commonly found in dogs. The genetic identity of microfilariae herein studied was also assessed by molecular amplification, sequencing and analysis of multiple ribosomal (Internal Transcribed Spacer region 2-ITS2) and mitochondrial (*cytochrome c oxidase subunit* and 12S) target genes. The phylogenetic history was inferred using sequences of a bar-coding dataset.

Results: Dermal microfilariae were distinct from those of other common filarioids of dogs being short (mean length of 186.7 µm), presenting a body flattened dorso-ventrally and rounded head, with a tiny cephalic hook. Genetic characterizations as well as the molecular phylogenetic history were concordant in supporting the identification at genus level as *Cercopithifilaria*.

Summary/Conclusions: Microfilariae here examined were distinct from *C. grassii* and resembled those of *Cercopithifilaria bairdii*, a little known filarioid described from Brazil. The research here presented suggests the existence of a *Cercopithifilaria* sp. infesting a dog from Sicily (Italy). Further studies are needed in order to better evaluate the taxonomical position and natural history of this little known filarial species of dogs.

Vector-borne infections in dogs from Hungary and Romania

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Background: Canine vector-infections, e. g. babesiosis, hepatozoonosis, *Dirofilaria immitis* heartworm disease and ehrlichiosis are of increasing importance in Europe due to import of dogs from the Mediterranean region, Portugal and South-East Europe. Additionally, pet owners take their dogs along on holidays to endemic countries in Europe. Nevertheless is data on the occurrence and distribution of CVBDs comparably limited for south-eastern Europe. Therefore we present data on CVBDs in 29 dogs presented for various reasons in a Veterinary Clinic in Bucharest, Romania, as well as from 187 imported dogs from Romania (109) and Hungary (78) imported to Germany submitted for routine diagnostic in our laboratory. All samples were processed in the years 2009-2010.

Methods: EDTA-blood samples were examined by Giemsa-stained blood/buffy coat smears, Knott-Test, DiroChek®-ELISA, PCR and/or indirect methods (IFAT) for the presence of vector-borne infections.

Results: Ten different pathogens were detected in a total of 216 examined dogs: *Babesia canis canis* (93 dogs), *B. c. vogeli* (5), *B. gibsoni* (4), *Leishmania* spp. (2), *Hepatozoon canis* (4), *Dirofilaria immitis* (3), *D. repens* (1), *Acanthocheilonema reconditum* (1), *Anaplasma phagocytophilum* (4), *Mycoplasma haemocanis* (16).

Six dogs from the Clinic were positive for *B. canis* ssp. in Giemsa-stained blood smears.

Serological examination by IFAT gave evidence for *Ehrlichia canis* infection in one dog, antibodies against *Leishmania* spp. were detected in 4 dogs from Romania.

B. c. canis was the most prevalent pathogen in all dogs (43.1%).

Conclusion: A broad range of arthropod-borne infections were detected in dogs from Romania and Hungary. While canine babesiosis due to large *Babesia* spp. is widely recognized in these countries, this study also identified *B. gibsoni* by PCR. Interestingly, four dogs from Romania were positive for *H. canis* by PCR, a pathogen previously not identified in Romania. Additionally six dogs were found positive for *Leishmania* spp. infection by PCR (2 dogs from Hungary) and/or IFAT (4 dogs from Romania). Dogs with borderline titres have recently been identified in Hungary as well as phlebotomine vectors, although considering dogs in our study it cannot be ruled out that they were previously imported to Romania or Hungary.

Future research should elucidate the possible (re-)emergence of *Leishmania* spp. in these countries.

Bacterial and protozoan pathogens of veterinary and medical importance in Romanian ticks

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Background: Ticks are important vectors of many different pathogens (bacteria, virus, protozoa etc), which can affect both animal and human health. From Romania, a growing number of Canine Babesiosis cases has been reported recently. However, little is known about the prevalence and distribution of *Babesia* spp. and of other bacterial tick-borne pathogens in the diverse tick fauna occurring in Romania.

Methods: Questing and feeding ticks were collected in 8 different geographic locations in Romania, either from the vegetation or from infested animals. All ticks were screened individually with conventional PCR methods for *Rickettsia* spp. and *Babesia* spp., followed by sequencing for species identification. With real-time PCR, *Ixodes ricinus* were additionally screened for *Anaplasma phagocytophilum* and *Borrelia* spp. and *Rhipicephalus sanguineus* for *Ehrlichia canis* and *A. platys*.

Results: A total of 382 ticks were collected. Of these, 35 were questing ticks and the remainder was collected from naturally infested cattle, sheep, goats, horses or dogs. Tick species were identified as *Hyalomma marginatum marginatum* (n=135), *Ixodes ricinus* (n=134), *Rhipicephalus sanguineus* (n=50), *Dermacentor marginatus* (n=48), and *R. bursa* (n=15).

Altogether 37 ticks (9 *D. marginatus* and 28 *I. ricinus*) and 32 ticks (1 *D. marginatus*, 11 *H. m. marginatum*, 20 *I. ricinus*) showed products of the expected length in the PCRs for *Rickettsia* spp. and *Babesia* spp., respectively. Preliminary sequencing data revealed sequences identical or closely related to *R. helvetica*, *R. monacensis*, *R. slovacica*, *R. raoultii*, *Theileria equi*, *T. sergenti/buffeli*, *T. orientalis*, *B. occulatans* and *B. microti*.

So far, seven *I. ricinus* were positive for *A. phagocytophilum*, and three for *Borrelia* spp.. One *R. sanguineus* was positive for *E. canis*, but none for *Babesia* spp. and *A. platys*.

Summary/Conclusions: The results of the present study show a wide distribution of very diverse bacterial and protozoan pathogens in the Romanian tick fauna, with the potential of causing human and veterinary diseases and point towards the need for further in-depth study.

Natural infections of *Aelurostrongylus abstrusus* in cats in Germany

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Background: Infections with the metastrongyloid nematode *Aelurostrongylus abstrusus* in cats have been sporadically reported from Germany and other European countries. The adult lungworm resides in the distal aspects of the bronchial tree, bronchioles and alveolar ducts and is known to cause severe verminous granulomatous pneumonia in cats. Recent studies from Denmark, Germany and Italy have shown that prevalence rate for *A. abstrusus* might have increased in the last years. The aim of the study presented was to assess the prevalence and regional geographical distribution of natural infections with *A. abstrusus* in cats in Germany.

Methods: Between September 2009 and March 2011 veterinary surgeons in Germany were asked to look for cats tentative for a lungworm infection with symptoms of respiratory diseases, i.e. coughing, dyspnoea, increased breathing rate, bronchopneumonia, as well as weight loss, sneezing, nasal discharge or with general poor condition. A total of 325 fecal samples were collected and examined by zinc chloride / sodium chlorid flotation and Baermann funnel technique. The collected data were analyzed by a geographic information system (GIS).

Results: Infections with lungworms were diagnosed in 22 of 325 examined cats (6.8%). 10 *A. abstrusus* positive cats were located in Baden-Wuerttemberg, 4 in Lower Saxony, 2 in Bremen and North Rhine-Westphalia each, and 1 in Bavaria, Mecklenburg-Western Pomerania, Saxony and Schleswig-Holstein at a time. Larvae of *A. abstrusus* were found in fecal specimen examined in September (1), October (1), November (1), December (3), January (8), February (5) and March (3). Most of the lungworm infected cats showed coughing (11) and dyspnoea (11), followed by increased breathing rate (7), weight loss (6), bronchopneumonia (4), general poor condition (4), sneezing (3) and nasal discharge (2).

Conclusions: The collected data indicate that *A. abstrusus* is endemic in cats in Germany. *A. abstrusus* is located in terms of extensive clusters mainly in southern and northern parts of the country. A seasonal distribution of lungworm positive cats with most cases during the winter seems to be most likely. The majority infected cats showed severe clinical symptoms characterized by dyspnoea and coughing. Due to the high number of lungworm-positive cats veterinarians should consider infections with *A. abstrusus* as a differential diagnosis in cats with symptoms of respiratory diseases in Germany.

First Report of *Trypanosoma cruzi* Infection in Naturally Infected Dogs from Southern Bahia, Brazil

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Chagas disease is a zoonosis caused by a hemoflagelated parasite that belongs to the genus *Trypanosoma* (Order *Trypanosomatida*; Family *Trypanosomatidae*) and it is transmitted by blood-feeding triatomine-bugs, through blood transfusion, vertical transmission, by the contaminated feces from infected triatomine-bugs or oral ingestion. This disease is reported from the borderline of the US to the southern region of the Latin America and affects around 20 million people worldwide. The severe acute infection, most of the time, is lethal and those cases generally are observed in the poorest areas in the American continent. In Brazil the disease is endemic in all states, mainly in areas surrounded by native rainforests. Dogs and wild animals represent important reservoirs to the parasite in the ambient and those can develop the same pathologies as humans do. This study aimed to verify the occurrence of *Trypanosoma cruzi* in dogs from Vila Operaria district, Buerarema municipality, southern Bahia, Brazil. The district is situated in an endemic region for Chagas diseases and has a strictly rural structure, surrounded by native rainforest and the main economic resources are from the cocoa farm production. A total of 272 dogs were included in the study, 158 (58,08%) from the urban area and 114 (41,92%) from the rural areas of the Vila Operaria district. The animals were initially clinically evaluated in order to identify any signs consistent with the disease. Subsequently, blood samples were obtained by venipuncture (5 ml) for molecular test. The primers utilized in the PCR reaction were 5'-AAATAATGTACGGGGGAGATGCATGA – 3' and 5' – GGGTTCGATTGGGGTTGGTGT – 3'. From the 272 dogs tested, only two animals (0.73%) were positive for parasite DNA. Those animals are from two distinct rural areas in the district. This is the first diagnosis of *Trypanosoma cruzi* infection in naturally infected dogs from Bahia State, Brazil.

Serological and Molecular Survey for Detection of *Leishmania Braziliensis* in Naturally Infected Dogs in Southern Bahia, Brazil

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Leishmaniasis is a group of diseases caused by a digenetic protozoan that belongs to the genus *Leishmania* (Order Kinetoplastida, Family Trypanosomatidae). American cutaneous leishmaniasis (ACL) is a zoonotic disease and in the American continent, has already been reported from the southern U.S. to northern Argentina. In Brazil, there are reports of this disease in all states. Among the dermatropic species of *Leishmania*, the most important in Brazil is *Leishmania braziliensis*. The ACL is traditionally observed in the wild environment, however, cases of the disease has been frequently reported in domestic animals, mainly dogs. This study aimed to verify the occurrence of leishmaniasis in dogs in the district of Vila Operária, Buerarema county, southern Bahia, Brazil. The district has a strictly rural structure and is inserted near the bordering areas of dense forests of the Atlantic rainforest, living basically on the cocoa agriculture. It is considered an endemic area for human leishmaniasis, and in 2009, 43 new cases were diagnosed in a small resident population, according to data from the Municipal Health Secretary. Dogs included in this study were initially clinically evaluated in order to identify gross lesions consistent with the disease. Subsequently, blood samples were obtained by venipuncture (5 ml) for serological and molecular techniques. Serological tests were performed with the kit IFI - Indirect immunofluorescence - canine visceral leishmaniasis, which according to the literature, cross-react with others species of *Leishmania*. The molecular tests were performed using primers with sequence 5' - GGGGTTGGTGTAATATAGTGG - 3' and 5' - CTAATTGTGCACGGGGAGG - 3'. From the total 272 animals tested, 136 (50.0%) had positive results for IFI test, being 80 (58.83%) animals living in the urban areas and 56 (41.17%) in rural areas (cutoff titer 1:40). However, by the method of PCR, only 10 animals (3.7%) were positive for *L. braziliensis*. Only four dogs positive in PCR and IFI tests. The results confirm a high prevalence in the region and the process of urbanization of the disease.

Keywords: *L. braziliensis*; IFI; PCR; *Canis familiares*.

Prevalence of gastrointestinal helminths in cats admitted at the Veterinary Policlinic of the University of Zulia, Venezuela

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Background: Gastrointestinal parasitism is one of the main causes of morbidity in domestic dogs and cats. Some of these helminths represent important public health problems. In Venezuela, little information on the prevalence of gastrointestinal helminths in cats is available. The objective of this study is to determine the prevalence of gastrointestinal helminths in cats admitted at the Veterinary Policlinic of the University of Zulia, Venezuela.

Methods: Fecal samples were collected directly from the rectum of 64 cats that were admitted at the Veterinary Policlinic of the University of Zulia during one year. These fecal samples were processed by the method of fecal flotation with saturated saline solution. For the purpose of this study, cats were divided into four age groups (1: cats younger than one year old, 2: between one and two years, 3: between two and four years, and 4: older than four years old).

Results: The general prevalence of gastrointestinal helminths in cats was 46.9%. The following helminths were observed: *Ancylostoma* spp. (29.7%), *Trichuris* spp. (10.9%), *Toxocara* spp. (7.8%) and *Physaloptera* spp. (6.3%). Significant statistical differences of the prevalence values were not observed among sex (42.9% in females cats and 50% in males cats) neither among racial groups (48.1% in mixed-breed cats and 41.7% in pure-breed cats). General prevalence in cats by age was: cats younger than 1 year old, 52.6%; between 1 and 2 years, 50%; between 2 and 4 years, 35.7%; and older than 4 years old, 47.1%. Statistical differences were not observed between ages. *Toxocara* spp. was only found in animals younger than 1 year old and *Trichuris* spp. was detected in animals older than 2 years.

Conclusions: The prevalence of gastrointestinal helminths in cats admitted at the Veterinary Policlinic of the University of Zulia was high. There were not differences in the prevalence by age, sex and breed. However, *Toxocara* spp. was only found in young animals and *Trichuris* spp. was detected in old animals.

Efficacy of emodepside plus toltrazuril suspension (Procox® oral suspension) against prepatent and patent *Isospora* spp. infection in dogs

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Background: Emodepside plus toltrazuril suspension was developed to provide a new treatment option for dogs where a nematode and *Isospora* spp. infection is suspected or diagnosed. Especially young dogs in groups or in changing environments can be infected with both parasite types. Three controlled, blinded GCP laboratory studies were conducted to evaluate the efficacy of the new product against *Isospora canis* and *Isospora ohioensis*-complex.

Methods: 75 unweaned pups were experimentally infected with approximately 60.000 sporulated oocysts of *I. canis*, 80.000 sporulated oocysts of *I. ohioensis*-complex or a combination of 20.000 *I. canis* plus 11.000 *I. ohioensis*-complex. In each study one group was treated during prepatency (2-4 days post infection) while dogs in a second group were treated individually when they had become patent for the respective coccidia species (*I. canis*: 11-13 days post infection, *I. ohioensis*-complex: 6-13 days post infection). The dogs were treated with the minimum therapeutic dose of 0.45 mg emodepside and 9 mg toltrazuril per kg bodyweight. Daily faecal oocyst counts from both groups were compared to placebo-treated control groups to determine efficacy.

Results: Dogs treated during prepatent *I. canis* or *I. ohioensis*-complex infection showed significantly lower oocyst counts for up to 12 days compared to the control group. Oocyst counts were reduced by 90.2% to 100% while the control group continued to exhibit an adequate infection except for one study where efficacy against prepatent *I. canis* infection faded after 12 days post treatment. Following treatment of patent *I. canis* or *I. ohioensis*-complex infections, significantly lowered oocyst counts were observed for up to 9 days compared to the control group. Faecal oocyst counts were reduced by 91.5% to 100%. In all three studies the number of days with diarrhoea was significantly lower when dogs were treated during prepatent *Isospora* spp. infection compared to the control groups. No adverse drug reactions were observed during the studies.

Summary/Conclusions: Emodepside plus toltrazuril suspension provides an effective treatment against prepatent and patent *I. canis* and *I. ohioensis*-complex infections in dogs.

Efficacy of emodepside 0,1 % / toltrazuril 2 % suspension (Procox®) against mixed experimental *Isospora felis*/*Isospora rivolta* infection in cats

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Background: The coccidia *Isospora felis* and *Isospora rivolta* are intestinal parasites occurring world-wide in domestic cats. In young cats, they can be detected with higher prevalence. There is no licenced product available for this indication, therefore the effects of toltrazuril in the new combination product Procox® oral suspension for dogs containing 0.1% emodepside and 2% toltrazuril, were studied

Methods: Eighteen kittens were experimentally infected each with a total of 1×10^5 oocysts of a mixture of *Isospora felis* and *Isospora rivolta*. In the infectious material the quantitative relation of *I. felis* and *I. rivolta* was about 1 : 5. Following a three days period after infection, two groups of 6 kittens were treated during the prepatent period with either a single dose of 9mg toltrazuril/kg body weight or 18mg toltrazuril/kg body weight. A group of six kittens without any treatment served as a control.

Results; On day 5 post infection the untreated kittens started the excretion of oocysts. Treatment with both toltrazuril doses significantly reduced oocyst excretion. Following a single dose with 18mg toltrazuril/kg body weight, the reduction of oocysts of both *Isospora* spp. was more pronounced in comparison to the lower dose with 9mg toltrazuril/kg body weight. The Procox® application was well tolerated and no adverse events were seen with any of the applied dosages.

Conclusion: When administered to kittens and as a single treatment during the prepatent period, Procox® is suitable to control the number of oocysts excreted in the faeces in case of an *Isospora felis* and *Isospora rivolta* infection.

Stress response in *Giardia* cysts

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Background: *Giardia*, one common cause of waterborne diarrheal disease, has two stages in its life cycle to meet changes in the surrounding environment; trophozoites and cysts. The cyst is the infectious, dormant life form and spread of the parasite into the environment occurs through feces of humans and animals. We have studied how the parasite copes with challenges that the cyst naturally encounter outside its host, either in the environment or during passage in water treatment plants. In particular, UV light treatment of the cysts and trophozoites has been investigated.

Methods: *Giardia* trophozoites were cultivated and encysted *in vitro*. Stress response and viability was studied during early encystation, during irradiation with UV light and chemical treatment. The different stressors were used on both trophozoites and on cysts. Reduction of cyst viability due to treatment was quantified by either excystation of cysts, or labeling with propidium iodine. Recovery from UV treatment was also measured using flow cytometry. UV treatment was done in a UV-crosslinker and irradiated at 10, 20, 50 och 100 J/m² followed by viability measurements. Both trophozoites and cysts irradiated at 20J/m² were collected for RNA sequencing, phosphorylated histone H2A antibody staining and excystation efficacy of cysts. In addition, DNA replication during encystations was studied in detail and measured by flow cytometry.

Results: UV treatment up to doses of 100J/m² does not kill *Giardia* trophozoites, whereas a dose of 50J/m² inhibit excystation of cysts. Antibodies against phosphorylated histone H2A stain all UV treated cells, both cysts and trophozoites. In addition, a majority of the cysts in the control stain at late encystations (16-20 hours), at the same time as DNA replication occur.

Conclusions: Surprisingly, trophozoites are less sensitive to the treatments. UV light is effective to kill *Giardia* cysts at higher doses. An active DNA replication and/or repair system could be an explanation for the lower sensitivity of trophozoites. This knowledge will lead to greater understanding of how the surrounding environment affects the cyst. Ultimately, this knowledge could also be used to improve water hygiene and to limit the spread of waterborne parasites.

Evaluation of zoonotic parasites in dogs in a rural area of Portugal

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The dog as host of zoonotic parasitic diseases is a source of public health concern and has a relevant socioeconomic impact, mainly in rural areas, whenever ruminants and dogs cohabit.

In previous studies with slaughtered pigs, *E. granulosus* was found in the rural area of Cantanhede (Genotype G7). Therefore, the objective of this study was to determine the regional prevalence of *E. granulosus* and other gastrointestinal parasites in dogs of this region, owned by small ruminant farmers.

The dogs owned by the officially listed small ruminant farmers were the sampled population. Therefore, 301 dog faeces were sampled in 234 farms.

Coprological analysis showed that 58.8% (n=177) of the sampled dogs had parasitic elimination. From these, 78.5% (n=139) had zoonotic parasites - *Toxocara canis* and/or *Ancylostomidae*, with 13.6% for *Toxocara canis* infection. Other parasites were observed, such as *Trichuris vulpis* (50.8%), *Taeniidae* eggs (2.8%), *Capillaria* spp. (1.1%), *Spirometra* spp. (0.6%) and *Isospora* spp. (6.8%).

The overall prevalence for *Toxocara canis* was 8%, with the highest mean egg elimination (712.5 eggs/gram) and 1.7% for *Taeniidae* eggs. *Ancylostomidae* had an overall prevalence of 40.9% and a mean egg elimination of 255.9 eggs/gram. *Trichuris vulpis* had an overall prevalence of 29.9% and a mean egg elimination of 209.6 eggs/gram.

The *Taeniidae* eggs were separated by Percoll gradient and PCR analysis was accomplished for egg identification. None of them were proved to be *E. granulosus* eggs.

This data collection is essential information to local veterinarians for development of strategies for treatment and parasite control as well as for public health authorities, concerned with the monitoring of the zoonotic infections in dogs.

Cutaneous leishmaniasis in a guinea pig (*Cavia porcellus*) diagnosed in Passo Fundo, state of Rio Grande do Sul, Brazil

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Introduction: Leishmaniasis is a zoonotic disease caused by *Leishmania* spp., which parasites the host's macrophages and is transmitted by the bite of sandflies of the genus *Lutzomyia* spp. or *Phlebotomus* spp. This study reports a clinical case of a guinea pig infected by *Leishmania* spp.

Material and methods: A 4-year-old white male guinea pig (*Cavia porcellus*) weighing 950g was admitted to the Veterinary Hospital of Universidade de Passo Fundo (UPF) in February 2010. The clinical examination revealed crusty ulcerated skin nodules in the right external ear. The biopsy specimen was sent to the Laboratory of Veterinary Pathology of Universidade Federal de Santa Maria, RS. The case was notified to the State Health Department, given its potentially zoonotic nature. The lesions were surgically excised for histopathological analysis and for characterization of *Leishmania* by a sensitive polymerase chain reaction (PCR) assay at Instituto Oswaldo Cruz, Rio de Janeiro. Other surgical procedures were necessary for the removal of new nodules that developed after the first surgery. Blood smears were obtained by the Laboratory of Veterinary Pathology of UPF in order to identify amastigotes in the macrophages.

Results: The histopathological examination revealed infiltration of the skin by a group of macrophages whose cytoplasm was full of long (1 x 3 µm) structures compatible with *Leishmania enriettii* amastigotes. These structures were also found in the dermis and epidermis. The diagnosis was of nodular and diffuse granulomatous dermatitis associated with intrahistiocytic and extracellular protozoa. The surgical removal of lesions was not effective as relapses were frequent. The diagnosis of leishmaniasis is based on clinical findings and on the visualization of amastigotes, which was also confirmed by the blood smears. PCR was negative for *Leishmania* sp. and no DNA could be found in the analyzed samples.

Conclusion: The present study shows the importance of different tools in the definitive diagnosis of leishmaniasis. The species could not be identified, but earlier reports of cutaneous leishmaniasis in *C. porcellus* have been associated with *L. enriettii*.

Incidence of Compatible Injuries with Hidatidosis in Plants of Task in the Province of Corrientes

Program of control and prevention of the hidatidosis in the province of Corrientes

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Introduction: The sanitary monitoring in the task plants has assured the quality and innocuity meat, safeguard the health of the population with respect to the pathologies that represent a risk for the same. Currents, in accordance with the Federal Law of Meats of Argentina qualified the faenadoras plants in the province.

The veterinarians of the plants registered the seizures, among others those of compatible injuries with hidatidosis. From the data collected during last the 5 years, with respect to the compatible injuries with hidatidosis he was decided to work in a project that used this information to the benefit of the control and the eradication of the disease.

Materials: Lists of monthly registries of tasks and seizure, each plant of task.

Computerized program: Control system of Tasks and Seizures.

Methods: Relief and compilation data. Tabulation and group of data. Graphical representation. Measurement of data. Statistical inference. Prediction.

Results: The sanitary monitoring in the faenadoras plants, allowed to evaluate the tendency and the provincial of this pathology, throwing like results that the departments with greater incidence are Mercedes, Paso de los Libres y Empedrado. It verified the presence of the same in other departments.

Conclusions: The registry of the seizures and its causes, in it task plants were an important tool in the monitoring epidemiologist, it allowed the monitoring of the diseases presents/displays in animal and it was used like tool for the control and monitoring of the zoonoses. This study allowed to the design and the beginning of an Eradication and Control program of the Hidatidosis in the Province.

Epidemiology of gestational toxoplasmosis in five municipalities in Parana State, Brazil

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Background: The prevalence of toxoplasmosis varies greatly between countries due to climatic and cultural differences of the population, so it is important to know the epidemiological and economic order to develop effective strategies to reduce the risk of infection. The aim of this study was to compare epidemiological data of pregnant women attending Basic Health Units in the municipalities of Londrina, Rolândia, Cambé, Cascavel and Palotina, during implementation of "Health Surveillance Program of Toxoplasmosis Acquired during Pregnancy and Congenital".

Methods: Every pregnant woman answered a questionnaire for the detection of variables associated with infection by *Toxoplasma gondii*.

Results: We analyzed 2226 pregnant women and the prevalence of IgG antibodies anti-*T. gondii* was found to be 50.5% in Londrina, 54.4% in Rolândia, 46.4% in Cambé, 53.0% in Cascavel and 61.3% in Palotina. In Londrina, the variables associated with the infection were: living in rural areas, more than one pregnancy, education level and income per capita. Rolândia was an association with residence in rural area, age group, more than one pregnancy, education level and income per capita. In pregnant women of Cambé was association between education level and income per capita and the Cascavel with age group, more than one pregnancy and income per capita. In Palotina association was found between education level, more than one pregnancy, the presence of cat and eating colonial salami.

Conclusions: The implementation of the program in these counties to gather data in epidemiological, associated with the geographic, demographic and cultural, revealed different factors associated with infection by *T. gondii* in each, emphasizing the need for specific studies in populations. The study revealed that 48.3% of pregnant women are susceptible to infection with *T. gondii* and that the program can be implemented in any municipality in, regardless of their characteristics and health service installed on it until there is a commitment of managers and professionals involved.

Histopathology and parasite burden of golden hamsters experimentally infected by *Leishmania chagasi* and treated with resin-oil *Copaifera reticulata* and ethyl acetate fraction of *Ricinus communis*

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Phytotherapy has been used in several health areas, and one of the steps of the validation of natural products is *in vivo* tests. The aim of this study was to evaluate the efficacy *in vivo* of resin-oil of *Copaifera reticulata* (ROCr) and ethyl acetate fraction of *Ricinus communis* (EAFRc) against *Leishmania chagasi* in golden hamsters experimentally infected. Hamsters were intraperitoneally infected by 1×10^7 parasites/0.1ml promastigotes of *L. chagasi* and then were divided into seven groups of eight animals. These animals were treated for 30 days (D0 to D30). Oil and fraction were administered in two doses - 100mg/kg and 50mg/kg. Positive control used 60mg/kg Glucantime®; negative control received no treatment; and another negative control of hamsters was not infected. After 15 days of treatment (D15) and 30 days pos-treatment (D60), two animals or six animals per group, respectively, were euthanized. Samples of liver and spleen were collected for histopathology and parasite burden count. Histopathological results of every animal treated, regardless of time or drug utilized, showed that cellular immune response was stimulated. This fact was evidenced by white pulp hyperplasia in cuff region. In the liver the most obvious sign was inflammatory granulomatous formations. Foci of hemorrhage were most evident in the untreated group. All treatments reduced spleen parasite burden, the most effective being the ones that used 100mg/kg ROCR and EAFRc. Their efficacy was of 49.5% and 53.7% in D15 and 74.7% and 71.2% in D60, respectively. However, they differed from Glucantime® 91.7% in D15 and 97.4% in D60. *Leishmania* burden in the liver was similar for the two plants used in the dose of 100mg/kg, ROCR 80.2% in D15 and 72.7% in D60 and EAFRc 71.4% in D15 and 71.2% in D60. Nevertheless, results did not differ from Glucantime® 92.2% in D15 but were differ from positive control 96% in D60. These results suggest that natural products have stimulated immune response, which may have assisted in reducing parasite burden of spleen and liver. Yet they showed lower efficiency compared with reference drug, Glucantime®.

Validation of a rapid immunochromatographic test Dual Path Platform (DPP®) for serodiagnosis of Canine Visceral Leishmaniasis

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Visceral leishmaniasis is a major public health problem in the world and the dog is the main reservoir. In Brazil a new test to diagnose infected dogs is being tested. To evaluate the immunochromatographic test (DPP®) it was compared with Indirect immunofluorescence assay (IFI - Bio-Manguinhos®) and enzyme-linked immunosorbent assay (ELISA - Bio-Manguinhos®), which were used as gold standard. We used 103 dogs divided into three groups with different clinical signs suggestive of visceral leishmaniasis: asymptomatic (n = 35), oligosymptomatic (n = 31) and symptomatic (n = 37). Blood samples were collected from cephalic vein to obtain serum to achieve IFI and ELISA, it was also collected a drop of blood from tip of ear of each dog to test DPP®. Data analysis was performed on total sample (103 dogs) and group of different clinical signs using a contingency table 2 x 2 and analyzed by GraphPad PRISM™ 5.0. DPP® test on total sample, regardless of symptoms, showed sensitivity of 56.10% and specificity of 100% when compared to IFI (kappa = 0.34) and values of sensitivity and specificity of 95.12% and 88.71%, respectively when compared with ELISA (Kappa = 0.82). When DPP® was used in the diagnosis of asymptomatic dogs the sensitivity was of 12% and the specificity of 100% compared to IFI (kappa = 0.07) and sensitivity and specificity of 100% and 94.12%, respectively, compared to ELISA (Kappa = 0.47). Compared with oligosymptomatic dogs, DPP® had sensitivity of 52.38% and specificity of 100% compared to IFI (kappa = 0.41) values and sensitivity and specificity of 100% and 86.96%, respectively, compared to ELISA (Kappa = 0.76). For symptomatic animals DPP® had sensitivity of 88.89% and specificity of 100% compared to IFI (kappa = 0.29) and sensitivity and specificity of 93.75% and 60.00% respectively, when compared to ELISA (kappa = 0.53). In conclusion, DPP® test showed good levels of sensitivity and specificity when ELISA was used as the gold standard in the total sample and a high degree of sensitivity and specificity compared to ELISA and IFI on symptomatic dogs, but lower grades for oligosymptomatic and asymptomatic dogs.

First record of *Angiostrongylus cantonensis* (Chen, 1935) in *Rattus rattus* and *R. norvegicus* in Belém, Pará, Northern Brazil

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Background: *Angiostrongylus cantonensis* is a nematode parasite of the pulmonary artery of rodents (*Rattus rattus* and *R. norvegicus*, mostly), that are the definitive hosts, and different species of mollusks as intermediate hosts. In human, the parasite migrates to central nervous system, where it causes a local inflammatory process (eosinophilic meningitis), and are considered an endemic disease in Asian countries. In Brazil, cases were reported in Espírito Santo and Pernambuco States.

Methods: Host samples were collected in Belem - PA - Brazil, in Guamá, Montese and Jurunas districts, who presents highest numbers of human leptospirosis cases in this region. We captured a total of 50 rodents from January 2009 to August 2010. Rodents were necropsied and the helminths examined by light and scanning electron microscopy for taxonomic identification.

Results: Males and females nematodes measured about 1cm were found in the pulmonary artery of two *R. rattus* and two *R. norvegicus* from Guamá and Jurunas districts. After morphological and morphometric analysis they were identified as *A. cantonensis*.

Conclusions: This is the first record of *A. cantonensis* in rats in the city of Belém. These data contribute to mapping of this nematode and serves to alert for endemic diseases control institutions for risk of transmission of zoonosis to local population and visitors.

First Record of *Calodium hepaticum* (Bancroft 1893) (sin. *Capillaria hepatica*) of *Rattus rattus* and *R. norvegicus* in Belém-Pará, North of Brazil

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Background; *Calodium hepaticum* (Bancroft 1893) (Nematoda: Trichuridae) is a parasite of hepatic parenchyma of mammals, including humans, having rodents of genus *Rattus* as first reservoir, where the prevalence could be 100%. Human infections occur generally by accidental ingestion of water and food with embrionated eggs of *C. hepaticum*. After attain in liver, the parasite reach maturity, deposit eggs and remain till death. Eggs dispersion in environment occurs with host death and disintegration of his carcass. Cannibalism between rodents are considered the mainly way of cycle maintaining of *C. hepaticum*. Although human cases are considered rare, reports are in worldwide and could indicate an enhanced frequency of cases, beyond sometimes could be undetected, by non identification of eggs in faeces, or could be confounded with another hepatic diseases, tumors or fibrosis. Specific diagnosis occurs primarily in grave cases, in majority, fatal. The presence of dense populations of synanthropic rodents, close relationship with human species, environment conditions of great part of poor population, made worse by insanitary practices, are enhancers factors for the dissemination of this species.

Methods: Fifty hosts samples were collected by traps, in three districts of Belém with highest reports of human leptospirosis (Guamá, Montese and Jurunas), from January, 2009 to August, 2010. After necropsy, liver were analysed for helminthes, and that samples presenting irregular yellow spots were fixed in Formaldehyde 5%, dehydrated and included in Historesin[®]. After polymerization, sections of 3µ intervals were made in Leica UC6 ultra microtome, stained with Gomori's Trichrome, and observed by light microscopy.

Results: 23 rodents (10 *R. rattus* and 13 *R. norvegicus*) from three districts were founded parasited by *Calodium hepaticum*, presenting marked alteration of hepatic lobes and vases, beyond tecidual reaction represented by liberation of hemosiderin and septum fibrosis.

Conclusion: This is the first record of *Calodium hepaticum* in Belém rodents, contributing for mapping of this nematode occurrence and as a alert to endemic control institutes of risk for public health of this zoonosis to witch local population and visitors could be exposed.

DLA-DRB1 Gene Polymorphisms and Susceptibility of Dogs to Visceral Leishmaniasis

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Visceral leishmaniasis is a worldwide public health problem that is getting worse due to its urbanization and geographic expansion. Immunological factors influence the outcome of this zoonosis in the dog reservoir and host's genetics may play a role in the susceptibility/resistance to this infection. The major histocompatibility complex class II genes termed "the dog leukocyte antigen (DLA) system" has been pointed as an important candidate gene for susceptibility to canine visceral leishmaniasis (CVL), given the large number of diseases already associated to it in other species. We evaluated 434 samples from dogs, mostly mongrel, aging more than two years and living in an endemic area for visceral leishmaniasis (Araçatuba, SP, Brazil). Positivity for the presence of *Leishmania* kDNA in blood samples was observed in 44.2% of the dogs. After direct sequencing of exon 2 DRB1 gene in 103 samples so far analyzed 19 haplotypes were seen, seven of which are non-synonymous haplotypes already described in dogs. The most frequent of these haplotypes were DRB1*01502 (0.339%) and DRB1*00101 (0.227%). Haplotype DRB1*01502 reported to be associated to CVL susceptibility was more frequent in PCR positive dogs while DRB1*00101 haplotype occurred mostly in negative dogs. In the near future these preliminary results may confirm the participation of DLA-DRB1 gene in canine visceral leishmaniasis outcome.

***Leishmania (Viannia) braziliensis* in popliteal lymph node of a dog in a *Leishmania (Leishmania) infantum chagasi* vulnerable area in São Paulo State, Brazil**

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Background: Cutaneous (ACL) and Visceral (LVA) American Leishmaniasis incidences are increasing in human and canine hosts, especially LVA, which is expanding its range through different parts of Brazil, including São Paulo State. A recent outbreak of visceral canine leishmaniasis (LVC) with local transmission of *Leishmania (Leishmania) infantum chagasi* among dogs in Campinas, a city near the study site located in Monte Mor county, required intensification of local epidemiological vigilance in order to avoid introduction of LVA.

Methods: As part of the municipal Leishmaniasis Surveillance Program, dogs from a rural locality, where a human ACL case was detected, were investigated regarding *Leishmania spp.* infection. All three resident dogs had symptoms compatible with cutaneous leishmaniasis, presenting skin ulcers during clinical inspection, being sampled for blood and skin lesioned tissues. IFI *Biomanguinhos* and ELISA serology was conducted using antigen *FML (Fucose Manose Ligand)*, *S7 (HSP70 Heat Shock Protein 70 Kd)*, *Biomanguinhos (Leishmania major like)* and *rK39 Dipstick test*. Skin samples were evaluated through PCR aiming detection and identification of the infection causative species.

Results: Two out of three dogs presented detectable IgG anti-*Leishmania* only by the ELISA *FML* with all the other serological methods presenting negative results. Seropositive animals had *Leishmania (Viannia) braziliensis* DNA, but not *Leishmania (Leishmania) infantum chagasi* DNA, identified through PCR at the skin lesioned samples and popliteal lymphnode.

Conclusions: This finding confirms absence of *Leishmania (L.) infantum chagasi* in the area, confirms the susceptibility of the dog to infection by *Leishmania (Viannia) braziliensis* showing the circulation of the causative agent in the locality and also emphasizes the dog role as a sentinel for the risk of LTA disease occurrence in human beings. Since *Leishmania (Viannia) braziliensis* may be obtained from popliteal lymphnode biopsies and dogs infected with this species are not considered important reservoir hosts of ACL, differentiation between *Leishmania* species infecting the dog is needed, specially in sympatric areas where *Leishmania (L.) infantum chagasi* also occurs.

Chemical composition of *Eucalyptus staigeriana* and *Lippia sidoides* essential oils and insecticidal effects on *Lutzomyia longipalpis*

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Visceral leishmaniasis is currently a public health and urban health problem. *Lutzomyia longipalpis* is the most important visceral leishmaniasis vector in the Americas, Mathematical models of the three methods of control suggest that insecticide spraying and dog vaccination are better solutions to the problem than the destruction of serologically positive dogs. In this work, effects of *Lippia sidoides* and *Eucalyptus staigeriana* essential oils were evaluated on egg, larva and adult phases of *Lu. longipalpis*. Insects were collected in municipality of Sobral in the State of Ceará, Brazil. Five treatments with different concentrations were performed along with three negative controls, distilled water and dimethylsulfoxide (DMSO) (3%)/Tween 80 (3%), and a positive control, cypermethrin (0.196 mg/mL). Tests were carried out in plastic pots internally coated with plaster and filled with a substrate made of rabbit feces and crushed cassava leaves. Eggs, larvae and adults were sprayed with oils. Hatched larvae were counted for 10 consecutive days and observed until pupation. Insect mortality was observed after 24, 48 and 72 hours. Statistical analysis was performed by Tukey test at 5% probability. All tested oils inhibited egg hatching. *E. staigeriana* oil was the most effective, since *L. sidoides* at a concentration of 40 mg/ml presented effectiveness of 94.59% but *E. staigeriana* oil was 90.29% effective with 20mg/ml ($P > 0.05$). In assays with insect larvae, *L. sidoides* oil was most effective, since *E. staigeriana* at a concentration of 5.0 mg/ml presented effectiveness of 100% but *L. sidoides* oil was 100% effective with 1.2 mg/ml ($P > 0.05$). Insecticide effects on adult sandflies after 24h has been showed *L. sidoides* oil was most effective, since *E. staigeriana* at concentration of 5.0 mg/ml presented effectiveness of 99.62% but *L. sidoides* oil was 100% effective with 2.5 mg/ml ($P > 0.05$). Major constituents of the oils were, (+) limonene, Z-citral and E-citral (*E. staigeriana*) and Thymol (*L. sidoides*), respectively. These results demonstrate good potential for use of plants as an alternative control strategy for *Lu. longipalpis*.

Prevalence of antibodies anti-*Rickettsia rickettsii* in dogs from the Tapirapé indigenous community in Brazilian Amazon region: Preliminary report

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Background: *Rickettsia rickettsii*, the etiologic agent of Rocky Mountain spotted fever (RMSF), is the most pathogenic rickettsiae for humans and some animals, and is considered a re-emerging disease in Brazil. Indigenous population from Brazilian Amazon live in great contact with ticks and wild animals, and their dogs are used to hunt, increasing the risk of zoonotic diseases, such as RMSF. Little is known about the prevalence of *R. rickettsii* in these dogs raised by the indigenous population and used for hunting activity.

Methods: A cross-sectional study was conducted to determine the prevalence of anti-*R. rickettsii* antibodies in dogs from seven villages from the Tapirapé indigenous community, located in the state of Mato Grosso, Brazilian Amazon region. For this purpose, 110 blood samples were collected from all the dogs present in the villages. The sera were obtained and stored at -20°C until analysis. An indirect fluorescent antibody test was performed using crude antigens derived from *R. rickettsii* strain Taiaçu from Brazil, using a cut-off point of 1:64. The effects of sex, age and the use of the dog for hunting were analyzed using Fisher's exact test and $P \leq 0.05$ were considered significant.

Results: The overall prevalence of *R. rickettsii* found was 35.5% (39 positives /108 total). All the risk factors evaluated presented effect on the prevalence of *R. rickettsii*. Considering sex, male dogs were more prevalent than female (43.4% vs 17.7%, $P = 0.009$); more adult dogs (42.9%, $P < 0.001$) were seropositive than juvenile animals (0%). Dogs that were not used for hunting presented 15% of prevalence (6/40), lower than the rate founded in dogs used for hunting (47.8% - 33/69), possibly due to greater contact with ticks during hunt activity.

Conclusions: The preliminary results indicate that the dogs from Tapirapé indigenous community have past contact with a spotted fever group (SFG) rickettsiae that could be *R. rickettsii* or another SFG *Rickettsia* species, and can indicate that indigenous population maybe are at risk for spotted fever rickettsiosis. Thus, further analyses including serology for other rickettsiae and molecular detection will provide a better understanding of the epidemiology of rickettsiosis in the region.

Occurrence of parasites of zoonotic importance in stray dogs collected by the Center for Zoonosis Control (CCZ) of Lages, Santa Catarina

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For samples of zoonotic potential, dogs are definitive hosts and their feces are important way of contamination especially in children. The risk is not limited to the domestic area, but also in public areas through the free movement of dogs in the street. The present study aimed to evaluate the occurrence of zoonotic parasites in dog feces collected by Center for Zoonosis Control (CCZ) of Lages, Santa Catarina- Brazil. We analyzed 68 fecal samples by the techniques of Willis Mollay, Lutz and Faust et al. during the months from November to February 2011 from 37 male dogs and 31 female dogs (51 animals aged one year to five years and 17 older). From these animals 95.59% were collected in the outskirts of town. The positivity for eggs of parasites with zoonotic potential, regardless of the technique employed, was 82.35% (51/68). Regarding gender 58.82% of male dogs had some type of eggs in fecal material, since the females showed 41.18% positivity for samples zoonotic potential. Parasite eggs from *Ancylostoma* and *Toxocara* were analyzed using Mollay Willis technique with positivity 100% and 7.84% respectively, and with 56.82% and 3.92% for the technique of Lutz. *Giardia duodenalis* were analyzed in three samples by the technique of Faust et al. We also found *Dipylidium caninum* eggs and *Echinostoma* sp in a sample; this one was confirmed by the presence of adult specimens in the initial portion of the duodenum through the autopsy. This is the the first record of this parasite in Santa Catarina- Brazil. So, we observe the necessity of several techniques for better diagnosis of zoonotic parasites, as well as the importance of collecting these animals to zoonosis centers since the animals are roaming the streets and are important sources of parasitic infections for the population.

***Ancylostoma* spp. in sand of beaches of central zone of Sinaloa, México**

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Ancylostoma spp. is a zoonotic parasite, nematode of the family Ancylostomatidae, the most common are *Ancylostoma duodenale*, *A. braziliense*, or *A. caninum*; these worms are not usually affected by extreme environmental conditions so they can be a problem in any area of the world, it is seen in tropical and subtropical areas, can disseminated for defecate in public areas soil and sand of beaches, where they will eventually hatch into larvae that can be ingested or penetrate through the skin and migrate through the epidermis, these blood-feeding parasites produce larva migrans, also known as creeping eruptions or sandworm eruptions; and heavy infection can cause health complication for animals and humans representing a public health problem. The objective of this report was to determine the presence of *Ancylostoma* spp. in moist sand of beaches of central zone of Sinaloa, México by means of the study a number of representative samples of moist sand:479, determined by Thrusfield method (2005): $n=[t*SD/L]^2$. Where n=sample size, t=value of the normal distribution (Student t) for a 95% confidence level (t =1.96), L=accepted error or precision (5%), and SD=weighted disease prevalence (%); the total of 479 composite sample of sand was determined by random samplings, took surface moist sand scraping of 100 grams of sand for each sample and deposited it in plastic bags; were transferred to the laboratory of parasitology of the FMVZ-UAS to be analyzed by the sedimentation method (samples were homogenized, filtered with gauze, placed in a container with water, homogenized and allowed to stand for 15 minutes, the supernatant was decanted and the process repeated three times and finally the sediment observed in a microscope), the eggs or larvae of the species of *Ancylostoma* are identical and for differential diagnostic, the adults can be readily identified by virtue of the differences in their mouth parts. The result indicated that of the 479 composite samples of sand of the twelve beaches, 141 (29.44 %), were positive to larvae of *Ancylostoma* spp.. Concluded that the contamination of sand of beaches with larvae of *Ancylostoma* spp. represent risk for public health and the pets, and it would be necessary to implement control strategies and prevention of common sense measures as to suggest reduce the contact of skin with soil and to avoid walking barefoot, to avoid the presence of loose animals, to promote deparasiting and proper disposal of animal waste.

Diagnosis of *Echinostoma paraensis* Lie and Basch, 1967 (Trematoda, Echinostomatidae) in hamsters *Cricetulus griseus*

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Background: Parasites of the genus *Echinostoma*, characterized by presenting a complex biological cycle, involving two intermediate hosts, especially freshwater mollusks, and habitat restricted to the intestinal lumen of the definitive hosts of which are especially waterfowl, mammals, including man, reptiles and fish, representing, therefore, support both in human medicine and in veterinary medicine. In this study, we investigated the efficiency and sensibility of the Kato-Katz and Hoffman used for diagnosis of experimental infection of hamsters *Cricetulus griseus* by *E. paraensis*.

Methods: We examined 15 fecal samples from hamsters infected *E. paransei* used in the maintenance of the experimental cycle of the parasite in the Laboratory of Parasitology of Wild Mammals Reservoirs (LABPMR) of the Oswaldo Cruz Institute (IOC), FIOCRUZ, Rio de Janeiro, RJ. Stool samples were collected with the aid of plastic bags previously identified, kept under refrigeration for twenty-four hours before processing. The experiment was conducted in LABPMR where faecal samples (one gram) were homogenized, weighed and processed by techniques of Hoffman and Kato-Katz readings were performed in duplicate. The Kolmogorov Smirnov was used to verify data normality. The results were expressed as mean $\pm$ SEM and subjected to the Tukey test to compare means ($\alpha = 5\%$).

Results: The method of Hoffman was more sensitive when compared with the Kato Katz, once, that was positive in all samples examined (100%), while it registered positive in only 76% of fecal samples. Regarding the number of eggs retrieved, the Kato Katz method was less effective (62.05 ± 14.35) compared to Hoffman (165.60 ± 11.22), resulting in a decrease of approximately 62.53% of the total number of eggs observed.

Conclusion: The method of Hoffman showed greater sensitivity and efficiency when compared with the Kato-Katz for the diagnosis of *E. paraensis* of hamsters.

***Trypanoxyuris (Trypanoxyuris) minutus* (Schneider, 1866) (Nematoda, Oxyuridae) in *Alouatta guariba clamitans* Cabrera, 1940 (Primates, Atelidae) in Rio de Janeiro, Brazil**

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Little is known about the Brazilian primate parasitology, especially in Rio de Janeiro state. The *Alouatta guariba clamitans* (Red Howler Monkey) is a common primate in the Brazil southeastern and southern. Published studies about parasites helminths of red howler monkeys are generally the states of Rio Grande do Sul, Minas Gerais and Espírito Santo. This research aims to study the diversity of helminths in the gastrointestinal tract of animals from the mountainous region from Atlantic Forest, Rio de Janeiro. Necropsies were performed in two adult male monkeys after accidental death on highway BR 116, Rio- Teresópolis, which cuts the Serra dos Órgãos National Park (22° 24' - 23° 32' S, 42° 69' - 43° 06' WGR). On physical examination were not found signs of anorexia, alopecia and anemia. So far were found in the cecum and ascending colon part of 1218 individuals *Trypanoxyuris minutus* (Nematoda: Oxyuridae) in a Red Howler Monkey. In the other, were found 3.295 *T. minutus* and heartworm, as yet unidentified, in the peritoneal cavity and heart. The worms were fixed in solution Raillett Henry and clarified in phenol order to identify by Yamaguti (1961), Hugot & Vaucher (1985), and Vincent et al. (1997). The intensity of infection is higher with the finalization of the remaining parts of the intestinal tract, which is common for oxyurid and this species of primate, according to reports from other areas of Brazil. One must consider that despite good physical conditions, this primate is almost always isolated in fragments of Atlantic Forest, which enhances the hyperparasitized forced by overlapping use of the same areas, including other species, and the aggregated distribution of parasites apart from the expected course. This seems to be the first record of *T. minutus* for red howler monkeys in the region of Rio de Janeiro.

Helminthes parasites in dogs of fishing villages of central zone of, Sinaloa, México

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The moist dogs of beaches can act as reservoir or vector of zoonotic parasite potentially pathogens, and represent risk for health humans and pets that have contacted with contaminated animals. Eggs and larvae of helminthes parasites can disseminated in the soil of public areas, and resist to adverse environment conditions, capable of surviving for many months; the humans and pets can infect that have contact with contaminated dogs or soil. The objective of this report was to determine the prevalence of helminthes parasites in dogs of fishing villages of central zone of Sinaloa, México. The animals included in the study were 205 dogs determined by random sampling representative with both sexes and cradle described by the Thrusfield method (2005) was used: $n = [t \cdot SD / L]^2$. Where n = sample size, t = value of the normal distribution (Student t) for a 95% confidence level ($t = 1.96$), L = accepted error or precision (5%), and SD = weighted disease prevalence (%). On the basis of the technique described, for each dog feces sample were collected rectally by digital stimulus, into plastic bags, were transported under refrigeration at 4°C to the FMVZ-UAS, and processed by the flotation technique with sugar solution. The results indicated that of the 205 dogs analyzed 51 (24.87%) were positive for helminthes parasites, with the following distribution: *Toxocara spp* 20 (9.76%), *Ancylostoma caninum* 25 (12.19%), *Dipylidium spp*. 6 (2.93%). It is concluded that the contamination with *helminthes* is a considerable number and the proportion of animals testing positive continues to be an issue of importance in the community, frequently these dogs roam in and around the town and can distribute parasites, residents and visitors are not well informed or ignore the risk of diseases that dogs can transmit to them; it would be necessary to implement control strategies and education for the prevention of the infections

Diversity of helminthes eggs in *Cerdocyon thous* Linnaeus, 1766 feces: a comparison among two different Brazilian Biomas

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Cerdocyon thous is medium size wild canid, popularly known as “Crab-Eating Fox.” This South American species lives in a great diversity of environments, except Amazon forest and Patagonia. It can share pathogens with other canids, including the domestic dog, since philogenetically they are close together. This contact is still caused by the availability of food near human dwellings and the species tolerance to disturbed areas, raising the possibility of disease transmission to humans as well. These characteristics make it a model to study the diversity of helminthes in different habitats. The main objective of this work is to compare the diversity of intestinal helminthes among two different biomes: Caatinga, a semi-arid area in Northeastern region of Brazil, and the Pantanal, the world’s largest wetland. At this moment, we have analyzed sixteen feces samples of *C. thous*, eight from Caatinga (Piauí and Pernambuco states) and eight from Pantanal (Mato Grosso state). In both biomas, the samples were taken in wet and dry seasons. The samples were conserved in Railliet & Henry solution and we have used Lutz’s method of spontaneous sedimentation to detect eggs of helminthes. Fifteen slides of each sample of feces were analyzed in microscopy (400x). Caatinga samples showed nine morphospecies of helminthes eggs: Trematoda Digenea possible *Alaria* sp., Ancylostomatidae, Ascarididae, Strongyloididae, Oxyuridae, Physalopteridae, two types of Trichuridae, and one unidentified nematode species. Six samples were positive. In Pantanal samples, fourteen morphospecies of helminthes eggs were identified: Cestoda Pseudophyllidea possible *Spirometra* sp., *Toxocara* sp. (Nematoda: Ascarididae), Ascarididae, two types of Strongyloididae, two types of Physalopteridae, two types of Trichostrongylidae, three types of Trichuridae, and two unidentified nematode eggs. All samples were positive. The preliminary results show a great richness of intestinal helminthes eggs found in feces of *C. thous* in both biomes, but slightly richer in Pantanal. Probably, this fact is due the high humidity levels that favors dispersion and the viability of immature forms in the ground. The continuing study aims to relate the similarity between the helminthes species in domestic and wild dogs in these two biomes, and assess the risks of zoonoses.

Spotted Fever Group (SFG) rickettsia antibodies on horses in the State of Santa Catarina, South region, Brazil

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Brazilian Spotted Fever (BSF) rickettsiosis is mainly caused by *Rickettsia rickettsii*, but in the state of Santa Catarina, South region of Brazil, *R. parkeri* is probably an etiology agent involved in recorded cases in this State. Serum samples were obtained from 150 horses from two meso regions of Santa Catarina State (Serra Catarinense and Vale do Itajaí) and were tested by means of the indirect immunofluorescence test (IFAT) for antibodies against Spotted Fever Group (SFG) rickettsia (≥ 64). The overall prevalence was 18.66%. Cross reactions were observed in all samples in at least two SFG *Rickettsia* species. Individually, 25 (16.66%) samples were positive for *R. rickettsii*, 15 (10%) for *R. parkeri*, 22 (14.66%) for *R. amblyommi*, 23 (15.33%) for *R. rhipicephali*, 16 (10.66%) for *R. bellii* and 19 (12.66%) for *R. felis*. Only in two horses the serodiagnostic was conclusive, one for *R. bellii* and one for *R. rickettsii*, with titers 4096 and 512, respectively. The occurrence of antibodies against SFG *Rickettsia* on horses (sentinel for BSF) indicates the circulation of a SFG *Rickettsia* in the state of Santa Catarina.

Comparison of indirect immunofluorescence assay, enzyme-linked immunosorbent assay and Dual Path Platform (DPP®) immunoassay for the diagnostic of canine visceral leishmaniasis

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Visceral leishmaniasis (VL) is a zoonosis that can be fatal if left untreated. This zoonosis is characterized by infection of mononuclear phagocytes with *Leishmania chagasi*. Currently, the spread of this disease in Brazil indicates the inefficiency of VL control measures and confirms that the lack of an efficient and rapid diagnosis of the disease, especially in dogs, is a serious risk to public health and an ethical problem and animal welfare. This study compares the effectiveness of a new serological method, Dual Path Platform (DPP®), with the indirect immunofluorescence (IIF) and enzyme-linked immunosorbent (ELISA), the gold standard tests for canine VL diagnosis. Blood samples of 302 domiciled dogs of an endemic area in the city of Mossoró, Rio Grande do Norte State, Brazil, were collected. Serological tests were performed in the Laboratory of Immunomodulation and Protozoology of FIOCRUZ, located in the state of Rio de Janeiro, Brazil. Thirty-five (11.6%) dogs were seropositive in the DPP. From this total, thirteen (37.1%) were positive for both the ELISA and IIF; ten (28.6%) were positive only for IIF; and three (8.6%) samples were positive for ELISA. In the validation analysis of the DPP in relation to IFI, the specificity and sensitivity were 94% and 21% sensitivity, respectively. In relation to the ELISA, 93% specificity and 40% sensitivity were observed. The DPP showed a Kappa coefficient of 0.25 in relation to IFI and 0.5 in comparison to ELISA. According to the results, it is concluded that the DPP presents good specificity but low sensitivity, what can generate many false negatives and fewer true positives. Consequently, infected animals are not detected, i.e., the DPP is not reaching the expected goal. Regarding the Kappa coefficient, the data obtained indicate that the concordance between the tests vary from passable to regular. This is expected, considering that the crude antigen used in ELISA and IIF is different from the recombinant antigen rK39 used in the DPP. In addition, the standardization techniques of the tests are different.

Anti-*Trypanosoma cruzi* antibodies detection in dogs of Mossoró, Rio Grande do Norte, Brazil

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Chagas disease is a zoonosis infectious and parasitic caused by the protozoa *Trypanosoma cruzi*. The transmission modes are oral, accidental, vertical, and transfusional. Besides the man, the dogs are important hosts and are directly linked to most cases of human infection by *T. cruzi*. This study aimed to evaluate the presence of anti-*T. cruzi* antibodies in dogs from the city of Mossoró, Rio Grande do Norte, Brazil and to correlate the positivity with the possible factors of risk for the canine population. After owner consent, blood samples of 302 domiciled dogs were collected to obtain serum. The serum samples were submitted to indirect immunofluorescence (IIF) and enzyme-linked immunosorbent (ELISA) assays in Laboratory of Immunomodulation and Protozoology of FIOCRUZ, localized in state of Rio de Janeiro, Brazil. The specificity and sensitivity of tests were calculated as well as the level of agreement (Kappa coefficient) between them. Twelve (3.97%) dogs were positive for both the tests. The most of the positive animals were males (83.33%), 58.33% were guard dogs, 91.67% slept in peri-domicile, 66.67% lived with other animals and 91.67% lived in houses which had waste material and plants in the backyard. These results indicate that guard dogs which used sleep in peri-domicile with waste material and other animals are more exposed to the barber, and consequently to the risk of infection by *T. cruzi*. The ELISA specificity was 93.33% and its sensitivity was 15.58%, while the IFI presented 76.36% specificity and 44.44% sensitivity. The Kappa coefficient was 0.733 indicating a good agreement between both tests and confirming the hypothesis that the use of two techniques in Chagas disease diagnosis allows the implementation of a more reliable serology. The presence of canine trypanosomiasis in the urban area of Brazil and the risk factors identification for *T. cruzi* transmission to dogs can alert the population and authorities about the necessity of development of effective control strategies against Chagas disease.

The Repellent Effect of Permethrin (70 Mg/Kg) in a Tick and Flea Spot-On Formulation for Dogs against Haematophagous Mosquitoes

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Introduction: Climatic changes and global warming are seen as the main factors that will bring alterations to the prevalence of vector-borne diseases. Therefore, it is essential to count on the availability of tools that may allow to control or at least reduce the attack of some of these vectors, which can no longer be considered by animal owners a mere discomfort to their pets.

Materials and Methods: Eighteen Beagle dogs were divided into three groups of six. The groups were allotted at the start of the study into groups A, B or C. Group A was treated with Power ® Ultra (permethrin, 40 %; imidacloprid, 5,15 %; pyperonil butoxide, 3 %; Brouwer S.A) at a dose of 1,75 ml per 10 kg body weight, equivalent to 70 mg of permethrin per kg body weight. Group B was treated with Power ® Forte (permethrin, 33 %; imidacloprid, 8 %; Brouwer S.A) at a dose of 1,5 ml per 10 kg body weight, equivalent to 50 mg of permethrin per kg body weight. Group C remained untreated as control. The dogs were housed in collective closed kennels. The doors and windows were fitted with removable mosquito nettings. Each kennel was provided with a plastic framed light trap. The source of mosquito infestation was natural. The evening prior to the predetermined sampling day, and having let the dogs into each kennel, the window and door mosquito nettings were left open between 19 and 23 h, and the light-traps were left on. The next morning the dogs were let out of the kennels and study personnel entered for the capturing of the free flying mosquitoes by using an entomological net. Mosquitoes were sent to the laboratory for counting and classifying. The percentage of blood engorged mosquitoes in each kennel was the parameter used to assess the repellent effect of each product.

Results: The results showed a 65 % repellent effect for a 31 day period at the highest dose level. This formulation was better than formulation B, and both showed highly significant differences when compared to the untreated control group C.

Conclusions: Treatment A can be considered an additional tool for fighting mosquitoes, and hence, could be recommended for reducing the prevalence of diseases they transmit.

Repellent Effect against Haematophagous Diptera of a 70 mg/kg dose Permethrin Spot-on formulation

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Introduction: Climate change is an increasing reality. Changes in the prevalence of diseases are expected, particularly when vector-borne. Progressive tropicalization of temperate and even dry regions, lead to the extension of the borderline of initially tropical diseases. It is due to this that research of some ectoparasites not considered up to now must be broadened. This is the case for haematophagous insects related to dogs. In human medicine repellents are considered an important tool for the prevention against haematophagous-insect bites, and in this way reduce the chances of transmission of diseases. This strategy could be replicated easily in veterinary medicine. The purpose of the present study was to compare the repellence against mosquitoes of two spot-on permethrin formulations indicated for treatment of fleas and ticks in dogs.

Materials and methods: A total of 18 dogs, living in a canine refuge close to Posadas (Misiones, República Argentina), were selected for the study and divided into three homogeneous groups of six individuals each, according to weight and length of coat. Each treatment group was housed in a separate kennel. One group was treated at a dose of 70 mg/kg of permethrin ^a, a second was dosed at 50 mg/kg of permethrin ^b, and a third ^c remained as the untreated control. The repellence effect of both treatments was determined by comparing the accumulated number of blood engorged mosquitoes captured at three day intervals obtained using CDC traps, which were placed overnight in each kennel the evening prior to sampling. Sampling was performed the following day during the morning.

Results: The repellence effect obtained reached 80 % for those dogs dosed at 70 mg/kg ^a for at least 25 days, while for those dogs dosed at 50 mg/kg ^b, a little over 50 % during the same period of time.

Conclusion: The results show that the repellence activity of permethrin at 70 mg/kg when applied on dogs can be of use as an aide in the prevention of vector-borne diseases.

^a Power Ultra, Brouwer S.A.; ^b Power Forte, Brouwer S.A.; ^c Untreated control

Diagnosis of Imported Canine Filarial Infections in Germany 2008–2010

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Background: Filarial infections of dogs are attracting attention across Europe because of the risk of spread into previously non-endemic areas (e.g., *Dirofilaria repens*) and as emerging zoonotic agents.

Methods: The occurrence of filarial infections in German dogs has been analysed based on 8,545 samples collected either from imported animals or following travel into endemic regions. All samples were tested by means of modified Knott's test and heartworm antigen assay within the period 2008–2010. For discrimination by means of PCR and sequencing a total of 73 blood samples as well as two samples of adult worms were included.

Results: Heartworm antigen was detected in 127 samples (1.49 %; 95 % CI: 1.25–1.77 %), but only 38 dogs also had microfilariae in their blood samples. On the other hand, 125 animals (1.46 %; 95 % CI: 1.23–1.74 %) were only positive in the Knott's test. A mono-infection caused by *D. repens* was detected in 35 cases, while *D. immitis* was proven in 15 samples, with 6 of these showing a combination of *D. immitis* and *D. repens*. Imported *Dipetalonema dracunculoides* or *Acanthocheilonema reconditum* infections were diagnosed in 24 cases and in a single sample a co-infection of *A. reconditum* and *D. repens* was evident.

Summary/Conclusions: *D. repens* was the most common filarial infection imported and it was introduced into Germany from eleven European countries. Slovenia and Hungary are reported for the first time as endemic for *D. repens* and *A. reconditum*, respectively. Furthermore this study reports, to the best of our knowledge, for the first time import of *D. dracunculoides* from the Canary Islands, *A. reconditum* from Majorca, *D. immitis* from Corfu and co-infections of *D. repens* and *A. reconditum* from Spain as well as mixed infections of *D. repens* and *D. immitis* from Corfu, Sardinia and Bulgaria. Co-infections with other arthropod-borne infections as well as therapeutical follow-up were also considered. Selamectin was not able to clear microfilaraemia in dogs infected with either *D. repens*, *A. reconditum* or *D. dracunculoides*, whereas a topical moxidectin/imidacloprid formulation was able to eliminate microfilariae in one dog infected with *A. reconditum*.

Studies on the prevalence of haematozoa in dogs

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Background: Blood protozoan and rickettsial diseases caused by pathogens transmitted by ticks and other vectors are an important cause of morbidity and mortality in dogs wide across India. Investigations on the prevalence of haematozoa were conducted on 951 dogs of different breeds and of both sexes (607 males and 344 females), aged from 3 months to 15 years presented to Small Animal Clinics of Veterinary Teaching hospital of the University and three local private clinics at Ludhiana, Punjab, India .

Methods: The dogs were suspected to be infected with haemoprotozoa based on clinical signs and history. The detection, diagnosis and identification of haematozoa were done by direct observation of the protozoa in smears and morphometric studies. In *Ehrlichia*, seroprevalence was also recorded.

Results: Prevalence recorded for haematozoan infections was 14.09% (14% in males and 14.24% in females). Highest prevalence of infections was seen in the month of June (25.37%), followed by August (14.18%) and July (13.48%). Highest prevalence of haematozoan infection was seen in cross bred than pure breeds of dogs. Except *Babesia canis* which was more prevalent in the age group of 2 to 5 years, all other haematozoan infections were more prevalent in dogs of ages 6 months to <2 years. The most prevalent parasites were *Ehrlichia* spp. (7.80%), followed by *Babesia gibsoni* (3.17%), *B. canis* (1.26%), *Hepatozoon canis* (0.95%) and mixed infection (0.53%). Dot Immuno Comb assay for detection of *Ehrlichia* spp. was found most sensitive (93.33%) followed by buffy coat (36%) and thin Giemsa stained blood smear (28%). In one case, *Trypanosoma evansi* concurrent with *H. canis* and *E. canis* was seen. Anaemia, thrombocytopenia and hyper gammaglobulinemia were most common findings in all infections. Ticks collected from infected dogs were identified as *Rhipicephalus sanguineus*. Anaemia was severest in *Ehrlichia* spp. infected dogs followed by *B. gibsoni*, *H. canis* and *B. canis*. In all the dogs infected with haematozoa, neutrophilia and lymphopenia were seen.

Conclusions: It was concluded that it is necessary to conduct haemato-biochemical studies along with the diagnostic studies in suspected cases based on clinical signs and history to arrive at a conclusion for treatment and management of haematozoan infections in dogs.

A comparison of 2-(2'-hydroxyphenyl) benzazole isothiocyanate and fuchsin staining for determining *Cryptosporidium* spp. oocysts

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Cryptosporidium spp. is a coccidian parasite of humans, domestic livestock and wide range of species in worldwide. Fluorescent methods to detect parasites are of great interest in biology. 2-(2'-hydroxyphenyl) benzazole isothiocyanate (BzICT) shows high fluorescence emission and a large Stokes shift due to an intramolecular proton transfer (ESIPT) mechanism. A recently described in this work, BzICT was evaluate, for the first time in this experiment, to detect *Cryptosporidium* spp. oocysts in smears fecal, in comparison with the standard method by a modified Ziehl-Neelsen technique (mZN). BzICT solution consists of 3×10^{-3} molar of fluorochromes in ethanol as a solvent, and characterized by spectroscopic techniques. The positive controls showed *Cryptosporidium* spp. oocysts obtained with mZN staining. The total positives samples were 54, including wild mammals, swines and horses. The positives samples of captive wild mammals at the zoological park of the Zoobotanical Foundation of the Rio Grande do Sul state, southern Brazil were: *Alouatta caraya*, *Aotus nigriceps*, *Ateles chamek*, *Puma concolor*, *Coendou prehensilis*, *Dama dama*, *Cervus unicolor*, *Kobus ellipsiprymnus* and *Myrmecophaga tridactyla* as well as two samples to *Rhea americana*. The positive fecal samples to domestic animals were: 18 to swine and 25 to horses. The new protocol to smears fecal staining was: once dry, the slides were fixed with absolute methanol for 5 minutes; dried over a flame; 2-(2'-hydroxyphenyl) benzazole isothiocyanate dyes solution is placed on each slide and incubated for 5 minutes; decolorize twice in 0,5% chloridric acid in absolute methanol; rinse in tap water and air-dry the slide. The entire surface of the smears was examined for the presence of *Cryptosporidium* oocysts. The cover slips were observed under at 400x and oil immersion at 1000x magnification, in optical and epifluorescent microscope with fluorescent filter system. Images were captured using a Sony digital camera. The BzICT confirmed the presence of *Cryptosporidium* oocysts in fecal smears slides, but not satisfactory in comparison with mZN technique. The images showed strong background fluorescence, with mayor time consumed to identify the oocysts. Further studies involving this fluorescent solution would be necessary.

Seasonal dynamics of asymptomatic *Leishmania* infection in dogs in Spain

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Canine Leishmaniosis caused by the sandfly transmitted protozoa *L. infantum*, is a chronic, life threatening, zoonotic disease of dogs, which is endemic in Mediterranean countries. The parasite infects macrophages and recent PCR-studies in tissue from healthy dogs indicate that the prevalence of infection is 50-80%, much higher than traditionally considered, as seroprevalence is typically <30%. Investigating the dog's antibody and PCR status is important because most symptomatic dogs are seropositive and asymptomatic infected dogs are a reservoir of the parasite. In the present study we used commercial antibody ELISAs and a real time PCR test for kinetoplast sequences to investigate the prevalence of *L. infantum* infection in asymptomatic dogs in spring and autumn, coinciding with the beginning and end of the sandfly season. Serum antibody and blood, skin, lymphnode and spleen PCR tests, were carried out on single samples from 212 dogs from Murcia in southeast Spain. Seroprevalence was 21% and was 14% in spring and 27% in the autumn ($p<0.05$). Similarly, the percentage of dogs PCR-positive in at least one sample were 56%, 29% in spring and 66% in autumn ($p<0.001$). Percentages of PCR-positives according to sample type were: 7% in blood, 32% in skin, 43% in spleen and 46% in lymph node tissue. Logistic regression models confirmed the strong association between infection and season, and antibody and PCR prevalence were also independently associated to the dog's body weight and age; they were highest in dogs ≥ 19 kg, seroprevalence increased with age and PCR-prevalence was lowest in 1 year-olds, highest in 2 year-olds and intermediate in dogs ≥ 3 years-old ($p<0.05$). Results suggest that dog infection is seasonally variable depending on repeated exposure to infected sandflies and probably the host's immune response to the parasite. It would be useful from a control point of view to evaluate the extent to which latent infections can be decreased by reducing exposure to the vector and the parasite at critical times.



SESSION L

Keynote Lecture

- **Integrated parasite management and drug resistance – with an eye to the future** - J. A. van Wyk

Oral Communications

- **Redefining anthelmintic resistance in ovine nematodes** - L. Rinaldi
- **Detection of ivermectin resistance by larval development test – back to the past or step forward?** - M. Várady
- **Novel insights in the interpretation of the faecal egg count reduction test to monitor anthelmintic efficacy** - B. Levecke
- **A risk management model for nematode control and drug resistance on Australian sheep farms** - B. Hosking
- **A computer simulation study to evaluate resistance delaying control strategies with a novel combination anthelmintic product on UK sheep farms** - D. J. Bartram
- **Worming and anthelmintic resistance in English cattle** - G. Coles

Posters

- **Lamb productivity responses and benefits to monthly STARTECT treatment of grazing lambs exposed to mixed anthelmintic-resistant nematode parasite pasture challenge** - C. M. Miller
- **Dynamics of the Gastrointestinal Nematode Resistance to Ivermectin in a Fattening Cattle System** - C. O. Descarga
- **Targeted treatment and production efficiency based-targeted selective treatment: effects on productivity and ivermectin resistance** - F. Kenyon
- **Restoring phenotypic susceptibility to benzimidazol in resistant Haemonchus contortus populations in goats** - J. F. de J. Torres-Acosta
- **Performance of dairy calves subjected to a targeted selective anthelmintic treatment (TST) regime in their first season** - A. W. Greer
- **Developing and refining a performance-based targeted selective anthelmintic treatment regime for grazing dairy calves** - R. W. McAnulty
- **Field Efficacy of Multi-Dosage Forms of Fenbendazole in US Cattle Using the Fecal Egg Count Reduction Test** - D. H. Bliss
- **Comparative assessment of ivermectin, abamectin and moxidectin efficacies against resistant nematodes in lambs** - M. Lloberas
- **Macrocyclic lactones and Cooperia spp in cattle in Argentina. Persistence of status of resistance after five years without the use of these drugs** - O. S. Anziani
- **Is larval development test reliable enough to detect ivermectin resistance in the field?** - M. Dolinská
- **The efficacy of Trichlorphon and Naphtalophos against anthelmintic resistant nematodes of naturally infected sheep in Argentina** - O. Prieto
- **Comparative efficacy of Trichlorphon and a Trichlorphon – Ivermectin combination treatment against anthelmintic resistant nematodes of cattle in Argentina** - O. Prieto
- **The control of multiple anthelmintic resistance in grazing cattle of Argentina: results on the use of a rational programme during seven years** - C. Fiel
- **Cattle worms resistance to Ivermectin treatments: effects on production** - C. Fiel
- **Comparative haematological values of parasitised and nonparasitised cattle in Abeokuta Southwest, Nigeria** - F. A. Akande
- **Effect of avermectins on the performance of weaned Nelore calves naturally infected by ivermectin-resistant nematodes** - F. de A. Borges
- **Drug resistance in animal trypanosomes in the absence of drug pressure** - V. Delespaux
- **Comparison of the efficacy of new anthelmintics against multidrug resistant nematodes** - H. Sager
- **Frequency of cattle herds with ivermectin resistant nematodes in the sub-humid tropics of Mexico** - H. L. Canul-Ku
- **Five years of targeted selective treatment against gastrointestinal nematodes in a mixed sheep and goat herd in the tropics** - J. F. Torres-Acosta

Integrated parasite management and drug resistance – with an eye to the future

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The continuous escalation in parasiticide resistance (AR) and increasing costs of drug discovery and development have been a major impediment to maintaining effective parasite control, indicating that significant changes in drug use practices are needed. While novel drugs such as monepantel and derquantel will provide a reprieve, it is essential that best use is made of both these and the older drug classes to ensure that the mistakes of the past are not repeated. However, this may present some serious challenges, as a lack of adoption of integrated parasite management strategies indicate that many farmers are not convinced of the need to prevent the development of AR, or else are not aware of the recommended strategies. In large part this reflects insufficient input into extension, in turn related to the scarcity of experienced extension personnel.

Of key importance is the perception by farmers of excessive “inconvenience” or complexity of resistance management recommendations even where they are convinced of the threat to profitability imposed by AR and have sufficient knowledge to implement changes. A further barrier is the inconsistent availability of laboratory services and expert advice from consultants, which vary markedly both between and within countries. It is essential that once a convincing case is made for changes to parasite control routines, the solutions are practical to implement and support services are readily available. It is suggested that resistance management concepts should be translated into automated systems appropriate to different environments and methods of animal raising and farm management. Novel technological aids, including telemetry and electronic communication systems for predicting parasite risk situations and interpreting local information, together with protocols to identify individual animals requiring treatment could significantly increase the uptake of effective strategies, but a substantial research effort is required.

While the emphasis in the review is on helminths, attention should also be paid to prevention of clashes between different disciplines within parasitology regarding sustainability, by developing management or control systems for a given group with due consideration to other parasites susceptible to the same approaches. For instance, treatment with macrocyclic lactones in winter in a summer rainfall region for sheep scab eradication or in early spring in such a region for sympatric *Teladorsagia* spp. is likely to select for resistance in *Haemonchus contortus*. Management of treatment regimes is essential to avoid such unintended consequences.

Redefining anthelmintic resistance in ovine nematodes

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Background: The WAAVP methods (Coles et al, 1992) stated that anthelmintic resistance in nematodes of sheep is present where there is less than 95% efficacy and the 95% confidence limit is less than 90%. However, field observations have suggested that a higher percentage efficacy can be expected in populations of susceptible worms if a sensitive faecal egg count (FEC) technique such as FLOTAC is used. Therefore the efficacy of six anthelmintics was tested in sheep in southern Italy where there is a high level of refugia in the ovine population (dairy ewes are not treated) and little evidence for the development of any anthelmintic resistance.

Methods: Fresh faecal samples were collected rectally at the time of treatment and 14 days later and FEC were made using the FLOTAC method accurate to 2 epg. All animals were weighed prior to treatment and given the correct dose with a syringe. Tests were run with flocks of sheep (12 to 20 animals per group) using six anthelmintic groups administered orally, levamisole (Levacide, 7.5 mg/kg) in 8 farms, ivermectin (Oramec, 0.1 and 0.2 mg/kg) in 8 farms, moxidectin (Cydectin, 0.2 mg/kg) in 3 farms, monepantel (Zolvix, 2.5 mg/kg) in 8 farms, netobimin (Hapadex, 7.5 mg/kg) in 22 farms (composite samples) and albendazole, (Sverminator/Valbazen, 3.8 mg/kg) in 5 farms (composite samples).

Results: A very high efficacy was obtained with all anthelmintics tested as follows: levamisole 99.3% (98-100%), ivermectin 0.1 mg/kg 99.5% (98.0-100%), ivermectin 0.2 mg/kg 99.9% (99.3-100%), moxidectin 100%, monepantel 99.4% (range 97-100%), netobimin 99.1% (range 92-100%) and albendazole 100%.

Summary/Conclusion: The results demonstrate that the definition of resistance as less than 95% efficacy is too low and that values closer to 100% need to be agreed. This will enable the first signs of resistance to be detected earlier which is important if the development of resistance is being followed under different management conditions. It also will give a value for the first sign of resistance developing to the valuable novel anthelmintic, monepantel. Similar studies are required to redefine resistance in nematodes of cattle and horses and to establish it in other animals.

Detection of ivermectin resistance by larval development test – back to the past or step forward?

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Background: Resistance to anthelmintic drugs in gastrointestinal nematodes of sheep and goats has become a serious problem concerning all countries with developed sheep industries. To detect anthelmintic resistance (AR) *in vivo* and *in vitro* methods can be used. The most widely used test to assess anthelmintic efficacy is the faecal egg count reduction (FECR) test. Although this standardized test is valuable in detecting AR, *in vitro* tests are cheaper to perform and therefore more suitable for large surveys.

Methods: Larval development test is the widely employed *in vitro* methods for detection of AR in ovine nematodes. A number of versions of larval development test that can detect resistance to all classes of anthelmintics have been developed. There are two variations with either liquid or agar base being used. Both tests rely on the development of eggs to L3 larvae. The aim of this study was to evaluate the potential of the micro agar larval development test for definition of resistance to macrocyclic lactones in *Haemonchus contortus* of sheep. Single infection with 5000 L3 of two susceptible and five resistant isolate of *Haemonchus contortus* has been carried out. Micro agar version of LDT was performed and faecal samples were collected four times during patency. The effect of three macrocyclic lactone anthelmintics (ivermectin, eprinomectin, ivermectin-aglycone) were examined.

Results: The results of the test are interpreted as LC_{50}/LC_{99} values. The LDT showed good ability to distinguish between susceptible and resistant strains. In general, resistance factors (RF - value of LC_{50}/LC_{99} of resistant strain divided by the respective value of susceptible strain) for the LC_{99} values were higher than those obtained by comparing LC_{50} values. In this study the values of RF using ivermectin were less than those obtained by using ivermectin aglycone or eprinomectin. The highly resistant isolates were readily distinguishable from susceptible strains, particularly when using ivermectin aglycone and eprinomectin with $RF > 20$.

Conclusions: We will discuss the significant impact of the method to detect ivermectin resistance in gastro-intestinal parasites of sheep and limitations of the current test will also be review.

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Novel insights in the interpretation of the faecal egg count reduction test to monitor anthelmintic efficacy

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Background: The faecal egg count reduction test (FECRT) is the method of choice to monitor anthelmintic efficacy against gastro-intestinal nematodes. Guidelines on how to conduct a FECRT are available in both public and animal health. Yet, the level of understanding of factors inherent to study design, parasite and/or host species to support these guidelines is poor. Therefore, the objective of this study was (1) to assess the impact of these factors on the interpretation of the FECRT and (2) to propose recommendations for future monitoring programmes.

Methods: A simulation study was performed in which the FECRT was conducted under varying conditions of sample size, detection limit, mean faecal egg count (FEC) and aggregation of FEC. Classification trees were built to explore the impact of the above factors on the sensitivity and specificity of detecting a truly reduced efficacy of less than 90 and 95%.

Results: All combinations resulted in a reliable detection of reduced efficacy except for small sample sizes (6-20) combined with a high detection limit (≥ 12) and/or highly aggregated FEC ($k = 0.1$). As expected, an increase in sample size and a decrease in detection limit improved the conclusions drawn. Yet, these differences were small when moderate sample sizes (≥ 20) and high mean FEC (≥ 200 EPG) were combined. Moreover, for drug efficacies ranging from 87.5 to 92.5% and from 92.5 to 97.5% the FECRT did not allow reliable diagnosis of reduced efficacy of less than 90 and 95%, respectively, irrespective of the sample size, detection limit and aggregation of FEC.

Summary/Conclusions: This study confirms that the interpretation of FECRT is affected by a complex interplay of various factors, including the aggregation of FEC. Moreover, we identified study designs in which the reliable detection of reduced efficacy remains unreliable. Finally, some practical guidelines for evaluating anthelmintic efficacy in future monitoring programmes are proposed.

A risk management model for nematode control and drug resistance on Australian sheep farms

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Background: Mathematical models are powerful tools that provide guidance to scientists in complex areas such as veterinary parasitology. In host-parasite grazing systems, the evolution of drug resistance is determined by the extent that survivors of drug treatment contribute their genes to future generations, which is influenced by a number of factors such as anthelmintic exposure, efficacy, parasite biology, host immunity, grazing management and climate. Models enable scientists to bring these factors together in a structured framework allowing questions to be asked in timeframes not achievable if biological studies were undertaken. A number of computer models have been developed to simulate populations of gastro-intestinal nematodes in sheep at pasture. In Australia, Dobson et al. (2011a,b) recently described a multi-species model for sheep in Australian grazing systems. This model is not that accessible to veterinary parasitologists as it is Fortran-based and so a new model (Risk Management Model for Nematodes[®]) has been developed in a Microsoft Excel platform.

Methods: Risk Management Model for Nematodes[®] is a risk management model of sheep nematode parasitism and control strategies catering for different geographical zones and farm management practices within Australia. The model describes over 20-year simulation periods the effect that anthelmintic treatment regimens have on mixed population nematode burdens (*Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus colubriformis*, *T. vitrinus*), and the production and cost benefits associated with control. Five anthelmintic classes including the new amino-acetonitrile derivatives (e.g. monepantel) and spiroindoles (e.g. derquantel) are incorporated into the model. The rate and endpoint of resistance selection can be predicted for each anthelmintic class in each simulation. The model is being validated against the output of the Fortran-based multi-species described above.

Results: The model outputs are presented in easy-to-interpret graphics, that autocorrect as background data such as treatment time or anthelmintics used are altered.

Summary: It is expected that this model will be a very useful support tool to help define how new anthelmintics can be practically used on commercial farms, with the intent of slowing the development of drug resistance to them and the older anthelmintic classes.

References: Dobson, R.J. et al. 2011a,b *Australian Veterinary Journal* 89: in press.

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A computer simulation study to evaluate resistance delaying control strategies with a novel combination anthelmintic product on UK sheep farms

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Background; The increase in reports of anthelmintic resistance (AR) worldwide requires the development and evaluation of control strategies for gastrointestinal nematodes that optimize the use of anthelmintics. With the recent introduction of monepantel, an amino-acetonitrile derivative (AAD), and the prospect of a further new active derquantel, a spiroindole (SI), it is essential that potential control strategies involving them are evaluated prior to their introduction. This study was designed to evaluate the use of a novel combination product containing derquantel and abamectin, a macrocyclic lactone (ML), for use in nematode control strategies as recommended under SCOPS (Sustainable Control of Parasites in Sheep) in the UK. Computer simulation models, which attempt to account for host-parasite, product, flock management and climate interactions, can be extremely useful tools to underpin strategic research and evaluate control strategies aimed at minimizing the development of AR.

Methods: Evaluation of the novel combination product was conducted using a predictive computer, parasite simulation model developed in the UK utilising current SCOPS recommendations to evaluate farm-level performance data and resistance management principles. Model inputs included information on climate, pasture contamination, and lamb worm burdens based on faecal egg counts (FEC), as well as management details including flock size, pasture rotations, lambing date, and anthelmintic treatments.

Results: Results suggest a significant delay in the development of resistance to the new anthelmintic class molecule, when derquantel is used in combination with abamectin compared to use of the two drugs separately, either sequentially or in rotation, across a 40-year time period. This advantage was consistent under both high risk and low risk (SCOPS) management strategies, using data from UK field-based studies as model inputs.

Summary/Conclusions: Model simulation indicated a significant advantage of using derquantel in combination with abamectin. Development of AR to the new active was delayed even in the presence of existing ML resistance. Further, when used in conjunction with other SCOPS recommendations, the combination prevented further development of AR to the ML class of compounds. The results support the findings of other modelling studies conducted in New Zealand on the combination product.

Worming and anthelmintic resistance in English cattle

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Background: The first case of anthelmintic resistance to macrocyclic lactones in bovine nematodes (*Cooperia* sp.) in the northern hemisphere was described in Somerset, England in 1998 but it aroused little interest. After a decade with no monitoring of the development of the problem, investigations were undertaken to see how far it had progressed in the intervening period.

Methods: Fresh faecal samples were collected by 32 farmers from the ground avoiding inclusion of soil at the time of treatment and 14 days later and submitted by post. The initial counts used the McMaster method but due to low egg counts all subsequent counts used the FLOTAC method. Farmers' dosing techniques were checked on a number of farms and found to be correct. Rectal sampling was used by one farmer and during farm visits by staff. Lung worm larvae were detected by FLOTAC.

Results: The majority of farmers were treating when egg counts were low or negligible. As a results many started submitting samples before considering treatment and only treated at housing. Reduced efficacy to ivermectin and other macrocyclic lactones (MLs) was found on 73% of farms whether given as a pour-on or by injection. Failure of control was found for both *Cooperia* and *Ostertagia* but the numbers of farms with resistance to both species was not determined. Benzimidazoles failed to control *Nematodirus* on one farm and *Dictyocaulus* on a second.

Summary/Conclusion: It was clear that many farmers are treating when not necessary. Considerable sums of money could be saved by only treating when required. Resistance to MLs was apparent in gastro-intestinal nematodes on the majority of farms examined, but the percentage of farms with ML resistant *O.ostertagi* resistance is not known. How far resistance to the other anthelmintic groups is present is not known but finding of resistance to albendazole in two species of nematodes on just two farms examined suggests it could be more common than is realised. Clearly strategies must be adopted to reduce the use of anthelmintics in cattle and maintain their efficacy.

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Lamb productivity responses and benefits to monthly STARTECT treatment of grazing lambs exposed to mixed anthelmintic-resistant nematode parasite pasture challenge

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The productivity advantages of using the highly effective anthelmintic were demonstrated in lambs in the presence of a mixed species benzimidazole-resistance nematode infection on pasture. Ten farmlets, each with 30 lambs, were rotationally grazed for 5 months to maximise pasture utilisation and lamb productivity responses. STARTECT (derquantel + abamectin combination) was administered on 5 farmlets at monthly intervals and benzimidazole (albendazole) was used monthly on the remainder. Faecal Nematode Egg Counts (FEC) and larval cultures demonstrated resistance to albendazole in the resident nematode parasite population, dominated by *Teladorsagia* and *Trichostrongylus*, and in the early months, *Nematodirus*. Faecal Egg Count Reduction Testing (FECRT) showed the efficacy of the albendazole was highly variable, ranging between 0% and 93% (overall means of 48.4 % and 40.90% for *Trichostrongylus* and *Teladorsagia*, respectively). This was highly dependent on farmlet, month, FEC, and worm species present at the time. After the 2nd treatment (May) liveweights of the 2 treatment groups commenced to diverge and average body weight of STARTECT-treated lambs was significantly heavier (9kg) after 5 months. Overall live bodyweight gain was significantly greater for STARTECT-treated lambs than for albendazole -treated animals, 25.43 kg and 16.43 kg respectively; $p < 0.001$. As a result, this translated into heavier carcass weights, 23.36 kg and 18.64 kg, respectively; $p < 0.001$ on day 157. Analysis of the growth rates demonstrated that by the 3rd treatment 55% of STARTECT-treated lambs had reached an optimal target weight of 38kg for draft to slaughter; and 99% could have been drafted before the 4th treatment. In contrast, only 29% of albendazole lambs had reached this optimum weight by 3rd treatment and 77% by the 4th treatment. We conclude that the increased productive advantage of using highly effective drenches such as STARTECT in the presence of an anthelmintic resistance can translate into reduced labour and anthelmintic costs as well as improvements in pasture utilisation, productivity, cash flow and financial returns for the producers.

Dynamics of the Gastrointestinal Nematode Resistance to Ivermectin in a Fattening Cattle System

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Introduction: The resistance of the nematode *Cooperia* spp. nematode to ivermectin is widely spread in Argentine. A control plan based on aspects of general and specific antiparasitic management was implemented in a herd going to diminish this condition. The objective of the study was to characterize the development of antiparasitic resistance after 7 years of control.

Method: The study was carried out from 2003 to 2010 in a cattle beef herd at the Marcos Juárez Experimental Station of INTA. In 2003, it was demonstrated an efficacy below 80 % to reduce fecal worm eggs per gram (epg) by ivermectin and began a control plan based on: a) avermectins were no longer used; b) reducing 1/3 number anthelmintic applications with restriction of treatments between ends of spring and summer; c) evaluation of anthelmintic efficacy before the introduction of weaned calves; d) pasture utilization was extended from 2 to 4 years. Resistance was determined in winter/spring of 2003, 2006, 2009 and 2010 by a faecal egg count reduction test (FECRT) according to World Association for the Advancement of Veterinary Parasitology (WAAVP). The groups evaluated were LVM (levamisole 7.5 mg/kg, Ripercol ®), FBZ (febendazole 5 mg/kg, Axilur ®), IVM (ivermectin 0.2 mg/kg, Ivomec ®) and Control (untreated).

Results: The minimum and maximum values of epg of Control groups in pre-treatment were 154 ± 77 to 469 ± 347 . *Cooperia* spp. (28-81 %), *Ostertagia* spp. (8-42 %) and *Haemonchus* spp. (9-38 %) were the predominant nematodes. All FECRTs efficacy was high for FBZ (2003: 100 %; 2006: 97.1 %; 2009: 99.3 %; 2010: 98.6 %) and LVM (2003: 96.4 %; 2006: 98.2 %; 2009: 98 %; 2010: 97.7 %). The efficacy of IVM group showed an ascendent tendency from 2003 to 2009 (2003: 76.7 %; 2006: 84.1 %; 2009: 92.5 %; 2010: 89.7 %). *Cooperia* spp. was the predominant post-treatment genus of IVM group (2003: 85 %; 2006: 67 %; 2009: 100 %; 2010: 100 %).

Conclusions: The efficacy levels obtaining of around 90 % is significant but must be taken into account the precision limitations of FERCT for making ultimate considerations about low levels of differences. Despite the difficulties to estimate the possible susceptibility recuperation time, the tendency obtained is encouraging and needs to be further evaluated.

Targeted treatment and production efficiency based-targeted selective treatment: effects on productivity and ivermectin resistance

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Background: The development of anthelmintic resistance threatens the sustainability of livestock production worldwide. Targeted selective treatments (TST, treatment given to individual animals that will most benefit) and targeted treatment (optimised whole-group treatments) have been proposed as alternative sustainable control strategies for gastrointestinal nematodes.

Methods: A replicated field trial was conducted over 4 consecutive years to compare the effect of treatment regime on lamb performance and the development of ivermectin resistance. A production efficiency based TST approach, where lambs were treated if their weight gain was deemed to be unsatisfactory by a decision support system, was tested. The performance of groups of approximately 40 lambs following the TST approach was compared with three whole-flock treatment groups: a neo-suppressive treatment (NST) group where treatment was administered each month, a targeted treatment (TT) group where anthelmintic was given strategically throughout the grazing season and a metaphylactic/therapeutic (MT) group where treatment was given on the appearance of clinical signs of parasitism. Weight gain was recorded fortnightly throughout the grazing season (May to October) and drug efficacy was monitored after ivermectin treatment using the faecal egg count reduction test.

Results: The mean cumulative liveweight gain ($\pm$ SEM) was the same in the TST and NST groups at 23.11 ± 0.31 kg and 23.11 ± 0.35 kg, respectively, and this was similar to that in the TT group (23.26 ± 0.30 kg). The performance was reduced in the MT group, where the mean cumulative liveweight gain was 21.14 ± 0.29 kg.

The mean number of treatments administered per lamb was 2.75, 5.25, 3.25 and 2.25 in the TST, NST, TT and MT groups, respectively. Initially, efficacy of ivermectin treatment in all groups was 97%. Throughout the study slight reductions were observed in the TST, MT and TT groups, with mean efficacy (95% confidence interval) of treatment over the 4 years of the study being 90.2% (92.4-87.4), 96.1% (97.1-94.6) and 91.6% (93.7-88.7), respectively, whereas in the NST group mean efficacy had declined to 68.8% (75.2-60.7).

Summary/Conclusions: Production efficiency based-TST appears to provide optimal use of anthelmintic in terms of numbers of treatments and maintenance of efficacy and lamb performance.

Restoring phenotypic susceptibility to benzimidazol in resistant *Haemonchus contortus* populations in goats

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Background: The reversion of resistant parasite strains to susceptibility has been proposed as a possible solution to the problem of anthelmintic resistance in ruminant systems. Reversion has been attempted by replacing the resistant worm population with a susceptible one (using anthelmintic treatments). Such replacement of resistant populations could be difficult to implement. The aim of the study was to restore the phenotypic susceptibility to BZ in resistant *Haemonchus contortus* populations in goats.

Methods: Two strains of *H. contortus* were characterized phenotypically (egg hatch test, EHT), and genotypically (allele-specific PCR) as BZ-resistant (BZres) and BZ-susceptible (BZsus). Eight goats reared free of GIN infection were artificially infected with different proportions of the two strains. Each goat received 2000 L₃ of *H. contortus* in the following proportions: goat 1 (100% BZsus), goats 2 and 3 (75% BZsus), goats 4 and 5 (50% BZsus), goats 6 and 7 (25% BZsus) and the goat 8 (100% BZres). At 28 days post-infection, eggs from the feces of each animal were retrieved and evaluated with EHT. The percentage of inhibition of hatching (PIH) was plotted and the lethal dose 50 (LD₅₀) and 95 (LD₉₅) and 95 % confidence intervals (95% CI) were calculated. On day 49 post-infection, all the goats were re-infected with 2000 L₃ of *H. contortus* BZsus. After 28 days of the re-infection, the EHT was repeated to determine the PIH and the LD₅₀ and LD₉₅ (95% CI).

Results: After the first infection eight populations of *H. contortus* were formed: three BZsus populations (goats 1, 4 and 6) and 4 BZ-resistant populations (goats 2, 3, 7 and 8). After the second infection with 2000 BZsus L₃, the 4-resistant populations showed phenotypic susceptibility.

Conclusion: The re-infection with a BZsus strain of *H. contortus* restored the phenotypic susceptibility to BZ in four populations that were phenotypically resistant. Two hypotheses may explain the present results: a) a substitution of populations after the second infection or b) the crossbreeding of the established and the incoming population. The results suggest that the strategy of re-infection with a BZ-susceptible strain can be considered as a viable option for the phenotypic reversion to BZ-susceptibility in goats infected with *H. contortus*.

Performance of dairy calves subjected to a targeted selective anthelmintic treatment (TST) regime in their first season

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Background: Despite knowledge that suppressive anthelmintic treatments select strongly for anthelmintic resistance in parasite populations, little attention has been paid to the sustainability of anthelmintic practices in New Zealand dairy systems. Many dairy calves are frequently exposed to suppressive or neo-suppressive anthelmintic regimes in order to ensure animals reach live weight targets. Here we investigate a performance-based targeted selective treatment anthelmintic regime in grazing dairy calves during their first year of grazing.

Methods: Two commercial dairy herds carrying 107 (Herd A) and 100 (Herd B) Friesian and Friesian x Jersey calves in Canterbury, New Zealand were used. Animals were allocated to treatment groups that were exposed to either a neo-suppressive anthelmintic regime (NST) or a targeted selective anthelmintic regime (TST) in which anthelmintic treatments were only administered to those failing to reach pre-determined liveweight gain targets. Treatments started at around 4-months-of-age and continued until animals were 13-months-of-age.

Results: Overall, NST animals in Herd A received 6 treatments while NST animals in Herd B received 7 treatments. Compared with their NST counterparts, the TST regime resulted in a 72% and 47% reduction in total anthelmintic use for Herds A and B, respectively. Animal performance was slightly compromised by the TST regime with mean cumulative liveweight gain during the trial period reduced by 5% in both herds, being 174.7 ± 2.97 kg and 165.9 ± 2.67 kg in Herd A ($P=0.03$) and 93.0 ± 2.36 kg and 88.1 ± 2.45 kg in Herd B ($P=0.15$) for NST and TST, respectively. The concentration of nematode eggs in the faeces was generally low, with mean values for all animals being 43 and 97 eggs per g for Herd A and B, respectively, and were not affected by treatment ($P>0.05$).

Summary: Overall, this study provides clear evidence that a performance-based TST regime can provide an effective means of reducing anthelmintic use in grazing dairy calves compared with a neo-suppressive regime with a minimal impact on animal growth. Furthermore, the difference in performance between Herd A and Herd B clearly suggest that environmental factors that influence calf growth and/or animal age need to be taken into account in order to set target liveweight gains that will optimise anthelmintic use.

Developing and refining a performance-based targeted selective anthelmintic treatment regime for grazing dairy calves

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Background: Recent studies have highlighted the risk of anthelmintic resistance in dairy heifer calves due to use of neo suppressive drenching regimes. For these systems, effective parasite control and reduced anthelmintic use may be achieved through targeted selective treatment (TST) anthelmintic regimes. Conventionally, parasite control is based on monitoring faecal egg counts (FEC). However, recent investigations in sheep suggest that FEC is a poor indicator of which individuals are likely to benefit from treatment. This investigation sought to explore if the same phenomenon exists in grazing dairy calves and to determine if this information could be utilised to develop and refine targeted selective treatment options for dairy calves.

Methods: 106 Friesian and Friesian x Jersey calves that were exposed to a range of anthelmintic regimes were used. Individual FEC and live weights (LW) were monitored at four to six week intervals from around 4-months-of-age until 13-months-of-age. At each sampling time, the relationships between FEC and animal performance were analysed to determine if FEC provides a useful indicator as to which animals may be suffering production losses from parasitism.

Results: There was considerable variation in the distribution of FEC both within and between sampling times, with individual values ranging from 0-100 epg on one sampling occasion to 0-1400 at another. Regardless of sampling time, there was no association between FEC and animals liveweight gain (LWG) ($P > 0.05$ for all), thus animals with high FEC grew as well as those animals with low FEC. This suggests FEC is a poor indicator for animals that are suffering from parasitism. Furthermore, the growth of dairy calves under a suppressive anthelmintic regime demonstrated a strong linear relationship between calf LW and LWG ($LWG = (LW \times 0.0086) - 0.375$, $r = 0.72$) between 50 to 150 kg LW. For animals above 150kg LW, 700-800 g/d growth was a suitable target as animal performance plateaus until the winter when LWG was reduced due to restricted feed availability.

Summary: FEC is a poor indicator of animal performance. Performance targets for a TST regime up to 150kg live weight can be set based on live weight. For animals in excess of 150kg, 700-800 g/d growth is a suitable liveweight gain target for a TST regime until feed becomes restricted during winter.

Field Efficacy of Multi-Dosage Forms of Fenbendazole in US Cattle Using the Fecal Egg Count Reduction Test

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Background: The field efficacy of multi-dosage forms of fenbendazole in US cattle was determined using a Fecal Egg Count Reduction Test [FECRT]. A total of 114 FECRT trials were conducted in 20 major livestock states in collaboration with 60 veterinary clinics or consulting veterinarians. Another 38 FECRT trials were conducted using fenbendazole drench formulation in combination with different macrocyclic lactone injectable or pour-on products.

Methods: Cattle used for all trials were naturally parasitized calves or yearling cattle. At least 18 animals were sampled for each trial 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 was not informed of the treatment until the analysis was completed. Completed FECRT reports were sent off to a national cattle parasite database supported by Intervet/Schering-Plough Animal Health.

Results: A total of 4,844 samples were analyzed (2,427 samples collected prior to treatment and 2,417 samples collected post-treatment). The mean efficacy of a fenbendazole drench formulation was 99.8% (73 trials; 2,901 samples), of a fenbendazole medicated feed was 99.9% (18 trials; 1,010 samples), of a paste formulation was 99.3% (2 trials; 81 samples), of a medicated mineral formulation was 98.9% (6 trials; 223 samples), of a medicated liquid feed was 96.6% (10 trials; 388 samples) and of a medicated cube or block was 93.4% (5 trials; 201 samples). Administration of a fenbendazole drench concurrently with a macrocyclic lactone injectable or pour-on formulation demonstrated an efficacy of 99.6% (38 trials, 1,459 samples).

Summary/Conclusions: These results were generated under field use conditions with the supervision of the veterinary service provided by each participating clinic. The significant use of macrocyclic lactone in the US appears to have altered the parasite populations from dominate *Ostertagia* species to more *Cooperia* species. This shift could explain the reduced occurrence of Type II *Ostertagiasis* and enhancing fenbendazole's overall efficacy found under natural field conditions.

Comparative assessment of ivermectin, abamectin and moxidectin efficacies against resistant nematodes in lambs

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The macrocyclic lactones (ML) (ivermectin (IVM), abamectin (ABM) and moxidectin (MXD) are extensively used in parasite control in ruminants. Resistance has emerged in different gastrointestinal nematodes after several years of their intensive use. An advantageous efficacy pattern against resistant nematodes was previously obtained for the oral administration of ML compared to the subcutaneous route. Although the three ML acts by the same mechanism, some differences in the efficacy against resistant nematodes have been observed particularly after their oral administration to infected sheep. The aim of the current work was to compare the efficacy of IVM, ABM and MXD against IVM-resistant nematodes following their intraruminal administration to infected lambs.

Twenty four (24) lambs naturally infected with IVM-resistant gastrointestinal nematodes were used. Animals were allocated into four (4) experimental groups (n=6), including an untreated control and those intraruminally treated (0.2 mg/kg) with either IVM, ABM or MXD. Individual fecal samples for nematode egg counts were collected at 0, 6, 9 and 15 days post treatment. Post-mortem examination was done at day 15 after treatment. The recovered adult gastrointestinal worms were identified by species and counted. The faecal count reduction test (FECRT) and percentage of efficacy on each nematode species were obtained according the WAAVP guidelines. The Kruskal Wallis test was applied to the statistical comparison.

A high level of resistance against the ML compounds was observed. The FECRT showed a complete failure for both IVM and ABM (0% reduction), while the egg reduction after the MXD treatment reached 86.8%. The overall efficacy against adult nematodes showed differences among ML, ranging from 84.8% (IVM) and 90.2% (ABM) to 98.0% (MXD). *Haemonchus contortus* appeared as the most resistant species, where the failure of IVM (20% efficacy) and ABM (39%) contrasted with the 89.6% efficacy obtained after the MXD treatment.

The advantage of the oral/intraruminal compared to the parenteral route and the differential efficacy patterns observed among different ML should be seriously considered to optimize nematode control in the early stages of resistant development before a complete therapeutic failure is observed.

Macrocyclic lactones and *Cooperia* spp in cattle in Argentina. Persistence of status of resistance after five years without the use of these drugs

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Background: in March 2004, *Cooperia* spp resistant to ivermectin (fecal egg count reduction test < 45%) was detected in Holstein calves belonging to the experimental station of INTA Rafaela, Santa Fe, Argentina. From then on, use of macrocyclic lactones was supplanted by levamisole and benzimidazoles. In April 2009 a field study was carried out to: a) determine whether the non-use of ivermectin during the last five years could have affected the susceptibility of *Cooperia* spp to this drug and b) compare the efficacy of ivermectin and moxidectin against this nematode.

Methods: twenty four Holstein male calves (4 to 6 months age) were selected on basis of nematode eggs per gram (epg) of faeces (> 150) and allocated to comparable groups of 12 animals each ($p = 0.324$). On day 0, animals in the first group were treated subcutaneously with moxidectin 1% at a rate of 0.200 mg / kg body weight ("Alfa Cydectin" ® Fort Dodge, Argentina) while remaining calves received ivermectin 1% by the same route and dose ("Ivomec" ® Merial, Argentina). Fecal samples from each animal were collected on treatment day and 17 days later for epg count. Composite fecal cultures were prepared for each group for determination of parasite genera. The reduction of epg for each treatment was calculated using the following formula = $100 \times 1 - (T2/T1)$ where T1 and T2 represent average epg before and after treatment respectively. Epg distribution differences between groups were assessed by Wilcoxon test.

Results: Upon treatment, parasite genera present were *Cooperia* 59%, *Haemonchus* 39% and *Trichostrongylus* 2%. After treatment, all larvae obtained in both groups belonged to *Cooperia* spp. Epg reduction caused by moxidectin (62%) was higher ($p < 0.001$) than with ivermectin (2%) but effectiveness of both drugs was much lower than 90% considered as "cut off" and indicative of resistance.

Summary: *Cooperia* spp remained with the resistance status after five years without use of macrocyclic lactones. Cross-resistance between ivermectin and moxidectin was confirmed. Moxidectin may show greater relative activity than ivermectin, but still result in reduction rates hpg incompatible with an efficient control of these parasites

Is larval development test reliable enough to detect ivermectin resistance in the field?

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Background: To aid the diagnosis of anthelmintic resistance (AR), a range of in vivo and in vitro techniques have been developed. Amongst in vitro techniques the egg hatch test and larval development test (LDT) are the most widely employed. While LDTs differ as to whether they use liquid or agar culture media, the basic principle is the same in that the eggs are cultured to the infective larval stage in the presence of different drug concentrations. The test is claimed to detect resistance against benzimidazoles, however, the authors reported some problems with the detection of ivermectin resistance due to the lack of a normal dose-response curve and sensitivity of the test. The objective of this study was to assess the sensitivity of LDT for the detection of low levels of ivermectin resistance.

Methods: Fourteen lambs were infected with susceptible (7) and ivermectin resistant (7) isolates of *Haemonchus contortus*. Faecal samples for LDT were collected three times from 35 to 45 days post infection. Micro agar LDT was performed as described by Coles et al. (2006). Different proportions of resistant and susceptible eggs were incubated in ivermectin aglycone and percentage of resistant eggs ranged from 1.5% to 12.0 % of all eggs in the wells. After 7 days of cultivation the number of L3 larvae at each concentration level was determined. After treatment with recommended dose of ivermectin the faecal egg count reduction in both isolates has been evaluated.

Results and conclusions: The faecal egg count reduction test revealed two distinct populations of *Haemonchus contortus*, first fully susceptible and second highly resistant. The test was able indicate the presence of approximately 10% of resistant worms amongst a susceptible background population. The probability to positively diagnose only 2% of resistant worms within the population was approximately 50%.

The efficacy of Trichlorphon and Naphtalophos against anthelmintic resistant nematodes of naturally infected sheep in Argentina

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An anthelmintic efficacy trial in sheep harbouring resistant worms to anthelmintics was carried out in Argentina. Seventy lambs, 10 to 12 months old and 26 kg in average were selected from a flock which had been grazing on pastures naturally infected with trichostrongyles resistant to the main groups of anthelmintics. The lambs were allocated into 7 comparable groups of 10 animals each and were treated as follows: G1 and G2, Trichlorphon (Nevugon®) and Naphtalophos (Baymetin®) respectively, both orally at the dose rate of 50 mg/k.b.w.; G3, Ivermectin (Ivomec®) subcutaneous via at 0.2 mg/k.b.w., G4, Fenbendazole (Axilur®) orally at 5 mg/k.b.w., G5, Levamisole (Ripercol®) subcutaneously at 8 mg/k.b.w. and G6, Closantel (Galgosantel®) orally at 10 mg/k.b.w. The G7 remained as non treated control group. The groups of treatments remained on separated concrete floored pens and fed dry grass free of infective trichostrongyle larvae and fresh water *ad-libitum*. Faecal samples were collected at days 0 and 10 post treatment (p.t.) to estimate the clinical efficacy (FECRT). Six animals of each treatment group, were necropsied at day 10 p.t. for counting and identification of the worms from abomasum, small and large intestines and determine the absolute efficacy of each test compound (CET). The clinical efficacy of Trichlorphon and Naphtalophos was 74.5% and 91.5% respectively. For G3, G4, G5 and G6 the efficacy was 98.4%, 91.2%, 86.7% and 67.1% respectively. Trichlorphon and Naphtalophos showed to be highly effective (> 99%) against *Haemonchus contortus* ($P < 0.05$); Naphtalophos also shown to have a high efficacy on *Trichostrongylus axei* (99.3%), *Teladorsagia circumcincta* (97.8%), *Trichostrongylus colubriformis* (99.2%), *Cooperia punctata/curticei/pectinata* (90.4%), *Nematodirus spathiger* (89.2%) and *Oesophagostomum venulosum/columbianum* (93.7%). Fenbendazole and Levamisole showed a high efficacy against all nematodes (> 95%) with the exception of *T. colubriformis*. The efficacy of Ivermectin was low against *H. contortus* (23%) and *Cooperia spp.* (46.3%). Closantel showed a low efficacy on *T. axei* (64.4%), *H. contortus* (80.6%) and *T. colubriformis* (59.5%). In a context where anthelmintic resistance is a widespread phenomenon, the use of Trichlorphon -when *H. contortus* is involved- but mainly Naphtalophos, could be a therapeutic alternative to be incorporated in control worm programmes.

Comparative efficacy of Trichlorphon and a Trichlorphon – Ivermectin combination treatment against anthelmintic resistant nematodes of cattle in Argentina

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A controlled anthelmintic efficacy trial (CET) in naturally infected cattle with resistant worms was carried out in Buenos Aires province (Argentina). Twenty-four Holando Argentino calves of 6 months and of 150 kg were used. The animals were grazing on naturally infected grass and were selected according to their faecal egg counts (e.p.g.), identified and allocated into 4 comparable groups of 6 animals each. The animals in G1 were treated orally with Trichlorphon (TCF) (Nevugon®) at the dose rate of 50 mg/k.b.w. and those in G2 received Ivermectin (IVM) (Ivomec®) subcutaneous via at the dose rate of 0.2 mg/k.b.w. The calves of G3 were treated with a combination of the test compounds TCF+IVM using the same via and dose rate of administration than those of G1 and G2 respectively. The G4 remained as non treated control group. The groups of treatments remained on separated concrete floored pens and fed dry grass free of infective trichostrongyle larvae and fresh water *ad-libitum*. Faecal samples were collected at days 0 and 14 post treatment (p.t.) to perform the faecal egg count reduction test (FECRT) to estimate clinical efficacy. All calves were necropsied at day 14 p.t. for counting and identification of worms from abomasum, small and large intestines and lungs to determine the absolute efficacy (CET) of each test compound. The clinical efficacy of TCF was 69.2%, IVM 76.2% and for TCF+IVM 99.4%. The absolute efficacy of IVM and IVM+TCF was 100% (P<0.05) against *Haemonchus placei*, *Trichostrongylus axei* and *Ostertagia ostertagi*. TCF showed the same performance but, against *O. ostertagi* the efficacy was 84.7%. The efficacy of IVM against *Cooperia oncophora/punctata /pectinata*, *Trichostrongylus colubriformis* and *Nematodirus helvetianus* was 80.4%, 79% and 0% respectively; the efficacy of TCF was 95.7%, 86.2% and 100% and that of TCF+IVM was 99.6%, 94.1% and 93.8% (P<0.05). The efficacy of TCF, IVM and TCF+IVM was 100% against *Oesophagostomum radiatum* and *Trichuris spp.* (P<0.05). The efficacy of TCF against *Dictyocaulus viviparus* was 52% and 100% for IVM and IVM+TCF respectively (P<0.05). The overall analysis indicates that the combination TCF+IVM reached an efficacy of 99.5% being an alternative for the control of nematode infections including IVM resistant strains.

The control of multiple anthelmintic resistance in grazing cattle of Argentina: results on the use of a rational programme during seven years

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The results from a rational control program in fattening cattle naturally exposed to multiple anthelmintic resistant trichostrongyles are described. In 2003 around 140 calves died on grazing pastures as a consequence of high worm burdens, despite the use of monthly alternate treatments with Ivermectin (IVM) or Fenbendazole (FBZ). After this event, fecal egg count reduction test (FECRT) showed a clinical efficacy of 73% for ivermectin (IVM), 49.1% for fenbendazole (FBZ) and 90.4% for levamisole (LVM). Some animals were necropsied to perform an efficacy controlled test (ECT) and results showed that efficacy of IVM was 76.1% and 22.6% against *Haemonchus spp.* and *Cooperia spp.* respectively and that of FBZ was 0% against *Ostertagia spp.*, 28.3% for *Haemonchus spp.* and 24.2% against *Cooperia spp.* respectively. In order to reduce the impact of subclinical losses, a rational control program based on faecal egg counts, faecal cultures and epidemiological back grounds was established. LVM was selected as the anthelmintic to be administered when recommended. In 2004 the programme advised the administration of three treatments (April, July and September), two in 2005 (June and August), one in 2006 (May), one in 2007 (May), one in 2008 (April), no one in 2009 (dryness for a 3 years period help for that) and two in 2010 (June and September). In 2006 a light recovery of the efficacy of IVM and FBZ was recorded, although in a less extension against *Cooperia spp.* and *Ostertagia spp.* respectively. After seven years the results of a FECRT indicated a clinical efficacy of 78.3% for IVM, 93.1% for FBZ and 100% for LVM, whereas an ECT showed that the efficacy of IVM was 0% against *Haemonchus spp.*, 99% on *Ostertagia spp.* and 0% against *Cooperia spp.* On the other hand, FBZ showed an efficacy of 100%, 73.9% and 100% respectively. The implementation of a seven years rational control programme successfully stopped mortality, minimized subclinical losses in grazing animals and reduced drastically the number of anthelmintic treatments. On the other hand, population of helminths maintained the resistance to IVM (*Haemonchus spp.* and *Cooperia spp.*) and FBZ showed a light recovery of the overall efficacy. The effects of dryness on worm infections and the use of FECRT in the practice to manage the anthelmintic resistance in grazing animals are discussed.

Cattle worms resistance to Ivermectin treatments: effects on production

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During 2008 and 2009 years, ten field trials in the main cattle productive area of Argentina to evaluate the impact of worm resistance to ivermectin treatments were carried out. The trials involved castrated male calves of 10-12 months old which were grazing on improved pastures naturally infected with worm populations which had suspicious of resistance to anthelmintics. In each of the farms the calves were identified, weighed and assigned to 3 comparable groups of 20 animals each and were treated as follows: G1: ivermectin (IVM) subcutaneous via, at a dose rate of 0.2 mg/kilo body weight (k.b.w.); G2: moxydectin (MXD) subcutaneous via, at a dose rate of 0.2 mg/k.b.w. and G3: non treated control. Faecal samples were collected from animals at days 0 and 14 after treatments to establish the faecal egg count reduction test (FECRT) for each test compounds and for cultivation to determine worm species. Individual body weight was recorded at days 0 and 90 post treatment. Resistance to IVM was found in 10/10 farms and for MXD in 1/10 farms. The average of FECRT for G1 was 63.2% (min. 2.2% and max. 89.3%) and for G2 was 92.7% (min. 55% and max. 100%) respectively. *Cooperia spp.* was the predominant nematode in the all faecal cultures of the farms where resistance to IVM was established, it being the single worm recovered in 8/10 farms. *Haemonchus spp.* was present in coprocultures of 2/10 farms located in the subtropical area after the IVM treatments. In the first year of the study and over 90 days of grazing the differences of weight gain in average of G1 and G2 upon G3 were 6.3 kg and 13.3 kg respectively (Tukey, $P < 0.05$). During the second year, differences were 11.7 kg and 14.8 kg respectively (Tukey, $P < 0.05$). The overall analysis of the two years study determined an average weight gain difference of G1 and G2 upon G3 of 10.2 kg and 15.4 kg respectively (Tukey, $P < 0.05$). These results suggest that resistance of *Cooperia spp.* –mainly- to ivermectin treatments may affects the productivity of grazing animals.

Comparative haematological values of parasitised and nonparasitised cattle in Abeokuta Southwest, Nigeria

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The use of haematological parameters in assessment of the health status of domestic animals is of paramount importance in clinical diagnosis of haemoparasitism. A comparative study on different haemoparasitic diseases of cattle compared with non-parasitized cattle was performed using different haematological indices in Abeokuta, Southwest Nigeria. This was done to provide information on the differences in the haematological indices of different breed of cattle with and without haemoparasitic infection. The study was carried out between June and October 2009. Two hundred blood samples were used, which consist of 166 females (83%) and 34 males (17%), which are White Fulani (75%), Sokoto Gudali (7.5%), Red Bororo (9.5%), Muturu (0.5%) and Cross breeds (7.5%). The mean PCV in infected White Fulani, Sokoto Gudali, Red Bororo, and Cross breeds of cattle were significantly lower ($p < 0.05$) (23.40 ± 3.48 , 30.75 ± 4.59 , 24.80 ± 4.3 and 29.5 ± 3.91 respectively) compared with non-infected cattle (34.60 ± 5.74 , 38.66 ± 3.45 , 33.80 ± 5.52 and 37.56 ± 4.43 respectively). The mean total WBC were significantly higher ($p < 0.05$) in infected White Fulani, Sokoto Gudali, and Red Bororo of cattle (9.27 ± 3.20 , 8.48 ± 2.22 and 8.95 ± 2.39 respectively) compared with non-infected cattle (7.72 ± 3.68 , 5.32 ± 1.81 , 6.43 ± 3.98 respectively). Conversely, the WBC was significantly ($P < 0.05$) low in the Cross breed cattle (6.82 ± 1.98) compared with the other three breeds of cattle. There was no significant difference in the WBC and the differential leukocyte counts between the four breeds of cattle. But, the range of the % Eosinophils count was significant ($P < 0.05$) in the White Fulani cattle (0-9) compared with the other breeds of cattle. The haemoparasites detected in the stained blood films of cattle sampled in this study were *Anaplasma spp.* (12%), *Anaplasma spp* and *Babesia spp* (10%), *Babesia spp.* (8.5%), *Trypanosoma spp.* (6%), *Babesia spp.* and *Trypanosoma spp.* (5%), *Anaplasma spp.*, *Babesia spp.* and *Trypanosoma spp.* (5%) among others. Various health implications of these parasites like anaemia, low resistance to other diseases and reduced productivity among others were discussed. It was concluded that there is need for various control plan to improve the health of cattle and production in Nigeria among which are good husbandry practice, use of resistant breed, improved control of ectoparasites and education of farmers on the need to keep their flock disease free.

Effect of avermectins on the performance of weaned Nelore calves naturally infected by ivermectin-resistant nematodes

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Background: The use of anthelmintic drugs must follow technical criteria for best economic return, especially on farms where the nematode population is resistant. This study evaluated the effect of four commercial endectocides on the weight gain of weaned Nelore calves, maintained under grazing and naturally infected by ivermectin-resistant gastrointestinal nematodes

Methods: One hundred Nelore calves, fifteen months-old, were selected among 140 weaned animals. These animals were divided into five groups in a randomized block design, based on body weight at day zero and weight gain in pre-trial period: Group 1: 2.25% ivermectin (450 mcg / kg) + 1.25% abamectin (250 mcg / kg), group 2: 3.15% ivermectin (630 mcg / kg), group 3: 3.5% doramectin (700 mcg / kg), group 4: 1% doramectin (200 mcg / kg) and group 5: control. To estimate weight gain, individual weight was assessed on days -28, zero, +28, +56, +84 and +112, always with the animals on water and food restriction for 12 hours prior to weighing. Feces samples were collected on all observational dates for measurement of parasite burden by counting of eggs per gram of feces (EPG). It was observed infections by *Haemonchus*, *Cooperia*, *Trichostrongylus* and *Oesophagostomum*. The two first nematode showed to be resistant to ivermectin in a previous FECRT.

Results: After 112 days, cattle treated with 2.25% ivermectin + 1.25% abamectin showed an increase of live weight gain of 11.85 kg compared to untreated, 9.41 kg higher than those treated with doramectin 1% , 9.05 kg when compared to those treated with ivermectin 3.15% and 6.10 kg compared to those treated with doramectin 3.5%. It was observed no difference ($P>0.05$) between the weight gain of calves treated with 3.15% ivermectin or 1% doramectin. Only the treatment with the ivermectin + abamectin association resulted in weight gain superior to the untreated group on days +56 ($P<0.05$) and +112 ($P<0.001$) and reduced the EPG counts.

Conclusions: The commercial formulation containing ivermectin 2.25% and 1.25% abamectin showed higher residual period than the other evaluated avermectins and was the unique formulation that significantly increased the weight gain of the calves infected by ivermectin-resistant nematodes.

Drug resistance in animal trypanosomes in the absence of drug pressure

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Background: African animal trypanosomiasis, mainly due to *Trypanosoma congolense*, is a major constraint to animal health and production in sub-Saharan Africa, whose control is mainly based on use of trypanocides. This control method is now seriously hampered by emergence of drug resistance. Diminazene aceturate (DA) resistance has been shown to be correlated to a point mutation in the P2-type purine transporter responsible for DA uptake. Drug resistance inducing mutations have been shown to occur randomly in nature but found at low prevalence due to the mutationselection balance, since they tend to incur a fitness cost on the parasite. The purpose of this study was to identify the origin of drug resistant trypanosomes and more specifically, to determine whether drug resistance traits occur in wildlife trypanosomes which were never exposed to the drug and thus help in our understanding of the dynamics and spread of trypanosome resistance to DA.

Methodology: 12 *T. congolense* recently isolated from tsetse or wildlife in game reserves in Zambia and South Africa were screened for the mutation inducing DA resistance using *bclI*-PCR-RFLP as described by Delespaux *et al* (2006). For characterization of sensitivity to DA, the standardized mouse inoculation method described by Eisler *et al* (2001) was modified to include lower doses of 10 & 5mg/kg and detection using PCR at the end of the experimental period.

Results: The point mutation linked to DA resistance was shown to be present in all the strains. By microscopy, 100% (n=24), 0.08% (n=72) and 0% (n=72) of the mice relapsed at the doses of 5, 10 and 20mg/kg respectively. By PCR, 100% (n=24), 51.4% (n=72) and 38.9% (n=72) of the mice relapsed at doses of 5, 10 and 20 mg/kg respectively.

Conclusion: All the isolated strains are potentially resistant since they carry the point mutation which induces resistance. However, the mere presence of the mutation does not imply phenotypic resistance since all strains did not relapse microscopically. There were some relapses observed using the more sensitive PCR, with the affected animals remaining healthy, indicating presence of low parasitaemia and reduced pathogenicity. This indicates that use of DA in animals infected with genotypically resistant strains reduces disease severity and transmission. There is however an increased danger of selecting for more resistant strains.

Comparison of the efficacy of new anthelmintics against multidrug resistant nematodes

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There is an urgent need for new anthelmintic classes in areas where multiple drug resistance is present and classical anthelmintics are no longer active. Recently two new active ingredients became commercially available for the treatment of nematode infections in sheep, monepantel (Zolvix®) and derquantel, the latter formulated as a combination with the macrocyclic lactone, abamectin (Startect®).

In order to assess the potential of the new actives for the control and prevention of spread of anthelmintic resistance of gastrointestinal nematodes, recent field isolates with a history of multiple resistance were used for experimental infection of sheep. Three treatment groups were defined: (1) monepantel at 2.5 mg/kg bodyweight, (2) 2.0 mg/kg derquantel in combination with 0.2 mg/kg abamectin and (3) abamectin alone at 0.2 mg/kg bodyweight. Each active was applied in the commercially available formulation, i.e. Zolvix®, Startect® and Genesis®. A fourth group remained untreated and served as control. Several studies were performed to compare the efficacy of the three treatments against larval (L4) and adult stages. This was determined by fecal egg count reduction and by worm counts.

The results demonstrate that monepantel was fully effective against multi-resistant species *Trichostrongylus colubriformis* and *Haemonchus contortus*. In contrast, the combination of derquantel and abamectin was effective against *T. colubriformis* but was not effective against the larval stages of *H. contortus*.

Frequency of cattle herds with ivermectin resistant nematodes in the sub-humid tropics of Mexico

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Background. Ivermectin (IVM) resistant nematodes are becoming a common problem in cattle herds world-wide. The situation of IVM resistance in beef and dual purpose cattle herds under hot tropical conditions of Mexico has not been investigated. The objective was to determine the frequency of cattle herds with IVM resistant nematodes in a subhumid tropical zone of Mexico using the faecal egg count reduction test (FECRT).

Methods. Thirty three herds (28 beef and 5 dual purpose herds) were monitored in a period of 6 months (September 2008 to February, 2009). Only 14 of the 33 herds were included in the trial. The other herds had not enough animals with sufficient nematode eggs in the faeces to be included in a FECRT. Some farms were visited twice trying to find more animals with egg counts higher than 150 eggs per gram of faeces. In the 14 surveyed herds the calves were randomly distributed in two groups: a) Treatment groups received 0.2 mg of ivermectin (IVM) / kg BW sc on day 0, and b) Control group without treatment. Faecal samples were obtained from each animal on days 0 and 14 post-treatment. Reduction percentages (% R) and 95 % confidence interval (95%CI) were calculated. A questionnaire survey was applied in all the farms to describe the management of the different cattle herds.

Results. The frequency of cattle herds with IVM resistant nematodes was 78.6 %. The remaining herds were suspected of IVM resistance (21.4 %). Not a single herd was found with susceptible worms. All the surveyed herds used IVM from two to three times per year (mainly beginning and end of the wet season). The farm with stronger resistance used IVM for 11 consecutive years (% R = 0%, 95 % CI = 0 - 47%). The treatment scheme used in the cattle herds surveyed seemed to affected refugia even at this low treatment frequency. Genera of nematodes resistant to IVM were: *Ostertagia*, *Haemonchus*, *Cooperia* and *Trichostrongylus*. A considerable effort is needed to perform FECRT in cattle herds under hot subhumid tropical conditions.

Conclusion. Beef and dual-purpose cattle farms in the hot sub-humid tropics of Mexico (Yucatan) showed a high frequency of IVM resistant nematodes.

Five years of targeted selective treatment against gastrointestinal nematodes in a mixed sheep and goat herd in the tropics

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The aim of the study was to design and test a targeted selective treatment (TST) scheme that would reduce the number of adult female hair-sheep and goats treated with an anthelmintic (AH) in a herd kept under subhumid tropical conditions. The study was performed from January 2006 to December 2010 in a farm using tropical forest as the main source of food (20°52'07" N; 89°37'24" W). Rainfall in the area is concentrated from May to December (mean of 960 mm per year). The flock consisted of a commercial mix of Pelibuey with different proportions of Blackbelly and Katahdin sheep (average 60 females per year), and local Criollo Goats (average 120 females per year). Once a month, all the females were monitored using the TST scheme which includes the FAMACHA® card and a body condition score (BCS) scheme (where 1 was emaciated and 5 was fat). Animals showing mild to severe signs of anaemia (FAMACHA® 3, 4, 5) and/or a low BCS (1, 2) were faecal sampled to determine their faecal egg counts (EPG) using a McMaster technique. Animals with more than 750 EPG were treated with an effective dose of levamisol (dose according to the ruminant species). The same procedure was followed every month. Goats had 3 times more chances to score a low BCS (1, 2) than sheep (OR=3.27; 95%CI= 2.9-3.7; P<0.05). Also, goats had 10 times more chances to show a FAMACHA® 4-5 than sheep (OR=10.24; 95%CI=9.2-11.4; P<0.05). As a result, goats had two times more chances of being sampled to determine their EPG than sheep (OR=2.05; 95%CI=1.8-2.4; P<0.05). Nevertheless, the use of FEC helped to keep a similar proportion of goats needing an AH treatment compared to sheep (OR=1.24; 95%CI=0.95-1.63; P>0.05). On average, the proportion of animals needing no AH treatment in a whole year was similar between sheep and goats (62.5% and 57.3%, respectively). Animals needing one treatment per year were 27.3% and 27.7% for sheep and goats. Animals needing two AH treatments were only 8.6% in sheep and 10.6% in goats. Animals needing three or four treatments per year were scarce (1.33% and 5.4% in sheep and goats, respectively).



SESSION M

Keynote Lecture

- **Good Management Practices and Changing Targets: Current status of resistance issues with companion animal parasites** - D. D. Bowman

Oral Communications

- **Angiostrongylus vasorum in the United Kingdom - a nationwide postal questionnaire survey** - M. Fox
- **Rhipicephalus sanguineus (Ixodida, Ixodidae) as an intermediate host of a canine Cercophthifilaria species (Spirurida, Onchocercidae)** - E. Brianti
- **Quantification of Blood Consumption by Fleas (Ctenocephalides felis) on Dogs Treated with Dinotefuran, Pyriproxfen, and Permethrin (Vectra 3D®) based on Polymerase Chain Reaction (qPCR) Detection of the Canine HMBS Gene Sequence** - B.L. Blagburn
- **Molecular identification of phlebotomine sand flies by mitochondrial and ribosomal DNA polymorphism** - M. S. Latrofa
- **Distribution and efficacy of four different spot-on formulations of permethrin in dogs artificially infested with Dermacentor reticulatus** - S. Wolken
- **In Vitro Bioassay For Detection of Anthelmintic Resistance In Dirofilaria immitis** - R. Kaplan

Posters

- **Suppression of Dirofilaria repens Microfilariaemia in Naturally Infected Dogs Using a Single Spot-on Application of a Solution Containing Imidacloprid 10 %/Moxidectin 2.5 % (Advocate®, Bayer Animal Health)** - D. Traversa
- **Seroepidemiological survey for canine angiostrongylosis in dogs from Germany and the United Kingdom** - M. Schnyder
- **Efficacy of Procox® oral suspension (0.1% emodepside / 2% toltrazuril) against experimental nematode (Toxocara cati and Ancylostoma tubaeforme) infections in cats** - K. Krieger
- **Myiasis in a dog shelter in Greece: epidemiological and clinical features and therapeutic considerations** - G.C. Fthenakis
- **Ticks and associated pathogens collected from dogs and cats in Belgium** - E. Claerebout
- **Ticks and tick-borne disease in dogs in the UK** - F. Smith
- **Tracking the origin of an outbreak of Hepatozoon canis infection in a dog shelter in southern Italy: prevalence of infection in ticks during spring and summer** - F. Dantas-Torres
- **Starvation and overwinter of Rhipicephalus sanguineus in southern Italy** - F. Dantas-Torres
- **Enhanced Bioavailability of Albendazole Using Rapidly Disintegrating Tablets in Dogs** - S. G. Castro
- **Ixodofauna Wild Animal and Home of the State of Santa Catarina** - M. Sangaletti Lavina
- **Cat Flea Susceptibility to Imidacloprid: Results of a 10-Year Monitoring Initiative** - B. Blagburn
- **Efficacy and safety of concurrent use of an indoxacarb spot-on and a 4% deltamethrin-impregnated collar against flea and tick infestations on dogs** - F. Guerino
- **Field efficacy of a spot-on formulation of indoxacarb for flea control in dogs and cats** - A. Flochlay-Sigognault
- **Oral Efficacy of Fipronil in the Control of Ctenocephalides felis felis and Rhipicephalus sanguineus in Dogs in Brazil** - L. Grisi
- **Ectoparasitological Characterization in Dogs of Urban and Rural Area from Itaguaí Microregion, Rio de Janeiro, Brazil** - J. A. Rezende Vilela
- **Evaluation of the therapeutic and preventive efficacy of 2.5 % Moxidectin / 10% imidacloprid (Advocate®, Bayer Animal Health) in dogs naturally infected or at risk of natural infection by Dirofilaria repens** - K. Hellmann
- **Efficacy and safety of the combination imidacloprid 10% / moxidectin 1.0% spot-on (Advocate® spot-on for small cats and ferrets) in the treatment of ear mite infection (Otodectes cynotis) in ferrets** - R. Schaper

Good Management Practices and Changing Targets: Current status of resistance issues with companion animal parasites

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As we continue to practice parasite control through the routine administration of broad-spectrum heartworm and internal parasite preventive/treatment products and the routine application of pet-targeted flea and tick control, we must be fully aware of the potential of resistance developing amongst the parasites against which our control is directed. There have been various monitoring systems developed and put into place. One that has been ongoing for a good while has been the international monitoring of flea populations for resistance to imidacloprid utilizing the collection of flea eggs from suspected “resistant” populations on a given pet. Work has also been ongoing around the world to monitor the development of *Rhipicephalus sanguineus* to routinely used acaricides. Also, recently in the United States, there has been work towards developing the means of assessing the field efficacy of heartworm preventives through the monitoring of microfilariae, and now also infective-stage larvae, both in their response to macrocyclic lactones and also potential genetic correlates in “resistant” phenotypes. In the US and other parts of the world, people have also been looking at the ability of the intestinal parasites of dogs (Seemingly, there is no work as of yet on the internal parasites of cats.) to develop resistance to some of the products. Australians have performed work with *Ancylostoma caninum* that suggests that there are isolates that appear resistant to pyrantel. We also have had research with working greyhounds in the US that have shown that it is possible for *Toxocara canis* to be subjected to selection for resistance to a number of products. The routine use of ponazuril in many shelters for coccidia control is of concern and should be monitored closely for continued efficacy because of our knowledge of the development of resistance in the coccidia of chickens when significant selection pressure is applied in the field. We have excellent products that can be used to prevent and treat parasitic disease in pets, and although there remain considerable refugia within the many untreated dogs and cats of the world, we need to remain open to the possibility of resistance being a possibility and continue to monitor the success of treatment and prevention regimens.

***Angiostrongylus vasorum* in the United Kingdom – a nationwide postal questionnaire survey**

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Angiostrongylus vasorum, a metastrongyloid nematode parasite of canids, was first reported in dogs in SW England in 1982. Since then further cases have been recorded in Wales, SE England and, more recently, in W Midlands, N England and Scotland. The aim of the present study was to establish the distribution of the parasite in domestic dogs on a nationwide scale by means of a simple postal questionnaire survey that sought information on (1) the numbers of cases seen by small animal veterinarians over the preceding 12-months; and (2) the method(s) of diagnosis.

Questionnaires were sent to 3950 small animal practices during 2009 and descriptive statistics obtained for variables representing the location of each practice (rural, suburban, city/town), awareness of the presence of *Angiostrongylus* in the area, numbers of cases diagnosed (clinical signs only or using an additional diagnostic test) and type of test employed. Choropleth maps were created using Arc GIS 9.2 (ESRI 2006) to visualize the geographic distribution of practices that had seen angiostrongylosis cases. These represented, for practices located in the same county, the risk of having seen at least one or more than five confirmed cases over the previous 12 months. The spatial scan statistic was used to identify significant clusters of practices with a high probability of having at least one or more than five confirmed cases. This was implemented in SaTScan v8.1.1 using a Bernoulli probability model and Monte-Carlo randomisation, clusters for which $P < 0.05$ were considered statistically significant.

1419 (35.9%) practices returned a usable response, the majority of which were located in a city/town. Nearly one third of practices were aware of the parasite occurring within 5 miles, 20.7% had seen at least one case and 0.3% had seen >20 confirmed cases over the previous 12 months. The most widely used confirmatory tests were faecal examination and serology (95%, 92% of practices, respectively). Existing foci of infection were confirmed in SE England and S Wales; infection was also found to be widespread in Central England, though distribution was patchy in N England and Scotland. Using intensity and distribution of clinical cases as an indirect indicator of parasite distribution, this study has confirmed that *Angiostrongylus* is spreading beyond the traditional hyperendemic foci seen in the UK.

***Rhipicephalus sanguineus* (Ixodida, Ixodidae) as an intermediate host of a canine *Cercopithifilaria* species (Spirurida, Onchocercidae)**

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Background: The taxonomy, life cycle, and pathogenic role of filarioids of dogs presenting dermal microfilariae have been little studied. Following the recent retrieval of dermal microfilariae identified as *Cercopithifilaria* sp. in a dog from Sicily (Italy), this study was designed to assess the competence of the brown dog tick (*Rhipicephalus sanguineus*) as an intermediate host of this filarial species.

Methods: An experimental tick infestation was performed on an infected dog using 300 nymphs of *R. sanguineus*. After engorgement was complete, detached nymphs were collected and maintained in an incubator. In order to detect the occurrence and the larval development of filariae in the tick, pools of nymphs were examined by both microscopic dissection and molecular analysis at pre-fixed time-points (i.e., the same day of tick collection from the initial infestation and 10, 20, 30 and 50 days after tick collection).

Results: A total of 270 engorged nymphs were collected from the dog. Developing filarioid larvae were detected in 10 (10%) out of 200 ticks dissected. Third infective stage larvae were observed in 4 ticks, 30 days after tick collection. Twelve out of 181 samples molecularly examined were positive for *Cercopithifilaria* sp., thus giving an overall infection rate of 6.6%.

Summary/Conclusions: This study demonstrates that nymphs of *R. sanguineus* feeding on a *Cercopithifilaria* sp.-naturally infected dog can assume microfilariae which develop up to the third infective stage. The role of *R. sanguineus* as a vector of this *Cercopithifilaria* sp. should be confirmed by proving its ability for transmitting third stage larvae to a naïve dog during blood feeding.

Quantification of Blood Consumption by Fleas (*Ctenocephalides felis*) on Dogs Treated with Dinotefuran, Pyriproxyfen, and Permethrin (Vectra 3D®) based on Polymerase Chain Reaction (qPCR) Detection of the Canine HMBS Gene Sequence

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Prevention of flea feeding by flea control products can help prevent primary diseases in pets and transmission of disease agents to pets and people. We compared flea feeding on control dogs and dogs treated with dinotefuran, pyriproxyfen, and permethrin (Vectra 3D®) using qPCR for detection of a canine hydroxymethylbilane synthase (HMBS) housekeeping gene sequence. Sixteen Beagle dogs were placed in two groups of 8 dogs each. Eight dogs were treated topically with Vectra 3D® per label recommendations and 8 dogs were treated with excipients without active ingredients. All dogs were infested with 150 adult fleas at time 0. Fleas were removed from dogs at 5 min, 10 min, 15 min, 20 min, 30 min, 45 min and 60 min after infestation. Canine HMBS DNA was quantified by comparison to PicoGreen® assayed copies of the standard during FRET-PCR performed on a LightCycler® 1.5 real-time PCR platform with Software version 3.53. Minimum feeding, as determined by mean number of HMBS copies per 10 fleas, occurred on treated dogs at each time point through 20 min post infestation, compared to controls. Fewer HMBS gene copies were also amplified from fleas on Vectra 3D® treated dogs at 30, 45 and 60 min than from fleas removed from control dogs. Differences of means at each time point except for 30 min were significantly different ($p < 0.02$). Reduction in blood consumption at the 7 different time points ranged from 52% (15 min) to 95% (20 min). Overall reduction in blood consumption by fleas on dogs treated with Vectra 3D was 78%. Results indicate that Vectra 3D® significantly reduces flea feeding immediately after flea infestation.

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Molecular identification of phlebotomine sand flies by mitochondrial and ribosomal DNA polymorphism

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Background: Sand flies include a diverse group of insects that vary in geographic distribution, ecology and vectorial role of pathogens. Although different molecular techniques have been used to differentiate some species of sand flies, no molecular datasets for mitochondrial and ribosomal DNA gene sequences have been generated for the simultaneous comparison of *Phlebotomus perniciosus*, *Phlebotomus perfiliewi*, *Phlebotomus neglectus*, *Phlebotomus papatasi* and *Sergentomyia minuta* which are the most representative species in the Mediterranean region. The aim of the present study was to assess the genetic diversity within and among sand fly species above by mitochondrial DNA (mtDNA) encompassing regions within *cytochrome b* (*cytb*) and NADH *dehydrogenase subunit 1* (*nadh1*) genes and the complete internal transcribed spacer 2 (ITS2) ribosomal region.

Methods: Sand fly specimens were collected in two regions of southern Italy (Apulia and Basilicata regions) and morphologically identified at species level. A ~900 bp long fragment of the mtDNA and ITS2 region (~500 bp) were sequenced and characterized for the five sand fly species. A phylogenetic analysis was carried out to investigate the relationships between sand flies sequences of both genes with those available in GenBank™ by using the Neighbor-Joining (NJ) methods.

Results: Eight mitochondrial haplotypes and three ITS2 sequence types were found among the examined specimens, according to the different sand fly species. The mean interspecific difference in the mitochondrial sequences was of 16.1% with an overall intraspecific nucleotide variation from 0.1 to 2.8%. A higher interspecific difference (mean 25.1%) was recorded for the ITS2 sequence, with an overall intraspecific nucleotide variation up to 4.9%. Phylogenetic analysis of the *Phlebotomus* spp. sequences examined and of those available in GenBank™ was concordant in clustering *P. neglectus*, *P. perfiliewi* and *P. papatasi* with the same species collected from different geographic areas of the Mediterranean basin in four and three main clades for mtDNA and ITS2, respectively.

Summary/Conclusions: This study demonstrates the utility of multilocus sequencing and provides a dataset for the molecular identification of the most prevalent sand flies in Mediterranean region and defines their phylogenetic relationships.

Distribution and efficacy of four different spot-on formulations of permethrin in dogs artificially infested with *Dermacentor reticulatus*

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Background: Varying reports concerning the efficacy of different permethrin formulations can be found in the literature, some discussing an influence of different pharmaceutical excipients on the distribution dynamics and the acaricidal activity of permethrin. To investigate this effect, an *in vivo* laboratory study was performed.

Methods: Four different permethrin spot-on formulations with marketing authorization in Germany were tested under equal conditions in six beagle dogs each and compared to an untreated control group (cross-over-design). For an accurate comparison, treatment was done per kg bodyweight at the minimum recommended dose. 1, 14 and 28 days after spot-on application the dogs were infested with *Dermacentor reticulatus* ticks and samples were taken to determine permethrin concentrations in blood, skin and hair by UV-VIS HPLC. Ticks were applied in chambers attached to the thoracic wall near the spot-on application site and on the hind leg, at the greatest possible distance from the application site. Skin and hair samples were taken close to the infestation site. Efficacies were determined 6 hours after each infestation.

Results: A significant acaricidal effect was observed in all treated groups over the whole duration of the study with neither significant differences between the four spot-on formulations, nor between the two parts of the body. No permethrin could be detected in blood samples. Values in the *stratum corneum* did not differ significantly between samples taken from the back and those taken from the hind leg. Permethrin concentrations in the hair were similar on both body parts on day 1, but partially higher on the back on day 14 and 28. Concentrations decreased considerably over the duration of the study, but no significant differences were found between the four products.

Summary/Conclusions: All four permethrin spot-on products showed a high speed of kill on *D. reticulatus* throughout the study period without significant differences between the formulations or at different parts of the body. Likewise no significant differences in permethrin concentrations were found, indicating that the different solvents did not significantly affect the dynamics of distribution of permethrin.

In Vitro Bioassay For Detection of Anthelmintic Resistance In *Dirofilaria immitis*

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Background: The canine heartworm (*Dirofilaria immitis*) is a parasite of significant veterinary importance in dogs and cats. For more than 25 years, macrocyclic lactone (ML) anthelmintics have been used widely in prophylactic therapy to prevent heartworm disease. However, increasing reports of adult heartworm infections in dogs, where the administration of monthly prophylaxis is documented, indicate increasing failure of prophylaxis. This suggests that there is an increase in the frequency of owner noncompliance, there is some as yet unidentified factor that is reducing the effectiveness of the drugs, or most likely, that ML resistance has developed in the worms. Objectively verifying owner compliance is not possible; therefore there is a great need for a test that can detect resistance in heartworms. To address this issue, we developed a larval migration inhibition assay (LMIA) using the third-stage infective larvae (L₃) as the target stage for the assay, since this is a stage targeted by the ML treatments.

Methods: Mosquitoes are fed on blood containing *D. immitis* microfilariae, and L₃ are harvested 2 weeks later. L₃ are then incubated in culture with a range of ML drug concentrations. After 48 hours incubation, the L₃ are placed into migration chambers containing a 25μ mesh, and the ability of L₃ to migrate through the mesh is determined microscopically and quantified.

Results: Performing this assay with a laboratory strain of *D. immitis* (Missouri strain) using eprinomectin as a representative macrocyclic lactone yields a reproducible sigmoidal dose-response with an approximate EC₅₀ of 2 μM (95% CI = 1.61 to 2.42 μM). We are now in the process of testing several parasite isolates from well characterized failure of prophylaxis cases. We also are evaluating the assay using several other ML analogues.

Summary/Conclusions: Based on experiments using the susceptible Missouri strain of *D. immitis*, the LMIA appears to be a useful bioassay for assessing the susceptibility of *D. immitis* to ML drugs. Additional testing using suspect-resistant isolates should allow us to assess the ability of this assay to detect shifts in ML susceptibility that are associated with resistance.

Suppression of *Dirofilaria repens* Microfilariaemia in Naturally Infected Dogs Using a Single Spot-on Application of a Solution Containing Imidacloprid 10 %/Moxidectin 2.5 % (Advocate[®], Bayer Animal Health)

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Background: The mosquito-transmitted nematode *Dirofilaria repens* (Nematoda, Filarioidea) cause dermatitis, skin lesions and itching in dogs and pruriginous urticarioid patches, swellings and eosinophilia in humans. Cases of canine and human dirofilariosis by *D. repens* have recently increased in different countries, thus spurring new studies on therapy and control of the infection. Given the efficacy of the antiparasitic spot-on formulation containing imidacloprid 10 %/moxidectin 2.5 % (Advocate[®], Bayer Animal Health) in the microfilaricidal treatment of natural canine infection by *D. repens* using consecutive monthly treatments, the present study evaluated the efficacy of a single application of Advocate[®] in the elimination of *D. repens* microfilariae.

Methods: The study was carried out from October 2009 to April 2010 in an endemic area of Central Italy. Eighteen autochthonous dogs infected naturally by *D. repens* were treated in October 2009 with a single dermal administration of Advocate[®] spot-on solution, applied according to label instructions. Presence/absence of circulating microfilariae and microfilariaemia levels were evaluated monthly until April 2010 for each of the 18 dogs with the exception of one animal which died in March.

Results: The single administration of Advocate[®] showed intensity and extent efficacy of 99.6 % and 88.9 %, respectively, in suppressing microfilariaemia by *D. repens*. In particular, all dogs but two scored negative for *D. repens* to both Knott and PCR methods throughout the study period. Two dogs were again microfilaremic after two and three months from the treatment, respectively, but with low levels of microfilariaemia ranging from 10 to 260 microfilariae/ml.

Conclusions: The single dermal administration of Advocate[®] to microfilaremic dogs proved to be a valuable mean to eliminate larval *D. repens* up to six months, thus interrupting the biological cycle of the nematode. Further studies are warranted to evaluate the number of treatments with Advocate[®] which would be necessary during the mosquito season to achieve 100 % intensity and extent efficacy in suppressing *D. repens* microfilariaemia towards an effective mean to control subcutaneous dirofilariosis.

Seroepidemiological survey for canine angiostrongylosis in dogs from Germany and the United Kingdom

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Angiostrongylus vasorum is a parasite living in the right heart and pulmonary arteries of dogs and foxes. Infected dogs may present severe respiratory symptoms but also haematological and neurological signs have been described. *A. vasorum* was originally present in endemic foci in e.g. south-western France, Ireland and Italy. During the last decades, *A. vasorum* has increasingly been reported in extended areas in central and western European countries and also in Canada. In Germany, *A. vasorum* was described in south-western regions, while in the UK in particular southern parts experienced an increased number of cases. The current diagnosis of *A. vasorum* infections is mainly based on the detection of L1 in faecal samples by larval migration techniques. However, alternative diagnostic tools such as molecular methods or serological tests have been developed. In this study, two ELISAs were employed in a seroepidemiological survey with more than 8000 sera originating from Germany (4003) and the United Kingdom (4030), which were submitted to veterinary laboratories for various medical reasons. An ELISA, using mono- and polyclonal antibodies (sensitivity 95.7%, specificity 94.0%), was employed for the detection of circulating antigen of *A. vasorum*. Additionally, an ELISA detecting specific antibodies using excretory/secretory antigens of adult stages (sensitivity 85.7%, specificity 98.8) was used. Test thresholds were regionally determined. In Germany, where mainly western federal states were sampled, 0.3% (n=13) of the animals were positive in both ELISAs, whereas totally 0.5% (n=20) of the tested dogs were antigen-positive and 2.25% (n=90) positive for specific antibodies. Regions with antigen- and antibody-positive animals were overlapping and distributed through the whole western sampling area of the country. In the United Kingdom, where mainly the south of the country was sampled, 0.97% (n=39) of the dogs were antigen- and antibody positive. Totally, 1.32% (n=53) of the animals were antigen-positive, and 3.2% (n=129) were positive for specific antibodies in overlapping regions. This first large scale survey confirmed the endemic presence of *A. vasorum* in Germany as well as in the UK, and additionally cases outside the known endemic foci were detected.

Efficacy of Procox® oral suspension (0.1% emodepside / 2% toltrazuril) against experimental nematode (*Toxocara cati* and *Ancylostoma tubaeforme*) infections in cats

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Background: Procox® is a new oral suspension containing a combination of the nematocidal and coccidiocidal active principles emodepside (0.1%) and toltrazuril (2%). Two exploratory studies were performed to determine the optimum therapeutic dose of Procox® for the removal of experimental infection with mature adult *Toxocara cati* and *Ancylostoma tubaeforme* in kittens.

Methods: In the first study, 18 eight weeks old kittens were inoculated with 450 L3 larvae of *T. cati*. 56 days after infection, the kittens were allocated to three treatment groups. These were treated with 0.5mg emodepside/kg body weight (group 1), 0.25mg emodepside/kg body weight (group 2) and 0.1mg emodepside/kg body weight (group 3), respectively. In the second study, 10 eight weeks old kittens were inoculated with 350 L3 larvae of *A. tubaeforme*. Four weeks after infection, the kittens were allocated to two treatment groups and were treated with 0.1mg emodepside/kg body weight (group 1) or 0.25mg emodepside/kg body weight (group 2). In both studies all kittens received a reference treatment with Drontal® (230mg pyrantel embonate and 20mg praziquantel per tablet) at the recommended dose of one tablet/4kg body weight 5 days after treatment with Procox®. Anthelmintic efficacy was calculated by reductions in numbers of worms expelled following treatment with Procox® as compared with those expelled after reference treatment with Drontal®.

Results: In the *T. cati* study, emodepside was 99.9%, 100% and 96.5% effective at 0.5mg, 0.25mg and 0.1mg per kg body weight, respectively. Against *A. tubaeforme* emodepside was 95.7% and 100% effective at 0.1mg and 0.25mg per kg body weight. No adverse events were seen during either study.

Conclusion: Procox® is efficacious for the control of mature adult *T. cati* and *A. tubaeforme* infections in cats at a single dose rate of 0.25mg emodepside/kg body weight.

Myiasis in a dog shelter in Greece: epidemiological and clinical features and therapeutic considerations

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Objective and methods: We describe epidemiological and clinical features of myiasis in a cohort of dogs, living in the animal shelter of a welfare society in central Greece, where, despite repeated veterinary advice, no pharmaceutical treatments against fly-strike were used. The shelter was monitored over 3.5 years, corresponding to four fly activity seasons.

Results: Seven dogs were presented with myiasis; two dogs were presented twice with infestations in different localisations in their body. There were nine cases of strike in seven animals, giving an incidence rate (IR) of 0.0058 case per animal-month at risk. Six cases occurred between May and July and three between August and October, giving IRs of 0.0077 per animal-month and 0.0039 per animal-month, respectively ($P=0.35$). Five cases occurred in males and four in females, giving IRs of 0.0078 per animal month and 0.0043 per animal-month, respectively ($P=0.41$). In three cases lesions were localised in the thigh, in 2 cases in the ear canal, in 2 cases in the prepuce, in 1 in toes and in 1 in the vagina. Median time from entrance to shelter to infestation was 5 months; it was 1 m in three injured animals and 5 to 15 m in other dogs. Larvae from all animals were identified as 2nd or 3rd stage instars of *Wohlfahrtia magnifica*. Treatment included mechanical removal of larvae, cleansing of the area, administration of injectable moxidectin (0.4 mg/kg bw) and a broad-spectrum antimicrobial agent. Complete recovery was achieved within 1.5 months, except in cases of preputial infestation which required 2.5 months.

Conclusions: Incidence rate of the disorder was high, as no preventive measures against the disorder were taken. Injuries predispose to quick infestation of animals by fly-strike. Moxidectin, although not licenced against cutaneous myiasis, was found to be of help as part of the therapeutic regime.

Ticks and associated pathogens collected from dogs and cats in Belgium

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Background: The most common ticks on dogs and cats in North-Western Europe are *Ixodes spp.* *Ixodes spp.* are vectors of babesiosis, borreliosis and anaplasmosis. Recent reports indicated an expanding geographical distribution of *Dermacentor reticulatus* in Western Europe. *D. reticulatus* is an important vector of canine and equine babesiosis. Until now it was uncertain whether *D. reticulatus* populations had also established in Belgium.

Methods: A survey was conducted in 2008-2009 to investigate the presence of different tick species and their associated pathogens on dogs and cats in Belgium. Seventy-five veterinary practices were selected by stratified randomization to collect ticks from cats and dogs. All collected ticks were morphologically determined to stage and species level. Ticks were analysed for the presence of *Babesia spp.*, *Borrelia spp.* and *Anaplasma phagocytophilum* DNA by PCR, targeting the 18S rRNA, flagellin and ankA genes, respectively.

Results: 2363 ticks were collected from 647 dogs and 506 cats. *Ixodes ricinus* (76.4%) and *I. hexagonus* (22.6%) were the predominant species. *Rhipicephalus sanguineus* (0.8%) and *D. reticulatus* (0.3%) were found in low numbers on dogs only. All dogs infested with *R. sanguineus* had a recent travel history, but *D. reticulatus* were repeatedly collected from one dog without a history of traveling abroad. The presence of a population of *D. reticulatus* was confirmed by flagging. None of the *R. sanguineus* and *D. reticulatus* ticks were positive for *Babesia* DNA, but 1.3% of the *Ixodes* ticks were found positive for the potentially zoonotic *Babesia microti* or *Babesia venatorum*. In addition, 23.2% and 10.1% of the collected *Ixodes* ticks were positive for *A. phagocytophilum* and *Borrelia spp.*, respectively. *Borrelia afzeli*, *B. garinii*, *B. burgdorferi s.s.*, *B. lusitaniae*, *B. valaisiana* and *B. spielmanii* were detected.

Conclusions: *D. reticulatus* was confirmed to be present as an indigenous tick in Belgium. Several *Babesia* species and *Borrelia* species were detected in ticks in Belgium for the first time.

Ticks and tick-borne disease in dogs in the UK

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Background: Current concerns over the potential impacts of changing climate and increased global movement of people and companion animals on the distribution of ectoparasites highlight the need for an accurate baseline understanding of existing prevalence patterns.

Methods: In this study, the distribution and prevalence of ticks infesting domestic dogs in Great Britain were examined. A total of 173 veterinary practices were recruited to monitor tick attachment to dogs in their local area between March and October 2009. Practices selected five dogs at random each week from those brought to the surgery and undertook a thorough, standardised examination for ticks.

Results: A total of 3,534 dogs were examined; 810 dogs were found to be carrying at least one tick. *Ixodes ricinus* was identified in 72.1% cases, *Ixodes hexagonus* in 21.7% and *Ixodes canisuga* in 5.6% cases. Five samples of *Dermacentor reticulatus* were also identified, adding to the growing evidence that an established population of *D. reticulatus* now exists in southern England. No ticks were detected by 19% of the veterinary practices, 50% of the veterinary practices reported that at least 14.9% of the dogs seen were infested and 14.6% reported that more than 50% of the dogs inspected carried ticks. The estimated incidence of tick attachment was 0.013 per day in March (lowest) and 0.096 per day in June (highest). A number of risk factors affected the likelihood of tick attachment on dogs. Gundog, terrier and pastoral breed groups were more likely to carry ticks, as were non-neutered dogs. Dogs with shorter hair were less likely to have ticks, and dogs were most likely to carry a tick in June. The preliminary results of PCR analysis of the ticks collected for canine pathogens will be discussed.

Summary: This study is of interest because, unusually, it presents the results of a randomised sample of dogs and gives a prevalence which is higher than previously recorded in Great Britain.

Tracking the origin of an outbreak of *Hepatozoon canis* infection in a dog shelter in southern Italy: prevalence of infection in ticks during spring and summer

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Background: *Hepatozoon canis* is a common protozoan of dogs, being among the most prevalent tick-borne pathogens infecting dogs around the world. It is primarily transmitted by *Rhipicephalus sanguineus*, the brown dog tick. We collected ticks from dogs and from the environment in a dog shelter where an outbreak of *H. canis* infection was detected in October 2009.

Methods: *R. sanguineus* ticks collected during the spring of 2009 (March-May), from dogs kept in a shelter in southern Italy, were tested by PCR for the detection of a *H. canis*-18S ribosomal RNA gene fragment. In addition, larvae (n=200) and nymphs (n=100) eventually found on sticky traps placed in the environment during the summer of 2009 (July), as part of a study on phlebotomine sand flies present in this dog shelter, were also tested by PCR.

Results: Out of 267 ticks (21 nymphs, 99 females, and 147 males) collected from dogs and tested by PCR, 4 (1.5%) resulted positive (1 female and 3 males). As expected no larvae were positive. Remarkably, a relatively high infection rate (8.0%) was detected in nymphs.

Summary/conclusions: The low prevalence of *H. canis* infection in ticks was initially unexpected because an outbreak of *H. canis* infection was detected in the studied dog population five months later (October 2009). In turn, this result suggested that dogs acquired the infection by *H. canis* sometime during the summer, when the tick population in the studied shelter reached its pinnacle. This hypothesis was strongly supported by the finding of a high prevalence of infection among engorged nymphs collected from the environment, three months before the outbreak. These findings points out that dogs became infected during summer, when ticks were abundant and highly infected by *H. canis*. Further research is needed to better understand the relationship between tick population dynamics and *H. canis* infection in dogs, which might provide useful information for practitioners and dog owners towards the prevention of canine hepatozoonosis in endemic areas.

Starvation and overwinter of *Rhipicephalus sanguineus* in southern Italy

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Background: *Rhipicephalus sanguineus* is the most widespread tick in the world, being able to survive under different climate conditions. By as a matter of fact, information on the survival of *R. sanguineus* in nature is meager. Thereby, the objective of the present study was to assess the longevity of *R. sanguineus* under natural climate conditions in southern Italy. Additionally, the reproductive fitness of *R. sanguineus* after starvation and overwintering was studied.

Methods: From 20 June 2009 to 7 March 2011, ticks were followed up under environmental and laboratory conditions. In particular, larvae were separated in groups of 25 and placed in the environment (n=538) and in an incubator (26 ± 1 °C, 70 ± 10% RH, and scotophase) in the laboratory (n=625). Nymphs were separated in groups (from 4 to 14 per vial) and placed in the environment (n=71) and in the incubator (n=14). Again, 57 unfed adults were followed up in the environment and 43 in the incubator. Furthermore, 16 females (first generation) previously feed on a rabbit were also included in this study in order to evaluate their post-oviposition survival. Ticks were examined every day and the number of live individuals in each vial recorded.

Results: In the environment, unfed larvae, nymphs and adults were able to survive for up to 34, 40 and 385 days, respectively. However, the maximum survival of larvae (44 days), nymphs (54 days) and adults (584 days) was longer in the laboratory. Adult ticks that had overwintered under natural climate conditions were able to feed and give rise to viable larvae, after 40 weeks of starvation.

Summary/conclusions: It is demonstrated that *R. sanguineus* is able to overwinter in southern Italy, without any prejudice to its fitness. These findings advance our knowledge on the natural history of *R. sanguineus* in nature and provide new insights into the epidemiology of certain tick-borne diseases in southern Europe as well as into the risk for their introduction in northern European countries.

Enhanced Bioavailability of Albendazole Using Rapidly Disintegrating Tablets in Dogs

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New therapeutic alternatives using Benzimidazole compounds have been studied for improving its *in vivo* physiological dissolution and consequent plasma bioavailability. The aim of this work was to compare the plasma pharmacokinetic (PK) profiles of two different Albendazole (ABZ)-based oral formulations in dogs. Formulation A: ABZ-Poloxamer 188 (P188) as solids dispersion (SD) tablets and Formulation B: conventional tablets of ABZ.

Formulation A was prepared using 400mg of SD (ABZ 200mg), 732mg of LudiflashTM, 60mg of sodium crosscarmellose and 8mg of magnesium stearate. Formulation B containing only ABZ compressed powder. A previous *in vitro* dissolution tests were performed using 0,1N HCl, 50rpm at 37°C. The comparative PK trial was undertaken in a crossover two-phases study as follows: six health Retriever dogs (35-45kg) were randomly divided into two groups. Phase I, Group I: Three dogs, received a single dose of ABZ 25mg/kg of the Formulation A. Group II: three dogs used as control group, were dosed with 25mg/kg of ABZ formulated as conventional tablets (Formulation B). After 21 days of wash-out period the treatments were reversed and the experiment repeated as Phase II. Blood samples were taken over 24h post-treatment, and upon analysed by High Performance Liquid Chromatography. Mann-Whitney non parametric test was used for the statistical comparison.

The *in vitro* dissolution test showed a faster dissolution of Formulation A (t_{15} :52,37%) compared to Formulation B (t_{15} :40,17%). The later correlated with the *in vivo* PK study. The area under the concentration vs time curve values (AUC) obtained for the active metabolite Albendazole Sulphoxide (ABZSO), after treated with Formulation A, showed a significant ($P<0.001$) increase of 150% (AUC=39 μ g.h/mL) values, when compared with Formulation B (AUC=15 μ g.h/mL.). Increase of dissolution of Formulation A was also reflected on higher values of obtained maximum plasma concentration C_{max} (+160%) ($P<0.001$). Plasma period detection obtained for ABZSO was 24h for Formulation A when compared with Formulation B (18h). The dissolution rate and bioavailability of ABZ was improved after being formulated in SDs. This novel formulation may impact in a shorter therapeutic regimen, by which may be useful in clinical practice for the treatment of dog endoparasites.

Ixodofauna Wild Animal and Home of the State of Santa Catarina

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Ticks can transmit diseases such as spotted fever and Lyme disease to humans, and Ehrlichiosis, Babesiosis and Anaplasmosis to animals. It is known that there is a wide range of the hosts parasitized by *Amblyomma*, comprising the majority of mammalian orders. The birds are rarely parasitized by the adult stage, and frequently hosts of the immature stages of some species. Amphibians and reptiles are also among the hosts of this genus. Aiming to identify ticks, and their possible hosts report for the first time the occurrence of certain species of ticks in the state of Santa Catarina, Brazil are collected ticks from domestic and wild animals that pass through the screening process at the Veterinary Hospital of the Agroveterinary Science Center (CAV), animals found dead on the highways and the state animal of municipalities in five regions: Plateau Serrano, South, Florianópolis, Northern and Upper Itajaí Valley. The identifications are performed in the Laboratory of Parasitology and Parasitic Diseases Agroveterinary Science Center, according to the specific dichotomous keys. So far were collected in the Plateau Serrano samples *Rhipicephalus sanguineus*, *Amblyomma aureolatum*, *A. tigrinum* and *A. ovale* parasite of the dogs; *A. aureolatum* and *A. rotundatum* in puma, *A. aureolatum* parasitizing graxaim, cat, wild dog and lion bay; *A. dubitatum* in capybara, *Rhipicephalus (Boophilus) microplus* parasitizing deer and *Ixodes loricatus* parasitizing opossum, totaling 78 samples. In the southern region were collected specimens of the *A. aureolatum*, *A. ovale* and *R. (Boophilus) microplus* parasitizing dogs, and one sample of the *A. longirostre* collected in the environment, totaling 28 samples. In the Greater Florianópolis samples of the *R. (B.) microplus*, *R. sanguineus* and *A. aureolatum* parasitizing the dogs; *A. fuscum* in ferret and samples of *A. cajennense* and *Anocentor nitens* on the horses, totaling 35 samples. In the northern region was collected *R. sanguineus*, *A. aureolatum*, *A. tigrinum* e *A. ovale* parasite of the dogs and samples *Anocentor nitens* on the horses, totaling 22 samples. In Upper Itajaí Valley, samples of the *R. sanguineus*, *A. aureolatum* and *A. ovale* in the dogs *A. ovale* and *A. aureolatum* into a frog; *A. dubitatum* in capybara and *A. aureolatum* in the wild cat, totaling 29 samples. Calves were excluded from this study are known to suffer parasitism by *Rhipicephalus (Boophilus) microplus* throughout the state. Partial data demonstrate a wide distribution of the ticks throughout the state and in different species, the species *A. rotundatum*, *A. fuscum*, *A. longirostre* and *Ixodes loricatus* were identified by the first time ever in Santa Catarina.

Cat Flea Susceptibility to Imidacloprid: Results of a 10-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 HealthCare). Flea eggs representing different field isolates of *C. felis* were collected from the United States, United Kingdom, France, Germany, and Australia. From 2001-2010, 1,946 isolates were received; 1,421 were placed in the assay. Host species was available for 1,930 isolates - 1,329 isolates were from cats; 601 from dogs. Percent of isolates received by month were: January - 2%; February - 2%, March - 1%, April - 4%, May - 7%, June - 9%, July - 12%, August - 14%, September - 18%, October - 15%, November - 11%, December - 5% (no data for 4%). Flea eggs collected by month were highest from May-November (86% of eggs collected). Isolates were challenged with a diagnostic dose of imidacloprid (3ppm) applied to larval flea-rearing medium. Adult survival of 5% or greater (2002-2010) was observed for 24 isolates. Re-conduct of the assay using either a repeat challenge dose of 3 ppm of imidacloprid or a dose-response Probit analysis confirmed the susceptibility of these isolates to imidacloprid. Numerous challenges were encountered during sample collection, conduct of the assays, and interpretation of results. Challenges and solutions will be discussed.

Efficacy and safety of concurrent use of an indoxacarb spot-on and a 4% deltamethrin-impregnated collar against flea and tick infestations on dogs

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A 4% deltamethrin-impregnated collar (SCALIBOR®, Intervet/Schering-Plough Animal Health) is used to protect dogs from the bites of sand flies (*Phlebotomus* sp.), mosquitoes (*Culex* sp.), and ticks for up to six months with high persistent efficacy. Immediate efficacy of the collar against fleas is known to be inadequate. This study was designed to evaluate the efficacy and safety of concurrent use of an indoxacarb spot-on (ACTIVYL™, Intervet/Schering-Plough Animal Health) and a 4% deltamethrin-impregnated collar for the long term control of *Ctenocephalides felis* fleas and *Rhipicephalus sanguineus* ticks on dogs.

Thirty-two dogs were randomly assigned to four treatment groups (8/group): (Group 1) negative control; (Group 2) dogs fitted with a 4% deltamethrin-impregnated collar for six months; (Group 3) dogs treated with indoxacarb spot-on once every four weeks for six months; and (Group 4) dogs treated with indoxacarb spot-on once every four weeks and a 4% deltamethrin-impregnated collar applied for six months. Dogs were experimentally infested with 100 fleas and 50 ticks two days before treatment (Day 0) and then on various days throughout the study.

The immediate effectiveness against existing flea infestations on Day 2 was 76%, 100% and 96% for Groups 2, 3, and 4, respectively. The persistent effectiveness ranged from 75.2% to 96.3% in Group 2, 100% in Group 3 and ranged from 98.9 to 100% in Group 4 up to Day 177. Immediate effectiveness against existing tick infestations was determined on Day 2 as 77%, 16% and 93% for Groups 2, 3 and 4. Persistent efficacy of >94% against ticks was observed for Groups 2 and 4 for up to Day 184 of the study.

Indoxacarb applied monthly to dogs was 100% effective against fleas over the six month of the study. Concurrent treatment with an indoxacarb spot-on every four weeks and a single 4% deltamethrin-impregnated collar provided persistent efficacy of >99% against fleas and >94% against ticks for six months. No adverse reactions were observed in this study.

Indoxacarb spot-on can be used concurrently with a 4% deltamethrin-impregnated collar to provide high efficacy against fleas and ticks on dogs.

Field efficacy of a spot-on formulation of indoxacarb for flea control in dogs and cats

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Two clinical trials were undertaken in client-owned pets naturally infested with fleas, to confirm the efficacy of a novel spot-on formulation containing 195 mg/mL indoxacarb (ACTIVYL™, Intervet/Schering-Plough Animal Health), under field conditions.

In a multi-regional field study, thirty-three veterinary clinics in Europe (France, Germany, and Spain) enrolled 207 dogs and 197 cats, randomly allocated to receive three applications of indoxacarb or a commercially available fipronil spot-on (FRONTLINE spot-on, Merial) at the recommended doses at 4-week intervals. Clinical examinations and baseline flea counts were performed prior to treatment and were repeated on six occasions at two-week intervals for twelve weeks. Flea counts and efficacy calculations were performed as recommended by the WAAVP and European guidelines. In the indoxacarb groups, percent reductions based on geometric means were 97.7% and 95.6% in Week 2, and reached 99.8% and 99.4% in Week 12, in dogs and cats, respectively. In the fipronil groups, percent reductions were 94.3% and 92.4% in Week 2, and 96.9 % and 97.1 % in Week 12, in dogs and cats, respectively. Mean flea counts in the indoxacarb groups were significantly lower than in the fipronil groups over the entire study period, at the end of which the proportions of households with all pets free of fleas were 98.4% and 96.1% in the indoxacarb groups and 77.6% and 85.7% in the fipronil groups for dogs and cats, respectively. In both groups the percentage of dogs with flea allergy dermatitis decreased markedly over time, from 18.8% to 0% and from 22.5% to 1.2%, respectively in the indoxacarb and in the fipronil groups. In cats, this proportion was reduced from 11.8% to 0% and from 12.9% to 1.2%, respectively. There was no significant difference between the numbers of animals with flea allergy dermatitis in the two groups throughout the study.

An additional study was conducted under severe flea re-infestation conditions in Spain, in two client-owned households, each with 25 naturally-infested cats, which were treated three times at 4-week intervals with indoxacarb. The efficacy was evaluated as described above. Percent reduction was 97.5% as soon as the first visit following treatment (Week 2), and reached 100% from Week 4 to Week 12.

ACTIVYL™ was well tolerated in both studies.

Oral Efficacy of Fipronil in the Control of *Ctenocephalides felis felis* and *Rhipicephalus sanguineus* in Dogs in Brazil

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Fipronil has been used worldwide by topical application for control of *Ctenocephalide felis felis* and *Rhipicephalus sanguineus*. The efficacy of fipronil administered orally was evaluated against *C. felis felis* and *R. sanguineus* in artificially infested dogs. Twenty four Beagle dogs were divided in three treated groups, which received fipronil in gelatine capsules at single oral doses of 2, 4 and 6 mg. kg⁻¹ of body weight on day 0, the fourth group was kept as non medicated control. Dogs were infested weekly with 100 fleas and 50 ticks, two days before each assessment. The evaluations were done weekly, counting fleas and ticks using the comb test method, on days +2, +7, +14 and +21. The efficacy data registered for flea control using 2 mg. kg⁻¹ were 100% on day 2 and 83.79% on day 7. Dogs treated with 4 mg.kg⁻¹ showed reductions of 88.34% for the day +2 and 67.98% for the day +7. Data related to days 14 and 21 for both groups were lower than 60%. Efficacy on animals treated with fipronil at 6mg.kg⁻¹ orally was 97.88, 95.27, 88.75 and 71.43% on days +2, +7, +14 and +21, respectively. Very low efficacy results were registered against ticks, *R. sanguineus* : fipronil orally at 2mg.kg⁻¹ exhibited efficacy of 25.35% and 0, dogs treated with 4mg.kg⁻¹ orally, 29.58% and 0, and animals treated with 6mg.kg⁻¹ the efficacy were 43.67 and 23.28%, respectively, on days +2 and +7. The data showed that oral administration of 6mg.kg⁻¹ of fipronil can be effective in controlling flea infestation by *C. f. felis* in dogs up to 14 days, but it is not effective in the control of *R. sanguineus* in dogs.

Ectoparasitological Characterization in Dogs of Urban and Rural Area from Itaguaí Microregion, Rio de Janeiro, Brazil

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Ectoparasites are capable of transmitting pathogens to humans, domestic and wild animals. The parasitism change according to the environment. This study aimed to characterize the population of ectoparasites in dogs from Itaguaí Microregion, Rio de Janeiro State, Brazil. Questionnaires were performed in order to collect information about the dogs. The ectoparasites were collected, kept in isopropanol and identified by stereoscopy. Ectoparasitism was observed in 159 dogs, being 127 (79.87%) and 32 (20.13%) from rural and urban areas, respectively. Of the dogs parasited, 28.30% were defined bred and there was a predominance of short fur in animals of both areas (around 55%). The history of tick infestation was higher in urban areas (96.87%) than in rural areas (81.89%). The contact with other animals occurred in 82 (64.57%) and 6 (18.75%) dogs from rural and urban areas, being 87.80% and 66.67% with farm animals, respectively. The hygiene conditions of the environment were worse in rural areas, where 84.25% were cemented and/or with land, and 11.81% with grass, different from that observed in urban areas, where 96.87% were cemented. About the access to pasture and/or forest, occurred in 84.25% of the rural dogs and 25% from urban areas. The periodic treatment with ectoparasiticide was carried out in 87.5% of dogs in the urban and 69.29% in rural areas. *Rhipicephalus sanguineus* was the most prevalent specie among the specimens collected, found in 33.07% and 56.25% of the dogs in rural and urban areas, respectively, followed by *Ctenocephalides felis* (19.69% and 46.88%) and *Ctenocephalides canis* (2.36% and 12.5%). Some specimens were found only in rural areas, such as *Amblyomma cajennense* (11.02%), *Trichodectes canis* (3.94%), *Amblyomma ovale* (2.36%), *Amblyomma dubitatum* (0.79%). Nymphs of ticks were separated into *Amblyomminae* (7.09%) and *Rhipicephalinae* (1.57%). Larvae ticks were found in 3.15% of rural dogs. The results of the present study suggest a different way of parasitism in rural areas. Studies about the occurrence of ectoparasites are essential for developing appropriate control measures and evaluating the risk that animals and humans are exposed to.

Evaluation of the therapeutic and preventive efficacy of 2.5 % Moxidectin / 10% imidacloprid (Advocate®, Bayer Animal Health) in dogs naturally infected or at risk of natural infection by *Dirofilaria repens*

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The objective of this GCP compliant clinical field study was to evaluate the efficacy of the combination of Moxidectin (minimum dose of 2.5 mg/kg) and Imidacloprid (minimum dose of 10.0 mg/kg) spot-on (IVP, Advocate®) as a preventive and therapeutic treatment of natural infection by microfilaria of *Dirofilaria repens* in dogs in the Czech Republic.

There were two arms of the study, both negatively controlled. Animals infected with *Dirofilaria repens* microfilaria were randomly allocated to one of the two groups of the treatment arm; animals negative for microfilaria were randomly allocated to the prevention arm groups. All enrolled dogs were observed physically and blood was monthly sampled for *Dirofilaria repens* microfilaria counts for 18 months.

34 dogs were enrolled in the treatment arm of this study (IVP: 18, CP 16). The reduction of the log transformed microfilaria counts was significantly higher in the IVP group on day 28 ($P=0.007$), 56, 84, and 112 ($p<0.001$). All animals treated with the IVP were negative after a single treatment. In the control group 93.75% remained positive ($P<0.001$).

87 dogs were negative for microfilaria prior to allocation to the "preventive" arm (IVP: 49; CP: 38). One dog in the untreated control group became positive for *Dirofilaria repens* microfilaria, while none of the IVP treated dogs became positive.

Advocate® was effective in the treatment of dogs infected with microfilaria of *Dirofilaria repens*. Due to the low rate of natural infections the preventive efficacy was not established, but no dog treated with the IVP became positive.

Efficacy and safety of the combination imidacloprid 10% / moxidectin 1.0% spot-on (Advocate[®] spot-on for small cats and ferrets) in the treatment of ear mite infection (*Otodectes cynotis*) in ferrets

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Background: Ferrets are at risk of ear mite infection (*Otodectes cynotis*) from other infected ferrets or dogs and cats sharing the same environment. In this study the efficacy and safety of a treatment with the combination imidacloprid 10% / moxidectin 1.0% spot-on (Advocate[®] spot-on for small cats and ferrets) was tested in ferrets naturally infested with ear mites.

Methods: Thirty nine ferrets naturally infested with *O. cynotis* (23 males and 16 females, 15 ferrets being kept in a zoo and 24 ferrets being kept by pet-owners) completed a study over a period from December 2009 to March 2010. The study was performed as a multicenter, non randomized, non controlled (all study animals were treated) and non blinded clinical field study. It was conducted in 2 French veterinary practices. Four visits (Visit (V)1 / Day (D)0 = inclusion and first treatment, V2 / D14= second treatment, V3 / D28= possible third treatment, V4 / D56 / termination). The dosage applied was 1 pipet (designed for cats weighing up to 4 kg) per ferret administered as spot-on two or three times at 14 days intervals (at D0, D14, and possibly D28 depending on the results of the parasitological examination of the ears at D28). For ethical reasons, there was no untreated control group

Results: Two or three treatments administered in 14 day intervals to ferrets infested with ear mites provided 100% parasitological cure on D56. At D28 after two treatment applications (D0 and D14) 79.5 % (31/39) of animals were cured. Only 23 % (9/39) needed a third treatment on D28. Local symptoms (inflammation and pruritus) were consistently improved (50.6% improvement at D14, 81% at D28 and 97.7% at D56) as well as the abnormal cerumen production (14.7% improvement at D14, 77.7% at D28 and 100.0% at D56). No general symptoms were noticed during the study (general health and skin aspect).

Conclusions: Advocate[®] spot-on for small cats and ferrets) is an effective and safe treatment for ear mite infection in ferrets. Total cure is obtained after two or three treatment applications at 14 days interval.



SESSION N

Workshops

- **WAAVP guidelines for faecal egg count reduction tests (FECRT)** - R. Dobson
- **WAAVP Guideline on the efficacy testing of anticoccidials in mammals (pigs, dogs, cattle, sheep)** - A. Joachim
- **Guidelines for testing the efficacy of ectoparasiticides for fish** - M. Fisher
- **WAAVP Guidelines: Combinations of anthelmintics for livestock** - T. Geary
- **WAAVP guideline for the evaluation of drug efficacy against non-coccidial gastro-intestinal protozoa in livestock and companion animals** - T. Geurden

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WAAVP guidelines for faecal egg count reduction tests (FECRT)

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Background: WAAVP guidelines for FECRT were provided in the 90's (Coles et al, 1992, Vet. Parasitol. 44, 35-44). Issues that have been investigated since then include (1) the efficacy formula used and the type of mean, (2) the detection limit of the FEC methods, (3) low and aggregated FEC, (4) small group sizes, (5) inclusion of an untreated control group verses pre- and post-treatment counts of the same group, (6) drug administration formulations, (7) the calculation of confidence intervals and (8) the criteria to define reduced efficacy. Therefore, it has been suggested that an update of the current guidelines is warranted to improve the interpretation of FECRT.

Methods: Review of the relevant literature and computer simulation studies. The impact of the above issues on the detection of a truly reduced efficacy was assessed from previous and ongoing studies.

Results: Correct assessment of efficacy from a FECRT is affected by a complex interplay of factors inherent to both the study design (sample size and detection limit of the FEC method) parasite and/or host (level of excretion and aggregation of FEC). Moreover, it suggests that a common approach for performing FECRT over different parasite and host species is not desirable. There is a large variation in both the level of egg excretion and aggregation caused by factors inherent to different parasite and/or host species, demanding a different sample size or detection limit. For some host species pre- and post-treatment egg counts of the same animals are necessary for improved precision while for others the less expensive post-treatment egg counts of control and treated animals is adequate.

Conclusion/Summary: Current guidelines recommend a standard approach which inadequately considers host- and parasite-specific factors, leading in several cases to suboptimal designs resulting in inefficient usage of available resources or inconclusive results.

WAAVP Guideline on the efficacy testing of anticoccidials in mammals (pigs, dogs, cattle, sheep)

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Background: While a number of guidelines (GL) directed by the WAAVP exist on efficacy testing for anthelmintics and ectoparasitocides, as well as for anticoccidials in poultry, no harmonized framework or GL is available for the evaluation of the efficacy of anticoccidials in mammals (pigs, cattle, sheep, dogs). There is, however, a growing amount of knowledge on this topic from field and experimental studies and it appears appropriate to collect and evaluate the available data by an expert consortium.

Methods: The GL will deal with *Isospora suis* in pigs, *Isospora* (syn. *Cystoisospora*) spp. in dogs, *Eimeria* spp. in cattle and in sheep, and *Cryptosporidium parvum* in cattle.

Results: Although the available knowledge about coccidiosis of the aforementioned host species is variable and although the demands regarding coccidiosis control are different (food animals vs. companion animals; different epidemiology etc) the GL will provide a basis for the development cascade in anticoccidial testing. This will cover early phases of testing (pilot screening) as well the evaluation of different infection models and the conduct of field studies. Epidemiological considerations for experimental and field studies including animal welfare considerations are addressed, too.

Conclusion/Summary: An approach will be undertaken to define clinical and parasitological parameters and statistical units, discuss diagnostic issues for different host species. This GL will also address resistance monitoring and the development of new assays, especially quantifiable *in vitro* tests which are being developed for *C. parvum* but as yet unavailable for other *Isospora* or mammalian *Eimeria* spp.

Guidelines for testing the efficacy of ectoparasiticides for fish

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Background: WAAVP guidelines are available for testing treatments to control a range of parasites in terrestrial animals. However, to date, no WAAVP guidelines have been produced for aquatic farm animals. Aquaculture is the fastest-growing food producing sector, providing nearly 50% of the world's food fish. Intensive fish farming of a few key species is expanding worldwide. Intensification has resulted in an increase in the economic significance of ectoparasite pathogens in all stages of the culture process. Owing to the high value of this growing market, it has been possible to invest in the testing of pharmacological products against these parasites. It is timely, then, to produce a set of guidelines for testing aquatic ectoparasiticides.

Methods: The testing of ectoparasiticides for fish has focused on a relatively small number of parasites and fish species. Experience of these studies has been brought to the construction of a set of guidelines which have been generalised so as to apply to a wider range of ectoparasite pathogens in major types of intensive culture systems.

Results: The guidelines illustrate the major considerations necessary for poikilotherms in aquatic environments. They describe the general husbandry of the test fish and their welfare under test conditions. Whereas the general procedures follow those for terrestrial animals, they highlight the need to ensure replicate environmental conditions and the difficulties in achieving a standard dose, both in topical and oral treatments. However, an advantage is that it is possible to include much larger groups and sample sizes when using fish, thereby contributing to a robust statistical analysis.

Summary/conclusions: The guidelines are in progress and will be the first to deal with food animals in aquatic habitats. Many details have been included which are unique to aquatic studies. Whilst such studies are relatively few, the guidelines show that a high level of rigour is possible to achieve.

WAAVP Guidelines: Combinations of anthelmintics for livestock

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Background: Pressure to obtain maximum performance in livestock production has guided the use of anthelmintics in recent decades. A probably unavoidable consequence of intensive chemotherapeutic use is the selection of drug-resistant parasite populations. Such populations have been detected in livestock within a few years of the introduction of new anthelmintic classes. The increased frequency of resistance to broad-spectrum anthelmintics, in the absence of suitably broad-spectrum replacements, initially led to the introduction of combination products to attain the previously achieved therapeutic coverage. Now combination products are recognized by some, but not all, scientific groups as being essential to delaying the emergence of drug resistant populations. The aim of the proposed guidelines is to assess the scientific basis for regulating the introduction of combination anthelmintics globally, e.g., enhanced efficacy, synergistic effects, delaying and/or combating resistance. Other relevant questions that will be considered are: (1) what are the data-based reasons to restrict approval of anthelmintic combinations for routine use? (2) what circumstances would change the current regulatory status of combinations? (3) should combinations, particularly for new drug classes, be preferred in order to retard the development of resistance? (4) what data package would be required to justify regulatory approval of anthelmintic combinations? and (5) is it possible to develop and implement globally-applicable harmonized guidelines for such products?

Methods: Review the relevant literature including the use of drug combinations in other therapeutic areas; combination products or regimens are routinely used in many areas of chemotherapy.

Results: The options to justify combinations of anthelmintics, and possible concerns and potential arguments against combinations, are reviewed.

Conclusion/Summary: WAAVP guidelines for combinations of broad-spectrum anthelmintics are urgently needed.

WAAVP guideline for the evaluation of drug efficacy against non-coccidial gastro-intestinal protozoa in livestock and companion animals

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Background: Over the last few decades, WAAVP guidelines have been published in order to standardize the efficacy evaluation of parasitocides in livestock, poultry and companion animals, along with guidelines for coccidiostats in poultry. There are however no guidelines for non-coccidial gastro-intestinal protozoa in livestock and companion animals, despite the high prevalence, and the clinical, subclinical and public health relevance of these parasites. Recent studies underscore the need for effective drugs and relevant guidelines, with main focus on *Giardia duodenalis*.

Methods: Review of the current literature, and outcome of the discussion held at the symposium 'Giardia in veterinary Medicine: to consider or not to consider?' (WAAVP, Ghent 2007).

Results and discussion: The current guideline deals with many aspects of how to conduct controlled studies to evaluate the efficacy against non-coccidial gastro-intestinal protozoa, using experimental infection models (dose determination and dose confirmation), and efficacy studies in commercial facilities (field effectiveness studies). Furthermore, the selection of animals, housing, the infection procedure, the choice of diagnostic technique and data analysis are discussed. The primary parameter for drug efficacy is the reduction in parasite excretion and/or parasite infection load. As infection with most of these protozoa results in a high excretion of infective stages, as most excretory stages have a long survival time in the environment, and as the dose required for infection is low, a minimum efficacy of 99% is proposed against non-coccidial gastro-intestinal parasites. The secondary efficacy parameter is a significant difference in the proportion of infected animals between treated and non-treated groups. There is still debate on (1) whether a gastro-intestinal parasite count is needed in dose determination/confirmation studies, implying necropsy of animals, (2) whether to use arithmetic or geometric mean counts to calculate reduction between treated and non-treated groups, and (3) whether the proposed efficacy is sufficient



SESSION O

Keynote Lecture

- **Non chemical control of helminths in ruminants: adapting solutions for changing worms in a changing world** - H. Hoste

Oral Communications

- **Antagonistic effect of chitin-degrading microfungi on thick-shelled pig helminth eggs** - K. K. Katakam
- **Field assessment of the efficacy of Duddingtonia flagrans against infective stages of sheep, goat and horse nematodes** - M. Knox
- **Comparison of copper oxide wire particles, copper sulfate and anthelmintic treatment for controlling gastrointestinal nematode infection in lambs** - J. E. Miller
- **Effect of grazing a bioactive forage, in the control of gastrointestinal nematodes in sheep, in Uruguay** - A. Mederos
- **The effects of condensed tannins against cattle nematodes** - A. Novobilsky
- **Acaricidal Activity of Some Selected Pakistani Plants** - Z. Din Sindhu1

Posters

- **Selection of Corriedale sheep for resistance or susceptibility to nematode infection in Uruguay** - D. Castells
- **In Vitro Anthelmintic Activity of Some Tropical Plants Against Gastrointestinal Worms of Goats in Malaysia** - R. Nurulaini
- **Effect of sainfoin on lamb coccidiosis** - A. Saratsis
- **Systematic review-meta-analysis: effect of selected alternative treatments on gastrointestinal nematodes in sheep under field conditions** - A. Mederos
- **Kinetics of galectin-11 expression in resistant and susceptible ovine breeds during a Haemonchus contortus infection** - G. Sallé
- **Effect of the intake of jack bean (Canavalia ensiformis) on the control of gastrointestinal parasites and the growth of tropical lambs** - L. Ríos-de Álvarez
- **In-vitro predatory activity of nematophagous fungi from Costa Rica against parasitic nematodes of sheep and goats** - N. Soto-Barrientos
- **Condensed tannin from Acacia mearnsii reduces helminths infections on goat** - L. Martins Costa Junior
- **In vitro anthelmintic efficacy of Australian plant extracts against gastrointestinal nematode parasites of cattle and goats** - F. Moreno
- **In vitro efficacy of plant extracts and synthesized substances on Rhipicephalus (Boophilus) microplus (Acari: Ixodidae)** - L. D. de Barros
- **Effect of Havardia albicans supplementation on feed consumption and digestibility of sheep and the biology of Haemonchus contortus** - H. H. Galicia-Aguilar
- **Transmission electron microscopy observations of tannin-rich plants effects on Haemonchus contortus** - C. Martínez-Ortiz-de-Montellano
- **Effect of sainfoin or quebracho supplementation against adult Haemonchus contortus and Trichostrongylus colubriformis in sheep** - C. Martínez-Ortiz-de-Montellano
- **Survival of Ascaris suum eggs in raw and separated pig slurry stored at 5°C and 20° C under anaerobic conditions** - K. K. Katakam
- **Anthelmintic Activity of Some Plants with Particular Reference to Their Condensed Tannin Content** - Z. Iqbal
- **Within breed variability in natural resistance to gastrointestinal nematodes with special emphasis to Haemonchus contortus in two Pakistani sheep breeds** - H. Abubaker Saddiqi
- **Duddingtonia flagrans in nutritional pellets: effect of different storage conditions on the viability of chlamydospores** - J. A. Fitz-Aranda
- **Effect of storage time on the viability of Duddingtonia flagrans chlamydospores when fed to sheep using nutritional pellets** - J. A. Fitz-Aranda
- **Differences in the exsheathment rate of Haemonchus contortus larvae exposed to non conventional tannin-rich plant resources** - J. J. Vargas-Magaña
- **In vitro activity of Myracrodruon urundeuva ethanolic extracts on eggs and larvae of Haemonchus contortus** - M. Mayara de Aquino
- **Effects of clinoptilolite on Eimeria spp. infection in sheep** - Y. Alcalá-Canto
- **Prevention of equine cyathostomin infection by spreading Duddingtonia flagrans chlamydospores directly onto the pasture** - A. Paz-Silva
- **Antiparasitic Phytotherapies to livestock production** - A. López
- **Analysis of the effect of antigenic stimulus on the development of the nematophagous fungus Duddingtonia flagrans** - A. Paz-Silva

Non chemical control of helminths in ruminants: adapting solutions for changing worms in a changing world

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Gastrointestinal nematodes remain a major threat for ruminant production, welfare and health associated with outdoor breeding. Up to now, the control of these helminth parasites mainly relied on the strategic or tactical use of chemical anthelmintics. However, the expanding development and diffusion of anthelmintic resistances in nematode populations imposes to explore and validate novel (or rediscovered) solutions available to be combined for an integrated and more sustainable control. These solutions refer to three main principles of action.. Firstly; methods of grazing management aiming at reducing GIN infection by minimizing the contacts between the susceptible hosts and the infective larvae; Such methods which were described since the early 1970s benefit now from the expansion of computing-model based approaches. Secondly, the improvement of the host response against GINs relying either on the genetic selection between or within breeds of sheep or goats or on the manipulation of nutrition. This option benefits of the better understanding of the potential mechanisms, in particular in regard of the host immune response against the worms. Thirdly , worldwide studies dedicated to non conventional anthelmintics, relying on the use of either mineral or vegetal compounds, which permit to eliminate the worms or to strongly and negatively affect the parasite biology are also evoked.. The recent developments and pros and cons concerning these various options/principles are discussed.. Last some results illustrating how the integration of these different solutions can be efficient and applicable in different systems of production and/or epidemiological conditions are presented

Antagonistic effect of chitin-degrading microfungi on thick-shelled pig helminth eggs

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The rising demand for pigs produced in organic farming systems, which require access to outdoor areas and associated increased risk of gastrointestinal nematode infections, is creating growing interest in alternative control strategies. Also development of anthelmintic resistance due to extensive use of anthelmintics is a concern in intensive pig production. Use of chitin-degrading microfungi is one of the options. Extensive research has been conducted on biological control of plant pathogenic nematodes using such fungi, and some fungal species are commercially available for plant protection. In contrast, there are only limited laboratory studies conducted on eggs from nematodes which are animal parasites. The aim of present study is to investigate the antagonistic effect of chitin-degrading microfungi on thick-shelled pig helminth eggs (i.e. *Ascaris suum* and *Trichuris suis*) both in the laboratory as well as in the field. We have performed a pilot study of the ovicidal effect of *Paecilomyces lilacinus* alone and in combination with chitin supplement on *A. suum* eggs over 4 weeks in 2% water agar. Within 1 week more than 90% of the eggs were hatched in both cases within the Petri dish as compared to controls, indicating that the fungus is capable of degrading the chitin wall so the larvae are becoming free. Soon we will proceed for full-fledged study with 2 fungal species (*P. lilacinus* and *Pochonia chlamydosporia*) which were proven to have ovicidal activity against nematode eggs. This will be done in the laboratory using soil samples amended with *A. suum* eggs, under semi-natural (pasture plot) conditions and finally on heavily contaminated pastures.

Field assessment of the efficacy of *Duddingtonia flagrans* against infective stages of sheep, goat and horse nematodes

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Background: Nematode parasites are a common problem in domestic livestock production due to their impact on production and cost of control measures. In seeking possible agents for the biological control of the infective stages, considerable evidence indicates that the nematophagous fungus *Duddingtonia flagrans* may be an ideal candidate.

Methods: For goats and horses, faeces was collected from individual animals being offered the supplement containing a native Australian isolate of *D. flagrans* or a placebo supplement and the faeces placed on separate pasture plots. At 2 week intervals for 8 weeks, presence of larvae in any remaining faeces and surrounding pasture was estimated. For sheep, 2 equal groups of 20 infected sheep were allocated to separate pasture plots. One group (TRT) was then offered supplement containing *D. flagrans* for 60 days while the other (CON) was offered a placebo supplement for the same period. The sheep were then removed from the pasture plots and replaced by groups of 25 worm-free tracer sheep (TRT2 and CON2). After 22 days the tracers were moved to pens with slatted floors for 20 days and 10 from each group slaughtered for worm burden estimation.

Results: The *D. flagrans* supplement reduced larvae numbers in faeces by up to 100% and 95% for goats and horses, respectively. Similarly, larvae recovered from pasture were reduced by up to 84% and 100% for goats and horses, respectively. For sheep, faecal worm egg counts (FEC) were reduced >60% in TRT sheep compared with CON sheep. TRT2 sheep had 60% lower FEC than CON2 sheep at 42 days after entry to their respective pasture plots and 57% lower worm burden at slaughter. No adverse reactions to the *D. flagrans* supplement were observed in any supplemented animals throughout the trials.

Summary/Conclusions: The reductions in infective larvae on faeces and on pasture suggest very effective control of nematode larvae by *D. flagrans* in goats and horses. The sheep studies support this suggestion as *D. flagrans* substantially reduced the infectivity of pasture grazed by sheep. A commercial product will soon be available to farmers to aid in nematode parasite control.

Comparison of copper oxide wire particles, copper sulfate and anthelmintic treatment for controlling gastrointestinal nematode infection in lambs

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Gastrointestinal nematode infection is a serious constraint to sustainable small ruminant production in the southeastern US. *Haemonchus contortus* is the primary pathogen which can cause severe anemia and even death. Copper oxide wire particles (COWP) have been shown to control *H. contortus* infection. The use of copper sulfate (CuSO₄) has also been promoted for control. A recent study incorporating CuSO₄ in a feed supplement was not effective. This study was conducted to evaluate the efficacy of COWP (2 g/hd), CuSO₄ (1% solution drench, 35 ml/22.7 kg), anthelmintic [ANTH, levamisole (8 mg/kg) + albendazole (10 mg/kg)] and a combination of COWP and ANTH. Thirty-three (9 Katahdin and 24 crossbred, Gulf Coast Native x Suffolk) lambs were randomly allocated to the 4 treatment groups plus a control group (CONT). Each group had 7 lambs except the ANTH and COWP/ANTH groups had 6. Fecal egg count (FEC) was conducted on day 0, 7 and 14 after treatment. FEC was log transformed and a repeated measures ANOVA over time was conducted. Means were adjusted for the variables in the model. There was no significant difference between groups ($p>0.05$) on day 0. There was a significant interaction for treatment ($p=0.0043$), day ($p<0.0001$) and treatment x day ($p=0.0170$). There was no interaction for breed ($p=0.0992$). On days 7 and 14, there was no difference ($p>0.05$) between CONT, ANTH and CuSO₄ groups, and COWP and COWP/ANTH groups were significantly ($p<0.05$) lower than the CONT group. Results indicate that COWP alone or with ANTH were effective in reducing infection, but ANTH alone and CuSO₄ were not effective. Copper treatment in the form of COWP appears to be preferable to CuSO₄ for controlling infection in lambs. Also, the worm population appears to be resistant to the combination of levamisole and albendazole.

Effect of grazing a bioactive forage, in the control of gastrointestinal nematodes in sheep, in Uruguay

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Background: The bioactive forage *Lotus pedunculatus* has been reported to have a high content of condensed tannins (CT) with a potential direct effect against gastrointestinal nematodes (GINs). This study evaluates the effect of *Lotus pedunculatus* on GINs in grazing sheep.

Methods: A block experiment was carried out at Glencoe Research Station, INIA Tacuarembó, Uruguay, during August-December 2005. Two blocks were divided into two plots and sown with *Lotus pedunculatus* cv. Grassland Makus (LM), reported as having a high content of CTs, and *Trifolium repens* LE Zapican (White clover, WC) with low CTs. Naturally parasitized Corriedale male lambs were placed on the plots (n=21) stratified by body weight and fecal egg counts (FECs) and divided into three groups (n=7), one drenched with Polietilen glycol (PEG) to inhibit the effect of CTs; one with water (H₂O) (CTs active) and one with an anthelmintic (ANTH). The follow-up period was 110 days. Production traits were recorded and FECs were performed on individual animals. Infective larvae were recorded in animals and pasture. At the end of the trial a sub-sample was selected for GIN adult counts and speciation (n=48). Summary statistics and a mixed linear regression model using the log FECs was fit using SAS 9.2.

Results: Briefly, final arithmetic means FECs for the lambs grazing LM were 630, 576 and 7 for PEG, H₂O and ANTH treatments, respectively, and 1407, 1935 and 35 for the same treatments in lambs grazing WC. The total GINs adult arithmetic means were 15964, 14080 and 5185 for PEG, H₂O and ANTH treatments, respectively in lambs grazing LM, and 8598, 1192 and 4874 for the same treatments in lambs grazing WC. The main GINs identified in the necropsies were *Trichostrongylus colubriformis* and *Trichostrongylus axei*. The statistical analysis showed only a significant difference in log FEC between treatments in the ANTH groups (p<0.01), for both forages.

Summary: Although summary statistics indicated a tendency for the FECs to be lower in lambs grazing LM, the statistical analysis showed no difference in FECs or adult nematode intensity, by forage. Lamb performance in all treatments was good (data not shown), suggesting a positive effect of both forages on the resilience against GINs.

The effects of condensed tannins against cattle nematodes

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Background: The use of bioactive forages in ruminant production was suggested as a possible alternative control of gastrointestinal nematodes (GIN). One of the most investigated groups of plants with anthelmintic properties are tannin-containing legumes from the *Fabaceae* family. Direct anthelmintic effects of tannin-containing plants have already been shown in sheep and goat GIN, but not in bovine GIN. Thus, the aim of this study was to evaluate *in vitro* effects of three tannin-containing plants against bovine GIN.

Methods: Effects of *Onobrychis viciifolia*, *Lotus pedunculatus* and *Lotus corniculatus* condensed tannin (CT) extracts on *Cooperia oncophora* and *Ostertagia ostertagi* were determined by a larval feeding inhibition assay (LFIA) and a larval exsheathment assay (LEA). The role of condensed tannins in causing the anthelmintic effect was verified by adding polyvinylpyrrolidone in tested extracts.

Results: In LFIA, a significant inhibition of feeding of first stage larvae of *O. ostertagi* and *C. oncophora* was detected for all three plants in a dose-dependent manner. The *L. pedunculatus* extract, based on EC₅₀, was most effective against both nematodes, followed by *O. viciifolia* and *L. corniculatus* extracts. The effect of CT extracts upon larval feeding behaviour was correlated with CT content and procyanidin/prodelphinidin ratio. CT extracts of all three plants also affected the course of larval exsheathment of *O. ostertagi* and *C. oncophora* L3 larvae.

Conclusions: Our results indicate that extracts of *O. viciifolia*, *L. pedunculatus* and *L. corniculatus* have *in vitro* anthelmintic activity against *O. ostertagi* and *C. oncophora*. Anthelmintic properties of plant extracts are most likely associated with total concentration of CT and presence of prodelphinidin tannins. This is the first report on anthelmintic effects of CT against bovine nematodes.

Acaricidal Activity of Some Selected Pakistani Plants

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Background: Infestation with the common cattle tick *Rhipicephalus (R.) microplus* is one of the major constraints in profitable livestock production in tropical and subtropical parts of the globe. Cattle tick not only feed on blood of animal but also plays an important role in the transmission of diseases. Annual productivity losses attributed to *R. microplus* are greater than 100 million dollars in Australia and more than US\$ 498.7 million in India. Control of ticks largely depends on the use of synthetic acaricidal drugs, but ticks have striking ability to become resistant to the toxic effects of the chemicals. Cattle tick has developed resistance against DDT, organo-chlorines, a range of organo-phosphate and organocarbonates, amidines and synthetic pyrethroids. Thus, there is an urgent need for new tick control strategies and one solution lies in use of plant extracts for tick control, especially in developing countries. Objectives of this study were to evaluate the acaricidal effect of crude plant extracts against the common cattle tick, *R. microplus*.

Methods: Eleven plants were selected from North-West Frontier Province of Pakistan for this purpose and their aqueous-methanol extracts were prepared. *In vitro* trials were conducted in Australia using acaricide susceptible strain in syringe test (modified larval immersion test). Briefly, the nozzle end of a 3 ml syringe was cut off, approximately 10 mg of eggs were placed in the syringe and it was sealed with a double layer of nylon gauze (organza fabric). Prepared syringes were incubated until 14 d after hatching of eggs, when the bioassay was performed on the larvae. Lethal concentrations for 50% of larvae (LC₅₀), LC₉₀ and LC₉₉ values were calculated for each tested product.

Results: Out of 11, nine plants were found effective. These plants in order of their activity are *Ricinus communis* (LC₉₉=0.6%), *Cedrus deodara* (LC₉₉=6.8%), *Nigella sativa* (LC₉₉=11.939%), *Daphne mucronata* (LC₉₉=15.7%), *Artemisia brevifolia* (LC₉₉=17.9%), *Trachyspermum ammi* (LC₉₉=23.1%), *Zingiber zerumbet* (LC₉₉=49.5%), *Prunus persica* (LC₉₉=75.3%) and *Capsicum annuum* (LC₉₉=103.4%).

Conclusion: These results consolidate the belief that, plants could be a good candidate for search of new compound for tick control and management of resistance in future.

Selection of Corriedale sheep for resistance or susceptibility to nematode infection in Uruguay

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Divergent breeding lines of Corriedale sheep were selected for low (R-Line) or high (S-Line) faecal worm egg count (FEC) following natural mixed infection (mainly *Haemonchus contortus*) and maintained grazed together on natives pastures at "Dr. Alejandro Gallinal" Experimental Research Centre of SUL since 1998. The nucleus flock was established with sheep from the Corriedale Central Progeny Test after a screening for low or high FEC Estimated Progeny Difference (EPD/FEC) of 3545 progeny lambs. More than 150 ewes were mated on the R-Line and 120 ewes on the S-Line. At least five sires were used in each line annually. The nucleus was linked through reference rams to the Global Corriedale Genetic Evaluation Program GCGEP and maintained open to the importation genes. Three FEC measurements (FEC1, FEC2, FEC3) after natural challenge of different cycles (separate by an anthelmintic treatment) post weaning and production traits as well as a complete pedigree were recorded each year. Mean FEC2 (9 to 12 months of age) of the S-Line were 3,3 times higher than the R-Line in 2004, 1,6 times in 2005 and 2006, 2,7 times in 2007, 6,8 times in 2008 and 3,3 times in 2009. Mean FEC3 (13 months of age) of lambs born in 2009 were 2246 and 344 FEC for the S-Line and R-Line respectively ($p < 0.005$ for ANOVA after Log Transformation). In 2009 spring rise was measured on lactating ewes 73 days after lambing and the mean FEC of ewes of the R-Line was 395 and 660 for the S-Line. For the R-Line and S-Line the genetic trends were -0.07 and +0.04 per year. The breeding values were -0.50 for the R-Line and +0.60 for the S-Line in 2009. It is concluded that selection for high or low FEC in Corriedale sheep has achieved a 6.8-fold difference between the divergent lines. The differences were observed in lambs and in the ewes at spring rise.

In Vitro Anthelmintic Activity of Some Tropical Plants Against Gastrointestinal Worms of Goats in Malaysia

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Livestock farming has become one of important economy contributors in Southeast Asia. In Malaysia, total number of sheep and goat has increased from 538,538 heads in 2007 to 562,554 heads in 2008. However, one of the main problems limiting productivity in small ruminants is infectious diseases such as pneumonia and gastrointestinal helminth infections which causes mortality and morbidity in goats and sheep. Due to anthelmintic resistance, novel approaches to worm control using ethnoveterinary plant to reduce helminthiasis is an area of research interests to be explored. Many studies were conducted in vivo (in experimental animals) which is very costly and time consuming. Thus, this study was designed to further investigate a few potential plants in vitro as a possible natural anthelmintic for use in small ruminants. Two plants which consists of *Piper betle* leaves and papaya (*carica papaya*) seed, were tested in vitro using modified Larvae Mortality Assay against gastrointestinal infective larvae of Boer goat. *Haemoncus contortus* larvae was distributed at a concentration of 50 L3 (n=±50) per well in 96 multiwells plates, incubated with diluted aqueous extract of *Piper betle* leaves and papaya (*carica papaya*) seed at 500 ug/ml and 1000ug/ml at 20°C for 24 hours. Control test was done using PBS. Results showed that after 24 hours, there was a significant reduction of motile larvae at 65% and 81% for papaya (*carica papaya*) seed and *Piper betle* leaves leaves. The control wells showed no reduction in terms of motility with standard deviation at 5-10%. In this study, the larval mortality assay was modified based on the principle of egg hatch assay and larval migration test to study the plant efficacy as a natural anthelmintic against *H. contortus* larvae obtained from naturally infected goats. Therefore, from the results, it can be suggested that *Piper betle* leaves and papaya (*carica papaya*) seed can be a potential alternative to be used as an anthelmintic. However, further studies should be conducted in vivo to study the mechanism of action in animals.

Effect of sainfoin on lamb coccidiosis

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Background: Sainfoin (*Onobrychis viciifolia*) is a forage legume which, when fed to sheep, due to its unique tannin and polyphenol composition have been shown to present highly beneficial properties such as anthelmintic properties, increase protein utilization and prevent bloating.

The aim of the study was to screen the *in vivo* effect of sainfoin against lamb coccidiosis when fed as hay to infected lambs or their ewes.

Methods: The experimental set-up included feeding trials in lambs which preformed twice and each time included 3 groups of 8 lambs each assigned to: group A-L (receiving sainfoin-hay); group B-L (receiving lucerne-hay and no further treatment) and group C-L (receiving lucerne-hay and treated with toltrazuril at the recommended dose rate). The trial started at weaning of the animals and lasted for 2 months. Additionally, a feeding trial which included 31 pregnant ewes and all the lambs borne was carried out including: group A_E (15 ewes, receiving sainfoin-hay) and group B-E (16 ewes, receiving lucerne-hay). Those ewes received the respective diet 1 month before and 3 months after lambing. The sampling period for their lambs lasted 9 weeks starting at day 9 of age.

During all trials, lambs were sampled weekly and faecal consistency and oocyst excretion (opg) were recorded.

Results: In the first 2 trials significant reduction in the total amount of oocysts excreted (using mean AUC values) between groups A-L and B-L was recorded only in one of them and from week 3 until week 7 after the start of the trial. In contrast to that, when ewes were fed sainfoin hay the total amount of oocysts excreted by their lambs throughout the trial was reduced from 847,065 to 439,092 (48.1% reduction in comparison to the control), which was a significant difference ($p=0.01$).

Conclusions: The results suggest that sainfoin intake by the ewes before and after lambing is a better approach to achieve an effect against *Eimeria* spp in lambs than feeding the lambs directly. Hay intake by recently weaned lambs may not be sufficient to reach the needed bioactive compounds concentration in their gut to obtain an anticoccidial effect. This may also explain why when efficacy against coccidia by feeding lambs appeared that was after 3 weeks of treatment.

Systematic review-meta-analysis: effect of selected alternative treatments on gastrointestinal nematodes in sheep under field conditions

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Background: A systematic review (SR) is a research synthesis method that follows a structured methodology, minimizing biases and random errors. Meta-analysis (MA) is a statistical method for synthesizing the results from similar studies with the aim to produce a more precise single estimate of a treatment effect.

Methodology: The interventions selected for this SR were nutraceuticals, genetic resistance (GR), homeopathies, nutritional manipulation (NM), copper oxide wire particles (COWP), grazing management (GM) and biological control (BC). A SR protocol was designed a priori, validated and tested, and a search algorithm was applied to three electronic databases; one level of relevance screening was conducted by two reviewers. Quality assessment and data extraction were conducted in one step. Random effect MA and meta-regressions (MR) were performed with the natural logarithm of the difference in means (lnMD) between the control and treatment groups, for fecal egg counts per gram of wet feces (FEC), worm counts (WC) or fecal egg counts per gram of dry matter (FECDM) as the outcome. Treatment effect estimates (lnMD), were back-transformed to their count ratios (CR) for presentation of results.

Results: Fifty-one studies (21 controlled studies [CS] and 30 challenge studies [ChS]) and 85 unique treatment comparisons were included in this review. Overall, the significance of pooled effect estimates and associated heterogeneity values for treatments varied by study design and outcome type and significant heterogeneity was observed for many treatment-outcome combinations. Homogenous MAs for two CS of homeopathies using an FEC outcome resulted in a non-significant pooled count ratio of 1.60 ($P=0.21$). Three challenge studies of COWP using a WC outcome, showed a significant pooled CR of 1.68 ($P=0.06$).

Summary: This SR identified a large body of primary research on alternative treatments for sheep GINs. However, very few studies were conducted under the same conditions, using the same treatments and outcomes. Thus, there was a lot of heterogeneity between studies. The MA suggested that even though nutraceuticals reported significant heterogeneity, they were still the most promising intervention besides genetic resistance (results not show) for the control of gastrointestinal nematodes in sheep.

Kinetics of galectin-11 expression in resistant and susceptible ovine breeds during a *Haemonchus contortus* infection

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Background: Host galectins bind glycans on the surface of gastro-intestinal nematodes and form part of the host's innate immune response to these parasites. However, the specific roles of these lectins have not been yet elucidated: formation of glycoprotein cement around nematodes that precipitates their expulsion or early recognition of parasitic antigens. Martinik Black Belly (MBB) sheep are more resistant than Lacaune (LAC) breed in *Haemonchus contortus* primary infections. This study was designed to compare the expression of sheep galectin-11 during a primary infection of *H. contortus* between the two breeds. Potential inhibitory effect of galectin-11 enriched mucus on larval migration was also investigated.

Methods: Six-months old MBB (N=14) and LAC lambs (N=10), reared indoors, were orally infected with 10 000 *H. contortus* L3 and euthanized at 4, 8, 15, 21 and 30 days post-infection (p.i.). Four other lambs (2 MBB and 2 LAC) were not infected. Ovine galectin-11 mRNA expression was measured by RT-PCR in the abomasal mucosa. Presence of the galectin-11 protein was determined by western blot in abomasal mucus. Inhibitory effect of abomasal mucus on larval migration was evaluated by the bioassay described by Rabel et al., 1994.

Results: No galectin-11 mRNA expression was noticed in the non infected lambs. In most (but not all) MBB lambs, there was a significant peak of galectin-11 mRNA by day 8 p.i. followed by a regular decline until day 30. In contrast, significant galectin-11 mRNA levels were only reported by day 15 p.i in LAC lambs. Western blots revealed intense specific bands in MBB by days 8 and 15 p.i. and in LAC by day 15 p.i. only. Abomasal mucus of MBB lambs showed higher inhibitory effects on larval migration compared to LAC lambs whatever the status (infected or not), the period of infection and the galectin-11 expression.

Conclusion: Resistant animals showed early and sustained galectin-11 expression compared to susceptible animals. This expression was not related to the enhanced inhibitory effect of abomasal mucus in the resistant breed.

Effect of the intake of jack bean (*Canavalia ensiformis*) on the control of gastrointestinal parasites and the growth of tropical lambs

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Background: Many tropical types of forage are known to harbour anti-parasitic properties which may assist in the development of a cost-effective method of parasite control for resource poor farmers. In particular, *Canavalia ensiformis* (jack bean) is known to contain high levels of lectin (Con A). In our previous studies the semi-purified form of other lectins (PHA) has been demonstrated, both *in vitro* and *in vivo*, to have anthelmintic properties.

Methods: This study investigated the effect of supplementation with canavalia seed on the performance and parasite status of twenty West African male lambs that were naturally infected and maintained under foraging conditions. For eleven weeks animals were allocated to one of two groups (n=10) and continued to graze native forages and star grass [*Cynodon nlemfluensis*] during the day and offered a supplement of either 2% bodyweight of brewers grain in the morning (group C-) or offered the same supplement which also included 2g per kgLW canavalia [*Canavalia ensiformis*] seed (group C+). Animal live weight was recorded weekly and faecal samples collected weekly for the determination of the concentration of nematode eggs and oocysts in the faeces.

Results: Canavalia supplementation increased liveweight gains from -10 ± 9.2 g per d for C- animals to $+21 \pm 8.2$ g per d for C+ animals ($P=0.02$), resulting in a 2.2kg advantage in live weight in canavalia supplemented animals by the conclusion of the study. This was associated with an overall reduced concentration of nematode eggs and oocysts in the faeces, with mean back-transformed values being 2020 and 1370 nematode eggs per g ($P=0.002$), and 2170 and 1430 oocysts per g ($P=0.05$) for C- and C+ groups, respectively.

Summary/Conclusions: In conclusion, dosing with canavalia seed at this level provided significant increases in animal performance that were associated with a reduction on their parasite status.

***In-vitro* predatory activity of nematophagous fungi from Costa Rica against parasitic nematodes of sheep and goats**

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Background: Resistant nematodes to albendazole and ivermectine have already been reported in Costa Rica. The acquired resistance to anthelmintics by gastrointestinal nematodes, requires biological control be considered as an alternative. The most effective natural soil enemies of nematodes are nematophagous fungi.

Methods: In order to collect and identify predatory nematophagous fungi (PNF), samples were obtained from 51 farms distributed throughout the seven provinces of Costa Rica. The origin samples included: soil from different crops (potatoes, tomatoes, bananas, ornamental plants, squash and coffee); animal feces (cattle, sheep, goat and horse); soil and fallen leaves from forest; and plants with signs of nematode infection. Each sample was processed using three techniques for the extraction of fungi from soil: sprinkling technique, soil dilution and humidity chamber. Twenty four strains of nematophagous fungi were found in 19 farms; 83.3% of the fungi were isolated by sprinkling technique. The following fungi were identified: *Arthrobotrys oligospora* (n=13); *Candelabrella musiformis* (n=9); and for the first time there was isolation of *A. conoides* (n=1) and *A. dactyloides* (n=1) in the country. Moreover, 16 strains from *Trichoderma* (n=13), *Beauveria* (n=1), *Clonostachys* (n=1) and *Lecanicillium* (n=1) were obtained. In addition, pH of each possible fungal isolation source was measured, and it varied from 5.2 to 9.9, however PNF isolates fell within the range of 5.6 to 7.5. The PNF strains were cultivated in four different media for the production of chlamydospores: potato dextrose agar (PDA); corn meal agar (CMA); malt extract agar (MEA) and potato carrot agar (PCA). Out of these cultures, 95.8% of the strains formed chlamydospores primarily in the PCA. Of these strains, the prolific spore producers were subjected to ruminant artificial gastrointestinal conditions. A total of 14 fungi were tested, out of which 42.9% survived the digestive analysis. Neither *A. conoides* nor *A. dactyloides* were viable following the *in-vitro* gastrointestinal test.

Conclusions: The PNF isolated in this study demonstrated an action against ovine and caprine gastrointestinal nematodes and are candidates for use in biological control of these organisms. Among these microorganisms, *Candelabrella musiformis* appears to be the most promising fungi for use as a biological control agent in Costa Rica.

Condensed tannin from *Acacia mearnsii* reduces helminths infections on goat

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The use of condensed tannins from *Acacia* (*Acacia mearnsii*) can be used to control gastrointestinal nematodes of small ruminants. The aim of this study was to evaluate the use of condensed tannins of *A. mearnsii* to control gastrointestinal nematodes in goats. To showed the efficiency of this plant rich in tannins, an experiment was conducted to observe the anthelmintic effect of condensed tannins from acacia (*A. mearnsii*) on young goats infected naturally. To perform this experiment were selected twenty-four kids after weaning and age between 3 and 5 months, who were divided into three groups of eight animals, being 1) Untreated control group, 2) Group treated with anthelmintic commercial (Levamisole and Albendazole) and 3) Group that received weekly four grams of condensed tannin of *A. mearnsii*. During 192 days after the start of the experiment faeces were collected at intervals of seven days and the animals were weighed every two weeks. At the end of the experiment the animals were slaughtered and carcass data were observed (fat cover, scrotal circumference and weight of the scrotum, weight and length of the hot carcass and circumference of the ham). Was not observed anthelmintic efficacy of condensed tannin from *Acacia*, just shown that use of the extract can maintain the low egg count per gram of faeces (EPG), in this case with one count of less than 500 eggs. The final weight and average daily weight gain of goats fed with condensed tannins show differences significantly from the control group ($p = 0.059$ and $p < 0.05$, respectively). No significant difference was observed in weight of skin and subcutaneous fat, hot carcass weight, scrotal circumference and weight scrotal, length of carcass and circumference of the ham ($p > 0.10$).

***In vitro* anthelmintic efficacy of Australian plant extracts against gastrointestinal nematode parasites of cattle and goats**

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Infection of ruminant livestock by gastrointestinal nematodes (GIN) is a world wide problem and the use of synthetic chemical to treat GIN is creating problems for animal welfare, disease resistance and food quality. The study of the anthelmintic properties of plants rich in secondary metabolites (PSM) is a line of investigation that can provide ecologically-sound methods for the treatment of parasites. There is evidence that different types of PSM compounds like condensed tannins, terpenoids, saponins, polyphenols, alkaloids and glycosides can potentially act against nematodes. The purpose of this study was to test *in vitro* the possible direct anthelmintic effects of 24 Australian plant extracts, rich in PSM, upon the migration of infective larvae (L3) of *Haemonchus placei*, *Cooperia* sp. (cattle) and *H. contortus*, *Trichostrongylus colubriformis* (goats) in order to identify plants that can then be tested *in vivo*. Plant extracts were diluted in a range of 5, 15 and 30 mg/ml concentrations and their effects on L3 motility were evaluated using a larval migration inhibition (LMI) assay. The effect of plant extracts on parasite migration was analyzed by ANOVA as a factorial design with two factors: plant species and concentration. Multiple comparisons of least means were made to detect the best extract-concentration combination. Plant extracts reduced the larval migration of *H. placei* and *Cooperia* sp. Extract from *Allocasuarina torulosa*, *Neolitsea dealbata*, *Acacia holosericea*, *Acacia salicina*, *Callitris endlicheri* and *Casuarina cunninghamiana* were the most effective against these parasites ($P < 0.0001$). Plants extracts were less effective on L3 migration of *H. contortus* and *T. colubriformis*. Extracts from *Callitris endlicheri*, *Casuarina cunninghamiana*, *Acacia farnesiana*, *Acacia holosericea* and *Acacia nilotica* showed an important larval migration inhibition against *H. contortus* and *T. colubriformis* ($P < 0.0001$). *Callitris endlicheri* was the plant species that consistently inhibited the larval migration of every nematode species studied. These *in vitro* results suggest anthelmintic properties associated with some of the plant species we studied.

***In vitro* efficacy of plant extracts and synthesized substances on *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae)**

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Herbal drugs have been widely evaluated as an alternative method of parasite control, aiming to slow development of resistance and obtain low-cost biodegradable parasiticides. The aim of this study was to evaluate the *in vitro* efficacy on *Rhipicephalus (Boophilus) microplus* of *Carapa guianensis*, *Cymbopogon martinii*, *Cymbopogon schoenanthus* and *Piper tuberculatum* extracts and similar synthesized substances. In the immersion test, engorged females were collected from cattle and evaluated in five dilutions ranging from 10% to 0.030625%. In the larval test, around 100 larvae were placed on filter paper impregnated with the dilutions, ranging from 10% to 0.02%. The treatments and controls in both tests were done in three replicates. Phytochemical analysis of oils was performed by gas chromatography. The main compounds were oleic acid (46.8%) for *C. guianensis* and geraniol for *C. martinii* (81.4%) and *C. schoenanthus* (62.5%). The isolated and synthesized substances showed no significant effect on larvae and engorged females. *C. martinii* at a concentration of 5% and *P. tuberculatum* at 10%, showed the best efficacy on the engorged females, causing 75.60% and 91.97% mortality, respectively. The fact that geraniol is present in greater quantities in *C. martinii* explains its higher activity in relation to *C. schoenanthus*. There is still a need to validate the *in vivo* use of safe and effective phytoparasiticide substances. Efforts should be focused on developing formulations that enhance the efficacy of these extracts *in vivo* and lengthen the residual period.

Effect of *Havardia albicans* supplementation on feed consumption and digestibility of sheep and the biology of *Haemonchus contortus*

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Background: Tannin rich fodder may provide a nutraceutical effect. However, the plant secondary compounds may affect the digestibility of the diet. This trial evaluated the effect of the intake of *Havardia albicans* foliage on sheep apparent dry matter digestibility (DMD) and the *Haemonchus contortus* worm burden, worm female length and fecundity.

Methods: Fifteen hair sheep (16.0 ± 4.2 kg LW) rose free of gastrointestinal nematode (GIN) were used. Animals were fed a basal diet: grain concentrate (14.3% CP) and *Pennisetum purpureum* grass (90% and 10% respectively) and were kept in metabolic cages during the experiment. Animals were infected on Day 0 with 3000 infective larvae (L_3) of *H. contortus*. On day 28 post-infection (PI) animals were randomly distributed to three groups: I) Control (100% basal diet), II) HA+PEG (basal diet 60% + *H. albicans* 40%) + Polyethylene glycol (PEG) and III) HA (basal diet 60% + *H. albicans* 40%). The *H. albicans* foliage was supplied for 13 d including 7 days of adaptation (day 28 to 40 PI). The content of condensed tannins (CT), total phenols (TP) and total tannins (TT) was determined. Concentrate, grass and *H. albicans* intake ($\text{kg LW}^{0.75}$), DMD and *H. contortus* fecal egg excretion were determined. Animal were humanly slaughtered at day 41 PI to assess adult worm burden, female worm length and fecundity (numbers of eggs *in utero*).

Results: Foliage had 7.15% CT, 6.13 TP and 6.69% TT. Experimental groups had similar intakes. Intake of *H. albicans* was not affected by PEG but reduced the DMD of HA+PEG and HA groups ($P<0.05$). The control group excreted 30% more eggs than HA+PEG and 36% more than HA. No significant effects were observed because only 5 animals were compared per group. Worm burdens were similar in all groups but the *H. contortus* females in the HA group were shorter and had reduced fecundity ($P<0.05$) (250 individuals were compared in each group). Adding PEG (HA+PEG) cancelled the effect of *H. albicans* intake on the worm length.

Conclusion: A short period of *H. albicans* intake had an effect on the DMD of sheep. There was no evident effect on fecal egg excretion but it reduced *H. contortus* female worm length and fertility.

Transmission electron microscopy observations of tannin-rich plants effects on *Haemonchus contortus*

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Background: *In vivo* trials showed that goats and sheep fed with tannin rich fodders or extracts may show a reduced faecal excretion of nematode eggs. Also, the female worms in the tannin supplemented animals have lower fecundity and/or length. The objective of this trial was to assess the ultrastructural changes caused by two tannin-rich fodders (*Lysiloma latisiliquum* or *Onobrychis viciifolia*) or by quebracho extract (*Schinopsis spp*) on adult *Haemonchus contortus* to better understand the *in vivo* AH effects caused by the consumption of tannin rich materials.

Methods: Ten goats were experimentally infected with *H. contortus* (3000 L₃ larvae/ kg BW). When infections were patent (from D28 post infection) a pair of goats received *L. latisiliquum* fodder ad lib, a second pair of goats received *O. viciifolia* fodder ad lib and a third pair received quebracho extract (90 g/day) for seven days. Four goats were kept without a tannin rich feed (control). After the provision of the tannins, the animals were humanely slaughtered to withdraw the abomasums and collect their female worms, which were immediately fixed in a glutaraldehyde-cacodylate solution at 4°C. After preparation of sections, the methods of transmission electron microscopy (TEM) were used to detect possible ultra-structural changes of *H. contortus* in contact with TR materials compared to control worms.

Results: The intestinal cells of *H. contortus* were a target for the bioactive compounds of TR plants, causing acute swelling and vacuolization as well as mitochondrial damages. Similar lesions were also recorded in the muscular and hypodermal cells of the parasites exposed to tannins. Collateral trials using the same tannin rich materials and the same period of feeding showed a significant reduction en faecal egg counts, worm length and fecundity in the *L. latisiliquum* supplemented animals and a reduced worm fecundity with the other tannin rich materials.

Conclusion: These tannin rich materials generated ultrastructural changes that might alter the worm motility and nutrition of the worms. The latter may help to explain the consequences on the reproduction and size of *H. contortus* in previous trials.

Effect of sainfoin or quebracho supplementation against adult *Haemonchus contortus* and *Trichostrongylus colubriformis* in sheep

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This trial aimed at determining the possible direct and/or indirect anthelmintic effects of sainfoin hay and quebracho extract against adult *Haemonchus contortus* and *Trichostrongylus colubriformis* populations in sheep. Thirty, two-month old, Lacaune lambs were experimentally infected (D0) with 3000 L₃ larvae of *H. contortus* and 5000 L₃ larvae of *T. colubriformis*. The control group (C) received Lucerne hay (*Medicago sativa*) *ad libitum* as a tannin-free forage and a commercial concentrate feed. The quebracho (*Schinopsis spp*) group (Q) received the same diet of the control group and a quebracho oral suspension (90 g/day) from D28 PI. The sainfoin (*Onobrychis viciifolia*) group (S) received the control diet plus Sainfoin hay *ad libitum*. When the infection was patent, five animals of the S and Q groups respectively were exposed to the tannin rich (TR) sources for 7 days (D28 to D34) and were sacrificed on D35 with 5 animals of the C group (short term). The remaining 5 animals in the original groups continued their treatments until D45 (16 days exposed to tannins) (long term) when the remaining animals were slaughtered. Live weight (LW) and nematode faecal egg excretion (EPG) were measured twice a week, and the PCV obtained weekly. At necropsy, abomasa and small intestine contents were recovered to determine worm burdens, female worm lengths and fecundity. Histological samples were taken from abomasums and small intestines for mucosal inflammatory cell counts. Short or long term exposure to the tannin source had no significant effect on the LW change and PCV between groups. Similarly, EPG excretion and the inflammatory cell counts were not affected by TR supplementation. However, *H. contortus* and *T. colubriformis* worm burdens were significantly reduced by quebracho supplementation ($P < 0.02$). The fecundity of *H. contortus* was reduced ($P < 0.04$) in the S group. The *T. colubriformis* fecundity reduced with quebracho ($P < 0.05$). Exposure time to the source of tannins had no significant effect for both GIN species.

Survival of *Ascaris suum* eggs in raw and separated pig slurry stored at 5°C and 20° C under anaerobic conditions

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Pig slurry is traditionally stored raw but recent technological developments allow a separation into a solid and liquid fraction. In contrast to raw manure and solid wastes, little is known about the survival of pathogens in the liquid fraction. The present study determined the survival of *Ascaris suum* eggs in raw slurry and a separated liquid fraction over 10 months at 5° C and 20° C under anaerobic conditions. Nylon bags containing un-embryonated *A. suum* eggs were placed in raw slurry or the liquid fraction in 1 L bottles. Three replicate samples were obtained for each sampling yielding a total of 84 raw slurry and 84 liquid fraction samples. Samples were analyzed at days 0, 7, 14, 21, 28, 42, 56, 84, 112, 168, 238 and 308. Three un-exposed controls (nylon bags with un-embryonated eggs inserted in distilled water) were stored at both 5° C and 20° C for each sampling date. Viability of eggs was assessed by the percentage of embryonation after six weeks storage in 0.01N H₂SO₄ at 20°C. Egg viability started declining from day 84 in both raw slurry and the liquid fraction and at day 238, the viability of *A. suum* eggs was reduced drastically to 5% and 1% in raw slurry and liquid fraction samples maintained at 20° C respectively. In contrast, egg viability was 82 and 58% in liquid fraction and raw slurry samples maintained at 5° C. Throughout the study, the viability of eggs in controls was above 90% at both the temperatures. The study confirmed a negative correlation between temperature and egg viability in raw and separated slurry fractions. Further, it is concluded that *A. suum* eggs and potentially also other pathogens seem to have a higher survival in the liquid fraction of separated manure as compared to raw manure. This needs to be considered when separated liquid manure is used to fertilize crops.

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

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The anthelmintic resistant gastro-intestinal nematode populations constitute a major problem especially in small ruminants in the subtropics and tropics because of frequent treatment failures. Plants containing condensed tannins (CT) have been reported to cause direct and indirect anthelmintic effects. The indirect effect of CT may be through an increase in protein availability and extra protein could improve the host's ability to mount an effective response towards gastrointestinal parasites, during the expression of immunity. The CT could also complex with nutrients and inhibit nutrient availability for larval growth or decrease gastrointestinal metabolism directly through inhibition of oxidative phosphorylation causing larval death. CT extracted from several forages have been reported to disrupt the life cycle of nematodes by preventing their eggs from hatching and by preventing larval development to the infective stages. It has been postulated that they may impair vital processes such as feeding and reproduction of the parasite or may bind and disrupt the integrity of the parasite's cuticle. Tannins may also induce physiological changes in the gut of the host resulting in secretion of mucous and chemicals harmful to the parasite.

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 *Syzygium 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 monosperma* (91.7%), *Syzygium cumini* (88.5%), *Terminalia arjuna* (87.3%), *Zyziphus nummularia* (87%), *Acacia nilotica* (80.1%), *Albizia lebbek* (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*.

Within breed variability in natural resistance to gastrointestinal nematodes with special emphasis to *Haemonchus contortus* in two Pakistani sheep breeds

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Nematode parasites are the main animal health constraint in sheep production on pasture and cause severe economic losses in term of milk, meat and wool. Use of broad spectrum anthelmintics is the common worm control strategy through out the world. Owing to the development of resistance against anthelmintic drenches, a major research attempt has been underway to scrutinize substitutes to chemical control. In this regard, evaluation/selection of genetically resistant breeds to gastrointestinal nematodes is the practical and feasible option. Unfortunately, it is not widely practiced due to difficulty of assessing parasite resistance which generally depends on indirect criteria like parasitological (fecal egg count and worm burden at necropsy), haematological (packed cell volume, eosinophils in peripheral blood), tissue cellular counts (mast cells, eosinophils). This controlling strategy up to genetic makeup of different pure resistant breeds and their crosses restricted to Europe, Australia and USA and poorly reported in Asia. Keeping in view the long term benefits of this strategy, the present study was planned first time to evaluate between and within breed genetic variation of two local sheep (*Thalli* and *Kachhi*) breeds. For this purpose, a survey was conducted on the livestock farms to examine the within breed difference in different age and sex groups based on fecal egg count. Moreover, 25 lambs each of these breeds were artificially infected with 10,000 L3 of *Haemonchus contortus* and subsequently kept on contaminated pasture to assess within breed variability against haemonchosis. Within breed variation was a common entity of both breeds but it was significantly ($P < 0.05$) higher in *Kachhi* flocks. Incorporation of naturally resistant stock in breeding programs and genetic mapping would be useful study areas in Pakistan.

***Duddingtonia flagrans* in nutritional pellets: effect of different storage conditions on the viability of chlamydospores**

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The present work evaluated the viability of the *D. flagrans* chlamydospores incorporated to nutritional pellets (NP) under four different storage conditions for 54 days. The NP (each containing 4×10^6 chlamydospores) were distributed into four groups (n =48) with their respective controls (without chlamydospores): Group 1) stored on a shelf in-doors, Group 2) in the fridge (4 °C), Group 3) out-doors with roof and Group 4) outdoors with no-roof. The viability of chlamydospores in the NP was evaluated for 8 weeks. Six individual NP from each group were tested every week. One g of each crushed NP was added to the respective 2% water-agar plates containing 200 *Haemonchus contortus* L₃. Plates were incubated at room temperature for 8 days. The content of each plate was then transferred to Baermann apparatus for 12 h. The number of larvae recovered from all the chlamydospore treated groups showed a significant reduction (67 to 82%; $P < 0.005$) compared to their respective controls. Viable chlamydospores were detected for the 8 weeks tested. Results showed evidence that the procedures evaluated in the present study are effective in storage NP and keep the viability of *D. flagrans* chlamydospores and their predatory activity against *H. contortus* infective larvae. These results are crucial to design a method of conservation of NP containing *D. flagrans* chlamydospores to be used in the control of animal parasitic nematodes.

Effect of storage time on the viability of *Duddingtonia flagrans* chlamydospores when fed to sheep using nutritional pellets

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The objective was to determine the effect of storage time on the integrity and viability of *Duddingtonia flagrans* chlamydospores when fed to sheep using nutritional pellets (NP). Fifteen sheep were artificially infected with *H. contortus* 28 days before starting the trial (dose of 350 L₃/kg BW). Sheep were distributed into five experimental groups (n=3) consuming spores from NP with different storage time: T1: 10 days; T2: 5 days; T3: 1 day. The group T4 received spores directly into the mouth using an oral suspension. The group T5 was fed NP without spores (control). Each NP in groups T1, T2 and T3 contained 4 x 10⁶ spores. Sheep were daily fed with spores from days 1 to 5. The quantity of NP offered per sheep was based on the live weight to achieve a dose of 2x10⁶ chlamydospore per kg BW. The consumption of NP was recorded daily (days 1 to 5). From day 1 of NP consumption until 5 days after the last fungal dose the number of eggs and *D. flagrans* spores in the faeces were estimated daily using a McMaster technique. A qualitative *in vitro* test was performed for each animal on days 1, 5 and 7 to determine the viability of the spores after voiding the gastrointestinal tract (GIT). The NP were a good vehicle for chlamydospores and sheep left no refusal. The spore doses were easy to achieve. The spores from the NP were detected in faeces 24 h after the first ingestion and animals eliminated spores until 3 days after the last fungal dose (day 8). Spores in faeces kept their viability to trap *H. contortus* L3 on days 1, 5 and 7 of the study irrespective of the group. The NP could be used as a vehicle to administer *D. flagrans* chlamydospores because they can maintain the integrity and viability of the *D. flagrans* chlamydospores after they pass through the GIT of sheep.

Differences in the exsheathment rate of *Haemonchus contortus* larvae exposed to non conventional tannin-rich plant resources

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Introduction: Tannin-rich plants have showed efficacy against gastrointestinal nematodes (GIN) of sheep and goats. Most trials have tested temperate and tropical fodders used as conventional sources of ruminant feed. However, besides fodders, there are several possible non-conventional sources of tannins not used for feeding ruminants, e.g. plant by product materials resulting from industrial processes. The agroindustrial residues and/or the non-conventional fodder plants can also be a source of tannins to control GIN in ruminants. The aim of the trial was to determine the efficacy of tannins extracted from non-conventional tannin sources using an exsheathment assay with *Haemonchus contortus* L₃ larvae.

Methods: Water-acetonic (30/70) extracts of cocoa shell, cocoa husk, cocoa seed, coffee, white mangrove, red mangrove and black mangrove were used. Each of the extracts was lyophilized. Total phenols, total tannins and condensed tannins were determined for each extract. Respective larval exsheathment tests were performed for each extract. Four concentrations of the lyophilized extracts from each resource were used (150, 300, 600 and 1200 µg/ml and PBS). The efficacy was measured as a percent of decrease of the exsheathment using *H. contortus* L₃ larvae from France.

Results: All the tannin rich extracts inhibited larvae exsheathment at least by 70% at the highest concentration tested (1200 µg/ml). The white mangrove and coffee extracts showed efficacy from 150 µg/ml (inhibition was 89 and 60 % respectively). The cocoa seed, red mangrove and black mangrove extracts showed efficacy from 300 µg/ml (60, 86 and 68 % inhibition of exsheathment respectively). Finally, the cocoa shell and cocoa husk extracts showed efficacy from 600 µg/ml (42 and 41 % inhibition respectively).

Conclusion: The results showed that non-conventional tannin sources are capable to impair the exsheathment process in much the same way as other sources of tannins obtained from conventional fodder plants from temperate and tropical areas. Thus, these non-conventional sources of tannins can be studied as alternative tools to control GIN of ruminants.

In vitro* activity of *Myracrodruon urundeuva* ethanolic extracts on eggs and larvae of *Haemonchus contortus

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Gastrointestinal nematodes are the major cause of economic losses in sheep and goats in Brazilian northeastern. The control of these parasites is realized with synthetic anthelmintics. However, the development of resistance has stimulated the search for alternatives, such as the use of tanniferous plants. Therefore, the aim of this study was to evaluate *in vitro* the effect of extracts of leaves and stems obtained from *M. urundeuva* on egg hatching and larval exsheathment of *Haemonchus contortus* and to verify the role of tannins in these processes from the addition of polyvinyl polypyrrolidone (PVPP). Ethanolic extracts were obtained from the leaves and stems of *M. urundeuva* and tested at concentrations of 0.5 to 8 mg/ml in egg hatching test (EHT) and at concentration of 300 mg/ml in larval exsheathment test (LET). The experiments were accompanied by negative control (3% DMSO or PBS). The results were subjected to analysis of variance and analyzed by the Tukey or Kruskal-Wallis tests ($P < 0.05$), respectively. Phytochemical analysis of leaf extract detected hydrolysable tannins, flavones, flavonols, saponins and xanthonenes. The stems extract was positive for condensed tannins and flavononols. In the EHT, the extracts showed efficacy in all tested concentrations. The role of tannins on egg hatching was confirmed, because the efficacy of the extracts pre-incubated with PVPP was statistically similar to 3% DMSO. In the LET, the leaves and stems extracts completely inhibited the exsheathment. The exsheathment percentage was similar to the negative control ($P > 0.05$) in the leaves extract after pre-incubation with PVPP. Although the addition of PVPP to stems extract have not significantly reduced the percentage of larval exsheathment, the reduction was substantial, because the values obtained were different from the extracts not treated with PVPP ($P < 0.05$). These data demonstrate that the extracts of leaves and stems of *M. urundeuva* may be useful in controlling gastrointestinal nematodes of small ruminants and the tannins are involved in this process; however the activity of other substances should not be discarded.

Effects of clinoptilolite on *Eimeria* spp. infection in sheep

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Background: The effect of supplementation with clinoptilolite (hydrated sodium potassium calcium aluminum silicate; zeolite), a mineral from sedimentary deposits, in a natural infection with *Eimeria* in pregnant ewes was studied.

Methods: Rectal fecal samples were collected from ewes before supplementation with clinoptilolite to confirm coccidiosis by the McMaster technique and to set two groups of 6 animals each, with three replicates per group (3 experimental and 3 control groups). Animals were randomly assigned according to their *Eimeria* burden (mean 2806.25±468) by means of a table of random numbers to conform reasonably even groups. Detected species at the beginning of the study were *E. ovinoidalis* (62%), *E. parva* (15%), *E. intricata* (9%), *E. pallida* (7%), *E. faurei* (4%), *E. bakuensis* (2%) and *E. granulosa* (1%). Sheep were kept in pens with free access to water and feed. In-feed inclusion of 1.25% clinoptilolite was given to six ewes 30 ± 1.7 days before lambing and thereafter daily during 42 days. Six ewes remained as controls and were fed the concentrate without clinoptilolite. Fecal samples were collected from ewes and neonatal lambs, *Eimeria* oocysts counted and identified.

Results: On day 42, efficacy reached a significant ($P \leq 0.05$) reduction of 97% in oocyst output in supplemented ewes in contrast with control animals. Lambs born from supplemented ewes showed a reduction of 98% in oocyst output in a mean of 28 ± 2.14 days after birth. Oocysts kept under laboratory sporulation conditions in 2% aqueous potassium dichromate as standard for optimal sporulation. The proportion of sporulated oocysts was determined microscopically every 12 h. Exposure to clinoptilolite *in vitro* or *in vivo* did not affect significantly the sporulation of oocysts shed by naturally-infected sheep. However, 72% and 38% of sporulated oocysts isolated from supplemented and non-supplemented sheep, respectively, were deemed microscopically as damaged and seen to collapse when incubated in the presence of clinoptilolite. Oocysts from control ewes remained morphologically undamaged.

Conclusions: These results suggest that clinoptilolite could prevent naturally occurring coccidiosis in ewes and lambs under field conditions.

Prevention of equine cyathostomin infection by spreading *Duddingtonia flagrans* chlamydospores directly onto the pasture

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Background: Larvae belonging to several gastrointestinal nematoda exit the egg and develop in the environment to attain the infective third larval stage, which are ingested when the animals feed the pasture grass. This emphasizes the need for certain measures on the environment.

Methods: According to the fecal egg count (FEC) number of strongyles, chemotherapy was administered to 12 horses exceeding a cut-off value of 300 eggs per gram of feces (EPG), and consisted of the pour on application of ivermectin at a 1 mg kg bw dosage. In addition, chlamydospores of the nematode-trapping *Duddingtonia flagrans* were employed for the biological control of the free-infective larval stages (also called L3).

The mares were divided into three groups:

G-1: 6 received ivermectin

G-2: 6 treated with ivermectin and grazing in a pasture where *D. flagrans* chlamydospores were directly spread onto the ground ($2 \times 10^6 / \text{m}^2$).

G-3: 4 remained without chemotherapy and received a dose of 2×10^6 *D. flagrans* chlamydospores / kg bodyweight.

Results: In all the horses receiving ivermectin the overall mean FECR 2 weeks following the treatment with ivermectin was 100% for the ascarids and the strongyles.

The cyathostomin egg-output was suppressed by the 2nd week after IVM-treatment (wat) in G-1 and G-2. Eggs reappeared in the feces of both treated groups at the 8th wat. Significant differences in the egg-output were observed only among the IVM-treated and control equines, whereas no differences between the two IVM-dewormed groups were obtained. The horses belonging to the G-3 passed strongyle eggs in the feces throughout the study, achieving values higher than in the treated groups to the end of the experiment.

Conclusions: A combined strategy by the pour on chemotherapy of the grazing animals in concert with the spreading of spores directly onto the pasture provides a very useful tool to reduce both the parasitism in the animals and in the environment.

Antiparasitic Phytotherapies to livestock production

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The ethnoveterinary research has been scarce explored in comparison with the human one, particularly, the use of natural medicine and their application in animals. Livestock, one of the major markets for animal health drugs, urgently needs for antiparasitic drugs discovery and alternative methods to control parasite infections, considering the development of resistance against marketed drugs.

In this context, an interdisciplinary project currently on course, is addressed to the search of potential antiparasitic (endo and ectoparasites) phytotherapies, using in vitro biomodels as tools for the screening of anthelmintic and insecticide activity of natural samples. The rat parasitic nematode *Nippostrongylus brasiliensis* L4 (an in vitro model already reported by us) and the "horn fly" *Haematobia irritans* (as per the method provided in the literature) were used for that purpose. Validation of insecticide bioassay was carried out using serial dilutions of diazinon (reference drug) made in acetone ranging from 0.1 to 0.6 µg/mL final concentration, when one milliliter of each was applied in Petri dishes (6 replicates at least). Controls with acetone were included. Acetone was allowed to evaporate (2h), and twenty-five flies were placed in each dish. Mortality of horn flies in each dish was determined 2h after the introduction of flies by counting live and dead flies (obtaining 11, 68 and 85 % of dead flies at 0.1, 0.3 and 0.6 µg/mL). The Prism GraphPad 5.0 program was used to analyze the dose-mortality response of diazinon bioassays. From the insecticide screening of twenty-four natural samples at 1 mg/mL, highlight the insecticide activity of Aguaribay (*Schinus molle* L.) and *Serjania meridionales* (with 47% and 56% of dead flies, respectively). On the other hand, the ethanolic extract of *Phyllanthus niruri* (EC₅₀ 64.9 µg/mL) was selected from previous anthelmintic activity studies, for bioguided fractionation.

In the present work we show the validation of an insecticide bioassay and its use for the screening of natural samples. Also we describe the advances in the bioguided fractionation of *Phyllanthus niruri* using *N. brasiliensis* in vitro model with the aim to meet potential metabolites useful as phytochemical markers to validate plant material as phytotherapeutic. Particularly, two products were isolated and chemically characterized by mean of gas chromatography-mass spectrometry of derivatized carbohydrates, and spectroscopic methods.

Analysis of the effect of antigenic stimulus on the development of the nematophagous fungus *Duddingtonia flagrans*

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Background: *Duddingtonia flagrans* is a nematophagous fungus which develops traps to capture nematodes present in the soil. This is a fungus capable to produce a large quantity of chlamydospores able to resist the passage through the gastrointestinal tract of ruminants and monogastrics. It has been stated that the presence of nematode larvae near to the fungus constitutes the main stimulus for its development. In the current study the switching of *D. flagrans* chlamydospores after their exposition to antigens from different helminth parasites has been analyzed.

Methods: The Mexican strain of *D. flagrans* FTHO-8 (CENID-PAVET, INIFAP-MEXICO) was used and chlamydospores were produced, harvested and managed in Petri dishes. A total of 40 agar-wheat plates were cultivated with chlamydospores by placing a 8x8 mm inoculum. Four weeks later, the initial inoculum was removed and 100 µl excretory/secretory antigen of cyathostomins, *Ascaris suum*, *Fasciola hepatica* and *Oesophagostomum* were added. Each antigen was tested into 8 plates, and a similar number remained without antigen as controls.

The development of the fungus was assessed by measuring the growth rate and the number of chlamydospores during 4 weeks.

Results: Although a fast growth rate was observed in the plates treated with the antigens, no significant differences were shown regarding the control plates (without antigenic stimulation). By opposite, the number of chlamydospores resulted significantly higher in the presence of the parasitic antigens ($F= 7.371$, $P= 0.013$).

The analysis of the antigenic stimulation showed no differences in the growth rates throughout the study. The production of chlamydospores was higher in the plates receiving the antigens from cyathostomins and *Oesophagostomum* specimens ($F= 3.656$, $P= 0.019$).

Conclusions: The development of the nematophagous fungus *D. flagrans* can be enhanced by antigenic products released by helminth parasites. The excretory/secretory products collected from the gastrointestinal nematoda cyathostomins and *Oesophagostomum* show a higher capability than that from trematoda or ascarids for switching the production of chlamydospores.



SESSION P

Keynote Lecture

- **Pharmacological assessment of combined anthelmintic formulations** - L. Alvarez
- **Optimizing anthelmintic combinations to counter anthelmintic resistance** - A. Lespine
- **Combination anthelmintics – a tool for delaying the development of anthelmintic resistance?** - D.M. Leathwick

Oral Communications

- **Interaction of derquantel and abamectin: in vitro worm bioassays** - A. C. Kotze
- **Interaction of derquantel and abamectin: Effects at a nAChR in *Ascaris suum*** - R. J. Martin
- **Contrary responses of *Caenorhabditis elegans* neuronal receptor mutants to different anthelmintic classes** - S. Miltsch

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Pharmacological assessment of combined anthelmintic formulations

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The development of resistance to the available anthelmintic drugs is a seriously increasing problem. Combination of anthelmintic drugs from different chemical groups has been proposed as an alternative parasite control strategy where failure of individual drugs is documented. A drug interaction refers to the possibility that one drug may alter the intensity of the pharmacological effects of another drug given concurrently. The modified effect may result from a change in the relationship between drug concentration and response of the organism to the drug (pharmacodynamic interaction) or from a change in the concentration of either one or both drugs in the organism (pharmacokinetic interaction). Some of the following pharmacodynamic interactions could be expected following the co-administration of two anthelmintic molecules: indifference, antagonism, additive or synergistic effect. A synergistic effect is achieved when the combined effects of the drugs are significantly greater than the independent effects of each molecule. This type of interaction would be an ideal situation to deal against resistant parasites. However, most of the data obtained on the pharmacological assessment of different available anthelmintic combinations would indicate that only an additive effect is involved on drug combined activity. A pharmacokinetic interaction occurs when a given drug modifies the absorption, distribution, biotransformation and/or elimination of other drug molecule. The overall disposition kinetics, the pattern of tissue distribution and the active drug concentrations at the site of action may be either increased or decreased when the interaction occur. The impact of increased systemic exposure due by a drug-drug interaction on the improvement of clinical efficacy against resistant nematodes remains unclear. Work carried out in our Lab demonstrated that the co-administration of albendazole and ivermectin in sheep does not induce an adverse kinetic interaction. However, it seems clear that the magnitude of the pharmacokinetic interaction may depend on the route of administration under assessment. For instance, while the presence of ivermectin did not affect the plasma disposition kinetics of albendazole/metabolites after the intravenous administration of both drugs, a higher albendazole sulphoxide systemic availability was observed after the intraruminal administration of albendazole combined with ivermectin (subcutaneous injection), compared to that obtained for the albendazole alone treatment. The pharmaco-parasitological evaluation of different anthelmintic compounds (benzimidazoles, macrocyclic lactones, imidazothiazoles, salicylanilides) used to control resistant nematodes, is under assessment in our Laboratory and will be discussed in this presentation.

Optimizing anthelmintic combinations to counter anthelmintic resistance

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Combinations of anthelmintics may be considered for three reasons: (1) to increase spectrum, for example when a flukicide is combined with a nematicidal anthelmintic; (2) to increase efficacy and reduce selection for resistance; or (3) to increase efficacy against parasites already resistant to one or more anthelmintics. In all cases, the effect of one pharmaceutical on the pharmacokinetics and pharmacodynamics of a combination partner should be considered. Some anthelmintics, such as ivermectin and triclabendazole interact strongly with ABC transport proteins, so that their combination with other anthelmintics could modify pharmacokinetic behavior in the host and in the parasite, with resultant effects on efficacy and possibly toxicity. In addition to considering such modifications to transport functions, shared mechanisms of resistance and resistance genes, which can include transporters and other genes, should be considered to ensure that the combination favors enhanced efficacy and not selection for higher levels of anthelmintic resistance. In terms of reducing selection for resistance, combinations will be most beneficial: (i) when resistance mechanisms, to the different anthelmintics in the combination, are under independent genetic control, *i.e.* do not share common mechanisms of resistance/resistance genes; (ii) are functionally recessive at the dose rates to be used; (iii) when the genes for resistance are rare and the efficacy of each of the component compounds against the susceptible genotypes approaches 100%; and (iv) when the compounds used have similar persistence. Because transport mechanisms are important in many types of resistance and because many anthelmintics are excellent inhibitors or substrates of ABC transporters, there is potential for increasing efficacy against parasites, with appropriate combinations. However, this needs to be balanced against possibly selecting on the same genes involved in resistance. Increased efficacy could result from modifying anthelmintic pharmacokinetics in the host or by blocking parasite transporters involved in resistance expression. Besides possible interactions on transporters, some anthelmintics from different mechanisms of action classes may impose cross selection in genes involved in resistance, such as macrocyclic lactones selecting on β -tubulin which is involved in benzimidazole resistance. Combination of anthelmintics should be based on knowledge of resistance mechanisms and not simply on mode of action classes and is best started before resistance has been selected to any member of the combination.

Combination anthelmintics – a tool for delaying the development of anthelmintic resistance?

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The benefit of combination anthelmintics at delaying the development of resistance has been debated for many years. Early modelling studies, principally of insect populations, produced somewhat conflicting conclusions which has fuelled confusion regarding the use of anthelmintic combinations. However, all these early studies agreed that if the right conditions are met, combinations can be very effective at delaying the development of resistance.

This presentation will review the factors identified as prerequisites for combinations to slow the development of resistance, and then use examples from a simulation model of nematode infection in sheep to illustrate the importance of some of these factors. The most important variable appears to be the proportion of the worm population exposed to treatment with the benefit of the combination declining as the proportion of the population exposed to treatment approaches 100% - this emphasises the importance of maintaining worms 'in-refugia' in order to delay resistance development. The presence of resistance to one or more of the actives in the combination also reduces the benefit of the combination although some benefit can still occur. Other variables such as the dominance of the resistance genes under treatment, poly-genic resistance and side-resistance between anthelmintic classes don't negate the benefit of combinations as long as other factors are managed appropriately.

Results from a 3-year replicated field study will also be presented. Twelve farmlets, each of 4 paddocks, were contaminated with a mixture of resistant and susceptible isolates of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* before being stocked with lambs. Three replicates of 4 treatments were applied in a 2X2 factorial design which compared drench type (i.e. ivermectin cf ivermectin + levamisole) and proportion treated (i.e. 100% cf 90% of lambs drenched at each treatment). Resistance was monitored using larval development assays and slaughter of treated and untreated tracer lambs. In *T. colubriformis* development of resistance to ivermectin was delayed by the use of the combination and levamisole resistance did not increase. In *T. circumcincta* resistance developed rapidly, presumably due to non-viability of the susceptible isolate used to contaminate the pastures. Despite this there was some delay in the build-up of resistance to ivermectin, but, resistance to levamisole increased. These results are consistent with model simulations which reproduced the conditions of the trial.

Interaction of derquantel and abamectin: *in vitro* worm bioassays

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Background: Startect is a new drench containing derquantel (a spiroindole) and abamectin (a macrocyclic lactone). We investigated whether there was any interaction between the two actives using *in vitro* bioassays with adult and larval life stages of parasitic nematodes.

Methods: We performed *in vitro* bioassays with abamectin and derquantel alone, or in combination, against adult and larval *Haemonchus contortus* worms, as well as *Teladorsagia circumcincta* larvae. Drug effects on adult worms were assessed using feeding and motility assays. Larval assays measured the effects of drugs on the numbers of larvae developing to the L3 stage. Responses to each drug alone were used to calculate the expected response if the two drugs were acting in an additive fashion. This was then compared to the actual response to drug combinations in order to determine whether the combination was additive, synergistic or antagonistic.

Results: Addition of a constant level of derquantel to adult worm assays over a range of abamectin concentrations in feeding and motility assays caused the abamectin dose response to shift further to the left (greater toxicity) than would be expected if the two drugs were acting in an additive fashion. This suggests that some synergism occurred. Feeding assays with derquantel alone indicated that this compound stimulated uptake of the feeding substrate in a concentration –dependent manner (uptake up to 50 % greater than in controls). Larval assays also showed a feeding stimulation effect with *T. circumcincta* (development rates up to 50 % greater than controls), however these larval experiments gave contrary synergism / antagonism indications with *T. circumcincta* compared to *H. contortus*. These contradictory effects were most likely due to the interaction of worm feeding behaviour, drug uptake levels, and relative larval growth rates. Therefore this larval assay system was unsuitable for examining the interaction between the two drugs in a way that could reflect their potential interaction *in vivo*.

Summary / Conclusions: Our adult worm experiments suggest that derquantel and abamectin interact in a synergistic fashion *in vitro*. A possible explanation for this interaction is that the stimulation of pharyngeal pumping by derquantel leads to increased uptake of abamectin from the culture medium. However, the physiological basis for this feeding stimulation remains to be determined.

Interaction of derquantel and abamectin: Effects at a nAChR in *Ascaris suum*

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Background: Startect^R is a novel anthelmintic combination of derquantel and abamectin which selectively paralyses parasitic nematodes. It is hypothesized that the effects of derquantel and abamectin are synergistic. We investigated the effects of derquantel and abamectin on the somatic muscle nicotinic acetylcholine receptor of the parasitic nematode *Ascaris suum* muscle using electrophysiological techniques. Previously, we have identified that derquantel has a rapid potent antagonistic ($IC_{50} = 0.2 \mu\text{M}$) effect at the *A. suum* somatic muscle receptors: we had also found that it acts as a competitive antagonist.

Methods: We used a body flap preparation of *A. suum*. A two-micropipette current-clamp technique was used to record the membrane potential and input conductance from the bag region of the somatic muscle cells. Acetylcholine was applied at different concentrations for 10 seconds by microperfusion under the control of computer software and the membrane potential response measured to the different concentrations of acetylcholine. We investigated the longer-term (> 15 min) effects of application of $0.3 \mu\text{M}$ abamectin, $1 \mu\text{M}$ derquantel, and $0.3 \mu\text{M}$ abamectin + $1 \mu\text{M}$ derquantel on the responses to different concentrations of acetylcholine. We pretreated 7-11 preparations with each of the test compounds and compared the membrane potential responses in control preparations (no pre-treatment drug) with preparations that had been pretreated. We fitted the dose-response plots with GraphPad Prism Software to a standard log-dose equation to obtain EC_{50} and R_{max} values. We calculated the Bliss additive effect of abamectin and derquantel from the single drug experiments and compared the observations with the observed effects of abamectin + derquantel applied together and looked for antagonism or synergism.

Results: The effect of derquantel appeared competitive, with a parallel shift of the acetylcholine response from an EC_{50} of $4.5 \mu\text{M}$ to $10.3 \mu\text{M}$. The effect of $0.3 \mu\text{M}$ abamectin was non-competitive: the maximum response, R_{max} , reduced from 20 mV to 13 mV without a change in the EC_{50} . The combined effects of derquantel and abamectin reduced R_{max} to 8 mV and the EC_{50} to $10.4 \mu\text{M}$. Thus derquantel was a competitive antagonist and abamectin was a non-competitive antagonist (negative allosteric modulator). The Fractional Response (1-Fractional inhibition) plot, in the presence of derquantel alone, abamectin alone, and derquantel + abamectin, plotted against the acetylcholine concentration, showed that the effect of derquantel and abamectin together was greater than predicted by addition.

Summary/Conclusion: Derquantel is a potent competitive nAChR antagonist. Abamectin was a non-competitive antagonist and the combination of the two compounds showed effects greater than addition.
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Contrary responses of *Caenorhabditis elegans* neuronal receptor mutants to different anthelmintic classes

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Background: Due to the increasing development of anthelmintic resistance in nematodes worldwide it is important to search for anthelmintic substances with new modes of action and also to investigate the possibility to combine substances with possible synergistic effects. There might be the chance to take advantage of the fact that nematode populations which have developed resistance against one anthelmintic class might respond hypersusceptible to another. The aim of this study was to investigate responses of neuronal receptor mutants to different anthelmintic classes in a *C. elegans* development assay and to investigate possibly synergistic effects between two anthelmintic substances from different classes, emodepside and tribendimidine.

Methods: Different *C. elegans* strains were used in this study: The N2 Bristol wild type strain, two *unc-49* GABA receptor mutant strains, one *slo-1* mutant as well as five acetylcholine receptor (AChR) loss of function mutant strains. The response of these mutants to different anthelmintics was evaluated in a *C. elegans* development assay. First larvae from synchronized *C. elegans* populations were incubated for 54 hours with different concentrations of one anthelmintic or combinations of the two anthelmintics. Development rates following exposure to anthelmintics were compared between the different *C. elegans* strains.

Results: Mutants showing decreased susceptibility against emodepside, such as the GABA receptor mutants and the *slo-1* mutant react hypersusceptible to tribendimidine compared to the wild-type. In contrast tribendimidine resistant AChR mutants did not show hypersusceptibility to emodepside, but decreased susceptibility to emodepside. The *slo-1* mutant being highly resistant against emodepside reacts hypersusceptible against piperazin. Preliminary results suggest a potential synergistic effect between emodepside and tribendimidine at least at low concentrations of the latter.

Summary/Conclusion: Emodepside resistant *C. elegans* mutants such as the GABA receptor *unc-49* mutants (decreased susceptibility) and the *slo-1* mutant (highly resistant) react significantly more susceptible to tribendimidine as wild-type *C. elegans*. In contrast no hypersusceptibility to tribendimidine was observed in resistant AChR mutants exposed to emodepside. The *slo-1* mutants showed also hypersusceptibility to piperazin. Currently more detailed studies of potential synergistic effects between the two anthelmintics are underway.



SESSION Q

Keynote Lecture

- **Emerging perspectives in the research of Babesiosis and Anaplasmosis of cattle** - C. E. Suarez

Oral Communications

- **A campaign to eradicate bovine babesiosis from New Caledonia** - T. Hüe
- **Why is Southern African canine babesiosis so virulent? An evolutionary perspective** - B. L. Penzhorn
- **The glycosylphosphatidyl-anchored protein repertoire of Babesia bovis and its significance for erythrocyte invasion** - A. Rodriguez
- **Stable expression of green fluorescent protein and target disruption of Tpx-1 in Babesia bovis using the WR99210/hDHFR selection system** - M. Asada
- **Renatured landscapes as spots for tick-borne pathogens** - C. Silaghi
- **No acaricide resistance in ticks, still problems with Anaplasma phagocytophilum in treated lambs** - J. Chirico

Posters

- **Phylogenetic studies of Babesia spp. in felids in southern Africa** - A. M. Bosman
- **Wild cervids and potentially zoonotic Babesia in Belgium** - L. Lempereur
- **First report of indigenous Dermacentor reticulatus populations in Belgium and preliminary study on associated Babesiosis pathogens** - L. Lempereur
- **Canonical and variant histone repertoire in Babesia bovis and their possible relevance in epigenetic regulation** - M. Dominguez
- **Seroprevalence of Four Vector-Borne Infections in Dogs from Romania: a Countrywide Study** - A. D. Mihalca

Emerging perspectives in the research of Babesiosis and Anaplasmosis of cattle

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The *Babesia bovis* and *B. bigemina* apicomplexan protozoa in conjunction with the rickettsia *Anaplasma marginale* are intraerythrocytic pathogens that are responsible for the most prevalent and costly tick borne diseases (TBD's) of cattle worldwide. These organisms are historically associated as they can cause clinically related hemolytic diseases in cattle, are all transmitted by *Rhipicephallus (Boophilus)* ticks, and share an uncanny ability to evade the immune systems of the vertebrate hosts, causing persistent disease. In addition, acute babesiosis and anaplasmosis can be prevented quite effectively by combining tick control and vaccination with living attenuated organisms. However these methods of control have numerous limitations and improved approaches are needed. Importantly, immunizations of cattle with inactivated experimental *Babesia* and *Anaplasma* vaccines can elicit variable degrees of protection, indicating the feasibility for the development of inactivated or subunit vaccines. A new research toolbox that includes full genome sequencing combined with the improved ability to genetically modify the organisms is enhancing our understanding of their biology. An emerging paradigm is the use of recently developed *Babesia* and *Anaplasma* transfection methods for functional gene characterizations and for vaccine development. Promising recently identified subunit vaccine candidates are also emerging, including babesial proteases, putative rhoptry, microneme, and sexual stage antigens, as well as subdominant, conserved, *A. marginale* outer membrane major surface proteins. However, significant knowledge gaps on the role of key parasite molecules involved in cell invasion, adhesion, asexual and sexual reproduction, tick transmission, and evasion of the immune system, remain. A better understanding of the biology of these organisms and the protective immune responses will positively contribute towards the goal of developing improved immunological and pharmacological interventions against these elusive pathogens that are responsible for the most devastating TBD's of cattle. Importantly, the currently available research toolbox provides basic research instruments for helping close current knowledge gaps which will aid the design and production of effective vaccines and alternative pharmacological interventions.

A campaign to eradicate bovine babesiosis from New Caledonia

An innovative strategy to eliminate bovine babesiosis

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In December 2007, *Babesia bovis* was introduced to New Caledonia through the importation of cattle that had been vaccinated with a live tick fever (babesiosis and anaplasmosis) vaccine. Although the tick *Rhipicephalus (Boophilus) microplus* is common in New Caledonia, the territory had previously been free of tick-borne disease of cattle.

The index case was diagnosed on 5 March 2008 and epidemiological investigations identified 22 farms on which the introduction of *Babesia* was considered possible, involving approximately 2300 cattle in 'high risk' zones and 1600 in adjoining 'suspect' zones.

Rather than slaughtering infected herds or attempting to eliminate the tick vector, the campaign has been based on quarantine of affected properties, and aggressive tick control in conjunction with the treatment of high risk cattle with three consecutive doses of the anti-protozoal drug imidocarb dipropionate.

Several subsequent rounds of surveillance by ELISA and PCR showed a progressive and dramatic decline in seroprevalence among infected herds and the absence of new infections. All 22 properties were considered to be free of *Babesia* within 12 months of the start of the disease control program and a recent serological round, 2 years later, confirms that no asymptomatic cases stay on these farms.

These results indicate that this innovative strategy was effective in eliminating *Babesia* from infected herds and feasible as an eradication strategy on a moderately large scale.

This presentation will be based on a paper published in TTBD.

Why is Southern African canine babesiosis so virulent? An evolutionary perspective

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Canine babesiosis is a common, highly virulent disease in Southern Africa with even pups and juveniles being severely affected. This contrasts with bovine babesiosis, for example, where host, parasite and vector co-evolved and young animals develop immunity after infection without showing clinical signs. *Babesia rossi*, the main causative organism of canine babesiosis in sub-Saharan Africa, was first described from a side-striped jackal (*Canis adustus*) in Kenya. Although data are meagre, there is evidence that indigenous African canids, such as jackals and wild dogs (*Lycaon pictus*), can harbour the parasite without showing untoward effects. Dogs are not indigenous to Africa. The vast majority of dogs presented at veterinary facilities in South Africa represent recently introduced European, Asian or American breeds. The contention is that *B. rossi* is a new challenge to which these dogs have not adapted. With intensive treatment of clinical cases, natural selection is effectively negated and the status quo will probably be maintained indefinitely. It is postulated that *Babesia vogeli*, which frequently results in unapparent infections or mild manifestations in dogs, represents or is closely related to the ancestral form of the canine parasite, possibly originating from wolves (*Canis lupus*).

The glycosylphosphatidyl-anchored protein repertoire of *Babesia bovis* and its significance for erythrocyte invasion

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Glycosylphosphatidyl-anchored proteins are particularly abundant on the surface of pathogenic protozoans and might play an important role for parasite survival. In the present work the relevance of GPI-anchored proteins for erythrocyte invasion of *Babesia bovis*, one of the tick-transmitted causative agents of bovine babesiosis, was studied. We show that cleavage of GPI-anchored antigens from the merozoite parasite stage by phosphatidylinositol-specific phospholipase C significantly hampered invasion of erythrocytes demonstrating the importance of these molecules for parasite propagation. In addition, the repertoire of GPI-anchored proteins of *B. bovis* was defined with high fidelity by searching its genome with available web-based bioinformatic tools, such as “big-PI”, “Pred-GPI”, “GPI-SOM”, and “FragAnchor”. Altogether 16 GPI-anchored proteins were identified of which five represent the Variable Merozoite Surface Antigens (VMSAs) that have been intensively studied as vaccine candidates and diagnostic antigens. The remaining eleven have not been studied so far and their function is unknown. Fifteen of the identified GPI-anchored proteins contain 2 to 16 amino acid repeats indicating that they are likely involved in functions of recognition, adhesion, or transport. In comparison to non-repetitive regions, repeats were found to contain a highly significant increased frequency of proline, indicative of unstructured regions. Repeats were also estimated to be 2.27 times more hydrophilic than non-repeat regions. In accordance with the “smoke screen hypothesis”, the property of being unstructured and highly hydrophilic may suggest that repeats represent eminent antibody epitopes. The evolutionary relation of the identified GPI-anchored proteins was assessed by analysis of the genome location of their encoding genes and a reverse BLASTp test. Apart from the five paralogous merozoite surface antigen genes that have been reported to be clustered in a single genomic region, all other eleven genes encoding GPI-anchored proteins were found to be rather dispersed in the genome. While nine of these represent orphan genes, two were found to be each part of gene families containing four and three members that encode non GPI-anchored proteins, respectively. The majority of the GPI-anchored antigens reported in this work has remained unnoticed so far but may represent potential vaccine candidates for the development of subunit vaccines (*Supported by the EC-INCO grants 003691 and 245145, and INTA AERG232152*)

Stable expression of green fluorescent protein and target disruption of *Tpx-1* in *Babesia bovis* using the WR99210/*hDHFR* selection system

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Babesia bovis is an apicomplexan intra-erythrocytic protozoan parasite transmitted by ticks to cattle. Genome manipulation has been a valuable technique for elucidating gene function in many pathogens. While transfection of *Babesia spp.* has been less developed than that of other apicomplexan species. In *Plasmodium* parasites, stable transfection system has been developed by using human dihydrofolate (DHFR) gene, which confers drug resistance to WR99210 and pyrimethamine. In this study, We have achieved stable expression of green fluorescent protein (GFP) in *Babesia bovis* by using the WR99210/human dihydrofolate reductase (DHFR) gene selection system. A GFP-expression plasmid with a *dhfr* expression cassette (DHFR-gfp) was constructed and transfected into *B. bovis* by nucleofection. Following WR99210 selection and cloning with limiting dilution, a GFP-fluorescent parasite population was obtained and the fluorescent parasite was maintained for more than 5 months under WR99210 drug pressure. The DHFR-gfp was used to construct a small circular chromosome and to target gene disruption in the parasite. For construction of the small circular chromosome (DHFR-gfp-Bbcent2), the putative centromere region of *B. bovis* chromosome 2 (Bbcent2) was cloned and inserted into the DHFR-gfp plasmid. Addition of Bbcent2 to the DHFR-gfp plasmid improved its segregation efficiency during parasite multiplication and GFP-expressing parasites were maintained for more than 2 months without drug pressure. For targeted disruption of a *B. bovis* gene we attempted to knockout the thioredoxin peroxidase-1 (TPx-1) gene (a single-copy 2-Cys peroxiredoxin gene) by homologous recombination. To generate the targeting construct (DHFR-gfp-Bbtpx1KO), 5' and 3' portions of *Bbtpx-1* were cloned into the DHFR-gfp plasmid. Following nucleofection, WR99210 selection and cloning, a GFP-fluorescent parasite population was obtained. Integration of the construct into the *Bbtpx-1* locus was confirmed by PCR. The absence of *Bbtpx-1* mRNA and protein were verified by reverse transcription PCR and western blot analysis/ indirect immunofluorescence assay, respectively. This is the first report of targeted gene disruption of a *Babesia* gene. These advances in the methodology of genetic manipulation in *B. bovis* will facilitate functional analysis of *Babesia* genomes and will improve our understanding of the basic biology of apicomplexan parasites.

Renatured landscapes as spots for tick-borne pathogens

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Background: The highly abundant hard ticks *Ixodes ricinus* and *Dermacentor reticulatus* can carry several pathogens with impact on human and animal health. For example, *Rickettsia raoultii* may cause tick-borne lymphadenopathy (TIBOLA) in humans; a febrile disease in connection with *R. helvetica* has been reported. *Babesia* sp. detected may cause human babesiosis, even though cases in Europe are rare and occur predominantly in immunocompromised patients. *A. phagocytophilum* is the causing agent of granulocytic anaplasmosis in domestic animals and humans.

The distribution and coexistence of these two tick species as well as the prevalence of the tick-borne pathogens *Anaplasma phagocytophilum*, *Babesia* spp. and *Rickettsia* spp. were investigated in renatured landscapes in former brown coal mining sites in Germany.

Methods: Questing ticks were collected with the flagging method in spring and autumn 2009. After individual DNA extraction, both *D. reticulatus* and *I. ricinus* were screened for *Babesia* spp. and *Rickettsia* spp. with conventional PCRs, followed by sequencing, and the *I. ricinus* also for *A. phagocytophilum* with real-time PCR.

Results: Altogether, 2,070 ticks (814 *D. reticulatus*, 1,256 *I. ricinus*) were collected. Overall infection rates were: 67,4% *Rickettsia* spp. in *D. reticulatus* and 9,8% in *I. ricinus*; 4,5% *Babesia* spp in *I. ricinus* and 0% in *D. reticulatus*; 9.2% *A. phagocytophilum* in *I. ricinus*. The rickettsial species detected were *R. raoultii* exclusively in *D. reticulatus* and *R. helvetica* only in *I. ricinus*. The *Babesia* species detected were *B. microti*, *B. divergens*, and *Babesia* sp. EU1.

Conclusions: Renatured recreation areas with high prevalence of tick-borne pathogens provide efficient interfaces for transmission to humans and companion animals. The sympatric existence and high abundance of ticks together with the high prevalence of various pathogens may facilitate the emergence of new diseases. Thus, studies which thoroughly investigate renatured areas as new biotopes for hosts and vectors are warranted to allow a more clear understanding on the epidemiology of these pathogens.

No acaricide resistance in ticks, still problems with *Anaplasma phagocytophilum* in treated lambs

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Background: Reoccurring problems with the tick-borne bacteria *Anaplasma phagocytophilum* causing tick-borne-fever in lambs on pastures at the island of Gotland, Sweden, has been observed during the last decade. Infected lambs developed lameness, fever and enlarged lymph nodes. Some of them died within the first day after developing symptoms. Therefore, acaricide resistance in ticks was suspected as pyrethroids were extensively used in prophylactic treatments.

Methods: During two consecutive years, ticks were collected by flagging eight sheep pastures. Approximately 100m² was flagged. Collected ticks were identified and subsequently tested for resistance against alphacypermethrin, deltamethrin and flumethrin, i.e. licensed acaricides for tick prophylaxis in Sweden. Moreover, presence of *A. phagocytophilum* was identified in ticks by real-time PCR. Blood samples from lambs showing clinical symptoms of tick-borne fever, as well as spleen from fatal cases were also analyzed by real-time PCR to detect *A. phagocytophilum*.

Results: Over 8 000 ticks were collected whereas 6 358 were identified to species and development stage. Only two species of ticks, *Ixodes ricinus* (43%) and *Haemaphysalis punctata* (53%), were detected. No resistance against the pyrethroids was detected in the ticks tested. *Anaplasma phagocytophilum* was found in both tick species and in all development stages. Approximately 11% of the ticks collected during the first year harboured *A. phagocytophilum*, whereas 60% of the nymphs, regardless of species, were carrying the bacterium. No *A. phagocytophilum* was detected in ticks collected during the second year of the study.

Cases with verified tick-borne fever in lambs varied between the years. Out of 25 lambs that showed symptoms of the disease, 18 (86%) were positive for *A. phagocytophilum* during the first year, whereas the bacterium was detected in 20 (80%) out of 25 in lambs during the second year. Out of the six fatal cases sent for autopsy during the first year, *A. phagocytophilum* was identified in four lambs. During the second year, all affected flocks had fatal cases, but only three lambs were sent for autopsy. Two of them harboured *A. phagocytophilum*.

Conclusions: The problem caused by the tick-borne bacterium *A. phagocytophilum* in lambs was not caused by acaricide resistance in ticks. The varying number of ticks as well as the incidence of *Anaplasma*-infected ticks during the study did not explain the scattered occurrence of tick-borne-fever in lambs.

Phylogenetic studies of *Babesia* spp. in felids in southern Africa

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Background: Feline babesiosis, first described in domestic cats in South Africa in 1937, is regarded to be of great importance in the coastal regions of South Africa, although isolated cases also occur on the eastern highlands of Mpumalanga province. 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.

Methods: In this study, DNA was extracted from blood collected from various domestic, captive and freeranging felids from different southern African countries. The V4 hypervariable region of the 18S rRNA gene was amplified and the PCR products were analysed using the Reverse Line Blot (RLB) hybridization assay. RLB probes to detect *B. felis*, *B. leo* and *B. lengau* were used to screen samples collected from various felid species.

Results: Results showed that *B. felis* and *B. leo* occurred more frequently in the host from which they had initially been described, namely domestic cats, lions and cheetahs respectively, but were also detected in other felid species. A large number of samples reacted only with the *Babesia* / *Theileria* genus-specific probe. These samples were further analyzed using 18S rRNA gene sequence and phylogenetic analysis.

Summary/Conclusions: Phylogenetic studies confirmed the presence of *B. lengau* in cheetahs and *Hepatozoon* in lions. It also suggested the existence of a novel *Babesia* species in domestic cats.

Wild cervids and potentially zoonotic *Babesia* in Belgium

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Background: Molecular evidence for the presence of *Babesia* sp. EU1 and *Babesia microti* in *Ixodes ricinus* ticks collected on dogs and cats was recently described for the first time in Belgium. *Babesia* sp. EU1, renamed *B. venatorum*, was first described in two asplenic patients and in roe deer in Europe. There is little information regarding the prevalence of the different species of *Babesia* and their potential zoonotic impact on this continent. This study was designed to investigate the presence of specific *Babesia* DNA sequences in ticks collected from cervids in Belgium.

Methods: In 2008, ticks were collected from wild cervids (*Cervus elaphus* and *Capreolus capreolus*) found dead, hunted or killed for sanitary reasons in the context of the activities of the WildScreen Network in Southern Belgium. Ticks were kept in ethanol 70 % and identified through the use of morphological keys. DNA extraction was performed for individual ticks with the NucleoSpin tissue kit. Each extracted sample was evaluated for DNA quality and absence of inhibition with a PCR based on the 16S rRNA gene. Only positive samples were further analyzed for identification of *Babesia* spp. A *Babesia* spp. genus-specific PCR was applied; it is based on the amplification of a part of the 18S rRNA gene. Sequencing and blast analysis were performed to identify *Babesia* species.

Results: A total of 1044 adult ticks were collected and 1023 were validated and screened for *Babesia*. Twenty eight samples were found to be positive for *Babesia divergens* (8), *B. venatorum* (14), *B. capreoli* (2) and *Babesia* spp. (4) respectively. The infection rate was evaluated to be 2.7% (95% CI: 1.8 – 3.9).

Conclusions: This study confirms the presence of *Babesia capreoli* together with two potentially zoonotic *Babesia* species in Belgium, most notably *Babesia venatorum*, in ticks collected from wild ruminants. The estimated *Babesia* spp infection rate (2.7%) is in agreement with other previous publications *B. venatorum* and *B. capreoli* are known to have cervids as a host, but the question remains for the role of deer as a reservoir for *B. divergens*. Therefore, knowledge of the most common reservoir source for transmission of zoonotic *Babesia* sp. will be useful for modelling the risk potential of this infection to humans.

Firsr report of indigenous *Dermacentor reticulatus* populations in Belgium and preliminary study on associated Babesiosis pathogens

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Background: The occurrence of indigenous clinical cases of canine (Losson *et al.*, 1999) and equine (Mantran *et al.*, 2004) babesiosis in Belgium during the last two decades suggested that the vector of the pathogens responsible for these diseases, *Dermacentor reticulatus*, could be present in Belgium. Recent reports indicated an expanding geographical distribution of *Dermacentor reticulatus* in Western Europe but until now it was uncertain that indigenous *Dermacentor* populations were established in Belgium.

Methods: Four different locations throughout Belgium, identified as potential *Dermacentor reticulatus* sites, were monitored by flagging during 2010. Ticks were stored in 100% ethanol immediately after trapping and morphologically identified. Tick DNA extraction was performed according to a proteinase K protocol (20mg/ml) following by an ethanol precipitation and to avoid false-negative results, an additional PCR targeting the tick 16S rRNA gene was performed. Only tick-DNA positive samples were further analyzed for the presence of *Babesia* spp. A *Babesia* spp. genus-specific PCR was applied based on the amplification of the 18S rRNA gene.

Results: Two different tick species were identified, *Ixodes ricinus* and *Dermacentor reticulatus*. A total of 282 *D. reticulatus* adult ticks were collected from the 4 sites. Ticks were found mainly from early March until the end of May with a peak of activity in March.

Four out of 188 tick extracts remained negative for the 16S rRNA gene PCR even after diluting the samples 10 and 100 X. The remaining 184 DNA extracts yielded negative results for *Babesia*.

Conclusions: This is the first record of indigenous questing populations of *D. reticulatus* in several areas of Belgium. The low number of ticks examined for the presence of *Babesia* spp. specific DNA did not allow us to conclude about the carrier status of *B. canis canis* by this tick species. Additional studies should be carried out in order to define more accurately the distribution and vectorial capacity of this tick species in Belgium.

Canonical and variant histone repertoire in *Babesia bovis* and their possible relevance in epigenetic regulation

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Bovine babesiosis is a tick-borne disease which causes serious economic losses in most countries in tropical and subtropical regions of the world. Currently, outbreaks can be prevented by vaccination using live attenuated parasites. These vaccines have certain disadvantages such as the risk of reversion of the attenuated into the virulent strain causing considerable economic losses in some countries. We hypothesize that reversion occurs by epigenetic mechanisms. The existence of epigenetic control for virulence factors in *Babesia bovis* has been suggested but has not been demonstrated yet. Epigenetic control mechanisms that have been described in several protozoan apicomplexa and seem to be a conserved feature of this group are: (i) the modification of histones by acetylation and methylation, and (ii) the incorporation of histone variants into nucleosomes. In the present work, our objective is to demonstrate that the necessary epigenetic players exist also in *B. bovis*. In a first step, the repertoire of core and variant histones present in *B. bovis* was identified using the well defined histone sequences of *Plasmodium falciparum* and *Toxoplasma gondii* available in GenBank databases. Preliminary annotation was done by identifying corresponding orthologous histones in *B. bovis* using reverse BLAST test and altogether eight different types of histones could be identified.

To further corroborate the exact identity of these histones, phylogenetic trees were constructed using core and variant histone sequences from other apicomplexa. By this way it was possible to unequivocally classify *B. bovis* histones into the types H2A, H2AZ, H2B, H2Bv, H3 (2), H3.3, H3CEN. All *B. bovis* histones clustered with those of the closely related *Theileria* sp. Amino acid sequences of the N-terminal tail of H3 and H4 histones of *B. bovis*, *T. gondii* and *P. falciparum* were aligned allowing to verify the presence of highly conserved motifs that are known to be targeted by acetyl- and methyl-modifiers. The results suggest that in *B. bovis* the main epigenetic players are present and are highly likely targeted by epigenetic modifiers.

Seroprevalence of Four Vector-Borne Infections in Dogs from Romania: a Countrywide Study

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Background: The medical importance of vector-borne pathogens is in most of the cases restricted to humans and few domestic species (i.e. dogs, horses, cattle). Due to their ecology and habits, dogs are considered important sentinel species for several arthropod-transmitted diseases. The aim of our study was to evaluate for the first time in Romania, on a national scale, the seroprevalence of four main vector-borne infections in dogs: *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Ehrlichia canis* and *Dirofilaria immitis*.

Methods: Serum samples from 941 dogs were collected between July 2010 and March 2011 in 94 localities from 24 counties in various parts of Romania. All samples were analyzed immediately by a IDEXX SNAP[®]4Dx[®] ELISA test (according to the manufacturer's instructions). Data was analyzed by statistical and GIS methods according to general epidemiological customs.

Results: Antibodies against *Anaplasma phagocytophilum* were detected in 56 dogs (5.9%) in 23 localities all over Romania, being the most prevalent of the four infections tested in our study. *Dirofilaria immitis* antigens were found in 23 dogs (2.4%), all restricted to few localities in lowlands from southeastern and western Romania. Six dogs tested positive for antibodies to *Borrelia* C₆ peptide, all of them around the Transylvanian plateau. The least prevalent infection was with *Ehrlichia canis*, with only two positive dogs (0.2%) limited to a single locality in southeastern Romania.

Conclusions: Our results, correlated with hard-tick distribution data, show that anaplasmosis is co-distributed with its vector. Lyme borreliosis has a focal distribution due to its complex ecology and ehrlichiosis is limited to a small area, suggesting its vectors in Romania are *Rhipicephalus* ticks. Despite the large distribution area of its mosquito vectors, dirofilariosis is limited to habitats with high average temperature values.



SESSION R

Keynote Lecture

- **Molecular diagnosis and typing** - F. J. Conraths

Oral Communications

- **The CARLA Saliva Test: a new tool for measuring anti-parasite immunity in sheep** - I. Sutherland
- **Tritrichomonas foetus isolates from cats and cattle show genetic differences in two unrelated, highly discriminating loci** - C. F. Frey
- **Toxoplasma gondii infection in sentinel and free-range chickens from Argentina** - G. Moré
- **Proteomic analysis of excretory-secretory biomolecules from Trichinella spiralis/T. britovi L1 larvae and their immunochemical/molecular characterization as potential diagnostic markers** - M. Kašný
- **Size Fragment Analysis on a Capillary Sequencer as a Simple Method to Identify and Quantify GI Nematode Eggs** - D. Zarlenga
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Posters

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- **Galectin-11, a potential marker of resistance against gastrointestinal nematode infection in small ruminants** - S. Preston
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- **Comparison between immunohistochemistry and PCR in fetal tissues of heifers experimentally inoculated with Neospora caninum** - S.G. Caspe
- **Immunodiagnosis of fasciolosis in Guanacos (Lama guanicoe) using recombinant Fasciola hepatica procathepsin L1 protease** - S. Carnevale
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- **Isolation and molecular detection of Toxoplasma gondii in bats from the metropolitan area of São Paulo City, SP, Brazil** - A. D. Cabral
- **Molecular analysis of Anisakis larvae from Merluccius hubbsi in Argentina** - S. J. Krivokapich
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- **Development of a Nested PCR-RFLP of ITS-1 region to differentiate Toxocara canis and T. cati eggs** - C. Gonzalez Prous

- **Detection of *Bartonella* spp., *Ehrlichia* spp. and *Rickettsiales* of the spotted fever group in dogs and their vectors on Pirai, Rio de Janeiro** - N. S. Moreira
- **Genetic Diversity in Field Isolates of *Trypanosoma theileri* Assessed by Analysis of Cathepsin L-like Sequences Disclosed Multiple Genotypes Infecting Cattle in Thailand** - H. Garcia
- **Diagnostics of giant liver fluke (*Fascioloides magna*) and geographical distribution of fascioloidosis in the Czech Republic (Europe)** - M. Kašný

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Molecular diagnosis and typing

Tissue-cyst forming coccidian parasites as an example

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A wide array of molecular techniques has been developed for diagnosing parasitic infections and typing their causative agents. These methods include various conventional and realtime PCR techniques. Due to the generally high sensitivity of many PCRs, potential cross-contaminations of diagnostic samples must be avoided by an intelligent laboratory management and monitored using sample processing controls. False-negative results, which may be due to inhibition of the polymerase, can be detected by inclusion of appropriate internal controls. A variety of techniques has been developed to examine the molecular diversity of pathogens. For example, multi-locus microsatellite typing was established to study the distribution of different isolates or strains in final or intermediate host populations of *Neospora caninum*. Since this technique can also utilize tissue samples, it is not necessary to isolate the parasite first. Methods to analyse short nucleotide polymorphisms, multi-locus microsatellite markers or multi-locus PCR restriction fragment length polymorphisms (PCR-RFLP) have been established as powerful tools for the differentiation of major clonal lineages, e.g. types I, II and III of *Toxoplasma gondii*. For this parasite, molecular typing results allow also conclusions on the virulence of the respective isolates in mice, although their relevance regarding virulence in other species is often less clear. DNA sequencing has become a powerful tool to obtain typing data of the highest resolution. Available techniques will be presented and their advantages and disadvantages discussed.

The CARLA Saliva Test: a new tool for measuring anti-parasite immunity in sheep

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Infection with gastrointestinal nematodes (GIN) is a major constraint on the productivity of grazing livestock. The development of selection methods to quickly and accurately identify animals capable of developing an effective natural immunity to infection would contribute to the development of sustainable worm control programs. A carbohydrate larval surface antigen (CarLA), present on the infective-stage larvae (L3) of all trichostrongylid nematodes, is a target antigen for host antibody (Ab). The levels of various Ab isotypes in serum and/or saliva of field-grazed lambs were assessed by ELISA, and Ab titres compared with parasite faecal egg counts (FEC) and a range of animal productivity parameters. Levels of anti-CarLA IgA in saliva proved to be the most heritable ($h^2=0.3$), and had the closest genetic correlation with FEC ($r=-0.5$). Those animals identified as having 'high levels' of anti-CarLA IgA typically have 20-30% lower FEC than animals with low or undetectable titres. Furthermore, animals with 'high levels' of anti-CarLA IgA tend to have improved growth rates post-weaning, and have no tendency for increased breech-soiling. The assay performed well regardless of parasite genera present on pasture. The saliva assay has a number of key practical advantages over the use of FEC for selection purposes: animals can be identified without a requirement to withhold anthelmintic treatment; sampling is rapid and easy and there is a significantly reduced barrier to adoption within the farming community. Measurement of anti-CarLA IgA in saliva by ELISA offers a practical, rapid and easy method of selecting for natural immunity to GIN in sheep.

***Tritrichomonas foetus* isolates from cats and cattle show genetic differences in two unrelated, highly discriminating loci**

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Background: *Tritrichomonas foetus* as causative agent of venereal disease in cattle has not been detected in Switzerland for more than a decade. However, cats suffering from diarrhea quite often harbor trichomonads that are classified as *T. foetus* based on morphology and molecular evidence of the internal transcribed spacer region 2 (ITS-2). Very recently, it has been postulated that isolates from cats and cattle are genetically different and we wanted to verify this finding.

Methods: In our study, we compared the sequence of single cell clones of (i) isolates from cats suffering from diarrhea, (ii) reference strains of *T. foetus* isolated from cattle available from ATCC, and (iii) isolates of *T. foetus* from naturally infected cattle on two unrelated genomic loci, the ITS-2 locus and a novel locus encoding elongation factor 1 α (EF1 α). To determine the power of discrimination of both loci we included other trichomonad species in the analysis.

Results and Conclusions: In both loci, *Pentatrichomonas hominis*, *Trichomonas vaginalis*, *Trichomonas gallinae* and *Tetratrichomonas gallinarum* were clearly distinguishable from *T. foetus*. *Tritrichomonas suis* and *Tritrichomonas mobilensis*, however, were very similar to *T. foetus* sequences in both loci. Between *T. foetus* isolates from cats and bovines, small differences were detected. In the ITS-2 locus, the isolates from cats displayed one single nucleotide polymorphism (SNP) that was consistent throughout all isolates compared to the isolates from cattle. This finding confirms the finding of a recent study by Slapeta et al. (2010). In the novel EF1 α locus, two regions with consistent SNPs between cattle and cat isolates were detected. These initial results of an on-going investigation suggest that based on the two genomic loci, *T. foetus* isolates from cattle and cats can be discriminated between each other.

***Toxoplasma gondii* infection in sentinel and free-range chickens from Argentina**

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This study aimed at isolating and genotyping *Toxoplasma gondii* from serologically positive free-range chickens from Argentina, and to evaluate the use of sentinel animals during a short time period of exposure to determine environmental contamination with *T. gondii* oocysts. Two groups of chickens were used: I. 2-3 month-old broiler-type chickens reared as sentinel animals on the farms in two different seasons, and II. adult chickens reared on the same farms for more than one year. The sentinel animals (240 one-day-old chickens) and the food were donated to 5 farmers in Las Flores and one in La Plata. Seroconversion of 7.0% (8/114) and 5.7% (5/88) (with titers 100 and 200) was observed in sentinel broiler chickens reared for a period of 74 days (January – April 2010) or 68 days (August – November 2010) respectively, as shown by a *T. gondii* specific immunofluorescent antibody test. Fifty-three percent (17/32) of adult chickens were seropositive and showed higher titers than sentinel animals. Considering both sentinel study periods (summer-autumn; winter-spring), the monthly average seroconversion rate of free range chickens was approximately 3%. This suggests a widespread distribution of *T. gondii* oocysts in the environment which did not vary much over the year under study. Isolation of *T. gondii* from tissues (brain and heart) of serologically positive chickens was achieved from six of seven free-range adult birds (from 3 farms) with IFAT titres ≥ 200 . The isolated parasites were analysed by multi-locus polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). *T. gondii* isolates corresponding to three different genotypes were obtained: two different genotypes consisted of atypical allele combinations (4 isolates), and the remaining genotype showed exclusively clonal type II alleles (2 isolates). On each farm, all the isolates obtained corresponded to the same genotype. These *T. gondii* genotypes were previously described in cats, dogs, chickens and capybaras elsewhere in South America. Our observations suggest that a greater diversity of *T. gondii* strains exists in Argentina than in North America and Europe.

Proteomic analysis of excretory-secretory biomolecules from *Trichinella spiralis*/*T. britovi* L1 larvae and their immunochemical/molecular characterization as potential diagnostic markers

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Trichinellosis is important zoonosis caused by consumption of inadequately heat-processed meat containing *Trichinella* larvae. Preventive measures in term of food safety are based on periodic controls of meat - according to the European Union legislation the digestive method employing the magnetic stirrer is recommended for a direct prove of trichinellosis. Indirect - serodiagnostic methods (e.g. ELISA/Immunoblot) are used for monitoring of trichinellosis especially in areas with a negligible incidence of this parasitosis and also for the certification of pig breeds. In common indirect serodiagnostic tests the excretory-secretory products (ESP) of the *Trichinella* larvae are used as referent "complex"-antigen. The unspecified mix of ESP antigens is one of the factors limiting the specificity of indirect methods. Therefore, we have focused on identification of potential *Trichinella* L1 larvae antigenic biomolecules (proteins) from ESP and their preparation in recombinant form for further immunodiagnostic application. The ESP of *Trichinella spiralis* and *T. britovi* L1 larvae were separated by one- and two-dimensional polyacrylamide gel electrophoresis (1D and 2D SDS-PAGE). Separated proteins were transferred to polyvinylidene fluoride-membrane (PVDF) and incubated with sera from pigs experimentally infected by two trichinella species. The strongest antigen-antibody reaction was recorded in area of spots 43, 45 and 53 kDa. These protein-antigens were identified by mass spectrometry methods (MALDI TOF/TOF), the corresponding genes were cloned, prepared in recombinant forms and currently are immunodiagnostically tested.

Size Fragment Analysis on a Capillary Sequencer as a Simple Method to Identify and Quantify GI Nematode Eggs

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Background: Classic approaches for discerning GI nematodes from fecal eggs require cultivation to infective L3 followed by morphological examination. Over the years, a plethora of molecular techniques have surfaced, the majority of which are now predicated upon real-time PCR or PCR amplification followed by gel analysis. While adequate for diagnosis, most techniques fall short of quantifying mixed infections without substantial numbers of controls and/or standard curves. The goal of the work presented herein, was to develop a simple and rapid test for differentiating and quantifying mixed infections of GI nematodes, using fluorescently-labeled PCR products and a capillary-based sequencer.

Methods: Prior sequence data indicate that the ITS2 region of common cattle GI nematodes is sufficiently distinct in length to delineate among infecting genera. As such, conserved PCR primers that span the ITS2 and bind to the 3'-end of the 5.8S rRNA (forward) and the 5'-end of the 18S rRNA (reverse) were synthesized; at least one primer of each set was fluorescently-labeled with FAM. DNA from egg samples or infective L3 was isolated, PCR amplified, diluted directly in HI-DYE sequencing buffer containing LIZ 500 standard, then loaded onto an ABI 3100 sequencer adapted for size fragment analysis. Tests were first performed on monospecific infections to validate the ability to differentiate *Haemonchus*, *Ostertagia*, *Cooperia*, *Trichostrongylus* and *Oesophagostomum* using this technology. Secondly and as proof of principle, L3 from *Ostertagia ostertagi* and *Cooperia punctata* were mixed in 10% increments, and the DNA isolated and analyzed for relative infection levels using Gene Marker V1.85.

Results: Using the chosen primer set, amplified DNA gave the following size fragments: *Haemonchus* 364 bp; *Ostertagia* 371 bp; *Cooperia* 374 bp; *Trichostrongylus* 369 bp, and; *Oesophagostomum* 355 bp using primers 1244 and 1246. Analysis of environmentally-derived samples from animals infected with *Haemonchus* and *Cooperia* coincided well with coproculture and PCR data. Quantified peak intensities using predefined mixes of *Cooperia* and *Ostertagia* gave a linear response in the range of 10%-90%. Data showed that primer design is critical for quantitative analysis.

Summary: Preliminary information suggests that size fragment analysis is both diagnostic and quantitative on a relative scale. The methodology is scalable for large sample numbers. Ongoing work is continuing to validate the technology for quantifying parasite mixtures.

Genetic diversity of *Toxoplasma gondii* in Australian wildlife

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Infection with *Toxoplasma gondii* can lead to a wide spectrum of disease states, ranging from altered behaviour to severe, often fatal illness. Virulence depends, in part, upon the genotype of the parasite. Until recently, almost nothing was known of the genetic diversity of *T. gondii* in Australia. We have found that *T. gondii* is common and widely distributed among the native fauna of Western Australia, with the parasite isolated from over 25 species of native mammals and birds, with prevalences ranging from 40-100%. Based on our preliminary analyses by multilocus PCR-DNA sequencing at polymorphic genes, the majority of marsupial *T. gondii* infections were Type II-like or atypical strains expressing several novel alleles. Multiple infections with different genotypes of *T. gondii* were also commonly found in infected marsupials which may suggest that sexual reproduction occurs regularly in the life cycle of the parasite in Australia. Our data raises questions about transmission, virulence and origin of *T. gondii* in Australia.

Molecular Characterization of *Giardia* and *Cryptosporidium* in Alpacas (*Vicugna pacos*) from Peru

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Background: Alpacas (*Vicugna pacos*), as most other mammal species, are at increased risk of acquiring infections of diverse etiology during the first months of life, being diarrhea an important cause of morbidity and mortality in neonatal animals. *Giardia* and *Cryptosporidium* are two genera of protozoan parasites that cause significant economic losses in domestic animals. The aim of the present study was the detection and molecular characterization of *Giardia* and *Cryptosporidium* isolates from alpacas from the Andean region.

Methods: Faecal samples were collected from 274 alpacas selected at random from 12 peruvian farms located in the regions of Puno and Cusco. A direct immunofluorescence antibody test (IFAT) was used to detect *Giardia* cysts and *Cryptosporidium* oocysts. Molecular characterization was carried out by PCR amplification and sequencing of fragments of the ssu-rRNA and Beta-Giardin and ssu-rRNA genes from *Giardia* spp. and *Cryptosporidium* spp., respectively.

Results: A total of 137 samples (50%) were positive by IFAT and PCR, 135 samples (49.3%) for *Giardia* spp. and 12 samples (4.4%) for *Cryptosporidium* spp. In ten samples, co-infection by both pathogens was detected. Ninety two samples were positive for *Giardia* spp. by PCR. Sequencing of the amplicons showed the existence of *Giardia duodenalis* assemblage A in 67 samples; *G. duodenalis* assemblage E in 24 samples; and inconsistent results between the two molecular markers used in a further sample. *Cryptosporidium parvum* and *Cryptosporidium ubiquitum* were identified in two and one sample, respectively.

Conclusions: This is the first study performing molecular characterization of a high number of isolates of both parasites in alpaca, identifying also for the first time *G. duodenalis* assemblage E and *C. ubiquitum* in this host. The high prevalence of *Giardia* spp. detected together with the identification of *G. duodenalis* assemblage A and *C. parvum*, suggest that zoonotic transmission of giardiasis and cryptosporidiosis between alpacas and humans can occur.

Galectin-11, a potential marker of resistance against gastrointestinal nematode infection in small ruminants

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Background: *Haemonchus contortus* is a major gastrointestinal nematode (GIN) parasite of sheep, causing significant production losses. Primary control of *H. contortus* is by the use of anthelmintics but is an unlikely long term control method due to the development of parasite anthelmintic resistance. Alternative strategies include the breeding of animals naturally resistant/resilient to infection. Such selection is based on the phenotypic identification of resistant animals. Currently, faecal egg counts (FEC) are the most common method of determining infection levels but the expense and labour requirements for this technique limits its impact and an alternative test is required. Two molecules associated with the development of immunity, Galectin-11 (a carbohydrate binding protein recently identified in our laboratory) and mucosal IgA, were investigated as potential resistant selection tools in infected animals.

Methods: Merino sheep were infected with *H. contortus* and the infection was allowed to develop until parasite maturation. FEC and PCV were measured throughout the trial. Worm burden and abomasal mucus were collected on day of kill (day 57). The levels of galectin-11 in the mucus were analysed by Western blot and quantified by densitometry. IgA was measured by ELISA coated with adult excretory/secretory products.

Results: Differences in these molecules between sheep with disparate parasite burdens were evident. Interestingly, spearman correlations indicated negative relationship between galectin-11 ($r=-0.7$, $p<0.05$) and IgA ($r=-0.8$, $p<0.05$) existed in comparison to cumulative FEC.

Conclusions: These results indicate that mucosal secretions of IgA and galectin-11 have a protective function during *H. contortus* infections. Further studies will investigate whether these relationship between mediators and parasitological parameters found in the abomasal mucus can be measured in peripheral samples (nasal mucus, serum, faeces) as phenotypic measurements more applicable for parasite resistance. Functional studies of galectin-11 and IgA using an *in vitro* migration assay and by immunohistochemistry are ongoing.

Recombinant antigens for serological diagnosis of bovine cysticercosis

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Bovine cysticercosis diagnosis is mainly performed by post mortem examination in Brazil. Development of reliable diagnostic methods may ensure an improvement in epidemiological investigations as well as allow the monitoring of treated animals. We have performed preliminary ELISA tests with defined recombinant *Taenia* antigens using sera from 29 naturally infected bovines, 2 experimentally infected animals and 11 animals negative for cysticercosis. The antigens were HP6, a *Taenia saginata* oncosphere adhesion antigen thought to participate in oncosphere penetration of the intestinal wall, and Ts8B2 an excretion-secretion product of *T. solium* metacestodes. The simultaneous use of these two antigenic targets may be a way to differentiate active infection from exposure without subsequent differentiation to the metacestode. The best results were achieved using Immulon 2HB[®] microplates coated with recombinant proteins at 10µg/mL, 5% skimmed milk as blocking solution, serum samples diluted 1:400 and anti-bovine IgG conjugated to peroxidase 1:10.000. Although differentiation between active infection and contact was not formally proved, the Ts8B2 allowed a better differentiation between positive and negative samples than the HP6 recombinant protein.

***Toxoplasma gondii* distribution in tissues of naturally and experimentally infected lambs in France**

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Background: The consumption of raw or undercooked meat products containing *Toxoplasma gondii* **tissue cysts** is one of the main ways of contamination for humans. The aim of the present study was to adapt a real time PCR technique for the study of the distribution and the quantification of *T. gondii* in the tissues of infected lambs.

Methods: Direct detection of *T. gondii* was performed by mouse bio-assay and real time-PCR in 9 tissue samples (skeletal muscles, tongue, brain, lung, intestine, heart) from 3 lambs orally fed with 10³ oocysts of a *T. gondii* type II strain (ME49) and from 3 naturally *Toxoplasma*-infected lambs. To evaluate the real time PCR technique, we analysed fresh and frozen tissues.

Results: We detected *T. gondii* only in the mice bio-assayed with the experimentally lambs. In all the lambs, the distribution of the parasite was ubiquitous with a significant tissue tropism and individual variation of the parasitic burden. In the experimentally infected lambs, the skeletal muscles had the highest parasitic burden whereas the lungs and intestine had the lower parasitic burden. Among all skeletal muscles, deltoids and quadriceps had the highest burden. In the naturally infected lambs, the brain and the diaphragm had the highest parasitic burden whereas the intestine had the lower parasitic burden. The fresh samples had a significant higher parasitic burden than the frozen ones ($p < 0.0001$).

Conclusions: The present study demonstrated a variable distribution, not only among different tissues, but also within the same tissue. These results confirmed the potential role of ovine meat as a source of *T. gondii* for humans. The detection of the parasite in the diaphragm looks relevant for the control of food safety and for the improvement of toxoplasmosis risk assessment.

Patterns of *Neospora caninum* antigen recognition at different IFAT titers in sera and their fetuses of experimental inoculated heifers by Western blot analysis

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The aim of this study was to evaluate *Neospora caninum* tachyzoite antigen recognition by antibody response in heifers experimentally challenged with *N. caninum* isolates (Nc 1 and Nc Spain 7) and in their fetuses. For this purpose, 13 naïve pregnant heifers were divided into three groups and were experimentally challenged intravenously with either 1x10⁸ tachyzoites of Nc-Spain 7 (group A, n=7), Nc-1 (group B, n=4) isolates and PBS (group C, n=2) at 65 days gestation. Titers of sera were determined by Indirect Fluorescent Antibody Test (IFAT). The comparison of tachyzoite antigen recognition was carried out in abdominal fluids from fetuses and sera from heifers of different groups. Besides, the pattern of antigen recognition into the two isolate tested groups in relationship between IFAT titers, were evaluated. Sera from group C and abdominal fluids from their fetuses were used as negative controls. Similar patterns of antigen recognition (17-18, 30, 34-35, 37, 60-62, 75-77 and 260-270 kDa signal) were established for both *Neospora* challenged groups at IFAT 1:800 titer. Differences were not found in antigen recognition among different IFAT titers in every challenged group. In the fetal fluids, only the 17–18 kDa antigen was found in all the positive fetuses. Moreover, in three out of four fetuses of group A other additional bands (31, 35, 62 kDa) were recognized. Sera of control heifers and fluids of their fetuses and sera of the pre-inoculated time did not bind to any *N. caninum* antigen. Only titers of 1:800 could be compared between challenged groups because the IFAT titers were different in each group. At this titer, the patterns of antigens recognized were similar in the sera of heifers of inoculated groups (A and B). However, when we compared the fluids from the fetuses of heifers inoculated with Nc-Spain 7 more bands were recognized. In conclusion, in agreement with natural cases of abortion, we found that only the 17 kDa antigen was recognized in all the positive samples examined (sera and fetal fluids), even when no other antigens were detected.

Comparison between immunohistochemistry and PCR in fetal tissues of heifers experimentally inoculated with *Neospora caninum*

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Neospora caninum is an apicomplexan parasite that causes abortion in cattle. The diagnosis in fetuses is based on the detection of histopathologic *N. caninum* compatible lesions (HP) and the identification of the parasite by immunohistochemistry (IHC) or its DNA by PCR. The aim of this study was to compare the IHC to nested-PCR (ITS-1) to identify the presence of the parasite in tissues of fetuses of heifers inoculated experimentally. For this purpose, these heifers were inoculated at 64 days of gestation (DG) and their fetuses were recovered at 100 DG. A total of 28 tissues (brain: 5, placenta: 8, liver: 8, heart: 7) out of 8 fetuses of experimentally infected heifers with microscopic lesions were processed by these techniques. Twenty four tissues of fetuses (brain: 6, placenta: 6, liver: 6, heart: 6) out of 6 non inoculated heifers without histopatological lesions were used as negative control. The concordance between IHC and PCR was determined. Fourteen of the 28 tissues (50%) were positive by IHC, all (n=24) negative control tissues were negative. The relationship between the number of positive fetuses by HP and those positive by IHC showed a statistically significant association ($p < 0.05$). On the other hand, 26 of the 28 tissue tested (92.8%) were positive by PCR. The relationship between the number of fetuses positive by HP and those positive by PCR showed a statistically significant association ($p < 0.005$). The concordance between nested-PCR and IHC was 76%. When we compared the number of individual tissues positive by IHC and PCR tests, no statistically significant differences were found ($p > 0.05$). In the present study, a greater percentage of positive cases was detected by PCR 26/28 (92.8%) in comparison to the IHC test 14/28 (50%). Despite the sensibility differences, both techniques had shown the same specificity (100%). Even though both tests were useful to confirm the infection, in this study, the PCR test showed higher sensibility and should be preferably used for diagnosis purposes.

Immunodiagnosis of fasciolosis in Guanacos (*Lama guanicoe*) using recombinant *Fasciola hepatica* procathepsin L1 protease

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Background: Little is known about *Fasciola hepatica* in South American camelids, alpacas (*Lama pacos*), llamas (*Lama glama*), vicuñas (*Vicugna vicugna*) and guanacos (*Lama guanicoe*), however it is known they are highly susceptible to fascioliasis. Prevalences ranging from <1% to 59% in endemic areas have been recorded. The aim of this work was to evaluate an indirect enzyme immunoassay as a tool for the diagnosis of *F. hepatica* infection in guanacos.

Methods: The study included 50 blood and fecal samples from guanacos from Rio Negro province, Argentina. Twenty-three samples were collected from guanacos grazing in endemic liver fluke areas, and 27 samples from animals from a fluke free area. Feces were analyzed for *F. hepatica* eggs presence and counting (eggs per gram of feces). Serum samples were analyzed by an indirect ELISA, employing a recombinant procathepsin-L1 of *F. hepatica* as antigen and anti-goat IgG horseradish peroxidase-labeled immunoglobulin as secondary antibody. Sera and secondary antibody concentrations were determined assaying serial dilutions from 1:50 to 1:400 for sera and 1:125 to 1:1000 for the conjugated antibody. The coprological assay was used as the gold standard. To analyze the data and determine the cut off value the ROC curve analysis was performed.

Results: The best assay concentrations resulted in 1:100 dilution for animal sera and 1:250 dilution for the secondary antibody. From the 23 positive guanacos, detected through the coproparasitological analysis, 20 resulted positive (sensitivity 89.96%) to the ELISA test, meanwhile from the 27 negative ones, the ELISA detected 25 as negatives (specificity 92.59%). The ELISA values were expressed as absorbance units at 410 nm and the calculated cut-off value was 0.313 (95% CI= 0.246-0.336). The predictive positive value was calculated in 90.91% and the predictive negative value was 89.29%.

Conclusions: The ELISA test evaluated in this study is a relatively sensitive and specific diagnostic tool to detect long term chronic *F. hepatica* infections, being the first report on the immunodiagnosis of fasciolosis in guanacos. Further work should be carried out with a large number of samples to validate the assay as a diagnostic tool.

Isolation and molecular characterization of *Toxoplasma gondii* strains in slaughtered pigs from Argentina

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Pork is regarded as one of the most important sources of *Toxoplasma gondii* infection for humans. The aims of this study were to isolate and genotype the parasite from naturally infected pigs from Argentina. Pigs (n=124) from outdoor and indoor farms without rodent and cat control were sampled at slaughterhouses from Buenos Aires province during 2008-2010. In addition, two *T. gondii* isolates previously obtained from pigs, named PIG10 and PIG15, preserved by passages in out-bred mice were tested. Fifty grams of diaphragm and tongue from pigs were obtained and conserved at 2-8°C and two grams were frozen at -20°C. For antibody detection, pig sera and meat juice were analysed by indirect fluorescent antibody test (IFAT). Fresh tissue samples from pigs with titers $\geq 1:25$ in sera or $\geq 1:10$ in meat juice were inoculated into mice, which were bled 27 days post inoculation for antibody detection by IFAT. DNA was extracted from all pig samples, from tissues of serologically positive mice and from the two previously isolated *T. gondii* strains with a commercial kit. Diagnostic PCR was performed with primers B22-B23, TOX4-5 and TOX5-8. Antibodies were detected in 49/104 pig sera and in 2/20 meat juice samples. Two and 3 of 113 mice were positive at 1:25 and 1:100 dilution by IFAT, respectively. Tissue cysts were not microscopically detected in ground brains from mice. *T. gondii* DNA was not identified in pig muscles, or in brains from inoculated mice. DNA samples from PIG 10 and PIG15 isolates were examined by multilocus PCR-RFLP, and showed clonal type II alleles at loci SAG2, BTUB, GRA6, SAG3, c22-8, L358, PK1 and c29-2. At the Apico marker, PIG 10 isolate showed type II, and PIG 15 isolate type I alleles. Although antibodies were present in pig sera, *T. gondii* DNA was not detected in tissues. Probably the parasite load was not high enough to infect mice or to be detected by PCR. To our knowledge this is the first report on *T. gondii* genotyping in domestic pigs from Argentina.

Diagnosis of Cuterebriasis in cats using ELISA with crude Maggot-Derived Antigen

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Cuterebra, a genus of North American Dipterans, utilizes rodents and lagomorphs as obligatory parasitic hosts for larval development. The larvae can also develop in cats where the maggots often occur in furuncular cutaneous lesions. Unfortunately, in cats developing maggots may migrate to atypical sites, e.g., the CNS, causing serious disease that is often fatal. In the northeastern U.S., the life cycle is typically univoltine, with most cases occurring each year between June and October. Diagnosis has typically been by maggot extraction from the skin or, in the case of visceral infections, via presentation, signs, radiographs, or more recently, computed tomography. To aid in the diagnosis of this infection, work has been ongoing on the development of an ELISA that utilizes crude antigens from maggots harvested from cats and rabbits. Here we report the development of an indirect ELISA using crude *Cuterebra* antigen to detect parasite-specific IgG and IgM in cats with known (45), suspected (23), or no known exposure to the infection consisting of serum from laboratory-reared cats (28) and serum samples from France (39) where the parasite is absent. Cats with maggots gave positive results on the ELISA in both IgG and IgM, while cats without cuterebriasis were negative. Moreover, some cats with neurological signs, but no observed maggot, were ELISA positive. Changes in *Cuterebra* specific IgG and IgM antibodies over time was demonstrated using successive serum samples from cats with observed maggots or with neurological signs.

Seroprevalence of *Toxoplasma gondii* in Iberian pig sows

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Background: The Iberian pig is an autochthonous porcine breed traditionally developed in extensive systems in South-Western Spain. Pigs infected with *Toxoplasma gondii* are considered an important source of infection for humans in several countries and infection rates vary in pigs raised indoor versus outdoor farms. The objective of the present study was to investigate the seroprevalence of *T. gondii* infection in Iberian pig sows raised in extensive and intensive management conditions.

Methods: Serum samples were collected from 2,000 Iberian pig sows belonging to 13 farms in year 2010. Mean farm size was 500 sows and 20% were sampled in each farm. Three types of management systems were included: traditional extensive outdoor (5 farms), intensive with outdoor access (4) and conventional intensive indoor (4). The presence of serum specific antibodies to *T. gondii* was evaluated by two validated commercially available tests: an indirect enzyme-linked immunosorbent assay (ELISA, ID Screen Toxoplasmosis Indirect, IDVet, France) and a direct agglutination test (DAT, Toxo-Screen DA, BioMérieux, France). ELISA results were expressed as average sample to positive (SP) values and SP values above 50% were regarded as positives. DAT results were considered positives at dilutions of 1:32 or higher. Prevalence data were related to management and facilities of the farms.

Results: Serum antibodies to *T. gondii* were detected in 152 sows (7.6%) by at least one of the techniques used. The mean seroprevalence rate of toxoplasmosis in Iberian pig sows oscillated between 5.3% (ELISA) and 6.6% (DAT). A good degree of agreement was found between both tests (kappa value=0.7). The higher (8.4%) mean individual prevalence rate by ELISA was in sows under traditional extensive outdoor system and poorest facilities. Sows reared under intensive management with outdoor access showed a prevalence of 5.4%. The lowest prevalence rate (1.1%) was found in sows under conventional intensive indoor system and highest facility level.

Conclusions: The results from this study suggest that the prevalence of *T. gondii* antibodies among Iberian pig sows seems to be moderate-low and some herds with high within-herd prevalence rates. *T. gondii* prevalence was related to the facilities and management of the farm.

Isolating a new North American strain of *Ancylostoma braziliense* from cats using molecular techniques

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In the past, acquiring a new *Ancylostoma* species isolate generally necessitated either euthanasia of the source animal and/or collection of adult worms post-treatment for morphological identification. More recently, a new field isolate of *Ancylostoma braziliense* was obtained from dogs without the need for necropsy or treatment by using a combination of fecal screening and molecular identification methods. Here, we report the successful use of this same protocol to isolate a new North American strain of *A. braziliense* from cats. Sugar centrifugal flotation performed on fecal samples from 40 cats in Florida identified 26 cats as being positive for *Ancylostoma* eggs. DNA was extracted from these eggs, followed by PCR-RFLP using the restriction enzyme HinF1. A total of four samples thus were determined to be *A. braziliense* mono-infections. Fecal cultures were initiated in Florida and then driven back to Ithaca, NY, where the larvae were harvested using the Baermann technique. Larvae were passaged first through a laboratory-reared Beagle dog in order to eliminate any *Ancylostoma tubaeforme* which may have been present in the feces but not detected by molecular methods. Eggs shed by the dog were confirmed to be *A. braziliense* by PCR-RFLP and DNA sequencing. Larvae cultured from the dog's feces then were used to infect a laboratory-reared kitten, which became patent 15 days post-infection. Again, eggs were confirmed to be *A. braziliense* by PCR-RFLP and DNA sequencing. The new isolate currently is being maintained in cats for future studies. The Beagle dog and kitten utilized for the strain isolation itself have since been dewormed and placed into adoptive homes.

Next-generation sequencing of mitochondrial genomes from lungworms – application of the datasets to assess phylogenetic relationships

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The advent of next-generation sequencing (NGS) offers unique opportunities for the high-throughput sequencing, assembly and annotation of mitochondrial genomes. Such genomes are a rich source of genetic markers for population genetic, systematic and evolutionary studies of parasites and a wide range of other organisms. Recently, Jex and colleagues (2010) showed that the major suborders within the order Strongylida (e.g., the Metastrongylina, Strongylina and Trichostrongylina) each resolved as distinct, monophyletic clades, which contrasted a recent phylogenetic study using nuclear ribosomal DNA sequence data (Chilton and colleagues, 2006), in which no monophyletic clades were formed for species within the Strongylina or the Trichostrongylina. Recent results supported the distinct placement of the lungworms (*Metastrongylus pudendotectus* and *M. salmi*) relative to all other suborders of the Strongylida, consistent with the hypothesis that the most basal evolutionary dichotomy within the Strongylida divides the order according to the predilection site in the host. However, comprehensive mitochondrial genome sequence datasets have been lacking for lungworms, and, thus, the relationships among various species of lungworms could not be assessed. The present study characterised the mitochondrial genomes of seven different lungworms (*Aelurostrongylus abstrusus*, *Angiostrongylus vasorum*, *Dictyocaulus viviparus*, *D. filaria*, *D. eckerti*, *Metastrongylus elongatus*, *Parafilaroides* spp. and *Protostrongylus* spp.) and inferred the phylogenetic relationships of these and other related, strongylid nematodes (with differing predilection sites in their hosts) using large amino acid sequence datasets.

Jex et al., Nucleic Acids Res 38: 522, 2010.

Chilton et., Mol Phylogenet Evol 40: 893, 2006.

DNA barcodes as a tool for identification of biting midges, potential vectors of bluetongue virus

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Background: Biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) are the insect vector of economically important veterinary diseases such as African horse sickness virus, Epizootic hemorrhagic disease virus and bluetongue virus (BTV). Bluetongue is caused by a double stranded RNA virus and infects ruminants. Transmitters of the BTV in Europe are *Culicoides imicola* and potential vector species *Culicoides obsoletus*, *Culicoides scoticus*, *Culicoides dewulfi*, *Culicoides chiopterus* and *Culicoides pulicaris*. However, identification of these based on morphological features are difficult. The use of DNA barcodes has been proposed as a tool for rapid identification to species for many groups of insects. Hence, a study was undertaken to construct DNA barcodes for all morphologically determined species in the Swedish collections.

Methods: In order to construct DNA barcodes for species collected in Sweden, mitochondrial cytochrome oxidase subunit I (COI) was chosen as marker. Specimens were sequenced from a small piece of tissue and the head, wings and genitalia were mounted and determined to species according to relevant keys. All sequences were compared against data available in Genbank.

Results: In total, 235 specimens of *Culicoides* morphologically identified to be 37 species were used. DNA barcode approach based on sequences of COI gene could identify 35 species among these. However, two pairs of closely related species, *Culicoides festivipennis* and *Culicoides clastrieri* could not be separated based on DNA barcode approach as well as *Culicoides manchuriensis* and *Culicoides salinarius*. Out of 35 species identified with barcodes nine were available in Genbank for comparison, of these eight matched well. However, specimens of *Culicoides lupicaris* did not cluster together with available sequences, though this species is known to be versatile. Each potential vector species possessed distinctive sets of COI haplotypes which discriminated well among species. However, *C. obsoletus* showed one specimen which had high divergence from other specimens. Furthermore, two species, *Culicoides newstedti* and *Culicoides halophilus* are listed as synonyms but showed high sequence divergence which indicates that they are separate species.

Conclusions: The use of COI barcodes as a tool for species identification of biting midges can differentiate 90% of species studied, for some closely related species a less conserved region such as ribosomal ITS spacer needs to be employed.

Diagnosis, Bioassay and Isolation of *Toxoplasma gondii* in horses from slaughterhouses

Isolation of *Toxoplasma gondii* in horses

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A case of congenital toxoplasmosis in France, in a mother reinfected by ingesting imported horse meat, probably from South America (Elbez-Rubinstein et al., 2009), led us to conduct this study. The aim was to investigate the presence of *Toxoplasma gondii* in horses' brains and antibody in sera. The animals were from six Brazilian states. 398 samples of brain and blood were collected in two slaughterhouses with inspection service. The sera samples were subjected to indirect fluorescence antibody test (IFAT) for *T. gondii* (IFAT ≥ 64) resulting in 46 (11.6%) positive. In the bioassay, 14 (3.5%) samples were positive to *T. gondii*, 12 (3.0%) to IFAT and two with presence of tachyzoites or brain cists in mice. Out of the 14 positive animals in the bioassay, 20 biological samples were subjected to PCR, obtaining positive results in three (14.3%). Isolates were genotyping by PCR-RFLP using SAG1, SAG-2, alt. SAG-2, SAG3, BTUB, L358, GRA6, C22-8, C29-2, PK1, Apico, CS3 markers. The isolates were not amplified in all markers, probably, because of low DNA concentration. Thus, we used 18S rDNA marker PCR-RFLP to evaluate closely related apicomplexan parasites (*Hammondia*, *Neospora*, *Sarcocystis*), and those strains were considered *T. gondii*. In total, 60 (15.0%) samples were positive to *T. gondii*.

Seroprevalence of *Toxoplasma gondii* in Domestic and Wild Animals from Fernando de Noronha Archipelago, Brazil

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Background: Fernando de Noronha is an archipelago of 21 islands and islets in the Atlantic Ocean, state of Pernambuco (Brazil) and has a varied biodiversity, including alien species or sinantropic animals such as rodents, cattle egrets, teju lizards, horses, cattle, sheep, goats, chickens, dogs, and cats. The objective was to determine seroprevalence of *Toxoplasma gondii* in domestic and wild animals from Fernando de Noronha archipelago, Brazil.

Methods: Between July 2007 to May 2010, blood samples were collected from 764 animals (533 domestic animals, and 231 wild or synantropic animals). Sera were tested by the indirect fluorescence antibody test (IFAT) and/or both by the modified agglutination test (MAT).

Results: Antibodies to *T. gondii* were found in 80 (80.0%) of 100 chickens (*Gallus domesticus*), in 3 (3.0%) of 100 cattle (*Bos taurus*), in 59 (60.8%) of 97 sheep (*Ovis aries*), in 9 (81.8%) of 11 goats (*Capra hircus*), in 7 (43.7%) of 16 horses (*Equus caballus*), in 70 (59.3%) of 118 cats, in 36 (39,6%) of 91 dogs (*Canis familiaris*), in 13 (38,2%) of 34 black rats (*Rattus rattus*), and in 157 (79,7%) of 197 cattle egret (*Bubulcus ibis*).

Summary/Conclusion: This is the first study on the seroprevalence of *T. gondii* in many species of Fernando de Noronha, Brazil. Results indicate endemic infection by this zoonotic parasite animal fauna in this part of Brazil.

Serosurvey and risk factors for *Toxoplasma gondii* infections in pigs of northern Germany

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Background: Although toxoplasmosis is a reportable disease in both animals and humans in several states of the European Union, the system for routine monitoring or reporting is considered to be inadequate and there is a lack of representative data on *Toxoplasma gondii* infections in livestock.

Methods: We developed a semi-automated test system that can be used in monitoring and surveillance programmes for pigs. The test is based on a commercially available preparation of the native surface antigen 1 of *T. gondii* (TgSAG1), and was validated and standardized with 450 serum or plasma samples from pigs inoculated orally with different doses of *T. gondii* (strain DX). Specific antibodies were detected from 7-14 days after infection until the end of the observation period (16 weeks). No cross-reactions were observed with heterologous sera (*Isospora*, *Sarcocystis*, *Trichinella*, *Campylobacter*, *Salmonella*, *Yersinia*). The test was used to analyse 13251 plasma samples from pigs maintained on 846 farms in Lower Saxony, northern Germany.

Results: Antibodies to TgSAG1 were detected in 2.5% of fattening pigs, 2.8% of piglets, 7.8% of sows, and 19.4% of boars. At least one animal was seropositive on 23.6% (200/846) of the farms, but only 10.7% (38/354) of farms with fattening pigs had seropositive animals. Risk factor analysis, which was carried out for 4335 pigs on 260 farms for which a detailed questionnaire was filled-in by the farmer, showed that *T. gondii* infection in pigs was influenced by: farm type, farm size, origin of replacements, number of cats, feeding practices, feed storage, floor type, cleaning methods, and personal hygiene.

Conclusions: The results of this study are important for the development of efficient control strategies and prevention of *T. gondii* infections in pigs, and consequently for the reduction of *T. gondii* transmission from pigs to humans.

Identification of strongylid species by PCR from crude egg preparations without DNA isolation

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Background: Although both rapid qualitative and laborious quantitative methods for detection of trichostrongylid nematode eggs in faeces of ruminants and horses are available, the identification of parasite species is still not straightforward and either relies on larval cultures requiring experienced staff or on DNA extraction followed by conventional or real-time PCR. Here, a direct PCR was developed allowing species/genus identification by conventional PCR using eggs directly obtained from flotation analysis for quantification.

Methods: Eggs were quantified from goat, sheep, cattle and horse faeces using FLOTAC method. For species determination, eggs were purified from the flotation solution by sieving through a 20 µm nylon mesh and collected in microcentrifuge tubes in water. After three cycles of freezing and boiling, samples were centrifuged, supernatants were collected and used for PCR. PCR primers were designed to give very short amplicons (80-150 bp) in order to maximise sensitivity. Species-specific primers were from the ITS-2 ribosomal DNA region. Pan-strongylid primers either flanked a highly variable region in the ITS-1 or minimally variable regions in the 18S and 28S RNA. PCR was performed with Phusion Hot-Start II High-Fidelity DNA Polymerase (Finnzymes) which turned out to work in the presence of faecal PCR inhibitors in the samples.

Results: Using DNA extracted from individually species-identified adult worms, six primer pairs specific for six important ruminant trichostrongylid parasites were tested to optimise PCR conditions for highest sensitivity and specificity. Species/genus-specific primers were able to identify even minor contaminations of parasite populations in egg populations and PCR products were routinely obtained from faeces containing between 20 and 50 eggs per gram. Moreover, pan-strongylid primers were able to obtain PCR products from crude egg preparations not only of ruminants but also of horses. In these cases, direct sequencing of PCR products was performed to identify at least the most predominant nematode species.

Summary/Conclusions: The direct PCR method presented here allows the rapid and inexpensive identification of major gastrointestinal nematodes in ruminants for diagnostic and/or routine laboratory purposes such as confirmation of contamination-free passage of parasites. For questions needing quantitative data on species composition, PCR products obtained could also be used for further analyses such as RFLP, reverse-line-blots or pyrosequencing.

Recombinant protein production by the HITag system. Evaluation of the immunogenicity and diagnosis interest for toxoplasmosis

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Toxoplasma gondii is a worldwide protozoan parasite that causes toxoplasmosis, a disease of veterinary and human concern, causing neonatal malformation or abortion and severe pathology in immunocompromised individuals.

Toxoplasma gondii oocyst wall protein (TgOWP) has a very important role in the parasite structure and is responsible by the high viability of the cysts. This protein was initially described as similar to COWP proteins of *Cryptosporidium parvum*.

The aim of this work was the production of a recombinant protein homologous to TgOWP protein. The encoding gene has approximately 1846 pb and codes for 499 amino acids.

The new TAG system uses a proteic fragment, called H fragment (from helminth), made from small polypeptides, with the purpose of increasing the protein expression, or heterologous proteic fragments, in expression systems of fusion proteins. This system was applied to the production of the recombinant protein HTgOWP, as well as to the polyclonal sera anti-HTgOWP. Therefore, it was possible to express, isolate and evaluate the immunogenicity of the recombinant protein and demonstrate that the immunoserum produced against this recombinant protein allows the detection of the parasite cysts.

In this way, the present TAG was patented under the registration number N° PCT/PT2009/000075, with the advantages of increasing the recombinant antigens production and its immunogenicity, allowing its isolation and potentiating the use of any recombinant protein production process and immunosera. This invention can be potentially applied to all expression systems of described recombinant proteins.

Concerning HTgOWP, it can be used in human and animal toxoplasmosis diagnosis and for environmental detection of cysts, namely in water and wastewater.

Prevalence of antibodies to *Sarcocystis neurona*, *Toxoplasma gondii* and *Neospora caninum* in opossums (*Didelphis* sp.) from Bahia, Brazil

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Opossums (*Didelphis* sp.) are synantropic wild animals which are frequently found in rural and urban environments in Brazil. They are hosts of numerous parasites, including *Sarcocystis neurona*, *Neospora caninum* and *Toxoplasma gondii*, which are cyst-forming protozoa of veterinary or zoonotic importance. Little is known about the exposure of opossums to the mentioned pathogens in South America. The objective of this study was to determine the prevalence of antibodies to *S. neurona*, *N. caninum* and *T. gondii* in South American Opossums (*Didelphis albiventris* and *D. aurita*), using indirect fluorescent antibody tests (IFAT) with serum dilutions of 1:40, 1:30, and 1:30 as cutoffs, respectively. Blood samples of 120 animals, 102 *Didelphis albiventris* and 18 *D. aurita*, were collected in two cities in Bahia State (Salvador and Salinas da Margarida). Six out of 120 (5.0%) sera, and 11 out of 120 (9.2%), were positive for *T. gondii* and *S. neurona* antibodies, respectively. No sample was positive for *N. caninum*. We conclude that *D. aurita* and *D. albiventris* are exposed to *S. neurona* and *T. gondii* in the studied regions. This is the first study to demonstrate antibodies against *S. neurona* and *T. gondii* in *Didelphis* sp. in the State of Bahia. All animal procedures were approved by the Institutional Bioethics Committee (Escola de Medicina Veterinária da Universidade Federal da Bahia).

Molecular identification of *Cryptosporidium* from dogs in Argentina

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There are many reports on the survey and prevalence of *Cryptosporidium* infections in pet dogs worldwide; however, genetic identification of the isolates has been reported in few studies. In Argentina, no genetic epidemiological survey of companion dogs has been performed. The aim of the present study was to evaluate *Cryptosporidium* infection in dogs by PCR and to perform molecular characterization of the isolates. Fecal samples (n=128) admitted to the parasitology service of a private laboratory were randomly collected between February and November 2009. Samples were tested for the presence of *Cryptosporidium* spp oocysts using the sedimentation-concentration method of Ritchie and Ziehl-Neelsen staining technique. Genomic DNA of *Cryptosporidium* from positive samples was extracted using a QIAamp Stool Mini Kit (Qiagen) and used as a template for polymerase chain reaction (PCR). PCR for genotyping was performed with primer pairs targeting *Cryptosporidium* 18S ribosomal RNA (18S rRNA) and sequenced. Sequences obtained were compared with those registered in GenBank by BLAST analysis. *Cryptosporidium* DNA was detected in one of 128 dogs (0.8%). The isolate was identified as *Cryptosporidium canis* (*C. canis*). This is the first report of *C. canis* detection by PCR and sequencing, which may be useful tools for the diagnosis and epidemiological studies of *Cryptosporidium* infection in dogs in Argentina.

Detection of *Cryptosporidium parvum* in calves fecal samples using real time polimerase chain reaction

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Cryptosporidium parvum is the ethiological agent of diarrhea and high mortality in cattle and clinical disease in immunocompetent and immunosuppressed humans. The present research aimed the development of a real time polimerase chain reaction (qPCR) assay using a TaqMan probe specific for the actin gene of *C. parvum* and its comparison with the nested PCR assay for the 18S rRNA gene of *Cryptosporidium* spp., in fecal samples from calves. A total of 209 fecal samples from calves aged between 1 and 6 weeks old were collected and stored in 5% potassium dichromate until processing. Oocysts were purified using centrifugal sedimentation in water-ether, following genomic DNA extraction. A standard regression curve with an efficiency of 99.7% and slope of 3.328 was accomplished using serial dilutions (10^9 to 10^3 copies) of cloned fragments of actin gene of *C. parvum*. Molecular analyses were carried out by nested PCR for *Cryptosporidium* spp. and qPCR with a TaqMan probe specific for *C. parvum*, respectively for amplification of fragments of the 18S rRNA gene and of the actin gene. Positive amplification was observed in 83 (39.3%) and 152 (72.7%) samples, respectively for nested PCR and qPCR. Kappa coefficient (0.367) at 5% significance level showed a slight agreement between both assays. Analytical specificity of qPCR using genomic DNA from *C. andersoni*, *C. baileyi*, *C. bovis*, *C. canis*, *C. ryanae*, *C. serpentis* and avian genotype II showed that qPCR was specific for *C. parvum*.

***Sarcocystis aucheniae* in llamas of the Argentine Puna: Molecular identification and development of diagnostic methods**

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Background: Macrocysts are a frequent finding in the meat of llamas (*Llama llama*) of the province of Jujuy, Argentina, preventing its consumption and commercialization. The identification of the causative agent and the development of simple diagnostic methods can bring a significant contribution to the rural economy of the affected areas.

Methods: Macrocysts were isolated from pieces of llama meat, and aliquots of their content were (a) microscopically examined; (b) subjected to DNA extraction and (c) resuspended in cell lysis buffer. Samples were used for PCR amplification of the 18S hypervariable region. Amplicons were cloned and sequenced. Sequences were analyzed by BLAST and compared with those of different *Sarcocystis* species by Neighbor Joining. Samples were analyzed by SDS-PAGE and immunoblotting, using sera of llamas from the provinces of Jujuy and Buenos Aires. Two protein bands that immunoreacted with several Jujuy sera were selected, purified by SDS-PAGE and electroelution, quantified and used as antigens to develop ELISA tests. One positive and one negative llama sera, as determined by immunoblotting, were used to optimize test parameters. Selected conditions were those that gave maximal ratios between the absorbancies of the positive and the negative sera.

Results: Microscopic examination of llama meat macrocysts showed the presence of abundant protozoans with gliding motility. According to the bioinformatic analysis of the obtained 18S amplicons, parasites belong to the *Sarcocystis aucheniae* species. The selected immunoreactive bands were of 15 and 72 kDa (SA15 and SA72). The optimal ELISA conditions for both antigens were: 0.4 ug of protein per well; a serum dilution of 1/800; 10% skim milk/0.2% Tween-20/PBS as blocking agent; and TMB as substrate, allowing 15 min color development and stopping the reaction with orthophosphoric acid. Both proteins resulted adequate as ELISA antigens, although the positive/negative ratios were slightly higher with SA15.

Conclusions: This is the first molecular identification of *Sarcocystis aucheniae* infecting llamas of Argentina. In addition, the developed pilot ELISA tests based on immunogenic cyst proteins appear as a promising solution for the detection of infection in living llamas, which may result in better animal management and an aid in the monitoring of future eradication campaigns.

***Toxoplasma gondii* antibodies in bats from the metropolitan area of São Paulo City, SP, Brazil**

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Toxoplasmosis is a parasitic zoonosis caused by *Toxoplasma gondii* (*T. gondii*) protozoan that has a worldwide distribution. The destruction of natural ecosystems has caused several problems to humans and other animals; it is important to investigate the close relationship among bats, humans and domestic animals. The aim of this study was to determine the frequency of *T. gondii* IgG antibodies in bats using the indirect fluorescent antibody test (IFAT > or = 1:16) and the modified agglutination test (MAT > or = 1:25). To perform IFAT, a heterologous IgG anti-bat produced in sheep was used. During 2010, sera of 166 bats from the metropolitan area of São Paulo city were collected and analyzed. The results showed that 12.65% (21/166) were seropositive to *T. gondii* by IFAT (titers from 1:16 to 1:256), including *Tadarida*, *Molossus*, *Artibeus*, *Platyrrhinus*, *Glossophaga* and *Desmodus* genus, and 10.84% (18/166) by MAT (titers from 1:25 to 1:50), including *Tadarida*, *Molossus*, *Artibeus*, *Platyrrhinus*, *Glossophaga*, *Molossops*, *Myotis* and *Desmodus* genus. Four seropositive samples by MAT were confirmed by IFAT. The concordance between the techniques, by Kappa (95% CI) was 0.1, resulting in a low agreement between IFAT and MAT techniques. This difference is due to haemolysed sera, frequently obtained from small bats, leading to false positive by MAT but not interfering in the IFAT. Thus, it was verified that bats are in contact with *T. gondii* during their lifetime and play a role in toxoplasmosis epidemiology.

Isolation and molecular detection of *Toxoplasma gondii* in bats from the metropolitan area of São Paulo City, SP, Brazil

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Bats are widely distributed in Brazil. It is important to investigate the close relationship among bats, humans and domestic animals. Thirty years ago *Toxoplasma gondii* (*T. gondii*) was isolated from four bats (*Phyllostomus hastatus hastatus*) in Brasília, DF. Little is known of isolation and molecular detection of *T. gondii* infection in bats. The objective of this study was to isolate *T. gondii* in bats from the metropolitan area of São Paulo city, SP, Brazil, during 2010. It was used the technique of peptic digestion of heart and pectoral muscle of bats with bioassay in three-mouse groups. Each bioassay was conducted for only one bat or as pools of two or three bats. Two hundred and forty-two bioassays were performed, obtaining two isolates. Twelve to twenty-three days post-inoculation, mice from two groups had clinical signs and died. Tachyzoites were found in lungs and both groups had cysts in their brains. Polymerase chain reaction (PCR) analysis also confirmed the presence of *T. gondii* in brain, and heart and pectoral muscle pool in one bat from each of these two groups. *Molossus molossus* (insectivorous) and *Desmodus rotundus* (hematophagous) were *T. gondii* B1 gene-positive. These results show bats take part of the epidemiology of *T. gondii* in Brazil. As the next experimental step, genotypic characterization of *T. gondii* using the Restriction Fragment Length Polymorphism-Polymerase Chain Reaction (PCR-RFLP) will be performed.

Molecular analysis of *Anisakis* larvae from *Merluccius hubbsi* in Argentina

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Background: *Anisakis simplex* is a complex of nematodes parasites including three sibling species (i. e. *A. simplex sensu stricto*, *A. pegreffii* and *A. simplex C*), responsible for the human anisakiosis caused by ingestion of third stage larvae (L3) present in raw or undercooked fishes. The complex have a wide word distribution and infect a broad range of marine species. The goal of this work was to assess the prevalence of *Anisakis* L3 from commercial hakes (*Merluccius hubbsi*) of Argentina and the molecular identification at species level of these larvae by the analysis of ribosomal and mitochondrial markers.

Methods: In the period 2006-2008 fourteen hakes collected from fish markets of two cities of Argentina, Mar del Plata and Buenos Aires, were examined for the presence of anisakid larvae in visceral organs and whole body cavity. The prevalence and mean intensity of infection with this parasite were evaluated. Larvae of *Anisakis* coming from hakes were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay of the internal transcribed spacer (ITS) regions and 5.8 subunit rRNA gene. Additionally, a phylogenetic analysis of the mitochondrial cytochrome oxidase subunit 2 (cox2) DNA sequences from these larvae was performed, using Maximum parsimony (MP) and Maximum likelihood (ML) methods.

Results: All fourteen hakes examined were found infected with *Anisakis* larvae with a mean intensity of infection of 6.5. The totally of L3 larvae analyzed by RFLP-PCR of ribosomal regions generated a band pattern similar to *A. pegreffii*. Likewise, phylogenetic tree of cox2, recovered under both MP and ML criteria, grouped the nucleotide sequences from these larvae with *A. pegreffii* .

Summary/Conclusions: The results from the present work shown that *A. pegreffii* larvae have a high prevalence in hakes destined for consumption in Argentina. Even though human infection for this parasite was not officially reported, these data point out a potential public health risk of anisakiosis in this country.

Isolation and genotyping of *Toxoplasma gondii* from *Oryctolagus cuniculus* in Argentina

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Toxoplasma gondii (Apicomplexa) is a worldwide distributed protozoan that affects mammals and birds and causes an important zoonotic disease. Rabbits are highly susceptible to acute toxoplasmosis. The aim of this study was to isolate and genotype *T. gondii* from rabbits in Argentina. An abortion outbreak and death of adult rabbits were observed in a farm from La Plata, Argentina. The farm had 21 rabbits which were fed with grass and home made feed and were raised for human consumption. A kitten had been introduced into the farm and rabbits ate the grass from the ground where the cat walked. A female rabbit that had aborted was necropsied and gut, liver, spleen, lung and mesenteric lymph node samples were collected for histopathologic and immunohistochemical analysis, and a serum sample was taken to detect antibodies to *T. gondii* by indirect immunofluorescent test (IFAT). Central nervous system samples were inoculated intraperitoneally in four Swiss mice. Tachyzoites were obtained six days post-inoculation (pi) from peritoneal fluids of mice. The mice died 13 days pi with clinical signs of acute toxoplasmosis. Isolated tachyzoites were cultured in Vero cells. DNA was extracted from cell culture derived tachyzoites and was analysed by multi-locus polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) for genotyping. At histopathology necrosis foci were observed in all samples and cyst were detected in spleen. The rabbit antibody IFAT titer to *T. gondii* was 1:12800. *T. gondii* DNA was detected by PCR, and the isolate corresponded to clonal Type III genotype according with PCR-RFLP analysis of nine markers. This genotype was previously obtained from wild and zoo animals in Argentina, and it was pathogenic for inoculated mice. This is the first genotyping of *T. gondii* from *Oryctolagus cuniculus* in Argentina.

Use of indirect fluorescent antibody test with anti-swine conjugate for detection of *Toxoplasma gondii* antibodies in collared peccary (*Pecari tajacu*)

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The collared peccary (*Pecari tajacu*) is a widespread mammal that inhabits tropical and subtropical American continent. It is related to pigs, and is also called musk hog or the Mexican hog. We previously reported 89% seroprevalence of *Toxoplasma gondii* in feral peccary using the modified agglutination (MAT) test. Because of this high seroprevalence and because it is a common prey for wild felids, the peccary is considered epidemiologically important in the life cycle of *T. gondii*, especially in the Amazon region. The use of indirect fluorescent antibody test (IFAT) for wild species is limited because of the lack of specific commercial conjugates for most of species. In the present study we tested the use of anti-swine conjugate to detect *T. gondii* antibodies in collared peccary through IFAT. One hundred and eight serum samples from captive collared peccary of both sex and different ages were tested for *T. gondii* antibodies using the commercial anti-swine conjugate (Sigma-Aldrich®, USA). Tachyzoites of the RH strain of *T. gondii* and a cut-off of 1:32 were used in the IFAT. For comparison, MAT (cut-off 1: 25) was used as the gold standard. From the 108 animals, 54 were positive by MAT with titer range from 1:25 to 1:12,800. By the IFAT, 48 samples were positive with titer range from 1:32-1:8,192; all of these samples were also MAT positive. All negative samples obtained by MAT matched with the result of the IFAT. Comparing both test and considering MAT as gold-standard, the sensibility of IFAT was 89%, the specificity was 100%, accuracy 94%, positive predictive value 100% and negative predictive value 90%. The positive samples on MAT that present negative results on IFAT had titers of 1:25 to 1:100. Thus, anti-swine conjugate can be used effectively for IFAT for *T. gondii* antibodies detection in collared peccary.

***Toxoplasma gondii*: serology, isolation and genotypic characterization from pigeons (*Zenaida auriculata*) from Londrina, Brazil**

Genotypic characterization of *Toxoplasma gondii* from pigeons

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Toxoplasma gondii is an obligate intracellular protozoan that has a worldwide distribution and infects most warm-blooded animals. Pigeons, as well as other birds, were used as the indicator for environmental contamination. The aim of the present study was to evaluate the prevalence of anti-*T. gondii* antibodies and genetically characterize isolates from pigeons (*Z. auriculata*) from Londrina, Parana state, Brazil. Fifty pigeons were captured in traps in the city of Londrina. Blood and tissue samples (lung, heart, pectoral muscle and brain) were collected to perform serology test and mouse bioassay, respectively. The prevalence of anti-*T. gondii* antibodies was obtained by the modified agglutination test (MAT), and titers ≥ 16 were considered as positive. After pepsin digestion of the organs three mice were inoculated for the isolation of *T. gondii*. Isolates were genotyped by PCR-RFLP using SAG1, SAG-2, alt. SAG-2, SAG3, BTUB, L358, GRA6, C22-8, C29-2, PK1, Apico, CS3 markers. Thirty (60%) out of 50 animals were positive with titers of 16 (46%), 64 (8%) and 256 (6%). Six *T. gondii* strains (12%) were isolated, and one of them was a new genotype. Two isolates were characterized as genotype II, and the other three were not amplified in all markers. We used 18S rDNA marker PCR-RFLP to evaluate closely related apicomplexan parasites (*Hammondia*, *Neospora*, *Sarcocystis*), and all strains were considered *T. gondii*. The prevalence of anti-*T. gondii* antibodies observed here, probably indicated a high level of environmental contamination. The present study is the first to isolate *T. gondii* from *Z. auriculata* and showed this species as important intermediate host of *T. gondii*. A new genotype and type II strains, an uncommon strain in Brazil, were observed in the present study. In conclusion, this study confirmed the diversity of *T. gondii* strains in Brazil.

Parasite load and MCP-1/CCL-2 expression in dogs with Visceral Leishmaniasis

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Chemokines seem to be implicated in T-cell amplification of the inflammatory response, an important step for protective host defense in visceral leishmaniasis (VL). Monocyte Chemoattractant Protein 1 (MCP-1/CCL-2) is a CC chemokine that is produced in large amounts by different cell types including monocytes, natural killer cells, lymphocytes and dendritic cells. The purpose of this investigation was to evaluate the parasite load in spleen and liver of *Leishmania chagasi*-infected dogs and the CCL-2 expression in spleen samples. Nineteen dogs from Araçatuba / São Paulo, endemic area of VL, were included in the study. The parasite load determination was realized by quantitative PCR (qPCR) and the expression of CCL-2 chemokine was analyzed by Reverse Transcription – Polymerase Chain Reaction (RT-PCR), using specific canine *primers*. Five animals (26,31%) were ELISA assay positive and nineteen (100%) were positive for qPCR assay. The standard curve designed for parasite quantification showed linearity with a correlation coefficient of 0,98 and slope -3.101. The parasite load was higher in spleen than liver samples and it varied from $4,2 \times 10^3$ to $1,8 \times 10^8$ parasites/g of tissue in spleen and from $1,7 \times 10^4$ a $2,0 \times 10^8$ parasites/g of tissue in liver. The CCL-2 expression occurred in all the spleen samples (n=19). Our studies demonstrated that qPCR is an important assay to determine parasite load in spleen and liver samples. The CCL-2 chemokine has promising effect in immune response of dogs with VL.

Occurrence of anti-*Toxoplasma gondii* antibodies in wild felids and canids in Brazil

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Toxoplasmosis is one of the most widespread and prevalent disease in the world, affecting practically all endothermic animals, including human beings. Little is known of the role of wildlife, especially wild felids, in the natural epidemiology of *Toxoplasma gondii* infection in Brazil. The objective of the present study was to determine the occurrence of anti-*T. gondii* antibodies in free-living wild canids and felids in three biomes in Brazil: Cerrado (Grande Sertão Veredas National Park, Minas Gerais), Atlantic Rainforest (Poços de Caldas, Minas Gerais), and Pantanal (Corumbá, Mato Grosso). From July 2009 to September 2010, 14 felids (7 jaguars, *Panthera onca*, 3 ocelots, *Leopardus pardalis*, 4 cougars, *Puma concolor*), and 6 canids (4 maned wolf, *Chrysocyon brachyurus*, 2 crab-eating fox, *Cerdocyon thous*) were captured and their sera were tested for the presence of antibodies to *T. gondii* by the modified agglutination test (MAT, 1:25 or higher). Sera were initially screened at 1:25 dilution, and positives were tested further in 2-fold serial dilutions. All 20 animals tested had anti-*T. gondii* antibodies with titers ranging from 200 to 3200 for *P. onca* and *P. concolor*; 800 to 1600 for *L. pardalis*; 25 to 400 for *C. brachyurus*, and 200 to 800 for *C. thous*. This 100% seroprevalence of anti-*T. gondii* antibodies in felids and canids indicate endemic nature of this parasite in all 3 geographic areas of Brazil studied.

Development of a Nested PCR-RFLP of ITS-1 region to differentiate *Toxocara canis* and *T. cati* eggs

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Background: Toxocariasis is an endemic zoonosis produced by nematodes of the genus *Toxocara* with high impact in public health. Although there are many reported species, *T. canis* and *T. cati* whose final hosts are dogs and cats respectively, have most epidemiological importance. The aim of this study was to develop a molecular tool to identify these two *Toxocara* species from the environment and in contaminated soils.

Methods: A bioinformatic analysis was performed from the internal transcribed spacer regions 1 (ITS-1) of ribosomal DNA from *T. canis* and *T. cati* obtained from the GenBank sequence database to develop a specific nested polymerase chain reaction-restriction fragment length polymorphism (nested-PCR-RFLP). Primers were designed to amplify a band of 306 bp for *T. cati* and 309 bp for *T. canis* and two restriction enzymes, *Mse* I and *Taq* I, were selected to generate differentiated patterns so as to discriminate both species.

First, the nested PCR-RFLP technique has been tested using larvae of *Toxocara* obtained from adults culture and then using embryonated eggs from dogs and cats stools. Eggs were mechanically broken, larvae were picked up and DNA extraction was carried out from individual and pooled eggs. The study was performed in samples coming from different regions of Argentina.

Results: Consistently with *in silico* analysis, we obtained the amplification products and enzyme restriction patterns expected for both *Toxocara* species. Eggs from dogs generated bands of 30 bp, 108 bp and 171 bp for *Mse* I and bands of 45 bp, 120 bp and 144 bp for *Taq* I, whereas cat eggs analyzed with *Mse* I produced two bands of 27 bp and 279 bp and using *Taq* I only original band was observed due to the fact that this enzyme has no restriction site in *T. cati*.

Summary/ Conclusions: It can be concluded that the Nested PCR-RFLP is an accurate method to unambiguously discriminate eggs from *T. cati* and *T. canis*, and is proposed as a useful tool in the study of contaminated soil in public places frequented by dogs and/or cats.

Detection of *Bartonella* spp., *Ehrlichia* spp. and *Rickettsiales* of the spotted fever group in dogs and their vectors on Pirai, Rio de Janeiro

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Introduction: In the last two decades the number of publications about infections caused by *Rickettsia* and *Bartonella* has increased with the identification of new agents due, among other factors, increased human contact with animals and arthropods capable of transmitting disease, the increasing interest of the scientific community and the greater availability of new diagnostic techniques. Considering the importance of pets in the transmission of *Rickettsia* and *Bartonella* to humans, this study aimed to assess the movement of the spotted fever group rickettsia (SFGR), *Ehrlichia* spp. and *Bartonella* spp. in domestic dogs, clinically healthy on Ribeirão das Lages, Casa Amarela and Santanésia, neighborhoods from Pirai, Rio de Janeiro.

Methods: After signing an informed consent by the owner, blood and ectoparasites were collected from 189 dogs. The blood samples were used for serological analysis by commercial indirect immunofluorescence assays (PANBIO), with a titration cutoff point of 1:64, to detect anti-*Rickettsia rickettsii*, anti-*Ehrlichia canis* and anti-*Bartonella henselae*. Molecular analysis by polymerase chain reaction (PCR) were performed to detect the genome of SFGR, *E. canis* and *Bartonella* from the collected ectoparasites.

Results: From the 189 examined dogs sera, 45 (23.8%), 22 (5.82%) and 9 (4.76%), respectively, were reactive to *E. canis*, SFGR and *Bartonella* spp. From the ectoparasites collected from 186 dogs included in this study, 128 (68.8%), *Rhipicephalus sanguineus*, 19 (10.2%) *Amblyomma cajennense*, 1 (0.53%) *Anocentor nitens*, eight (4.3%) *Ctenocephalides felis* and 30 (16.1%), *Ctenocephalides canis* were identified. Through PCR amplification it was possible to detect the 386-bp fragment (genus *Rickettsia*) in 56.7% of the ectoparasites and the 414-bp fragment (genus *Bartonella*) in 42.2% of the ectoparasites. A preliminary phylogenetic analysis of the amplified product confirmed the movement of *Bartonella henselae* and *Rickettsia* spp. in ticks.

Conclusion: This study permits the understanding of the importance of dogs as sentinel animals and amplifiers of diseases transmitted by ticks and fleas as well as the movement of the spotted fever group rickettsia and *Bartonella* in the Médio Paraíba region.

Genetic Diversity in Field Isolates of *Trypanosoma theileri* Assessed by Analysis of Cathepsin L-like Sequences Disclosed Multiple Genotypes Infecting Cattle in Thailand

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Here we describe the first survey of *Trypanosoma theileri* in Thailand, a widespread and prevalent parasite of cattle transmitted by tabanid flies and considered potentially pathogenic. Investigation of 210 bovine blood samples by hematocrit centrifuge technique (HCT) revealed 14 samples with trypanosomes morphologically compatible to *T. theileri*. Additional animals were positive for *T. theileri* by PCR based on the Cathepsin L-like sequences despite negative by HCT. Results revealed a prevalence of $26 \pm 15\%$ (95% CI) of *T. theileri* infection in Thai cattle. Additionally, 12 blood samples positive for *T. theileri* were detected in cattle from other farms. From a total of 32 blood samples positive for *T. theileri* in cattle, seven were used to evaluate the genetic polymorphism through sequence analysis of PCR-amplified CATL DNA fragments. Eleven of the 19 CATL sequences analyzed showed be different. All CATL sequences from Thai samples clustered with sequences previously described for phylogenetic lineages TthI and TthII, supporting only two major lineages of *T. theileri* in domestic cattle around the world. The results disclosed an unexpectedly large and highly polymorphic genetic repertoire, revealing multiple genotypes of *T. theileri* circulating in Thai cattle, even within the same animals and farms.

Diagnostics of giant liver fluke (*Fascioloides magna*) and geographical distribution of fascioloidosis in the Czech Republic (Europe)

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Background: Giant liver fluke *Fascioloides magna* (close relative species to *Fasciola hepatica*) is attracting attention of wildlife managers, veterinarians and researchers because of its unusual body size (3-10 cm), high potential of pathogenic effect and continuous geographical spreading. Annually, the number of cases of animal infections (mainly hoofed game) caused by this fluke is monitored in Europe, including Czech Republic (with some foci of prevalence over 90%). The current diagnosis of fascioloidosis is primarily based on dissection of the host livers (to search for adult flukes) and on the unreliable coprological examination (to search for eggs), which is insufficient. The other diagnostic methods, especially the intravital-indirect ones, such as ELISA/Immunoblot or PCR-diagnostics could therefore represent an appropriate alternative. Together with the progressive development of reliable immuno- and molecular-diagnostic methods for fascioloidosis in our lab, we also distributed 1622 survey forms focused on monitoring of fascioloidosis to the community of "Czech Inspectors of Hunted Game" (CIHG)(years 2009-2010). We received back one fifth of forms (21.2%) containing positive or negative answers. The administrative units with particular CIHG who answered the form were geographically relatively equally distributed and therefore we believe that also the recorded distribution of *F. magna* within wildlife animals reflects the real situation in the Czech Republic. Although it was proved that the number of areas with fascioloidosis is still growing, the risk of pathogenic impact of *F. magna* on populations of free-living and farm-animals is generally underestimated.



SESSION S

Keynote Lecture

- **Applied ichthyoparasitology: what can parasites tell us about fish stocks?** - J. T. Timi

Oral Communications

- **Elucidating the lifecycle of endemic Leishmania in Australia** - A. M. Dougall
- **Isolation of Toxoplasma gondii from wild animals from Brazil** - S. N. Vitaliano
- **Gastrointestinal resistance in gastrointestinal parasites of exotic artiodactylids in zoological collections in the United States** - S. Howell
- **Onchocerca cervipedis (Nematoda: Onchocercidae) in moose and caribou at high latitudes of North America** - G. G. Verocai
- **Molecular and morphological identification of Sarcocystis rileyi from the mallard duck in the Czech Republic** - B. Koudela
- **Pathology of Profilicollis chasmagnathi in Olrog's Gull from Argentina** - L. La Sala

Posters

- **A newly discovered Vastrestrongylus species (Nematoda: Protostrongylidae) in ungulates at high latitudes of North America- Taxonomy and Phylogeny** - G. G. Verocai
- **Ectoparasites and Haemoparasites Prevalence in Wild Birds in Two Fishing Villages in Parana Coast - Brazil** - P. B. Mangini
- **Gastrointestinal parasitic fauna of the southern river otter (Lontra provocax), the american mink (Neovison vison) and dog in Chile** - T. Probst
- **Infection by Cryptosporidium spp. in Psittaciformes and Passeriformes from the state of São Paulo, Brazil** - A. A. Nakamura
- **Simultaneous infection by Cryptosporidium galli and Isospora sp. in Oryzoborus angolensis: pattern of oocyst shedding** - M. Vasconcelos Meireles
- **First record of Setaria tundra in Danish roe deer (Capreolus capreolus)** - H. L. Enemark
- **Parasitic survey in captive neotropical primates, Brazil** - M. Bohrer Mentz
- **Prevalence of parasites in Rattus norvegicus in animal house** - M. Bohrer Mentz
- **Ectoparasites of Tropidurus hispidus (Squamata: Tropiduridae) as bioindicators of human impact** - L. M. Costa Junior
- **Use of Polymerase Chain Reaction to detect piroplasms in wild felidae clinically healthy** - D. P. Rodrigues
- **Occurrence of ticks in wild mammals in the city of Santarém, Pará, Brazil** - H.S. Soares
- **Occurrence of ticks (Acari: Ixodidae) in indian hunting wild animals in the state of Mato Grosso, Brazil** - H. S. Soares
- **Light and Scanning Electron Microscopy of Sporocysts of Eurytrema coelomaticum (Giard et Billet, 1892) Looss, 1907** - J. Pinheiro
- **Ultrastructure of the Sporocysts of Eurytrema coelomaticum (Giard Et Billet, 1892) Looss, 1907** - J. Pinheiro
- **Infection status of freshwater fish, Carassius carassius with metacercariae of Clinostomum schizothoraxi in two basins of Wular Lake** - U. R. Zaragar
- **Molecular Detection of Borrelia burgdorferi s.l. in Tissues of Wild Carrier Hosts: Epidemiology, Genetic Diversity and Methodological Pitfalls** - A. D. Mihalca
- **Wildlife Hosts for Ticks of Genus Ixodes in Romania: New Data and Geospatial Approach** - C. M. Gherman
- **Giardia duodenalis in non-human primates: a Bayesian evaluation approach for the evaluation of four diagnostic assays in populations excreting different levels of cysts** - B. Levecke
- **Parasitosis in rabbits of Porto Alegre, Rio Grande do Sul, Brazil?** - M. J. Tweedie de Mattos

Applied ichthyoparasitology: what can parasites tell us about fish stocks?

J. T. Timi

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The aim of veterinary parasitology is to know different kinds of parasites for proper diagnosis, treatment and control of diseases, an elusive goal in the marine realm where, with the exception of some aquaculture practices, the control of parasitic infections is unfeasible. On the other hand, and from a biological perspective, an ecosystem is considered as healthy if it is one that is rich in parasite species, and all information about their biology, diversity, life cycles etc., can be applicable for conservation purposes or sustainable development of resources under exploitation. One of the applied aspects of ichthyoparasitology is the use of parasites as biological markers to provide information on the stock discreteness of their fish hosts. An accurate identification of any exploited fish population, and the delineation of its boundaries with adjacent populations, is a prerequisite for the implementation of policies that ensure an efficient and sustainable management of the resources. In this sense, the use of parasites as biological tags has gained recognition as being applicable to the problems of fishery management and is increasingly being used worldwide, constituting a promissory tool to predict harvest locations and even to help combating illegal fisheries.

Whereas several biological features of parasites (i.e. body size, lifespan and reproductive output) tend to produce similar population characteristics wherever the species become established, local environmental factors can regulate the survival and transmission success of infective stages, causing inter-population variations of parasite burdens. In other words, this methodology is based on the distribution patterns of parasites which, in the marine environment, are determined mainly by temperature–salinity profiles and by their association with specific masses of water. Analyses of distribution patterns of parasite species in relation to gradients in environmental (oceanographic) conditions in the South-west Atlantic showed that latitudinal gradients in parasite distribution are common in the region. These clines determine a differential composition of their communities at different latitudes, which makes possible the identification of different stocks of their fish hosts. Results of the successful use of biological tags for stock discrimination and fish migratory behaviour are shown, with emphasis on both geographic and across fish species distribution of anisakid nematodes; the most important group of parasites displaying zoonotic and economic implications in marine fisheries.

Elucidating the lifecycle of endemic *Leishmania* in Australia

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In 2003 an unknown *Leishmania* species was isolated as the cause of cutaneous lesions in red kangaroos (*Macropus rufus*) located in the Northern Territory. This was the first identification of natural *Leishmania* infection in Australia and the parasite was characterised as a unique species.

The lifecycle of *Leishmania* typically involves a mammalian host and transmitting phlebotomine sand fly vector. This study aimed to investigate the lifecycle of Australian *Leishmania* by incriminating both a vector and reservoir of the parasite.

Traditional methods for *Leishmania* diagnosis were utilized in the investigation including *in vitro* culture, cytology, histology, and serology, as well as the development of a new specific Australian *Leishmania* real-time PCR. Native marsupials commonly found in the Darwin rural area were opportunistically sampled and screened for *Leishmania*. Serology and real-time PCR assisted in determining the parasite's geographic range and its natural mammalian hosts. New clinical cases of cutaneous leishmaniasis were identified and characterised in northern wallaroos (*M. robustus woodwardii*), a black wallaroo (*M. bernardus*) and agile wallabies (*M. agilis*). Overall, antilopine wallaroos (*M. antilopinus*) and agile wallabies presented with high exposure and tolerance to the parasite, implicating them as reservoirs.

To incriminate a vector, phlebotomine sand fly trapping was undertaken in two hot spot areas of known transmission and were subsequently screened for *Leishmania*. No association between *Leishmania* and sand flies was found. Subsequently, alternative vectors present in the Darwin rural area were investigated for infection giving startling results. For the first time, evidence linking the day-feeding midge, genus *Forcipomyia*, subgenus *Lasiohelea* as the biological vector of *Leishmania* is presented. This finding will have massive implications in the understanding of the *Leishmania* lifecycle worldwide.

Elucidating the complete lifecycle of *Leishmania* in Australia is vital to predicting the effects of an exotic introduction or an endemic expansion of the parasite. This knowledge is fundamental for ongoing disease surveillance and Australian biosecurity.

Isolation of *Toxoplasma gondii* from wild animals from Brazil

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Toxoplasma gondii (*T. gondii*) is an intracellular protozoan parasite that infects almost all warm-blooded animals, including humans. Although research indicates that wild animals are frequently positives for *T. gondii*, the role of wild life in this parasite's epidemiology is not well understood. The present study aimed to isolate *T. gondii* from free-living and captive wild birds and mammals from different locations from Brazil, by bioassay in mice. Brain and heart homogenates were submitted to peptic digestion and were bioassayed in groups of five mice per sample. Tissues of mice that died were examined for *T. gondii* tachyzoites. Mice were bled six weeks post-inoculation, and their sera were tested for anti-*T. gondii* antibodies by the modified agglutination test (MAT). Surviving mice were euthanized two months after the inoculation and their brains were examined for the presence of *T. gondii* tissue cysts. We isolated *T. gondii* in 18 wild animals (16 mammals and 2 birds) from five different locations from Brazil. *T. gondii* was isolated from one lineated woodpecker (*Dryocopus lienatus*), one maned wolf (*Chrysocyon brachyurus*), one hoary fox (*Pseudalopex vetulus*), one oncilla (*Leopardus tigrinus*), one burrowing owl (*Athene cunicularia*), one paca (*Cuniculus paca*), one opossum (*Didelphis marsupialis*), two six-banded armadillo (*Euphractus sexcinctus*), three nine-banded armadillo (*Dasypus novemcinctus*), three collared anteater (*Tamandua tetradactyla*), and three white-lipped peccary (*Tayassu pecari*). Sixteen of the 18 isolates were lethal to nearly 100% of inoculated mice. The isolate from the burrowing owl caused 50% mortality in mice, and an isolate from one six-banded armadillo did not cause the death of the infected mice. This study represents the largest collection of *T. gondii* isolates from wild animals in Brazil. Results are important because some of the mouse virulent strains with atypical genotypes have caused fatal disease in humans in French Guiana.

Anthelmintic resistance in gastrointestinal parasites of exotic artiodactylids in zoological collections in the United States

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Background: Gastrointestinal nematode (GIN) parasites are a significant health concern for both domestic and non-domestic ruminants resulting in significant morbidity and mortality. In exotic artiodactylids, the primary parasite species of concern in most collections are *Haemonchus* spp., which thrive in warm, humid climates. Historically, parasite control programs in zoological institutions have relied heavily on frequent anthelmintic treatments, without the benefit of proper diagnostic surveillance. Similar strategies in domestic small ruminants have resulted in a high prevalence of multiple-anthelmintic resistance, but it is not known if similar levels of resistance exist in zoological collections. The objective of this study was to evaluate the status of anthelmintic resistance in GIN parasites of exotic artiodactylids in zoological collections of the United States.

Methods: Fecal samples (n=66) from 11 different host species were received as routine diagnostic submissions for evaluation of anthelmintic resistance status from 2004 to 2010. Nine different zoological collections in Florida, California, Georgia and Ohio were represented in the survey; however, 6 of the 9 collections and the majority of samples (86%) were from zoological collections in Florida. For each sample, nematode eggs were isolated from feces and used to perform the DrenchRite[®] Larval Development Assay, which evaluates the susceptibility of GIN to benzimidazole, levamisole, ivermectin and moxidectin. Based on the measured dose response, an inference is made of susceptible, suspected resistant or resistant.

Results: In the majority of samples, the preponderance of L₃ were *Haemonchus* spp., thus this was the only parasite for which determinations of resistance status were made. All 66 samples (100%) evaluated for *Haemonchus* were resistant to benzimidazole, 26 samples (39%) were resistant to levamisole, 49 samples (74%) were resistant to ivermectin and 3 (8%) were resistant to moxidectin. Twenty-four samples (36%) demonstrated multi-drug resistance to all 3 classes of anthelmintics. The 3 samples (8%) demonstrating resistance to moxidectin also showed resistance to benzimidazole and levamisole, thus these represent cases of total anthelmintic failure (resistance to all 3 classes plus moxidectin).

Conclusions: Anthelmintic resistance is a serious concern for zoological collections in the United States. Parasite control must become more evidence-based; with regular surveillance of fecal egg counts and anthelmintic resistance.

***Onchocerca cervipedis* (Nematoda: Onchocercidae) in moose and caribou at high latitudes of North America**

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Background: The legworm, *Onchocerca cervipedis* Wehr & Dikmans, 1935, is a widespread parasite of cervids, occurring from Costa Rica to boreal regions of North America. Blackflies (Diptera: Simuliidae) act as vectors, infecting ungulates when feeding on blood. To date, the northernmost *O. cervipedis* reports have been in moose and woodland caribou from British Columbia and moose from northern Alberta, Canada, and southeastern Alaska, USA. Although disease attributable to these parasites is known in *Odocoileus* spp., such has yet to be clearly demonstrated among other cervid hosts.

Objectives: Report the occurrence of *O. cervipedis* in moose and caribou at high latitudes of North America, and present associated molecular and pathological findings.

Methods: Three moose harvested in the Sahtu Region, Northwest Territories (NT) presented with firm subcutaneous nodules (approximately 2 cm) on proximal metacarpus. Cysts revealed filarioid-like nematodes. Similar lesions were also found in moose and caribou from localities in southwestern, southcentral and interior Alaska. Nematodes were removed from cysts and preserved in 70% ethanol. Specimens were cleared in lactophenol, examined microscopically, and identified according to Wehr and Dikmans (1935). Genomic DNA was extracted from specimens from NT moose. PCR was performed using nuclear and mitochondrial primers. Tissue sections were stained with hematoxylin-eosin.

Results: Specimens were identified as *O. cervipedis*, and deposited as vouchers in the United States National Parasite Collection. Sequences were the first generated for this species, and will be deposited in GenBank.

Histopathology revealed periosteitis and multifocal granulomatous cellulitis containing intra-lesional larvae.

Conclusions: Our findings extend the distribution of *O. cervipedis* to high latitudes of North America. Effects of this parasite in caribou and moose are not well understood. Suitable simuliid vectors are known to occur in Alaska and NT. Vector abundance and biting rates, determinants of transmission to ungulate hosts; might be influenced by climate change and lead to disease emergence, as observed for a related filarioid, *Setaria* spp. Studies on parasite and vector ecology are necessary to predict future range expansion and understand the impact on cervid hosts.

Molecular and morphological identification of *Sarcocystis rileyi* from the mallard duck in the Czech Republic

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Background: Protists of the genus *Sarcocystis* are parasites with a requisite two-host life cycle based on a prey-predator (intermediate-definitive) host relationship. Herbivorous and omnivorous animals (prey) usually serve as intermediate hosts in the muscles of which asexual stages (sarcocysts) form. The species with macroscopic cysts often observed in wild ducks is *Sarcocystis rileyi*, which causes the disease known as “rice breast disease” in waterfowl from North America. The striped skunk is the only known definitive host for *S. rileyi*. There are two recent reports (from Poland and Lithuania) only of *S. rileyi* infection in ducks from other continents except North America.

Methods: We employed light microscopy, electron microscopy, and DNA sequencing for studying samples of muscle containing macroscopic cysts in two naturally-infected mallard duck (*Anas platyrhynchos*) which had been shot by hunters in the Czech Republic.

Results: According to molecular and morphological results, macroscopic cysts examined corresponds to *Sarcocystis rileyi*.

Summary/Conclusions: This is the first documented case of *S. rileyi* in the Czech Republic. However, definitive host of this species has not been determined yet.

Pathology of *Profilicollis chasmagnathi* in Olrog's Gull from Argentina

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Introduction: Acanthocephalans could be pathogenic to marine birds. Infections by *Profilicollis chasmagnathi* have been associated with mortality in chicks of Olrog's Gull (*Larus atlanticus*), a vulnerable species from the Atlantic Coast of Argentina, Uruguay and Brazil. However, there is still an important gap on the knowledge regarding pathological aspects of these infections. Here, we present new pathological findings associated with infections by *P. chasmagnathi* in Olrog's Gull chicks.

Methods: Freshly dead chicks ($n = 110$) of Olrog's Gull were salvaged at a colony in the Bahía Blanca estuary, Argentina. Chicks were necropsied within 4 hr of collection, and their gastrointestinal tract was removed and fixed in 10% formalin. Infected regions were dehydrated in ethanol, embedded in paraffin, sectioned at 5 μm , stained with hematoxylin/eosin, and examined under light microscopy.

Results: Areas adjacent to the parasite attachment sites had atrophic villi, mucosal sloughing, and ulcerations. Acute changes consisted of eosinophilic infiltration of the lamina propria, lymphangiectasia, hyperemia, congestion, hemorrhage, and hyperplasia and necrosis of crypts. In superficial penetrations, there was intense fibroblast proliferation, and infiltration with macrophages, lymphocytes, and eosinophils. In mild penetrations, the parasite's proboscis was surrounded by chronic inflammatory infiltrate represented by lymphocytes, macrophages, giant cells, and eosinophils. In deep penetrations, the presoma of the worm was associated with granulomatous peritonitis, which was observable as fibrous nodules on the serosal side of the peritoneum. The space between the proboscis and the serosa was infiltrated by lymphocytes, plasma cells, macrophages, giant cells and eosinophils. Complete perforations (without granuloma formation) by immature worms were observed in chicks approximately 5 days of age.

Conclusions: Our results showed advanced pathology associated with *P. chasmagnathi* infections in Olrog's Gull chicks from very early in their post-hatching period. The presence of complete perforations by immature *P. chasmagnathi* in very small chicks underlines their high pathogenicity in the birds.

A newly discovered *Varestrongylus* species (Nematoda: Protostrongylidae) in ungulates at high latitudes of North America- Taxonomy and Phylogeny

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Background: An unknown species of Protostrongylidae Leiper, 1926 has been discovered in caribou, moose, and muskoxen from Alaska to Labrador. Nematodes were initially characterized relative to other protostrongylids through comparisons of DNA sequences from the second internal transcribed spacer (ITS-2) of first stage larvae (DSL) in feces (Kutz et al., 2007). No taxonomic description was possible because adult nematodes were not isolated.

Objectives: Develop taxonomic and phylogenetic data for a newly discovered lungworm species in ungulates at high latitudes of North America.

Methods

Collection: On April, 2010 adult nematodes were collected from the lungs of three muskoxen in Nunavik Region, northern Quebec. Intact nematodes and fragments were fixed in 70% ethanol.

Taxonomy: Specimens were cleared in phenol, and examined microscopically. Measurements and drawings were performed. Genomic DNA was extracted from worm fragments in three muskoxen. PCR was performed using the primers NC1 and NC2. Sequences at the ITS-2 locus were compared with those from DSL of the undescribed protostrongylid (Kutz et al. 2007). Molecular data from Kutz et al. (2007) and detailed morphological comparisons were taken in consideration to establish identity and putative taxonomic position. Phylogenetic analysis, based on morphological characters, was performed using the program Phylogenetic Analysis Using Parsimony (PAUP 4.0), having Elaphostrongylinae as outgroup.

Results: A new species is referred to Varestongylinae Boev, 1968 and *Varestrongylus* Bhalerao, 1932 based on integrated molecular and morphological data, and a phylogenetic context for the genus. Analyses indicate a sister-species relationship with *Varestrongylus alpenae* (Dikmans, 1935), a lungworm of white-tailed deer from Eastern North America.

Conclusions: A new species of *Varestrongylus* was confirmed in caribou, muskoxen and moose from North America. The Nearctic distribution of this *Varestrongylus* and *V. alpenae* suggests that speciation occurred within North America, following expansion from Eurasia.

Its population structure will be investigated using nuclear and mitochondrial markers to develop insights about life-history patterns, historical biogeography and coevolution with an assemblage of ungulate hosts. Studies of biology and ecology are underway to investigate potential impacts on host health and dynamics.

Ectoparasites and Haemoparasites Prevalence in Wild Birds in Two Fishing Villages in Parana Coast - Brazil

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Background: Biodiversity evaluation and its relationship with health are focused by conservation medicine; parasites are considered elements of biodiversity and play a role as sentinels of environmental health. This assay reports the interaction between assembly of birds, their parasites and landscape in fishing villages.

Methods: In order to detect this interaction, five landscape variables were evaluated: community size; houses density; forest loss; forest path and signs of wildlife hunt presence; and presence of domestic animals. Sixty eight birds (17 animals from eight guilds in Ararapira village and 51 animals in seven guilds in Superagui) were captured with mist nets to clinical evaluation, blood samples collection and ectoparasites collection. The blood smears were stained with Giemsa and the ectoparasites were fixed in alcohol 70% in adhesive tapes. Six morphotypes of ectoparasites and one of haemoparasite were found in Ararapira and sixteen morphotypes of ectoparasites and three hemoparasites in Superagui.

Results: The birds from Ararapira that showed alterations in clinical status had significantly higher ($p < 0,05$) ectoparasites infestation. In Superagui this relation had no significance, showing that the presence of a pathogen is not directly related to the disease development in this site. The absence of ectoparasites were seen in 17,7% of the birds in Ararapira and in only 11,8% in Superagui. The ectoparasites found were *Trombiculidae*, *Argasidae*, *Analgidae*, *Epidermoptes bilobatus*, *Macronyssidae*, *Menoponidae*, *Proctophyllodidae*, *Pteronyssus* sp., *Pterodectinae*, *Pterodectes* spp., *Trouessartia* spp., *Trouessartia mangaratibensis*, *Trouessartia rosterii*, Mallophaga and no identified mites. The haemoparasites were microfilariae, *Leucocitozoon* sp., *Haemoproteus* sp. and *Plasmodium* sp. The prevalence of ectoparasites were more significant in Superagui ($p = 0,01$). *Trombiculidae*; *Pterodectes* sp. and *Trouessartia* sp. represent 82,35% of ectoparasites in Ararapira, and 61,82% in Superagui.

Conclusions: Superagui showed less abundance and diversity of birds and higher parasites prevalence and diversity than Ararapira. It was seen that animals from Ararapira have better environmental condition, higher bird biodiversity and less anthropic influence and showed less parasite diversity and prevalence (seven types in 83,3% of animals) than Superagui (19 types in 88,2% of animals). These results express the importance in adding biodiversity and landscape data in wildlife health evaluation and also reveal significant correlation between assembly of birds structure, their parasites and anthropic landscape alterations in fishing villages in Parana coast.

Gastrointestinal parasitic fauna of the southern river otter (*Lontra provocax*), the american mink (*Neovison vison*) and dog in Chile

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The present study identified the gastrointestinal parasites using coprological analyses of three native carnivores in the southern Chile, the threatened southern river otter (*Lontra provocax*), the domestic dog (*Canis lupus* var. *familiaris*) and the introduced american mink (*Neovison vison*). Our objectives were to describe parasitic groups of these species, parasite overlap between species and effect of dogs presences in parasite presence in wild species. We collected fecal samples in a site where all species are sympatric and other site where only otter and minks are present. We collected a total of 84, 49, and 34 fresh fecal samples from otters, minks and dogs respectively. Samples were stored in 10% formalin and then analyzed using the sedimentation-flotation technique. In dogs 70.6% of the samples were positive to parasite. In otters 50% of the samples were parasite positive when dogs were present and 53% in dogs absences. In minks 42.1% of the samples were parasite positive in the dog-inhabited area and 27.3% in the non dog-inhabited area. But no significant differences in parasite prevalence were observed in both mustelids for both areas. The parasites were classified into four groups: Nematodes, Cestodes, Trematodes and Protozoa. The otter presented the highest parasite richness (19 species of parasites) followed by the mink (15 species of parasites) and finally the dog (8 species of parasites). To estimate the parasite group overlap between species, the Schoener's index was utilized. The higher overlap was detected in the case of carnivores that use similar habitats (mink/dog and mink/otter). In addition, dogs had a higher parasite overlap with minks from the area with dogs presence (0,54) than with the no presence area (0,33). Considering the limited sample size of this study, our results suggest a potential effect of dogs presence in parasite composition in native carnivores, future studies should determine the effect of invasive species in parasite dynamics in natural wild populations specially considering threatened species.

Infection by *Cryptosporidium* spp. in Psittaciformes and Passeriformes from the state of São Paulo, Brazil

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Cryptosporidiosis is considered a major protozoan infection in birds, with several reports of infection in the respiratory tract, proventriculus, intestine and bursa of Fabricius, resulting in economic losses and mortality. Three species of *Cryptosporidium* are considered valid in birds: *C. baileyi*, *C. galli* and *C. meleagridis*. Besides these species, there are several genotypes genetically distinct from the species of *Cryptosporidium* described in birds, as avian genotypes I, II, III, IV and V. The aim of this study was the detection of *Cryptosporidium* spp. in Psittaciformes and Passeriformes from wild animal rehabilitation centers in the state of São Paulo, Brazil. A total of 757 fecal samples was collected and stored in 5% potassium dichromate solution at 4°C until processing. Oocysts were purified in Sheather's sugar solution following genomic DNA extraction. Molecular analysis was performed using the polymerase chain reaction (nested-PCR) to amplify a fragment of the 18S subunit of the ribosomal RNA gene. The amplification of *Cryptosporidium* DNA fragments was observed in 47 (6.21%) samples from rufous-bellied thrush (*Turdus rufiventris*), saffron finch (*Sicalis flaveola*), green-winged Saltator (*Saltator similis*), rufous-collared sparrow (*Zonotrichia capensis*), blue-fronted parrot (*Amazona aestiva*), *Sporophila* sp., chopi blackbird (*Gnorimopsar chopi*), white-necked thrush (*Turdus albicollis*), double-collared seedeater (*Sporophila caerulescens*), buffy-fronted seedeater (*Sporophila frontalis*), pale-breasted thrush (*Turdus leucomelas*), common waxbill (*Estrilda astrild*), shiny cowbird (*Molothrus bonariensis*), creamy-bellied thrush (*Turdus amaurochalinus*), plain parakeet (*Brotogeris tirica*), red-cowled cardinal (*Paroaria dominicana*), white-bellied seedeater (*Sporophila leucoptera*), vinaceous parrot (*Amazona vinacea*), orange-winged parrot (*Amazona amazonica*), great kiskadee (*Pitangus sulphuratus*), chalk-browed mockingbird (*Mimus saturninus*), *Icterus* sp. and ultramarine grosbeak (*Cyanoloxia brissonii*). Studies regarding the occurrence and the molecular characterization of *Cryptosporidium* in birds are important, since the zoonotic potential and the clinical relevance of the avian *Cryptosporidium* species and genotypes are not completely understood.

Simultaneous infection by *Cryptosporidium galli* and *Isospora* sp. in *Oryzoborus angolensis*: pattern of oocyst shedding

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Several species of birds are susceptible to infections by *Isospora* spp. or *Cryptosporidium* spp. in the epithelium of the gastrointestinal mucosa, with the presence of subclinical, acute or chronic infection. There are no published data on the pattern of shedding of *Cryptosporidium galli* oocysts in single infections or in simultaneous infection with *Isospora* sp. in birds. The purpose of this study was to characterize the pattern of oocyst shedding in two lesser seed-finches (*Oryzoborus angolensis*) with simultaneous natural infection by *C. galli* and *Isospora* sp.. The samples were collected each day, in two periods: from 8:00 a.m. to 2:00 p.m. and from 2:00 p.m. to 8:00 p.m., until the death of the birds, during 34 days (bird 1) and 23 days (bird 2), and stored in potassium dichromate 5%. The oocysts were purified by centrifugal flotation in Sheather's sugar solution and screened by the negative malachite green staining. The data analyzed were the number of oocysts of *C. galli* observed in the two periods of fecal samples collection, and the daily peak of shedding of oocysts of *C. galli* and *Isospora* sp. ranked as scores from 1 to 4. The two birds had higher shedding *C. galli* oocysts in the period from 8:00 a.m. to 2:00 p.m.: bird 1 (average of 40.6 oocysts per slide) and bird 2 (average of 16.2 oocysts per slide). In the period from 2:00 p.m. to 8:00 p.m. there was an average of 14.4 and 10.2 oocysts per slide, respectively in birds 1 and 2. The results of the scores of oocyst shedding revealed a greater amount of oocysts of *Isospora* sp. in the period from 2:00 p.m. to 8:00 p.m.. For both birds, the shedding of *C. galli* oocysts was constant, ranging from score 1 to 4, during all the period of sample collection. There was an increase in the shedding of oocysts of *Isospora* sp., with a predominance of score 4, in the days preceding the death of the birds. This is the first study related to the pattern of shedding of oocysts of *C. galli* in birds and its relationship with the shedding of *Isospora* sp. oocysts.

First record of *Setaria tundra* in Danish roe deer (*Capreolus capreolus*)

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No previous finds of the mosquito-borne filarioid nematode *Setaria tundra* have been reported from Denmark, although it was described decades ago in Swedish and Norwegian reindeer as well as in roe deer from Germany, Bulgaria and more recently also from Italy and Finland. *Setaria* spp. are usually considered harmless inhabitants of the abdominal cavity of ungulates causing only focal areas of mild chronic peritonitis. However, in recent years *S. tundra* has been associated with an emerging epidemic disease resulting in severe morbidity and mortality for both reindeer and moose in Finland.

The Danish find of *S. tundra* was from a fawn shot in October 2010 near Randers, in the eastern part of Jutland. At slaughter several (>20) approximately 5 cm long, slender, white worms were observed in the peritoneal cavity. Morphology of the worms, revealed by light microscopy, correlated to that of *S. tundra* described by Rejewsky (1929) and Nikander et al. (2007). Sequences of the mitochondrial 12S rRNA and *cox1* genes, 454 and 595 base pairs respectively, were 99.5-99.7% identical to previously published *S. tundra* isolates from France and Italy.

Roe deer are thought to be asymptomatic carriers of *S. tundra*, and may be connected to the spreading of this parasite. In reindeer heavy worm burdens of *S. tundra* have been found to cause severe peritonitis and negatively affect body condition score. Thus in the light of the possible climatic changes which could result in warmer, more humid weather in Scandinavia and thereby larger numbers of mosquitoes, it is important to monitor this vector-borne parasite. This will not only increase the understanding of factors promoting its expansion but also help to predict disease outbreaks.

Parasitic survey in captive neotropical primates, Brazil

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Neotropical primates (Primates: Platyrrhini) require special attention in their captive husbandry and management. Besides viral and microbiological infections, parasites have been identified as critical components to consider in conservation biology, but little information is available concerning intestinal parasites in these animals.

Objective: the aim of this study was to provide baseline data on faecal parasites in captive neotropical primates.

Material and Method: a parasitological survey was conducted in the Brazilian primates collection at Mini-Zoológico Municipal Palmira Gobbi Dias (Porto Alegre city) and Mantenedor de Fauna Arca de Noé (Morro Reiter city) in the state of Rio Grande do Sul, Brazil. The population and genera of the primates were: 45 marmosets (*Callithrix*); 44 capuchins (*Cebus*); 20 howler monkeys (*Alouatta*); 6 squirrel monkeys (*Saimiri*), 3 woolly monkey (*Lagothrix*) and 11 night monkeys (*Aotus*). A total of 55 individual fecal samples, representing all groups, were collected at the moment of their arrival, during the clinical examination. The material was identified and preserved in formol 10%. Flotation and sedimentation methods were used to analyse faecal samples in the Parasitology laboratory of Department of Microbiology, Immunology and Parasitology of Health Basic Science Institute / Universidade Federal do Rio Grande do Sul (ICBS/UFRGS), Porto Alegre city, Brazil.

Results: 19/55 samples, belonging to *Cebus* genre, were positive to helminth eggs: Superfamily Strongyloidea 12/19 (63%), *Hymenolepis* 4/19 (21%), *Strongyloides stercoralis* 2/19 (10%); *Ascaris* sp. 1/19 (6%); *Ancylostoma* sp.; 1/19 (6%). No protozoans were found in any genera studied.

Conclusion: the exchange of disease is a concern for wildlife conservation. The results of this study provide an important baseline for future investigations of gastrointestinal parasites in this primate community.

Prevalence of parasites in *Rattus norvegicus* in animal house

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Although the improvement of sanitation in housing systems resulted in a marked reduction in pathogenic agents in laboratory experimental animals, in a colony with no effective sanitary barriers the incidence of parasites is constant, causing several nutritional changes. This also interferes with the development and physiology of the animals. Currently, conventional colonies of rats use drugs for the control and eradication of parasites.

Objective: The aim of this study was to verify the prevalence of parasites in a conventional animal house of *Rattus norvegicus*, Wistar lineage.

Material and method: 404 fecal samples were collected from adult rats, distributed in 4 rooms of a conventional colony of *Rattus norvegicus*, Wistar lineage: at the first and second rooms there are sexually mature rats properly paired to breed, at the rooms three and four there are males and females respectively. The fecal samples were collected in pool from the cages in 10% of the colony, once a week, during 9 months. The samples were identified and kept under refrigeration until processed at the laboratory of Parasitology of Microbiology, Immunology and Parasitology Department, Health Basic Science Institute of Universidade Federal do Rio Grande do Sul (ICBS/UFRGS), Porto Alegre city, Brazil. For the parasitic diagnostic it was used two methods: flotation (Willis, 1938) and sedimentation (Hoffmann, Pons and Janer, 1934). **Results:** from the total number of samples collected, 137/404 (34%) were positive for the genus *Syphacia* 109/137 (70%) and *Eimeria* 41/137 (30%).

Discussion: *Syphacia* sp. (Nematoda:Oxyuridae) is a common helminth parasite in animal house. Otherwise, very little is known concerning the prevalence, pathogenicity, clinical signs and control of the protozoan *Eimeria* spp. (Coccidia: Eimeriidae): most are rare or not reported in laboratory rats.

Conclusions: this study registered *Rattus norvegicus* infected with *Eimeria* spp. and *Syphacia* sp. The parasitological monitoring in animal house is very important to improve the sanitation status and minimize the effects of natural parasitic diseases on research results.

Ectoparasites of *Tropidurus hispidus* (Squamata: Tropiduridae) as bioindicators of human impact

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The destruction of habitat could negatively affect biodiversity and the study of environmental impact caused by man can be assessed using bioindicators. In recent years, there has been a growing interest in parasites as indicators of human impacts on the environment. Nevertheless, studies on parasites as bioindicators in terrestrial systems are scarce and mostly focus on helminth of amphibians and/or mammals. *Tropidurus hispidus* occurs in many environments, including disturbed areas, enabling a study of parasites in both environments preserved or disturbed areas. This study aim evaluate the use of mites infesting *T. hispidus* as bioindicators of human disturbance. Samples were collected at 20 sampling points (10 in conservation areas and 10 in peridomestic areas) located in Maranhão state, northeastern Brazil. At least 20 lizards were collected at each sampling point were sacrificed with anesthetic, preserved in ethanol 70% and fell in the Herpetological Collection of the Federal University of Maranhão. To examine whether the intensity of infestation varied between disturbed and conserved areas was used Analysis of Covariance (ANCOVA), using the type of area (if disturbed or not) as dependent categorical variable. Of the 194 lizards collected in non-impacted, 135 (69.6%) were infested by *Eutrombicula* sp. and 120 (61.8%) by *Hirstiella* sp. In disturbed areas, the 207 lizards examined, 55 specimens were infested by *Eutrombicula* sp. (26.6%) and 187 (90,0%) by *Hirstiella* sp. The intensity of the two groups of mites varied with the anthropogenic (*Eutrombicula* sp.: $F = 18.75$, $p < 0.0001$; *Hirstiella* sp.: $F = 32.43$, $p < 0.0001$). The intensity of mites *Eutrombicula* sp. was greater in non-disturbed ($22:57 \pm 31.52$) than in disturbed areas ($10:18 \pm 20:13$). However, mites *Hirstiella* sp. occurred in a greater extent in disturbed areas (67.4 ± 104.5) than in preserved areas (28.8 ± 50.39). Thus, mites may be used as bioindicators of environmental impacts caused by humans.

Use of Polymerase Chain Reaction to detect piroplasms in wild felidae clinically healthy

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Introduction: The study of the diseases has an important place in strategies for conservation of wildlife. Studies show that wild cats can be affected by hemoparasites, and often turn into their natural reservoirs. Many zoos have errant pets, which may be a new source of contamination for captive wildlife. The aim of this study was to report the occurrence of piroplasms in wild felines at the Rio de Janeiro's zoo (RIOZOO), comparing the microscopic diagnosis with Polymerase Chain Reaction (PCR).

Methods: On this study 32 wild felines were used, regardless of sex or age, from the following species: *Leopardus geoffroyi*, *Leopardus pardalis*, *Leopardus tigrinus*, *Leopardus wiedii*, *Lynx lynx*, *Felis chaus*, *Felis serval*, *Panthera leo*, *Panthera pardus*, *Panthera onca*, *Panthera tigris aetaica*, *Puma concolor*, *Puma yagouaroundi*. The blood samples were obtained during routine management, consisting of peripheral blood (ear tip smear) and whole blood, which were stored with EDTA for the search of piroplasms by PCR and complete hematological analysis. The PCR assays were done on the Instituto Biomédico of Universidade Federal Fluminense (UFF). The ear tip smears were stained with Wright and analyzed at the Veterinary Laboratory of UFF for the presence of hemoparasites.

Results: Three slides of ear tip (9.37%) showed intraerythrocytic forms of piroplasms indistinguishable from *Cytauxzoon* spp. and *Babesia* spp. The molecular evaluation by PCR revealed that 15.62% were positive using the primers PIRO A and PIRO B for *Babesia canis*, being three samples from *Panthera onca* and two from *Leopardus pardalis*. These samples were also positive for *Cytauxzoon felis* using primers *C. felis*-F and *C. felis*-R. No positive results were found for *Theileria*. In order to testify the PCR results, the five samples were sent to sequencing, revealing *C. felis* on all samples. No hematological changes were observed in all 32 samples evaluated.

Conclusion: It was concluded that *Cytauxzoon felis* is present in healthy felines from RIOZOO, and that the PCR assays were more sensitive on detection of that parasite when compared to microscopy of peripheral blood smears. Yet, PCR results should be confirmed by sequencing its products.

Occurrence of ticks in wild mammals in the city of Santarém, Pará, Brazil

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The species of ticks that parasitize domestic animals are well studied, however little is known about the species that infested wild animals, mainly in relation to its taxonomy, biology, ecology, geographical distribution, common hosts and vectorial pathogens capacity. During October 2010, ticks were collected in free-living wild mammals in the city of Santarém, Pará state, Brazil. In total 82 adults and 32 nymphs were collected from 2 spotted paca (*Cuniculus paca*), 2 nine-banded armadillos (*Dasypus novemcinctus*) and 9 white-lipped peccary (*Tayassu pecari*). Ticks were identified using *Amblyomma* taxonomic keys for adults and nymphs. From the 8 collected nymphs from *C. paca*, 2 were identified as *Amblyomma coelebs* and 6 as *Amblyomma naponense*; the 19 collected nymphs from *D. novemcinctus* were identified as *Amblyomma humerale*; from the 82 collected adult ticks from *T. pecari*, 2 males and 4 females were identified as *Amblyomma oblongoguttatum*; the remaining 44 males and 32 females were identified as *A. naponense*; from the 5 collected nymphs from white-lipped peccary, 2 were identified as *A. coelebs* and 3 as *A. naponense*. According to the literature, there is a report of a nymph of *A. coelebs* parasitizing *T. pecari* in Madre de Dios, Peru. The find of 2 nymphs of *A. coelebs* in *T. pecari* is in agreement with previous report of this species of tick using this animal species as host for nymphal stage. This study constitutes the second report of nymphs of *A. naponense* in *C. paca* and the second record of nymphs of *A. humerale* in *D. novemcinctus* in Brazil, however this is the first report of nymphs of *A. coelebs* in *C. paca* and nymphs of *A. naponense* in *T. pecari* in Brazil.

Occurrence of ticks (Acari: Ixodidae) in indian hunting wild animals in the state of Mato Grosso, Brazil

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Ticks are arthropod ectoparasites, of the class Arachnida, of worldwide distribution, parasitizing terrestrial vertebrates, amphibians, reptiles, birds and mammals. They are important for public and animal health for transmitting infectious agents and causing injuries to their hosts during blood feeding. During the month of September 2010, ticks from Indian hunting wild animals in the state of Mato Grosso, Brazil, were collected. Ticks were collected from a snake (*Bothrops moojeni*), a nine-banded armadillo (*Dasypus novemcinctus*), a white-lipped peccary (*Tayassu pecari*), a Brazilian giant tortoise (*Chelonoidis denticulata*), a rusty-margined guan (*Penelope superciliaris*), a bare-faced curassow (*Crax fasciolata*), a razor-billed curassow (*Pauxi tuberosa*), a wild-mouse (*Cerradomys scotti*), a Brazilian agouti (*Dasyprocta azarae*) and a collared peccary (*Pecari tajacu*). Ticks were identified using a dissecting microscope and taxonomic keys. In total, 30 adult ticks, 178 nymphs and 246 larvae from wild animals were collected, as follows: one female of *Amblyomma rotundatum* and three nymphs of *Amblyomma humerale* from *B. moojeni*; one *Amblyomma pseudoconcolor* male from *D. novemcinctus*; six males, three females, and eight nymphs of *Amblyomma cajennense*, and a female of *Amblyomma oblongoguttatum* from *T. pecari*; 12 males of *A. humerale* from *C. denticulata*; one nymph of *A. cajennense* and a larva of *Amblyomma* sp from *P. superciliaris*; 32 nymphs of *A. cajennense* from *C. fasciolata*; one nymph of *Amblyomma naponense*, 46 nymphs of *A. cajennense* and 243 larvae of *Amblyomma* sp from *P. tuberosa*; two nymphs of *Amblyomma ovale* from *C. scotti*; one nymph of *Haemaphysalis juxtakochi*, five nymphs of *A. cajennense* and two larvae of *Amblyomma* sp from *D. azarae*; two males and two females of *A. naponense*, one male, one female and 79 nymphs of *A. cajennense* from *P. tajacu*. This present study constitutes the first report of nymphs of *A. humerale* parasitizing snake *B. moojeni* in Brazil. The tick *A. humerale* has already been reported in Brazil in lizard *Plica plica*, *Plica umbra* and *Kentropyx calcarata*. The present record of *A. humerale* in *B. moojeni*, reinforces previous findings of this species of tick using reptiles as hosts for nymphal stage. The present study reports, for the first time in Brazilian territory, nymphs of *A. cajennense* in *P. superciliaris*, *C. fasciolata* and *P. tuberosa*, nymph of *A. naponense* in *P. tuberosa* and nymph of *H. juxtakochi* in *D. azarae*.

Light and Scanning Electron Microscopy of Sporocysts of *Eurytrema coelomaticum* (Giard et Billet, 1892) Looss, 1907

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Background: *Eurytrema coelomaticum* is a fluke that infects ruminants in South America, Europe and Asia. The morphology of the mother and daughter sporocysts of *E. coelomaticum* obtained from *Bradybaena similaris*, the first intermediate host, is described for the first time by light and scanning electron microscopy.

Methods: The larvae were obtained from snails *Bradybaena similaris* experimentally infected, fixed 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4. For light microscopy (LM) observations the fixed larva were mounted in glass slides, observed and measured using an Olympus BX51 and the images were captured with an Olympus DP12 camera or a Zeiss Axiovision system. For SEM the fixed larva were washed three times in 0.1M cacodylate buffer, pH 7.4, post fixed in 1% osmium tetroxide and 0.8% potassium ferricyanide, and washed again in the same buffer. The larva were dehydrated in a crescent ethanolic series and critical point dried using CO₂ (Baltec CPD). In some experiments, the dried specimens were mechanically fractured. The sporocysts were mounted on metallic stubs and golden coated (Pinheiro et al., 2004). The observations were made using a scanning electron microscope Zeiss DSM962 or a Jeol SEM5310, operating at 20kV. The images were obtained using the SemAfore software.

Results: The intermediate host was exposed to *E. coelomaticum* eggs and after 30 days the mother sporocyst was found in the coelom adhered to the intestine wall. This sporocyst was a rounded or elongated mass (0.1078 mm), with numerous germinal balls in it, which cells have nucleus with prominent and dense nucleolus centrally located and a folded tegument with no specializations. The daughter sporocysts obtained from dissection of infected snails have varied shape, one hollow tapered region with many transversal and longitudinal striations, named anterior end. The expelled daughter sporocyst presented an oval sac-like central region with a small anterior and a posterior longer filament-like prolongation. The measures of the expelled sporocysts are presented and compared to previous descriptions.

Conclusions: By the first time the morphology of the mother and daughter sporocysts of *E. coelomaticum* was analyzed under LM and SEM and details of this topography were showed, contributing to enrich the morphological characterization of these larval stages, as an important taxonomic tool. These results were discussed.

Ultrastructure of the Sporocysts of *Eurytrema coelomaticum* (Giard Et Billet, 1892) Looss, 1907

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Background: The digenetic trematode *Eurytrema coelomaticum* is a parasite of pancreatic ducts of ruminants. The ultrastructure of the mother and daughter sporocysts of *E. coelomaticum* was analyzed.

Methods: The larvae were obtained from snails *Bradybaena similaris* experimentally infected. The collected larvae were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4 for 24 h. For TEM the fixed larvae were washed in 0.1M cacodylate buffer, post fixed in 1% osmium tetroxide and 0.8% ferrocyanide, washed again in the same buffer, dehydrated in a crescent series of acetone, infiltrated and embeded in epoxy resin (Polybed). Semi-thin sections were stained with toluidine blue and observed under a Zeiss Axioplan light microscope; images were captured with an MRc5 AxioCam digital camera and processed with the Axiovision program. Ultrathin sections were stained with uranyl acetate and lead citrate (De Souza, 2007) and observed in a Zeiss 900 Transmission Electron Microscope at 80kV. The images were obtained using ITEM Software.

Results: The mother sporocyst was attached to the coelome of the intestine wall intimately adhered in some regions. It presents a highly folded tegument with granules and the body wall was composed by an outer syncitial layer, basal lamina, and circular and longitudinal muscle layer. Below was the cell body (cyton) with the nucleus. The daughter sporocysts obtained by dissection exhibited many granules and secretory vesicle in the outer layer indicating an intense secretory activity. The body wall presented the same layers of the mother sporocysts, but the outer syncitial layer invaginated and an amorphous layer was present between the syncitial and circular muscle layers. The protonephridial excretory system was viewed. The anterior and posterior end of the expelled sporocyst exhibit a degenerated structure, but biological activity still occurred in these regions. The swollen middle of the body was filled by a lamellar structure formed by degenerating membranes, but the excretory system was preserved. The endocyst wall was fibrillar and filled by cercariae and amorphous, membranous and secretory material inside it.

Conclusions: By the first time the internal organization of the mother and daughter sporocysts of *E. coelomaticum* was described and the changes occurred through the larval development from mother to daughter sporocyst were described. These results were discussed.

Infection status of freshwater fish, *Carassius carassius* with metacercariae of *Clinostomum schizothoraxi* in two basins of Wular Lake

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Background: The water quality has got impact on the density of aquatic organisms, including parasites. At present water bodies of Kashmir valley are under lot of stress from man induced pollution. The deteriorating water quality may increase the infection rate in fish because the immune system of host is at stake. The aim of this study was to assess the present infection rate of metacercariae of *Clinostomum schizothoraxi* in two basins differing in water quality and to assess the impact of sex and condition factor on the parasitic infection.

Methods: The Wular Lake is the largest fresh water lake of the Kashmir Valley and has been designated as a Ramsar site in 1992. Geographically the lake is located at an elevation of 1580m between the coordinates of 34°15'N to 34°25'N and 74°03' E to 74°04'E. In the present study two basins were selected in the lake, Watlab and Sopur (Ningal) and two sites were selected in each basin. The two basins show differences in water quality as per previous reports. Physico-chemical characteristics of water were investigated on monthly basis as per APHA (1998) and CSIR. The present study was conducted to determine the prevalence of metacercariae of *Clinostomum schizothoraxi* in Wular Lake, Kashmir Himalaya. A total of 76 fish samples were collected from the survey between September 2008 and July 2009.

Results: The physico-chemical features of lake showed seasonal change in the water quality with better quality in winter and more degradation in summer. The water temperature ranged between 5°C – 28.8°C. The lake showed changes in physico-chemical features in two basins with high level of eutrophication/contamination in Sopur (Ningal) basin in comparison to Watlab basin. The results showed significantly high prevalence (%=18.8; $p<0.05$) in the basin with high eutrophication/contamination level in comparison to less eutrophic basin (%=12.8). The overall mean prevalence of *Clinostomum schizothoraxi* in two basins was 31.3% in combined sex. No statistically significant differences were observed in relation to sex (females, 28.6%; males, 34.1%, $P>0.05$). Condition factor (k) of infected fish (1.74 ± 0.05) was slightly higher than in infected fish (1.73 ± 0.05).

Summary/Conclusions: In conclusion, according to the results of this study, metacercarial infection increased with the decrease in water quality. Therefore metacercarial infection in *Carassius carassius* can provide supplementary information on the pollution status of water body. This information can be also useful for fishery resource managers for devising proper way out for controlling this infection.

Molecular Detection of *Borrelia burgdorferi* s.l. in Tissues of Wild Carrier Hosts: Epidemiology, Genetic Diversity and Methodological Pitfalls

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Background: *Borrelia burgdorferi* sensu lato is the causative agent of Lyme borreliosis, the most widespread vector-borne disease in the cool-temperate regions of the Northern hemisphere. The medical importance of this pathogen is generally restricted to humans and few domestic species. But as for many vector-borne diseases, the key to the understanding of the epidemiology of Lyme borreliosis consists in revealing the ecological relationships that exist between pathogens, vectors and wildlife hosts. Thus, our aim was to evaluate the prevalence, geographical distribution and genetic diversity of *B. burgdorferi* s.l. in various wild vertebrates from Romania. We also discuss methodological pitfalls regarding the best tissues to be collected from reservoir hosts for molecular detection of the Lyme diseases agent.

Methods: Between June 2010 and April 2011, tissue samples (skin, heart, liver, spleen, kidney, urinary bladder) have been collected from 99 dead animals belonging to 26 species from various localities in Romania. From each tissue sample, the DNA was extracted individually using Qiagen DNeasy Blood & Tissue kits. Molecular detection of *B. burgdorferi* s.l. was achieved by nested PCR using OspA primers. Genospecies identification was accomplished by RFLP.

Results: *Borrelia burgdorferi* s.l. DNA was detected in 23 animals belonging to 8 species (Mammalia: *Apodemus agrarius*, *Crocidura suaveolens*, *Capreolus capreolus*, *Mustela lutreola*, *Vormela peregusna*, *Vulpes vulpes*; Aves: *Parus major*; Reptilia: *Lacerta agilis*). The most prevalent genospecies was *Borrelia afzelii*, followed by *B. garinii* and *B. burgdorferi* s.s. Out of 10 positive mammals, the cardiac tissue was positive in 90% of the cases, the liver in 40% and the spleen in 20%.

Conclusions: Our report is the first world record of *M. lutreola* and *V. peregusna* as carrier host for the Lyme disease agent and the first study on the presence of *B. burgdorferi* s.l. in carrier hosts from Romania. The most suitable tissue for PCR detection of this tick-borne pathogen was the heart.

Wildlife Hosts for Ticks of Genus *Ixodes* in Romania: New Data and Geospatial Approach

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Background: Ticks of genus *Ixodes* have a worldwide distribution, and according to the latest taxonomical review, there are 243 extant species considered valid. Of these, 13 species were reported in Romania. Clarifying the host preferences of a vector tick is essential for understanding the epidemiological features of associated diseases. Few studies are available in Romania on the distribution and host preferences of ticks of genus *Ixodes*, most of them focused on domestic mammals. The aim of the present study was to bring new data on the host spectrum of genus *Ixodes* in Romania and to provide updated GIS-based maps on their distribution in Romania.

Methods: Between February 2010 and March 2011, 330 individuals of wild vertebrates (Mammalia, Aves, Reptilia) were sampled within the frame of a large-scale epidemiological study on reservoir hosts for Lyme borreliosis. Ticks were identified to genus level according to published keys. All ticks belonging to genus *Ixodes* were further identified to species level, using morphological criteria. For all collections, GPS data was recorded.

Results: From the 330 examined individuals, 116 (35.1%) (in 21 species) were harboring 837 hard ticks (mean intensity 9.3). Of these, 66 individuals (56.9%) (in 19 species) harbored ticks of genus *Ixodes*. The total number of *Ixodes* ticks collected was 413. Identification of ticks from genus *Ixodes* revealed the presence of 4 species: *I. ricinus* (94.4%), *I. vespertilionis* (4.6%), *I. hexagonus* (0.7%) and *I. arboricola* (0.3%). The most abundant species, *I. ricinus* was found on 16 host species. Statistical analysis showed that immature stages of *I. ricinus* prefer small vertebrates as rodents, birds and reptiles ($p < 0.001$) while adult stages prefer carnivores and ungulates ($p < 0.01$). Birds infected with *I. ricinus* were hosts only to immature stages, while reptiles and mammals harbored all stages.

Conclusions: Our study reports eight new hosts for *I. ricinus* in Romania and one new host for each *I. vespertilionis*, *I. arboricola* and *I. hexagonus*. This is also the first synoptic GIS-based review of hard-tick distribution and host associations in Romania.

***Giardia duodenalis* in non-human primates: a Bayesian evaluation approach for the evaluation of four diagnostic assays in populations excreting different levels of cysts**

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Background: *Giardia* is one of the most prevalent gastro-intestinal parasites in captive nonhuman primates (NHP). Although the knowledge of diagnostic properties is essential for a reliable estimation of the prevalence in epidemiological surveys, no studies have focused on the evaluation of different assays for the diagnosis of *Giardia* in NHP. In the present study, the prevalence and the test properties of four detection techniques were estimated in NHP populations excreting different levels of cysts using a Bayesian approach.

Methods: *Giardia* cyst excretion intensities in 124 groups of non-human primates (NHP) housed at nine zoological gardens and one sanctuary in Belgium and the Netherlands were examined using an immunofluorescence assay (IFA). In addition, the prevalence of *Giardia* and the test properties of microscopic examination (ME), IFA, ELISA and PCR were estimated in 58 groups excreting different levels of cysts.

Results: The results revealed a high variation in cyst excretion intensity between groups, ranging from 1 to 4574 cysts per gram feces. A Bayesian analysis indicated that PCR (70.8%, 95% confidence interval [38.2-82.3]) and IFA (64.1% [37.3-75.1]) were the most sensitive techniques, followed by ELISA (40.7% [23.2-51.5]) and ME (23.0 [12.1-33.2]). All techniques were highly specific (> 95%). With the exception of PCR, the assays' sensitivity increased with higher levels of cyst excretion, while the specificity of all techniques remained unchanged.

Summary/Conclusions: The high variation in the intensity of excretion of cysts that was observed, suggests that the quantification of cyst excretion may help to identify groups at risk, consequently improving the management of *Giardia* in captive NHP. The results also indicate that IFA is the preferred diagnostic assay, since it is both a quantitative and sensitive technique, only missing infections with low cyst excretion intensities. Furthermore, the results demonstrate that the intensity of cyst excretion results in variable sensitivity, which may bias studies on the epidemiology of *Giardia*.

Parasitosis in rabbits of Porto Alegre, Rio Grande do Sul, Brazil?

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The helminthiasis in rabbits influence directly the health and weight gain in animals, damaging commercial creations. This study was carried out to determine the occurrence of helminth infections among rabbits in commercial breeding of RS Brazil. The animals were derived from properties with commercial farming system. Animals of both sexes were aged two months to a year. The animals' feces samples were collected on a property in the municipality of Porto Alegre, RS where they are kept in outdoor cages suspended with floor screen, each containing three animals per allocation. These are treated every 60 days with piperazine-36%. The fecal samples were processed in the laboratory of Helminthology of FAVET-UFRGS, through methods of Willis-Mollay and Dennis Swanson & Stone. Fecal samples of 115 animals were analyzed for the identification and quantification of eggs. Results showed that 50% of the samples were infected by *Syphacia* sp.; 33,3% of *Paraspidodera* sp. and 16,6% of *Strongyloidea*. The results demonstrate that there is a need to conduct an effective parasite control in order to prevent economic losses caused by parasitism in rabbits.



SESSION T

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Oral Communications

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Vaccines against cysticercosis and hydatid disease

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Highly effective vaccines have been developed against infection with the larval stages of *Taenia solium* (TSOL18) and *Echinococcus granulosus* (EG95) in the parasites' intermediate hosts. The vaccines are sterile, stable preparations containing a recombinant protein expressed in *Escherichia coli* together with the adjuvant Quil A. Two immunizations a month apart typically leads to >95% protection against an experimental challenge with the parasites. Field trials have demonstrated that the vaccines are effective against naturally acquired infections. Both vaccines are entering their final stage of development prior to being generally applied for reducing transmission of *T. solium* and *E. granulosus* in order to reduce the incidence of human neurocysticercosis and hydatid disease. Vaccine production technology has been developed for commercial-scale manufacture of the EG95 protein and the vaccine has received registration for commercial use in China (200?) and Argentina (2010). Development of vaccine scale-up technology for TSOL18 is on-going.

A novel strategy has been applied to enhance the effectiveness of the TSOL18 vaccine. This strategy utilizes the application of anthelmintic at the time of vaccination, removing any viable *T. solium* cysticerci pre-existing in an animal's muscle tissues prior to vaccination. Adoption of this strategy in a field trial of the vaccine in Cameroon led to the complete elimination of transmission of the parasite by the vaccinated animals involved in the trial. A similar strategy is not available at present for *E. granulosus* because no suitable anthelmintic treatment is known which could be used in a similar way. Research is underway investigating the use of anthelmintic or anthelmintic combinations against *E. granulosus* infections in lambs towards developing a treatment that could be used to complement the use of the EG95 vaccine.

Although a number of vaccine field trials have already been undertaken, or are underway, with TSOL18 or EG95, there remains a need for carefully controlled scientific evaluations of both vaccines when implemented as part of disease control programs. Such evaluations are necessary in order to demonstrate the vaccines' effectiveness in reducing the incidence of human disease, in minimizing the length of time necessary for control measures to be effective and to determine their sustainability for maintaining disease control.

Launching of the first recombinant vaccine against Hydatidosis in Argentina

Development of a new tool to protect intermediate hosts

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Echinococcosis by *Echinococcus granulosus* is a worldwide zoonotic disease whose epidemiology and control is often considered to be a veterinary matter. Therefore, a recombinant vaccine has been developed for prevention of infection of the intermediate hosts by Heath & Lightowlers in 1995. CEVAN-ICT Milstein belonging to CONICET, together with the University of Melbourne and AG Research made possible to produce a pilot scale vaccine that utilizes the recombinant oncosphere protein, EG95. In February 2011 Providean Hidatil EG95®, was approved by Argentina's Animal and Plant and Food Safety Agency (SENASA) as the first recombinant vaccine for use in sheep and goat. Tecnovax S.A. obtained the registration certificate for the new product and carried out the scaling up and production. The efficacy of Providean Hidatil EG95® was compared with the recombinant Australian vaccine and the bioequivalence was demonstrated.

Methods: EG95 gene was cloned from mRNA *Echinococcus granulosus* eggs of the tapeworm parasite of dogs.

The semi-purified recombinant EG95-GST protein expressed in bacteria was combined with Montanide ISA 70 plus saponin as emulsifying agent. Three groups of sheep (n=10) were immunized subcutaneously on days 0, 30 and 365 with different formulations. Group 1 was immunized with Providean Hidatil EG95® (50µg/dose). Group 2 received the Australian formulation (50µg Eg95-GST plus 1 mg QuilA). Control sheep received no antigen. Efficacy of Providean Hidatil EG95® was proved by monitoring the serological response of sheep in comparison with sera from sheep immunized with the Australian formulation.

Results and Conclusions: Providean Hidatil EG95® induced high titers of specific IgG antibodies in sera from immunized sheep that persist for more than one year and did not show significant differences with those induced by the Australian vaccine. This trial demonstrates that Providean Hidatil EG95® is bioequivalent to the Australian formulation. The production of the first licensed recombinant veterinary vaccine is a prominent fact in our country. The inclusion of this practical tool in the program for controlling hydatidosis will allow to prevent the illness in the intermediary hosts through elicited seroprotection and the subsequent reduction of the offering hydatid cysts.

***Taenia solium* taeniosis and cysticercosis in a rural community of Eastern Zambia-A community based study**

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Background: *Taenia solium* is a neglected zoonosis, with neurocysticercosis being the most common cause of acquired epilepsy in endemic areas. In Zambia, multiple studies have indicated high prevalences of porcine cysticercosis, with estimates up to 64.2%, and presence of risk factors in most rural areas. However, there are no published data on the status of the infection in humans in the country. The objective of this study was to determine the prevalence of taeniosis and cysticercosis in humans in a rural community in eastern Zambia.

Methods: In a community-based study, stool and serum samples were collected from all willing participants. A questionnaire was administered to the participating households, which were also geo-referenced. Taeniosis was diagnosed by coprology and by polyclonal antibody based copro-antigen enzyme linked immunosorbent assay (copro Ag ELISA). Cysticercosis was diagnosed by detection of circulating antigens using the B158/B60 Ag ELISA. Collected tapeworm segments were analysed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results (preliminary): A total of 264 households from 21 villages participated, from which 709 stool and 706 serum samples were collected. One stool sample (0.14%) was positive for *Taenia* spp on coproscopic examination while 13 (1.8%) positives were detected by copro-Ag-ELISA. Thirty six serum samples tested positive on Ag ELISA, resulting in an estimated prevalence of human cysticercosis of 5.11%. The recovered proglottids were confirmed to be *T. solium*. The following risk factors were present: lack of meat inspection, consumption and selling of infected pork, free-range pigs, poor sanitation, and absence of toilets in 43.8% of the households. Preliminary statistical analysis revealed that age was significantly associated with human cysticercosis (OR = 1.32; 95%CI = 1.16 – 1.50).

Summary/Conclusions: This study indicates the presence of *T. solium* taeniosis and human cysticercosis in the study community, as well as factors necessary for its transmission. It also indicates the urgent need for more comprehensive studies for a better understanding of the epidemiology of the parasite, which should ultimately lead to adapted control measures.

Bovine cysticercosis in cattle slaughtered in southern Chile: Prevalence, distribution and viability of *Cysticercus bovis* in cattle naturally infected

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Objective: The purpose of this work was to determine the prevalence, cyst viability, preferential sites of bovine cysticercosis, as well as its number per animal.

Methods: To accomplish these objectives a number of 198260 bovines from three major abattoirs in southern Chile, were examined from January to December 2010. To determine the preferential sites of *Cysticercus* in cuts carcass (33 tissue sites) of naturally (n=20) infected cattle, tissues from all sites were cut into approximately 0,5 cm thick slices and the total number of parasitic cysts determined for each site.

Results: The examination of various organs of 198.260 cattle showed that 1.158 (0,58%) were infected with *Cysticercus*. The prevalence varied significantly between PFCs, age groups, class, category and sex, but not varied season and origin of the animals. The anatomical distribution of *Cysticercus* to the inspection were 58,37% in heart, 40,40% in masseter muscles, 0,95 in tongue, 0,14 in esophagus and 0,14 in carcass. While the anatomical distribution of *Cysticercus* to the total dissection were 42,03% in heart, 15,94% in masseter muscles and 42,03 in cuts of carcass (Tenderloin, Point end Brisket, Knuckle, Runmp Heart, Osso bucco, Silverside, Cube Roll Cover, Ribs, Topside, Flanck Meat, Flanck Steak, Eye of Round, Bolar, Thin Skirt). Of the total of carcasses positives for *Cysticercus*, 89,19% of them were infected by only one cyst, 8,78 with two, 1,35 with three and 0,68% more than four. According to the classification concerning viability, the occurrence of metacestode was 88,89% nonviable (dead), 4,27% viable (alive) and 6,84% corresponded to other pathologies. From the *Cysticercus* alive, 70% of them were located in the masseter muscles, 25% in the heart, 5% in the tongue; whereas from those dead, 63,43% were located in the heart's muscles, 35,82% in the masseter muscles and 0,75% in the tongue, also 100% *Cysticercus* nonviable were found in carcass.

Conclusions: The prevalence of *Cysticercus* varied between PFCs, age groups, and sex. Most of the *Cysticercus* found in inspection and all of the dissection were nonviable. The heart was confirmed as the preferred site for detection of bovine cysticercosis based on high cyst frequency of infection. More extensive examination of the heart is recommended to improve detection of infected animals.

Characterization of hydatid cyst fluid from ruminants and humans by SDS-PAGE

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Polypeptide analysis of purified hydatid cyst fluid (HCF) from ruminants (sheep, goat, cattle, buffalo and camel) and human beings was performed by sodium dodecyl sulphate polyacrylamide gel (12%) electrophoresis (SDS-PAGE) under reduced conditions. HCFs from different species revealed variable segregation pattern of proteins not only within the different hosts of the parasites but also in different types of HCF. In fertile HCF, five to three protein bands were observed in different intermediate hosts of the parasite viz., buffalo (269, 166, 89, 59 and 25kDa), goat (46, 29, 22 and 18kDa), sheep (209, 138 and 63kDa), cattle (269, 89 and 59kDa), camel (195, 166 and 123kDa) and human beings (195, 138, 21 and 6kDa). In sterile HCF of different species, less number of protein bands was found as compared to fertile cysts. The two protein bands were observed in sterile HCF collected from camel (195 and 141kDa), cattle (27 and 18 kDa), buffalo (43 and 27kDa) and human (30 and 21kDa), whereas only one polypeptide band found in sheep (123kDa) and goat (78kDa). SDS-PAGE analysis of hydatid cyst fluid collected from different intermediate hosts of *Echinococcus granulosus* exhibited variable profiles of proteins. The present study suggests that SDS-PAGE alone is not a reliable diagnostic tool both in ruminants and human, it should be coupled with recent molecular techniques.

Mefloquine – a novel treatment for Alveolar Echinococcosis

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Alveolar echinococcosis is caused by the metacestode stage of the fox tapeworm *Echinococcus multilocularis* and causes severe disease in the human liver and occasionally other organs, which is fatal if treatment is unsuccessful. The present chemotherapy is based on benzimidazoles and it has been found to be ineffective in some instances, parasitostatic rather than parasitocidal, and usually involves the lifelong uptake of very big doses of drugs. New treatment options are needed but seldom developed due to the rare occurrence of the disease. Results of an in vitro screening indicated that mefloquine, usually applied in the prevention and treatment of malaria, showed promising results. Mefloquine was administered orally and intraperitoneally for eight weeks to animal models infected with metacestodes. A potential synergy with albendazole was also investigated. Treatment with mefloquine administered intraperitoneally presented a significant reduction in total parasite weight similar to albendazole oral treatment. Whether the action of the drug is indeed parasitocidal remains to be investigated. Oral administration of mefloquine had no impact perhaps due to a low absorption of mefloquine at lower doses.

The mechanism of action of mefloquine against the malaria parasite is not yet clear. Mefloquine is believed to prevent heme polymerization in the food vacuoles of the *Plasmodium* parasites. There accumulation of non-polymerized toxic heme would then be responsible for the parasite's death. Such a mechanism would not explain the action of the drug in *Echinococcus multilocularis* metacestodes, as this does not consist of an intracellular parasite and is not known to feed on haemoglobin. Affinity chromatography of metacestode extract on a mefloquine-bound matrix resulted in specific binding of *E. multilocularis* ferritin, which was identified by mass spectrometry analysis. Ferritins are globular proteins responsible for the storage of intracellular iron in a non-toxic form, as well as controlled iron transport. Free iron is toxic to cells as it acts as a catalyst in the formation of free radicals from reactive oxygen species. The binding of ferritin by mefloquine might induce changes in the intracellular iron metabolism of the parasite, causing its death. This mechanism has still to be confirmed. Nevertheless, mefloquine is an interesting candidate for the treatment of alveolar echinococcosis as it is a compound which is already approved for use in humans.

The Efficacy of Immunomodulators in Albendazole Therapy of Alveolar Echinococcosis

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Background: The larval stage of *Echinococcus multilocularis* causes alveolar echinococcosis, the most dangerous autochthonous parasitosis in Europe, predominantly transmitted by foxes as the definitive host. The *E. multilocularis* larval cysts affect the liver of intermediate hosts such as small mammals or man. The chemotherapy of alveolar echinococcosis with available anthelmintics has only the parasitostatic effect. The therapy is complicated with generalized immunosuppression caused by the parasite itself. Application of immunomodulative substances could improve the host immunostatus during *E. multilocularis* infection and limit the growth of the parasite.

Methods: The experiments were carried out using 150 mice of BALB/c strain: Group 1 - mice without the infection and the treatment (control), Group 2 – mice infected with *E. multilocularis*, Group 3 - mice infected with *E. multilocularis* and treated with albendazole (ABZ), Group 4 - mice infected with *E. multilocularis* and treated with ABZ and liposomized muramyltripectide phosphatidylethanolamine (L-MTP), Group 5 - Mice infected with *E. multilocularis* and treated with ABZ and transfer factor (TF). The concentration of serum cytokines IFN- γ and IL-5 and the production of superoxide anion (O_2^-) in peritoneal macrophages were examined during 26 weeks after the infection. The treatment efficacy was determined by the measurement of the weight of *E. multilocularis* cysts in hosts. Statistical differences were assessed using Kruskal-Wallis ANOVA, followed by post hoc Tukey's HSD test.

Results: The combined L-MTP+ABZ therapy induced a long-term development of protective Th1 response (the highest serum concentration of IFN- γ ; $P < 0.01$), on the contrary, Th2 response (cytokine IL-5) was inhibited till the end of the experiment. L-MTP+ABZ stimulated the production of O_2^- for 10 weeks ($P < 0.01$). Stimulation of cellular immunity of mice with alveolar echinococcosis with L-MTP and an interaction with ABZ's antiparasitic effect resulted in the greatest and long-term reduction of growth of *E. multilocularis* cysts in the host from week 10 p.i. till the end of the experiment ($P < 0.01$, $P < 0.05$). The serum level of IFN- γ after TF+ABZ therapy was significantly higher within therapy ($P < 0.01$) and during this time the significantly ($P < 0.05$) inhibited IL-5 synthesis was detected. The O_2^- production was stimulated after TF+ABZ therapy for 10 weeks, too. The combined therapy TF+ABZ resulted in the greater parasite restriction ($P < 0.05$) in comparison to ABZ therapy, but did not reach the cyst reduction after L-MTP+ABZ therapy. Results of alternative therapeutical strategies with use of immunomodulators are promising and might contribute to higher efficacy of the anti-echinococcus treatment.

Anti-hydatic activity of Ginger and Ginseng against *Echinococcus granulosus* metacestode: an *in vitro* study

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Background: Human echinococcosis is caused by infection with the metacestode stage of *Echinococcus granulosus* (*E.g.*). It constitutes a serious public health problem in various parts of the world, particularly in Algeria. We have previously reported that IFN- γ effectively killed *Echinococcus granulosus* protoscoleces *in vitro* by iNOS (inducible nitric oxide synthase) induction (Amri et al., 2007). In the present study, our aim is to investigate the bioactivity of two commonly used herbal products; Ginger (*Zingiber officinale*) and Ginseng (*Panax ginseng*) against *E. g.* cyst components.

Methods: Protoscoleces (PSC) and cyst wall (CW) were obtained from lung hydatid cysts of sheep. PSC and CW were cultured with crude aqueous extract of dried Ginger or Ginseng (1, 10 and 100 μ g/ml). Furthermore, we evaluated the activity of these two products (1 and 10 μ g/ml) in association with IFN- γ (100UI/ml) on PSC cocultured with mononuclear cells (PBMC) of hydatid patients (before and after surgery) and healthy donors. After 20h of incubation, cultures and cocultures supernatants were harvested to evaluate parasite viability. **Results:** Our results show that Ginger and Ginseng exhibited a dose- dependent cytotoxic effect on PSC and CW. In the PSC culture, both induce a marked morphological change with loss of the membrane integrity, the order of hooks, and the homogeneity of the calcareous corpuscles. Moreover, contractile movements of membrane and suckers disappear gradually followed by complete degeneration of PSC. In the CW culture, the germinal layer (GL) becomes darker and small areas of detachment from the laminated layer (LL) are observed. Finally, GL loses completely his integrity followed by LL. Under our conditions, PSC seemed more susceptible to Ginger than Ginseng in cultures and cocultures. Interestingly, synergistic scolical effects between Ginger or Ginseng and IFN- γ are also observed. Similar findings are observed in cultures performed with PBMC of presurgical and postsurgical patients and healthy donors.

Conclusions: Taken together, our results demonstrate that Ginger and Ginseng have an antihydatic effect *in vitro*. This effect is amplified in the presence of IFN- γ . All these data suggest the use of Ginger and Ginseng with or without IFN- γ in the treatment of *Echinococcus granulosus* infection. However, *in vivo* studies on an animal model (as rodents) should be done before using these new drugs for human treatment.

Characterization, fertility and vitality of bovine hydatid cysts in La Pampa province

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Hydatid cyst disease is caused by larval stage of *Echinococcus granulosus* in human and domestic animals. The aim of this work is to analyze the characteristics, fertility and vitality of bovine hydatid cyst. Ninety (90) hydatid cysts obtained from bovines (between 2 to 3 years old) slaughtered in a meat processing plant of La Pampa province were analyzed. Visceral location, size of the cysts, and fertility (presence or absence of protoscoleces) were evaluated. Vitality was determined by microscopic examination and staining 0.1% eosin. At the total cysts evaluated 95.5% were located in lung, 3.3% in the liver and 1.2% in both visceral locations. Cysts measured were between 1 cm to 6 cm of diameter. Related to fertility 57.78% (52/90) were fertile, 35.56% (32/90) infertile and 6.66% (6/90) absceded. Cysts with less than 3cm of diameter had a fertility rate of 38.5% and those with 3 cm of diameter or more had a fertility rate (72.5%) significant higher ($p<0.001$). Of the total fertile cysts 88.46% (46/52) presented vitality. The percentage of bovine hydatid cysts found was higher in lung than liver tissue and their fertility and vitality was higher in those hydatid cysts bigger to 3 cm.

Epidemiological aspects and morphological characterization of hydatid cysts in slaughtered ruminants from different areas of Romania

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Background: Cystic echinococcosis (CE), caused by the larval stages (hydatid cysts) of *E. granulosus* is one of the most important parasitic infections in livestock in the world, as well as a major zoonosis. In Romania, CE continuing to pose significant problems of public health and cause important economic losses by condemnation of parasitized organs in slaughtered animals. In the present study the prevalence and morphological characterization of hydatid cysts in slaughtered ruminants from three different areas of Romania were determined.

Methods: The study was conducted during of two years (2009, 2010) in abattoirs from three different regions of Romania. Hydatid cyst count and morphological characterization were realized based on routine meat inspection and examination of cysts from 10226 cattle and 15779 sheep. A number of 4350 hydatid cysts recovered from slaughtered ruminants were examined in laboratory for morphological characterization and determining of their viability.

Results: The prevalence of CE in the examined animals ranged from 25.4 to 97.1% in sheep and between 27.2 - 64.6% in cattle.

The most affected organs were liver and lungs; the cysts were found in both organs in 57.8% of cattle and 52.5% of sheep; 32.2% of the cattle and 25.7% of sheep presented cysts only in liver, while 6.2% of cattle and 17.9% of sheep had cysts only in lungs. Sporadic localization, in spleen (2.6% in cattle, 3.2% in sheep), kidney (0.9% in cattle, 0.7% in sheep), and heart (0.3% in cattle) were registered.

The most of hydatid cysts (51.03% from cattle and 50.53% from sheep) were small, measuring fewer than 2 cm. The medium cysts (2-5 cm) were present in 34.69% in cattle and 38.72% in sheep. The large cysts (bigger than 5 cm) were present in 14.28% in cattle and 10.75% in sheep.

The viability of hydatid cysts, based on presence of protoscoleces in microscopical examination was very different within the two ruminant species: 58.53% from the examined cysts in sheep were fertile, while in cattle only 0.36% from cysts were fertile.

Conclusions: The present study provides baseline data on the current status of cystic echinococcosis in ruminants in Romania, showing a widespread of the disease and the necessity of a rigorous parasitological control program.

Detection of constitutive Nitric Oxide Synthase in *Echinococcus granulosus metacestode*

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Background: Human echinococcosis is caused by infection with the *metacestode* stage of *Echinococcus granulosus*. It constitutes a serious public health problem in various parts of the world, particularly in Algeria. In cattle, the parasite develops into large cysts especially in the liver and lung. We have previously found the presence of nitric oxide (NO) in culture medium of protoscoleces after 20h of incubation. This result suggests a nitric oxide production by the parasite itself. In the present work, we wish to confirm this hypothesis by investigating the presence of Nitric Oxide Synthase (NOS) within the parasite.

Methods: We evaluated direct effect of NOSynthase regulators (substrate, inhibitor and cofactor) on protoscoleces *in vitro*. At the end of culture period (20h), medium of each culture was harvested for nitric oxide determination. Furthermore, we investigated the reactivity of protoscoleces with an anti-NOSynthase antibody by immunocytochemical test.

Results: Our results confirm NO production by the parasite itself. Interestingly, this production is regulated by NOS effectors. In fact, NG-monomethyl-L-arginine (L-NMMA), a competitive inhibitor of NOS, caused a significant decrease in NO production. Furthermore, the addition of L-Arginine (NOS substrate) and Tetrahydrobiopterin (BH4, NOS cofactor) to parasite culture increased NO production. Moreover, in immunocytochemical test, we found that reactivity was highly associated with the tegument of protoscoleces. Positive staining was also observed in the parenchyma.

Conclusion: Taken together, the results reported here show, for the first time to our knowledge, the presence of constitutive NOSynthase in *Echinococcus granulosus* protoscoleces. The role of this enzyme in the parasite–host interaction remains to be clarified.

Field Study: Evaluation of the Eg95 Vaccine against Ovine Hidatidosis

First Communication

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Introduction: The objective of the work was to evaluate the impact of the introduction of the EG95 vaccine to cut the transmission cycle of the ovine hidatidosis, assessing the technique feasibility under field condition in an endemic area of the Río Negro province, Argentina.

Material and Methods: Two areas were defined; the vaccination areas, composed by the Indian communities of Anecón Grande, Río Chico Abajo, Nahuel Pan and Manuel Choique, and the control ones Blancura Centro and Lipetrén (192 small farmers, 16615 ovine's, 404 dogs).

Diagnosis: Dog faeces (CoproELISA/Western Blot), soil (Polymerase chain reaction: PCR) and serum samples of 2-4 teeth lambs (ELISA/WB) of 109 random statistically representative farmers were collected. Necropsy of adult ovine and arecoline test in canines were also done to confirm the disease. Since December of 2009 the lambs received three doses of EG95 vaccine (University of Melbourne) at 30, 60 and at 365 days of life.

Preliminary Results: the prevalence in dogs, lambs and sheep were 11.3 (CoproELISA/WB), 34.3 (ELISA) and 62% (Necropsy), respectively. During the first year were vaccinated 2725 (1° dose) and 2427 lambs (2° dose) with a coverage percentage of 87.7 and 78.2% of animals, and 90.1 and 92.8% of farmers.

Discussion: The operation cost, the social characteristic of the community, and the trouble on the ground such us: deficient facilities, amplitude in the lambing period, sell of the animals and movement of the vaccinated lambs among farmers, are going to be analysed in order to include the vaccine as a tool in a control programme.



SPECIAL COMPANION ANIMAL'S PARASITOLOGY SYMPOSIUM

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New insights into the epidemiology and diagnosis of canine and feline lung nematodes

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The relevant lung worms of dogs are *Crenosoma vulpis*, *Angiostrongylus vasorum* and *Eucoleus aerophilus* (syn. *Capillaria aerophila*), whereas *Aelurostrongylus abstrusus* and occasionally *E. aerophilus* are of importance in cats. The presence of most of these parasites is sustained by reservoirs in wild carnivores. In contrast, reports of pet animals harbouring *Filaroides* spp. or *Oslerus osleri* are rare, and cases of *Paragonimus kellicotti* are observed regionally. *C. vulpis* is highly prevalent in wild canids. The key clinical sign in affected dogs is coughing and, therefore, non-parasitic etiologies are often differentially considered. Reports of previously restricted endemic areas of *A. vasorum* are increasingly complemented by case and prevalence studies outside these foci. In dogs infected with *A. vasorum*, the clinical signs include respiratory and neurological deviations with potentially lethal outcome. Aelurostrongylosis in cats is reported world-wide with varying prevalences, partially depending on the employed detection methods. Clinical signs are often overlooked. Infections with the metastrongylid nematodes *C. vulpis*, *A. vasorum* and *A. abstrusus* are associated with the ingestion of snails or slugs. The life cycle of *E. aerophilus* is less clear but is also suggested to be bound to intermediate hosts. Patent infections with metastrongylids can be diagnosed by the detection of first stage larvae in faecal samples, which are preferentially subjected to larval migration or FLOTAC techniques. Flotation methods allow detecting the characteristic *E. aerophilus* eggs shed by the adults. Adult *A. vasorum* are living in the circulation system, in particular in the right heart and the pulmonary arteries, and ELISAs for the detection of circulating antigens or specific antibodies have recently been developed. These methods may help to identify affected animals before the onset of patency and can be used for mass-screening of dog populations. Furthermore, PCR assays have been used for the detection of *A. vasorum* or *A. abstrusus* DNA in faecal or blood samples and they may be useful in surveys investigating the intermediate hosts. The following questions regarding the emergence of lung worm infections will be addressed: Is there an actual spread of the parasites and is this caused by the dissemination with infected wildlife, intermediate hosts or pet animal movements? Are affected animals increasingly been detected due to the newly developed diagnostic tools? Are veterinarians more aware of lung parasites and/or do pet owners increasingly agree with diagnostic processing and anthelmintic prophylaxis/treatment of their pets?

Neosporosis and toxoplasmosis pet animals

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Neospora caninum and *Toxoplasma gondii* are closely related protozoan parasites but they are biologically different. Dog and other canids are definitive hosts for *N. caninum* and neosporosis is primarily a disease of cattle and dogs whereas felids are definitive hosts for *T. gondii* and toxoplasmosis causes clinical disease in most warm blooded animals, including dogs, cats, humans. *N. caninum* is a primary pathogen in dogs and can cause clinical disease in dogs of all ages. Most cases of clinical canine neosporosis have been in congenitally infected dogs involving littermates, whereas congenital canine toxoplasmosis is rare. Paralysis of rear limbs, often with contracture, is the most consistent sign of neonatal neosporosis. A wide array of clinical signs have been reported in older dogs, often on immunosuppressive therapy Treatment of clinical neosporosis with currently available drugs, including clindamycin, is only partially effective. None of the drugs kill *N. caninum* tissue cysts. Co-infection of *T. gondii* could occur with *N. caninum* infection, and should be considered in differential diagnosis. Although antibodies to *N. caninum* have been reported in felids, and the domestic cat can be experimentally infected with *N. caninum*, there is no report of clinical neosporosis in cats. Pneumonia, and uveitis are common clinical signs of toxoplasmosis in cats. Cats can excrete millions of environmentally resistant oocysts in nature and they are usually asymptomatic during the oocyst shedding phase.

Update on the Diagnosis and Prevention of Vector-Borne Canine Diseases

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Pathogens transmitted by blood-sucking arthropods such as ticks, fleas, sand flies and mosquitoes cause some of the most widespread and severe diseases of animals and humans. Dogs are affected by a wide spectrum of viruses, bacteria, protozoa and helminths transmitted by arthropod vectors. Transmission of pathogens is often mediated via the vector's saliva during the course of a blood meal, however, other modes of vector transmission such as ingestion of the vector by the canine host as in hepatozoonosis may occur. Accurate and rapid detection of disease is paramount for the management of vector-borne infections, and for instituting preventative measures in order to disrupt the transmission of the causative agent and stop the spread of disease to additional hosts. In the case of canine vector-borne diseases, preventative measures may include the application of ectoparasiticides to kill or repel external parasites, vaccination of dogs to disrupt the establishment of infection following contact with the pathogen, and avoidance of exposure to ectoparasites to minimize contact with the pathogen vectors. Detection of the infection is not only helpful in establishing the diagnosis of a vector-borne disease but also in the follow up of treatment and its outcome. The diagnostic approaches for each canine vector-borne pathogen may vary in accordance with its life cycle, the infected tissues involved, the host immune response and the likelihood of confirming infection by a certain technique. Some vector-borne infective agents such as *Babesia canis* can be eliminated from the dog by successful medical treatment, whereas treatments against other agents such as *Leishmania infantum* may only substantially decrease the parasite load, improve the clinical manifestations of disease, and allow the immune system to keep infection under control. Topical external parasiticides and transmission-blocking vaccines are also helpful in decreasing the infectiousness of dogs to vectors such as sand flies, mosquitoes and ticks and diminishing the transmission of vector-borne canine pathogens to other naive hosts. Recent longitudinal studies on the prevention of vector-borne canine diseases in endemic areas have employed an array of diagnostic tests including quantitative serology and real-time PCR for the quantitative detection of pathogen DNA in the blood and other tissues. These studies have indicated that the use of topical parasiticides in spot on formulations or collars is efficient in markedly reducing the transmission of pathogens to other dogs and in cases of zoonotic infections such as visceral leishmaniosis, also to humans.
