

ABSTRACT BOOK



25th International Conference of the World Association
for the Advancement of Veterinary Parasitology.

16-20 August, 2015

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Abstracts

WAAVP 2015

16-20 August 2015

Arena & Convention Centre Liverpool UK

Posters

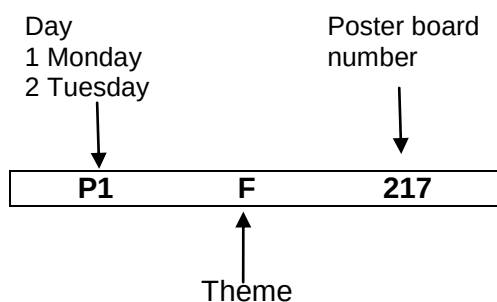
Posters will be on display from Sunday 16 August for the duration of the conference.

Theme	Posters	Posters with 2 minute oral presentation
A: Drugs and drug resistance Pharmacokinetics	P1A142-P1A182 P1A213-P1A216	P1A183-P1A212
B: Climate change, epidemiology and modelling	P1B001-P1B064	P1B065- P1B075
C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)	P2C249-P2C312	P2C313-P2C330
D: Vaccines and immunology	P2D400-P2D420	P2D421-P2D426
E: One health	P2E360- P2E388	P2E389-P2E399
F: Genes and genomics	P1F217-P1F240	P1F241-P1F243
G: Co-infection	P1G076-P1G086	P1G087-P1G091
H: Companion animal	P1H092-P1H134	P1H135-P1H141
I: Novel diagnostics	P2I331- P2I350	P2I351-P2I359
J: Genetics of host resistance	P2J244-P2J245	P2J246-P2J247
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Posters will be presented by authors at the following times:

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1	Monday 17 August	18:00-18:45	G: Co-infection
1	Monday 17 August	18:00-18:45	H: Companion animal
2	Monday 17 August	18:45-19:30	A: Drugs and drug resistance and pharmacokinetics
2	Monday 17 August	18:45-19:30	F: Genes and genomics
3	Tuesday 18 August	18:00-18:45	J: Genetics of host resistance
3	Tuesday 18 August	18:00-18:45	K: Lifelong education (UG and PG)
3	Tuesday 18 August	18:00-18:45	C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)
3	Tuesday 18 August	18:00-18:45	I: Novel diagnostics
4	Tuesday 18 August	18:45-19:30	E: One health
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4	Tuesday 18 August	18:45-19:30	Late breaking abstracts

Poster reference explanation



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Plenary Presentations

Monday 17 August 2015

10:30-11:30

Plenary 1

Parasites in a changing world

Lord Robert May

Department of Zoology, Oxford University

This talk will speculate on the kinds of new problems – or amplification of existing problems – in Veterinary Parasitology that are likely to arise as the planet warms to essentially unprecedented levels.

Tuesday 18 August 2015

11:00-12:00

Plenary 2

Exhausted CD4 T-cell response induced by *Anaplasma marginale*

Professor Wendy C Brown

*Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine,
Washington State University, Pullman, WA, USA*

Anaplasma marginale is a tick-transmitted, intra-erythrocytic rickettsial pathogen of cattle that causes anemia and mortality. Cattle that survive acute disease remain persistently infected with fluctuating high levels of bacteremia. Failure to clear infection is due in part to antigenically variant major surface proteins (MSPs) but we propose persistence also results from CD4 T-cell exhaustion during high pathogen load. Following *A. marginale* infection, cattle previously immunized with MSPs developed a progressive loss of T-cell proliferation and IFN- γ and TNF- α secretion. Exhaustion was reversed after clearing the infection with tetracyclines. Exhaustion led to deletion of tetramer^{+ve} CD4 T cells from peripheral blood, and although small numbers of antigen-specific CD4 T cells were detected in lymphoid organs and liver, functional T cells could not be recovered. We hypothesized that *A. marginale* infection induced regulatory T cells. Furthermore, we proposed that CD4 T-cell exhaustion following infection would require the presence of the T-cell antigen, used for immunization, on the infecting bacteria. Cattle were immunized with MSP1a containing a DRB3*1101-restricted T cell epitope and potential regulatory FoxP3⁺ and $\gamma\delta$ T cell subsets in blood and spleen were enumerated by flow cytometry following infection with either *A. marginale* expressing the T-cell epitope, or *A. marginale* subsp. *centrale* lacking the epitope. Loss of the MSP1a-specific T-cell response occurred only after infection with *A. marginale*, and was not associated with an increase in T cells with a regulatory phenotype. Current research is examining the induction of inhibitory T-cell receptors PD-1 and LAG-3 and the ligand PD-L1 following infection.

This research was supported by USDA-NIFA 2010-65119-20456 and NIH NIAID AI-053692

Wednesday 19 August 2015

11:00-12:00

Plenary 3

Novel interventions and enabling technologies for insect-borne disease prevention

Professor Janet Hemingway

Director of the Liverpool School of Tropical Medicine

As we attempt to eliminate a number of insect borne diseases progress is hampered by the lack of effective interventions, poor surveillance and inadequate implementation, often driven by a lack of capacity to deliver the programmes in the disease endemic countries. Product development partnerships, underpinned by public and philanthropic funding have stimulated a raft of new products that are now starting to come onto the market and will revolutionise our ability to control and prevent a number of diseases if properly implemented. Enabling technologies to facilitate implementation, such as IT systems, serious games and diagnostic platforms are also being rolled out to strengthen Capacity and improve surveillance. The beneficial impact of these technologies on a number of operational programmes will be described.

Thursday 20 August 2015

11:00-12:00

Plenary 4

"Evolutionary genomics and parasite biology"

Tim Anderson

Texas Biomedical Research Institute, San Antonio, TX, USA

The central problems in parasite control are evolutionary in nature. We control parasites by imposing massive drug selection - and parasites enthusiastically respond by evolving resistance. Hosts evolve protection against infection - and parasites evolve to circumvent these protections. We are now in a golden age for Parasitology with genomes in abundance, and developing molecular toolboxes. We are in a position to understand at the molecular level how parasites adapt, to work rapidly so we can redesign drugs and manage control interventions more effectively, and to gain fundamental insights into parasite biology. I will highlight several ongoing projects in my laboratory - on schistosomes and malaria parasites - to illustrate the value of applying evolutionary, population and quantitative genetics approaches to parasite biology.

Sponsors' Presentations

Monday 17 August 2015

11:30-12:30

Sponsor's session Bayer

Rhinos, dogs and Seresto® collars

Bruce Leslie

Regional Ranger, Kruger National Park, SANParks, South Africa

The dramatic increase in rhino poaching over the last seven years in South Africa is having a devastating impact on South Africa's natural heritage. 827 rhinos were killed for their horns in 2014 alone. Consequently, South African National Parks are constantly investigating ways in which to increase the detection and arrest rate of armed suspects, to counter the increasing poaching incidence. One of these interventions was the use of canines such as the Belgium Shepherds or "Malinois", being trained to track poachers as well as having the ability to detect firearms and ammunition.

These dogs have had a significantly positive influence in the fight against rhino poaching. However a number of challenges to maintaining these animals in healthy conditions have presented themselves, the biggest threat being that of potentially fatal vector-borne diseases such as canine babesiosis and erlichiosis.

Through a partnership with Bayer, all working dogs in the Kruger National Park have been supplied with Seresto® collars in order to repel tick vectors and avoid subsequent disease. The program has been a rousing success, with no incidences of vector-borne diseases occurring in any of the dogs wearing Seresto collars, helping to maintain them in top working condition.

Monday 17 August 2015

11:30-12:30

Sponsor's session Bayer

Prevention and control of Rocky Mountain Spotted Fever in the Southwestern United States

Casey Barton Behravesh, MS, DVM, DrPH, DACVPM

National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, USA

Rocky Mountain spotted fever (RMSF) transmitted by the brown dog tick (*Rhipicephalus sanguineus*) has emerged as a significant public health risk on American Indian reservations in Arizona. During 2003–2013, ~300 RMSF cases and 20 deaths were documented among Arizona's American Indian population. The high case fatality rate makes community-level interventions aimed at rapid and sustained reduction of ticks urgent. Beginning in 2012, a two year pilot integrated tick prevention campaign called the RMSF Rodeo was launched in a ~600-home tribal community with high RMSF rates. During year one, long-acting tick collars were placed on all dogs in the community, environmental acaricides were applied to yards monthly, and animal care practices such as spay and neuter and proper tethering procedures were encouraged. Tick levels, indicated by visible inspection of dogs, tick traps and homeowner reports were used to monitor tick presence and evaluate the efficacy of interventions throughout the project. By the end of year one, <1% of dogs in the RMSF Rodeo community had visible tick infestations five months after the project was started, compared to 64% of dogs in Non-Rodeo communities, and environmental tick levels were reduced below detectable levels. The second year of the project focused on use of the long-acting collar alone and achieved sustained tick control with fewer than 3% of dogs in the RMSF Rodeo community with visible tick infestations by the end of the second year. Homeowner reports of tick activity in the domestic and peridomestic setting showed similar decreases in tick activity compared to the non-project communities. Expansion of this successful project to other areas with *Rhipicephalus*-transmitted RMSF has the potential to reduce brown dog tick infestations and save human lives.

Tuesday 18 August

12:00-13:00

Sponsors' session: Merial

New trends in the control of parasites in dogs and cats

Chair person: Prof. Frederic Beugnet

1) The control of parasites: past and future

Speaker: Frederic Beugnet, DVM, PhD, Dip. EVPC

2) Broad spectrum approach to control cat parasites

Speaker: Dr. Eric Tielemans, DVM

3) Nexgard Spectra®: the addition of an internal spectrum to the new

generation insecticide/acaricide afoxolaner

Steffen Rehbein, DVM, Dip. EVPC

Major parasites infesting both cats and dogs are ectoparasites (fleas, ticks, and to a lesser extent mites, *Demodex*, *Cheyletiella*, lice), gastrointestinal worms (gastrointestinal nematodes and cestodes) and cardiopulmonary worms (heartworm and lungworms). These are all commonly found in companions animals, and in addition to their pathogenic effects on their host, many of them present a zoonotic risk. Their control is therefore of importance both for veterinary and public health. Deworming, far from being a banal procedure, must take into account numerous factors related to the host (species [cat vs. dog], age, lifestyle), parasites (lifecycles, risk environments) and medications (activity spectra of the drug substances, pharmaceuticals, protocols for use). The treatment protocols against worms are to be based on epidemiological considerations and awareness of the activity spectra of the anthelmintics. Depending on the animal species, age and lifestyle, one active substance may be more appropriate than another. On the basis of pharmaceutical behavior and the number of animals to be treated, some presentations are more appropriate than others. All of the foregoing are to be taken into account in the therapeutic procedure, whose medical and hygienic importance remains underestimated. But anthelmintics should not be considered alone, and in many case, the control of fleas and ticks must be integrated due to their prevalence and the high probability of co-infestations. This is why the use of veterinary antiparasitic drugs including both ecto- and endo-parasites, often names "endectocides", is increasing in pets all over the world. It is also more convenient for pet owners than combining different treatments. To answer to this need, Merial recently launched last year Broadline® spot on for cats, a combination of fipronil, (S)-methoprene, eprinomectin and praziquantel, and is launching this summer NexGard Spectra®, oral palatable chewable for dogs, containing afoxolaner and milbemyrcins oxime.

Thursday 20 August 2015

12:00-13:00

Sponsors' session: Elanco

Elanco: expanding horizons together

William Weldon PhD, Daniel E. Snyder DVM, PhD, Dipl. ACVM, ECVP, Christian Epe DVM, PhD, Dipl. ECVP

Join us for this one-hour symposium showcasing Elanco's vision: "Food and companionship enriching life" and how with the recent acquisitions by Elanco of Lohmann Animal Health and Novartis Animal Health, these milestones will help to drive more future value and innovation to our customers. The new Elanco will be able to provide more solutions with a larger portfolio (including parasiticides), more innovation and a greater global reach. The speakers will provide an overview of: **1)** an introduction of the new Elanco and why parasitology is a key part of its current and future vision; **2)** the experience of Elanco and Novartis in veterinary parasitology and ongoing and future support of scientific organizations such as WAAVP; and **3)** the New Elanco R&D organization by bringing two different R&D structures together to bring new innovation to the marketplace. In closing, the speakers will select and answer questions previously submitted as part of "*What are your BIG Questions?*" survey. This survey is intended to ask for your inputs on the many challenges and demands facing Veterinary Parasitologists and the field of Veterinary Parasitology across the world. Our ask to you is where should the efforts of Elanco research and development be focused? ***We want to expand horizons together.***

The session will be moderated by Dr. Jason Drake from Elanco Animal Health and features three speakers.

William Weldon PhD, Vice-President, Research and Development, Elanco Animal Health, Greenfield, IN

Daniel E. Snyder DVM, PhD, Senior Research Advisor, Research and Development, Elanco Animal Health, Greenfield, IN

Christian Epe DVM, PhD, Head of Parasitology, CRA Research Site, Research and Development, Elanco Animal Health, St. Aubin, Switzerland

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Keynote Presentations

Monday 17 August 2015

13:30-14:00

Keynote 1

Concurrent Session 1.1

A: Drugs and drug resistance

“Looking to the Future” of anthelmintic resistance research: harnessing the power of genomics and genetics to fill our current knowledge gaps

John Gilleard

Professor of Parasitology and Associate Dean, Research at the University of Calgary Faculty of Veterinary Medicine (UCVM).

Anthelmintic resistant parasites are an increasingly serious problem for animal health and production and an emerging threat to global human health. In spite of some progress, our understanding of the genetic basis of anthelmintic resistance is far from complete. For example, we still have a poor understanding of how many different mechanisms of resistance occur, how these mechanisms differ between parasite species and geographical regions, how resistance mutations emerge and spread in parasite populations, how different resistance mutations impact parasite fitness and how different management strategies affect the emergence of resistance. However, the genomic resources and tools needed to address many of these questions are increasingly available and affordable. Consequently the next few years promises significant progress in filling many of these current knowledge gaps. In this talk, I will review our current “state of understanding” of the molecular genetics of anthelmintic resistance for the small ruminant gastro-intestinal strongylid parasite *Haemonchus contortus*; the parasite species for which we know the most. I will provide an update on the recent progress being made in genomic and genetic studies and discuss how, although significant challenges remain, we are entering a new and exciting phase of discovery.

Monday 17 August 2015

13:30-14:00

Keynote 2

Concurrent Session 1.5

One health

One health and veterinary parasitology

Emily Jenkins

Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Canada.

One Health can be defined as a coordinated, collaborative, interdisciplinary and cross-sectoral approach that recognizes that the health of humans, animals and ecosystems is intimately connected. Veterinary parasitology has long recognized the need for a One Health approach to the diversity and complexity of parasite transmission among animals, people, and the environment. Too often, however, the risks of zoonotic transmission overshadow the benefits from close contact with companion animals and shared environments with wildlife. I'll discuss three case studies where the roles of animals as sources of human exposure to zoonotic parasites are not clear-cut. 1) *Echinococcus multilocularis* – if a dog develops alveolar hydatid disease in the forest, is anybody listening? 2) *Toxoplasma gondii* – it's not your cat that's making you crazy...3) *Giardia* – *Canis* too *familiaris*? Through these case studies, I illustrate some of the challenges facing veterinary public health and parasitology in engaging the broader One Health community, and the importance of a balanced risk-benefit approach to veterinary parasites as we “look to the future” in a One Health world.

Monday 17 August 2015

14:00-14:30

Keynote 3

Concurrent Session 1.4

D: Vaccines and immunology

Variability in *Neospora caninum* and its relevance to cattle infection

Luis-Miguel Ortega-Mora, Gema Alvarez-García, Esther Collantes-Fernández, Mercedes Gómez-Bautista, Pilar Horcajo, Javier Regidor-Cerrillo

Saluvet Group, Animal Health Department, Faculty of Veterinary Sciences Faculty, Complutense University of Madrid, Madrid, Spain

Neospora caninum is an apicomplexan cyst-forming protozoan parasite phylogenetically closely related to *Toxoplasma gondii*. It has a heteroxenous life cycle where definitive hosts (dog, coyote dingo and gray wolf) can shed unsporulated oocysts in their feces and the intermediate host, mainly cattle, harbors the asexual tachyzoite and bradyzoite parasite stages in their tissues. *N. caninum* transmission in cattle occurs through ingestion of sporulated oocysts and transplacental infection from the dam to the foetus during gestation. Transplacental transmission takes place as a consequence of an oocyst-derived primo-infection (exogenous transplacental transmission; ExTT) or after recrudescence of a persistent infection acquired *in utero* (endogenous transplacental transmission; EnTT). *N. caninum* infection may lead to abortion, stillborn or congenitally infected calves. It is unclear which host and parasite dependant factors play a major role in the outcome of the infection. *N. caninum* shares with *T. gondii* a world-wide distribution, ability to infect different intermediate hosts and capacity for genetic recombination in a definitive host that may give rise to genetic and biological diversity. So far, the molecular basis of the disease remains to be elucidated and studies on parasite variability and population structure are limited. Predominance of vertical transmission in cattle suggests the clonal propagation of this parasite and initial studies based on microsatellite sequences have shown evidence the geographic sub-structuring and predominant clonal propagation in some areas. In addition, studies on host-parasite interactions in *in vitro* culture systems revealed marked differences in lytic cycle kinetics, mechanisms to breach biological barriers for tachyzoite dissemination and stage conversion that can explain variability *in vivo*. Such variability has already been confirmed in mouse and bovine experimental models showing marked differences in pathogenicity, infection dynamics, tissue parasite distribution and induction of immune response. New approaches based on high-throughput sequencing methods and genetic manipulation tools together with well-defined ruminant models will be the key in unraveling the molecular basis of variability in *N. caninum* and its effect on cattle infection.

Acknowledgements: This research is presently funded by the Spanish Ministry of Economy and Competitiveness (AGL2013-44694-R).

Monday 17 August 2015

14:30-15:00

Keynote 4

Concurrent Session 1.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Tannin containing legumes as a model for nutraceuticals against digestive parasites in livestock

H.Hoste^{1,2}, J.F.J. Torres –Acosta³, C.A. Sandoval-Castro³, I. Mueller-Harvey⁴, S. Sotiraki⁵, H. Louvandini⁶, S.M. Thamsborg⁷, T.H. Terrill⁸

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Parasitic infections with gastrointestinal nematodes (GINs) still represent a worldwide major pathological threat in outdoor production of various livestock species. Because of the widespread resistance to synthetic chemical anthelmintics, there is a strong impetus to explore novel approaches for a more integrated management of the infections. The use of nutraceutical plants for controlling GINs is one of the alternative which has been widely studied since 20 years.

The objectives of this review are: **i)** to define and illustrate the concept of 'nutraceutical plants' in the context of veterinary parasitology based on data from the most studied GIN models in small ruminants, the tannin-containing legumes (Fabaceae); **ii)** to illustrate how the 'nutraceutical concept' could be expanded to other plants, other livestock production systems and other GI parasitic diseases, and **iii)** to explain how this concept is opening up new research fields for better understanding the interactions between the host, the digestive parasites and the environment.

Monday 17 August 2015
16:00-16:30

Keynote 5

Concurrent Session 2.1

A: Drugs and drug resistance

Anthelmintics: The best way to predict the future is to create it

Richard J. Martin, Alan P. Robertson, Saurabh Verma, Shivani Choudhary, Suhhanva Kashyap, Melanie Abongwa, Fudan Zheng

Dept of Biomedical Sciences, Iowa State University, Ames, IA 50011, USA

We can predict aspects of the future by creating new knowledge. When we look at drugs that are used to control parasites, we see that there is new knowledge about their mode of action and this knowledge is predicted to allow us to put combinations of drugs together more rationally to increase the spectrum of action and to slow the development of anthelmintic resistance. In this paper we comment on some recent observations of ours on the modes of action of emodepside, diethylcarbamazine and tribendimidine. Emodepside increases the activation of a SLO-1 K current inhibiting movement, and diethylcarbamazine has a synergistic effect on the effect of emodepside on the SLO-1 K current, increasing the size of the response. The combination may be considered for further testing for therapeutic use. Tribendimidine is a selective cholinergic nematode B-subtype nAChR agonist, producing muscle depolarization and contraction. It has different subtype selectivity to levamisole and may be effective in the presence of some types of levamisole resistance. The new information about the modes of action may aid the design of rational drug combinations designed to slow the development of resistance or increase the spectrum of action.

Monday 17 August 2015

16:00-16:30

Keynote 6

Concurrent Session 2.5

One health

25 years of the *Onchocerca ochengi* model: What have cows taught us about human river blindness?

Benjamin L. Makepeace¹, Germanus S. Bah^{1,2}, Stuart D. Armstrong¹, Alistair C. Darby³ and Vincent N. Tanya⁴

¹*Institute of Infection & Global Health, University of Liverpool, Liverpool, UK;* ²*Institut de Recherche Agricole pour le Développement, Regional Centre of Wakwa, Ngaoundéré, Cameroon;* ³*Institute of Integrative Biology, University of Liverpool, Liverpool, UK;* ⁴*Cameroon Academy of Sciences, Yaoundé, Cameroon*

Human onchocerciasis or river blindness is a devastating neglected tropical disease affecting 30 million people in sub-Saharan Africa. The microfilariae of *Onchocerca volvulus* are responsible for all of the major disease manifestations, comprising severe dermatitis, depigmentation of the skin, and visual impairment, which can lead to irreversible blindness. In the early 1990s, researchers from the University of Tübingen and the Liverpool School of Tropical Medicine recognised the enormous potential of a parasite of African cattle, *Onchocerca ochengi*, as an ideal “analogue” of human onchocerciasis. Many trials of macrofilaricidal drug and vaccine candidates in this system followed in northern Cameroon, but it was the unexpected response of adult *O. ochengi* to a decades-old antibiotic, oxytetracycline, that contributed to a revolution in our understanding of both the basic biology of filarial nematodes and their treatment. Subsequently, the *O. ochengi* system has provided deep insights into a fascinating symbiosis with *Wolbachia*, an endobacterium that was previously believed to only infect arthropods. Here, I will describe how a combination of classical histological techniques combined with next-generation sequencing and proteomics have enabled a gradual unveiling of the role of *Wolbachia* in *O. ochengi*, which almost certainly functions on several levels. Contrary to expectations, *Wolbachia*’s contribution to the symbiosis is not limited to metabolic provisioning, but extends to immune evasion, suggesting that the endobacteria have an important part to play in the extraordinary longevity of *Onchocerca* spp.

Monday 17 August 2015

16:30-17:00

Keynote 7

Concurrent Session 2.2

F: Genes and genomics

Comparative genomics of over 70 helminth genomes

James Cotton¹, Nancy Holroyd¹, Diogo Ribeiro¹, Isheng Jason Tsai^{1,5}, Eleanor Stanley¹, Avril Coghlan¹, Bhavana Harsha¹, Daria Gordon¹, Taisei Kikuchi³, John Martin², Philip Ozersky², Kym Pepin², Xu Zhang², Bruce Rosa², Rahul Tyagi², Xin Gao², Bruce Bolt⁴, Kevin Howe⁴, Makedonka Mitreva², Matthew Berriman¹

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Genomic data is available for a growing number of helminth species, including exemplars of most major clades of parasitic worms, but these data do not capture much of the diversity of helminth parasites. To address this, the international helminth genome initiative is attempting to expand our knowledge of helminth genomes. With a large group of collaborators, we have generated whole-genome shotgun sequence data and draft genome assemblies for over 50 parasitic nematode and platyhelminth species for which genomic data was previously unavailable, including parasites of medical and veterinary importance and some comparative species that occupy important phylogenetic positions, for example as outgroups to important groups of parasites. These data include the first genomic data for a number of important parasites such as equine strongyles, lungworms and Guinea worm. The genomes we include are demonstrably largely complete, but the fragmentary nature of the assemblies makes some analyses challenging. Despite this, we can see for the first time the broad outlines of genome evolution for parasitic helminths, identify gene families that are evolving in surprising ways and thus may have important functions, and assess how well *C. elegans*, and the better-known parasite species in each group, represent the diversity of helminths.

Tuesday 18 August 2015

09:00-09:30

Keynote 8

Concurrent Session 3.1

A: Drugs and drug resistance

Pharmacological challenges to extend the lifespan of existing and novel anthelmintics

Prof. Carlos Lanusse, Med Vet., Dr Cs Vet., Ph.D., Dip ECVPT

*Laboratorio de Farmacología, Centro de investigación Veterinaria de Tandil (CIVETAN),
CONICET-Universidad Nacional del Centro, Tandil, Argentina.*

In view of the increasing concern for the development of resistance, the use of pharmacology-based information is critical to design successful strategies for the future of parasite control in livestock. The magnitude of the investment in chemicals to control helminth parasites in domestic animals reveals the need for further scientific research on how to maximize drug use. Integrated pharmaco-parasitological research approaches have greatly contributed to optimize drug activity. The assessment of the drug disposition in the host and the comprehension of the mechanisms of drug influx/efflux/detoxification in different target helminths, has signified a relevant progress on the understanding of the pharmacology of anthelmintic drugs in ruminant species. However, additional scientific knowledge on how to improve the use of available and novel molecules is required to avoid/delay resistance development. Different pharmacokinetic-based approaches to enhance parasite exposure and the use of combination of drugs from different chemical families have been proposed as valid strategies to delay the development of anthelmintic resistance. The limited available information on the potential additive and/or synergistic effects occurring after co-administration of two (or more) drugs with different mode of action is unclear. The occurrence of potential pharmacokinetic and/or pharmacodynamic interactions between drug components highlights the need of deeper and integrated research to identify the advantages/disadvantages of the use of combined drug preparations. The data summarized in this 2015 WAAVP Keynote presentation emphasizes on how the use of pharmacology-based information may be critical to design successful strategies for the future of parasite control in ruminants, including significant issues needed to secure/extend the lifespan of the recently available anthelmintic molecules.

Tuesday 18 August 2015

09:00-09:30

Keynote 9

Concurrent Session 3.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

“Sustainable use of anthelmintics – how we administer drugs influences the development of resistance”

Dave Leathwick PhD

Agresearch Grasslands, Palmerston North, New Zealand

Anthelmintic resistance in gastrointestinal nematodes is now a global phenomenon affecting most species of grazing animals (e.g. goats, sheep, cattle, deer and horses), and considerable scientific effort has been put into understanding how resistance develops and how it can be delayed or managed. However, the development and marketing of anthelmintic products has evolved more or less independently of any considerations for the development of resistance. This presentation will consider the hypothesis that the way in which anthelmintic products are formulated and delivered plays an important role in the rate at which resistance develops to the constituent actives. All anthelmintic products are registered as effective against a range of parasite species based on measured efficacy against populations which are susceptible to the active ingredient at the administered dose rate. However, the rate at which resistance develops is strongly influenced by efficacy against resistant genotypes, in particular the heterozygotes during the early phase of selection when resistance genes are still rare.

The way in which drugs are formulated and delivered can affect the dose, variability in dose and duration of exposure of the target worms to the drug, and all these factors can potentially influence the rate at which resistance develops in the treated population. Different routes of administration (e.g. pour-on, injection, oral) result in very large differences in plasma profiles, but, in fact these may be largely irrelevant as plasma levels may be a poor indicator of the concentration of drug reaching the target worms. Formulations and routes of administration which result in persistent activity influence the reproductive advantage afforded to resistant genotypes, especially when drug concentrations decline to suboptimal levels over time. Combining different actives with similar spectra of activity into a single product has been shown to have benefits in reducing the survival of resistant genotypes. However, use of combinations raises the potential of drug-drug interactions and formulation effects which may also be important.

Tuesday 18 August 2015

09:00-09:30

Keynote 10

Concurrent Session 3.5

Novel technologies

Small RNAs in novel diagnosis and control of parasitic helminths

Collette Britton, Alan D Winter, Neil D Marks, Henry Gu, Tom McNeilly, Victoria Gillan, Eileen Devaney

University of Glasgow Institute of Biodiversity, Animal Health and Comparative Medicine, Henry Wellcome Building, Bearsden Road, Glasgow

microRNAs (miRNAs) and small interfering (siRNAs) are important regulators of post-transcriptional gene expression. With the availability of parasite genome data and interest in developing better diagnostic tests and control methods, these small RNAs are receiving increasing attention. miRNAs are 22 nucleotide non-coding RNAs which bind to target mRNA sequences, often in the 3' untranslated region (UTR), to reduce gene expression. Using RNA sequencing and microarrays we have profiled miRNAs expressed during development of the nematodes *Haemonchus contortus* and *Brugia pahangi*. Many of the miRNAs are unique, suggesting roles in adaptation and survival within the host environment. Others are present across species and may play important conserved functions in nematode development. In *H. contortus*, we have identified miRNAs showing specific temporal or spatial patterns of expression and focussed on those 1) developmentally regulated as larvae switch from free-living to parasitic stages 2) highly enriched in gut tissue 3) released from the parasite and present in excretory-secretory (ES) products. Our aim is to identify the target genes and functions of these small RNAs. miRNAs present in *H. contortus* ES products can also be identified in host gut lymph node and abomasal tissue, suggesting that they may influence host-parasite interactions and immune responses. In filarial and schistosome infections, miRNAs released into host plasma can be exploited as sensitive and specific diagnostic markers. Application of siRNAs to mediate RNA interference (RNAi) gene silencing and improvements to this technique to identify essential gene function will also be discussed.

Tuesday 18 August 2015

09:00-09:30

Keynote 11

Concurrent Session 3.4

H: Companion animal

Of the diagnostic challenges and the unwritten stories of dog and cat parasites

Domenico Otranto

Department of Veterinary Medicine, University of Bari, Bari, Italy

Is it still possible to discover new parasites of dogs and cats? Do we really know enough about them? To what extent do limitations in the diagnosis of dog and cat parasites represent an obstacle for a deeper understanding of their biology? Diagnosis in parasitology has a deep impact on animal health and welfare and, in some cases, public health. Although, over the last few years, advances in the diagnosis of parasitic diseases have largely paralleled knowledge of their biology, gaps in the diagnosis of cat and dog parasites still exist. For instance, difficulties in obtaining samples for research purposes (due to ethical issues or to the invasive nature of the sampling procedures), inaccurate sample storage and poor sensitivity of the commonly used techniques, may represent major obstacles in diagnosing parasitic diseases. Other hurdles are often associated with the biology of parasites (e.g., the intermittent presence in blood of tick-borne pathogens) or, simply, through the fact that some parasites of pets are largely ignored by the scientific community. This lecture will provide key examples of parasites of dogs and cats, which are currently considered of minor importance, also because of the limitations in their diagnosis. Among them, new or, in some cases, previously “misdiagnosed parasites” with overlapping morphological features, biology or ecology, represent a major challenge when trying to correctly diagnose “unknown parasites” (for which only occasional reports are available). Further research is needed in order to provide the scientific community with more reliable, cost-effective diagnostic tools, which ultimately, will assist our understanding of some mis- or less-diagnosed parasitoses.

Tuesday 18 August 2015

14:00-14:30

Keynote 12

Concurrent Session 4.2

G: Co-infection

Helminth-microbe co-infection: insights from a natural system

Vanessa Ezenwa

Odum School of Ecology and College of Veterinary Medicine, University of Georgia, USA

Co-infection with helminths can have profound effects on a host's response to microbes, and an increasing number of studies are investigating the consequences of worm-microbe co-infection in laboratory settings. To better understand the dynamics of co-infection in natural systems, we used a free-ranging population of African buffalo to explore the consequences of worm infection for bovine tuberculosis (BTB). We followed 200 animals for four years to test the effects of anthelmintic treatment on individual and population-level outcomes of BTB infection. We found that de-worming had no effect on individual risk of infection, but enhanced survival of BTB-infected individuals. Histological results from BTB-positive animals suggest that deworming reduces the severity of BTB in buffalo by reducing levels of bacterial dissemination. Importantly, the contrasting effects of anthelmintic treatment on BTB infection probability and mortality translate into enhanced fitness for the pathogen (*M. bovis*). This study reveals the potentially complex effects anthelmintic intervention can have on non-target microbial co-infections.

Tuesday 18 August 2015

16:00-16:30

Keynote 13

Concurrent Session 5.2

B: Climate change, epidemiology and modelling

ECONOHEALTH: placing helminth infections of livestock in an economic, environmental and social context

Johannes Charlier^{1,*}, Fiona Vande Velde^{1,2}, Mariska van der Voort³, Jef Van Meensel³, Ludwig Lauwers^{3,4}, Verolien Cauberghe², Jozef Vercruysse¹, Edwin Claerebout¹

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Livestock farming is central to global food security and to the sustainability of rural communities throughout Europe. Animal health management has a major impact on farming efficiency. Although animal health research has provided effective prevention strategies for the major enzootic diseases of livestock, these strategies typically provide solutions for single infectious diseases and they are often not adequately implemented due to farm-specific constraints. We propose a concept termed “ECONOHEALTH” which aims at including the economic and social context in our understanding of the factors that drive animal health. The concept is elaborated on using the example of the major helminthic diseases of cattle in temperate climate regions (gastrointestinal nematodes, liver fluke and lungworm). By considering major diseases simultaneously and placing disease-complexes in an economic and a social context, we believe that insights will be generated upon which more integrated, situation-adapted and thus more effective prevention strategies can be devised.

Tuesday 18 August 2015

16:00-16:30

Keynote 14

Concurrent Session 5.5

J: Genetics of host resistance

Improving host genetics for resistance to parasites: searching for optimum phenotypes in a changing climate

Eric R. Morgan and Hannah Rose

University of Bristol, School of Veterinary Science, Bristol Life Sciences Building, 24, Tyndall Avenue, Bristol, BS8 1TQ, United Kingdom

The long tradition of selective breeding of domestic animals for resilience to parasite infection has accelerated with intensifying threats such as drug resistance, and as new statistical and molecular tools become available. Progress has been best documented for resistance to gastrointestinal nematodes in sheep, with resistance traits being integrated into breeding programmes in the field, and significant efforts directed to enhanced detection of resistant genotypes. Several areas of debate have emerged, including the relative place of resistance and tolerance in parasite resilience and its economic benefits, and the potential for selective treatment approaches to underpin alternative pathways to genetic improvement. Notably, selection under current parasite challenge could be undermined by altered parasite epidemiology in future, especially as a result of climate change. We present results from predictive models of nematode free-living stages that compare the epidemiological benefits of reduced faecal worm egg output under different climatic and management scenarios. This approach could help to design optimal phenotypes for future farms, and hence inform breeding programmes through prioritisation of traits and markers. At the same time, increasing emphasis on refugia-based treatment strategies provide opportunities for farmer-led selection for parasite resilience, especially where more centrally developed genetic resources are unavailable. Efforts to develop host resistance or resilience in other host-parasite systems could benefit from similar strategic consideration of the potential impacts of climate change on epidemiology. Ultimately, breeding programmes for different parasitic and other diseases should be integrated so that their aims align in the best interests of sustainable livestock farming.

Wednesday 19 August 2015

09:00-09:30

Keynote 15

Concurrent Session 6.5

I: Novel diagnostics

Testing times: How can we move on from the feast or famine approach to diagnostic tool development?

Ted Bianco, Wellcome Trust

We live in an era of evidence-based medicine. A time when we expect to have a rationale for how we approach the treatment of ill-health. But neither human or animal medicine has fully come to terms with what it takes to produce the tools we need to diagnose many of the conditions requiring our attention in a suitably effective and efficient manner. That is to say, we have tests galore to diagnose a case of malaria, but use very few of them in practice. And we have few tests to diagnose early dementia, yet desperately need them. Until we hunker down to do what is needed rather than what is tractable, our test-kit catalogues will suffer from an excess of some tests and dearth of others. Focusing on the most significant unmet needs in diagnosis sounds like a call to arms that should appeal to the research community, the funders and industry alike. It is my contention that it is the researchers that must lead the charge to make this happen.

Wednesday 19 August 2015

09:30-10:00

Keynote 16

Concurrent Session 6.1

A: Drugs and drug resistance

Unravelling Triclabendazole resistance in the liver fluke, *Fasciola hepatica*

Jane Hodgkinson

Veterinary Parasitology, University of Liverpool

The liver fluke, *Fasciola hepatica* is an economically important trematode parasite pathogen of livestock that frequently impacts on the health and welfare of cattle and sheep worldwide and is regarded by the WHO as a re-emerging zoonosis. With predictions for further increases in the prevalence of infection due to a changing climate, increased animal movements and anthelmintic resistance, combined with its ability to modulate the host immune system and affect diagnosis and susceptibility to other pathogens, *F. hepatica* infection is likely to have a substantial impact on livestock production and human health in future.

The potential for anthelmintic resistance to be complex and multigenic in nature and involving as yet unidentified genes, has promoted whole genome approaches to identify drug resistance mechanisms. In this review, we report a classical genetic mapping approach to identify the major genetic loci involved in conferring triclabendazole (TCBZ) resistance in *F. hepatica*. In order to dissect the spread of genes associated with TCBZ resistance we have taken an experimental approach by crossing TCBZ susceptible (TCBZ-S) and TCBZ resistant (TCBZ-R) clones of *F. hepatica* and mapping TCBZ-R genes through subsequent F1 and F2 populations. To ensure use of isolates representative of the current field situation and to avoid issues associated with laboratory isolates we have identified TCBZ-R field isolates from the UK. We have used single miracidial:snail infections of *F. hepatica* to generate clonal parental lines of TCBZ-R and TCBZ-S parasites and confirmed their TCBZ resistance status in experimental infections in sheep. These parental clones have been used to derive F1 progeny using a panel of neutral microsatellite markers we developed to track the genotypes of those parasites that had undergone cross fertilization events between TCBZ-R and TCBZ-S parasites and to allow subsequent identification of F2 recombinants. A draft genome sequence for *F. hepatica* has been produced and in order to support SNP discovery to map regions of the genome associated with resistance the re-sequencing of genome-wide SNPs for five isolates will be reported. Ultimately, the project relies on comparing the frequency of SNP alleles derived from the resistant parental clone and linked to the TCBZ resistance locus (or loci) in pooled, phenotyped F2 recombinants. In vivo and in vitro phenotyping of F2 recombinants will be described and pooled genotyping on >20 replicates followed by analysis of allele frequencies to associate SNPs to TCBZ resistance is underway and will be discussed.

Wednesday 19 August 2015

10:00-10:30

Keynote 17

Concurrent Session 6.4

D: Vaccines and immunology

Progress towards a sub-unit vaccine for control of teladorsagiosis in sheep

Jacqueline Matthews

Moredun Research Institute, Edinburgh, UK

Despite decades of research, the development of commercially viable recombinant sub-unit vaccines against parasitic nematodes has been unsuccessful. We developed a strategy to identify antigens for inclusion in a vaccine to control *Teladorsagia circumcincta*, the major pathogen causing parasitic gastroenteritis in small ruminants in temperate regions. We did this by studying IgA responses directed at proteins specific to post-infective larvae and by searching for antigens that would have a potential immunomodulatory role at the host/parasite interface. Recombinant versions of eight molecules selected on the basis of immunogenicity, homology with vaccine candidates in other nematodes and/or with potential immunoregulatory activities, were tested in a vaccine formulation, administered in the context of Quil A adjuvant. Vaccinates were subsequently subjected to a repeated challenge infection designed to mimic field conditions and the levels of protection obtained in vaccinates compared to those in sheep administered with Quil A alone. A number of trials have now been performed with varying levels of immunity obtained in the different trials. The level of immunity induced varies amongst individuals and is particularly associated with the age of the sheep at vaccination, with significant levels of protection obtained in sheep over 6 months of age. This presentation will describe the vaccine components, the immunisation trials and the steps that we are currently taking to optimise the prototype in an attempt to mitigate the variation in responsiveness observed amongst different sheep.

Thursday 20 August 2015

09:00-09:30

Keynote 18

Concurrent Session 7.1

F: Genes and genomics

***Eimeria* genomics: where are we now and where are we going?**

Damer P. Blake

Pathology and Pathogen Biology, Royal Veterinary College, Hawkshead Lane, North Mymms, AL9 7TA, UK

The evolution of sequencing technologies, from Sanger to next generation (NGS) and now the emerging third generation, has prompted a radical frameshift moving genomics from the specialist to the mainstream. For parasitology, genomics has moved fastest for the protozoa, with sequence assemblies becoming available for multiple genera. Progress has commonly been slower for parasites of animals which lack zoonotic potential, but the deficit is being redressed with impact likely in the areas of drug and vaccine development, molecular diagnostics and population biology. Genomics studies with the apicomplexan *Eimeria* species clearly illustrate the approaches and opportunities available. *Eimeria* cause the disease coccidiosis, most notably in chickens where the global cost is thought to exceed US\$3 billion every year. Farmers commonly rely on chemoprophylaxis to control *Eimeria*, but drug resistance develops rapidly and is now widespread. Vaccines are available, but production capacity and relative cost limit uptake. New methods of control are required, but progress has been undermined by the lack of genomic resources. Now, more than ten years after sequencing started, a genome sequence assembly has been published for *Eimeria tenella*. Additional sequence assemblies for all other *Eimeria* species which infect the chicken and *Eimeria falciformis*, a parasite of the mouse, offer powerful opportunities for comparative analysis, enhancing development of novel diagnostic and control strategies. The current status of *Eimeria* genomic resources will be reviewed and their application to define population structure for *E. tenella* described, revealing an intriguing dichotomy between Northern and Southern populations characterised by distinctive spatial haplotype occurrence with evidence of both clonality and panmixia.

Thursday 20 August 2015

09:00-09:30

Keynote 19

Concurrent Session 7.2

B: Climate change, epidemiology and modelling

Looking to the future of geospatial health: strengths, weaknesses and challenges

Laura Rinaldi and Giuseppe Cringoli

Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, CREMOPAR Campania Region, Naples, Italy

Geospatial health is now a firmly established approach for geo-positioning, collating, exploring, visualizing and analyzing parasitological data in a spatially explicit manner at various scales (local, regional and area-wide scales). The future of geospatial health is already taking place now with a plethora of technologies, methods, software, models, tools and web-based platforms available for scientists, practitioners, decision-makers and stakeholders working in the field of epidemiology and surveillance of parasitic infections. However, effective health responses depend upon the ability of quality data and sustainable health systems to provide accurate and timely information for action. Use of new technologies supported by mobile-, visual- and electronic-based (m-, v- and e-health) approaches as well as improved strategies of diagnosis are considered research priorities to strengthen the use of geospatial tools for monitoring and modeling parasites of veterinary importance. The overall strengths, weaknesses and challenges of using geospatial tools in veterinary parasitology deserve a SWOT analysis. Finally, recommendations are needed on how to make best use of geospatial tools to have a meaningful impact on control and prevention of parasitic infections in the era of climatic and global changes.

Thursday 20 August 2015

09:30-10:00

Keynote 20

Concurrent Session 7.4

D: Vaccines and immunology

Immune modulation by *Teladorsagia circumcincta* and implications for control

Tom N McNeilly

Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, Midlothian, UK.
EH26 OPZ

Teladorsagia circumcincta is the primary cause of parasitic gastroenteritis in small ruminants in temperate regions. Immunity to this parasite is slow to develop, requiring continuous exposure over a number of weeks. This is consistent with an ability of the parasite to suppress host immune responses.

We are now beginning to unravel the mechanisms and molecules this parasite uses to manipulate host immunity. For example, we know that Foxp3⁺ regulatory T cells are recruited to the gastric mucosa during infection the parasite induces an early regulatory immune response which is partially overcome during more chronic infection. Furthermore, excretory-secretory (ES) products derived from fourth stage larvae (L4), the parasitic stage most targeted by the immune response, are profoundly suppressive of T cell activation and this is in part mediated by the regulatory cytokine interleukin-10. Proteomic analyses of L4 ES products have identified fractions highly enriched for suppressive activity, although attempts to identify the specific suppressive molecules within these fractions have been largely unrewarding. Detailed proteomic and transcriptional analysis of L4 parasites within either the mucosa or the lumen of the abomasum has been more successful: this approach has identified a number of potentially immunosuppressive molecules which are enriched in mucosal-dwelling L4, including TcK6, an ShK-domain containing protein which inhibits cytokine production by T cells, and thioredoxin peroxidase, an antioxidant with the potential to modulate macrophage function. Current work is now focused on testing these molecules as vaccine antigens, with the aim of inducing antibodies to neutralize their suppressive effects and thus enhance parasite-induced immunity.

Thursday 20 August 2015

14:00-14:20

Keynote 21

Concurrent Session 8.4

Lifelong Education

Establishing a Veterinary Parasitology Specialty—the North American experience

Anne M. Zajac,

Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg VA.

In 2011, veterinary parasitology received provisional specialty status from the American Veterinary Medical Association based on reviews by the American Board of Veterinary Specialists (ABVS) and Council of Education. Recognition of the parasitology specialty was the result of efforts by an organizing committee and charter members, who represented a broad range of veterinary parasitologists in academia and private industry. Organizational assistance was received from the American Association of Veterinary Parasitologists. Primary reasons for seeking specialty recognition were improving the profile of the discipline and promoting training of new veterinary parasitologists with demonstrated clinical expertise. Rather than a separate College, parasitology is included as a subspecialty within the American College of Veterinary Microbiologists, but maintains a separate exam committee and an independent Board exam process. The Parasitology Board exam consists of general and clinical portions, both in multiple choice format. The test is administered annually in December with the first test having been given in 2012. After a period of at least 4 years of provisional status, ABVS can be petitioned to grant full status to the specialty. Because traditional parasitology PhD research programs may not include a clinical component, several programs have been developed that include this training, including the National Center of Veterinary Parasitology (Oklahoma State University), which offers a multi-institution residency training program in clinical parasitology.

Thursday 20 August 2015

14:20-14:40

Keynote 22

Concurrent Session 8.4

Lifelong Education

Veterinary Parasitology – status quo and where to go

Kurt Pfister

President EVPC

Veterinary Parasitology is - according to the constitution of the European Veterinary Parasitology College (EVPC) - a discipline-oriented specialisation which basically focuses on parasites and their effects on animal health (incl. all the characteristic subdisciplines) in order to control animal parasitic diseases. The study of Vet. Parasitology also includes the prevention of parasitic zoonoses which are important for human health.

Accordingly, the objectives of EVPC are manifold, but the main include among others „to further the scientific progress in teaching and research in Veterinary Parasitology in Europe, to establish standards of training and experience for qualification as a European Specialist...., and to develop continuing education programmes in Vet. Parasitology in Europe“, etc.

Undoubtedly, some of these basic requests have been reached, but presently we are still quite far away from having arrived at the targeted goals, e. g. when looking at

- open positions of Chairs of Vet. Parasitology at several EU Universities
- the decreasing number of professorship at the Universities in parts of Europe in the recent past
- lack of Standard Residency Training Programmes for young Vet. Parasitologists
- insufficient numbers of examined Diplomates
- lack of continuing education programmes in Europe.
- often insufficient clinical relationship of our work, etc. etc....

So what to do?

EVPC is an accredited member College of the European Board of Veterinary Specialisation (EBVS) and has – as such -to fulfill their requests. When looking at them, there is clearly a need for Vet Parasitology Depts/working units and thus consequently for EVPC Diplomates to become more active in motivating/recruiting young parasitologists for completing their education by preparing/establishing for them "Standard Residency Training Programmes". Similarly, there is a great need to develop more intensively the clinical emphasis and a closer collaboration/relationship with the clinical field, as this is one of the major argument for being a member College of EBVS. In addition, there is a great need for establishing continuing education programmes for Diplomates in both, the clinical area as well as in the area of research.

An important issue for future directions about Veterinary Parasitology will be the outcome of a debate to be held with regard to both, the often lacking scientific staff and scarce financial resources. Therefore, additional, more open strategies should will have to be envisaged for our discipline at the Veterinary Faculty levels throughout Europe.

Thursday 20 August 2015

14:00-14:30

Keynote 23

Concurrent Session 8.5

I: Novel diagnostics

Technical developments and their implementation in ruminant routine diagnostics and monitoring programmes

Janina Demeler

Institute of Parasitology and Tropical Veterinary Medicine, Department of Veterinary Medicine, Freie Universität Berlin

For the effective management and control of helminths in ruminants, three key questions are usually addressed: which parasite species are present, which one is mainly responsible for disease and how resistant is the present nematode population. In recent years, the rapid increase in technological evolution enabled development and implementation of new methods in this field of parasitology. However, despite the existence of a range of new methods it has been shown that the transfer into routine diagnostics and field monitoring is rather low. New technical developments use three main columns which are either DNA-based, including real time amplification and/or next generation sequencing, involve multiplexing of assays or address the development of pen-side diagnostic techniques. A range of new methods has been reported for their use in scientific research. For the identification of the species present in faecal samples a direct faecal PCR has been reported for a range of hosts which is compatible to detection methods such as high resolution melt, pyrosequencing or RFLP. A multiplex-tandem real time PCR was described to sufficiently enable identification as well as quantification of nematode species present in faeces of sheep using the Easy plex system. Deep sequencing has been investigated regarding the potential to diagnose resistance within a population of nematodes. For the detection of benzimidazole (BZ) resistance individual pyrosequencing assays are now available for 5 different nematode species and have been evaluated in BZ-resistance field surveys (sheep & cattle) in European countries. The Luminex xMAP technology was employed to develop a multiplex immunological bead-based assay for the simultaneous detection of pasture borne nematode infections in bovine milk and serum samples. A step towards pen-side application was the design of a LAMP assay for the rapid and highly sensitive detection of *Haemonchus contortus* in faecal samples of sheep. Despite this range of new techniques, robust, reliable, rapid and low-priced methods to be implemented in routine diagnostics are still missing. This accounts for the assessment of individual and herd health status as well as for the identification and quantification of the parasite species responsible for disease. One major constrain is the acceptance of farmers to spend money on diagnostic, particularly if a direct benefit in terms of increased productivity is not obvious. This talk aims at highlighting recent development in diagnostic techniques as well as their potential for implementation in routine testing.

Oral Presentations

Monday 17 August 2015

Concurrent Session 1.1

A: Drugs and drug resistance

Ref: O001/0303

Selection for anthelmintic resistant *Teladorsagia circumcincta* in pre-weaned lambs by treating their dams with long-acting moxidectin injection

Dave Leathwick, Karl Fraser, Chris Miller

AgResearch, Palmerston North, New Zealand

The possibility that treatment of ewes with long-acting formulations of macrocyclic-lactone (ML) anthelmintics could select for anthelmintic resistant nematode populations in the suckling lamb through transfer of anthelmintic active in the ewe's milk was investigated. Ewes, carrying twin lambs, were treated pre-lambing with a long-acting formulation of moxidectin, a 100-day controlled release capsule (CRC) containing abamectin and albendazole or remained untreated as controls. Seventeen days later 7 ewes from each mob, along with their lambs, were relocated indoors. Blood and milk samples were collected at intervals and tested for the presence of moxidectin or abamectin. Lambs were dosed weekly with 250 infective stage larvae of *Teladorsagia circumcincta*; one lamb from each pair received larvae of a known ML-susceptible isolate while the other was given ML-resistant larvae. Following treatment with moxidectin, this drug was detected in the plasma of all ewes until about day 50, and in their lambs until about day 60. Following treatment with a CRC, abamectin was detected in the plasma of all ewes until the last sampling on day 80 and in the plasma of all their lambs until about day 60. Drug was detected in the milk from all treated-ewes until day 80. Although egg counts were low, lambs from treated ewes had significantly lower egg counts than those from untreated ewes. There was no difference between treatments in the worm burdens of lambs given ML-resistant larvae, but the burdens of susceptible parasites in lambs whose mothers had been treated with moxidectin were significantly (70%) lower than those in lambs in the other treatment groups given the same isolate. Treatment of ewes pre-lambing with long-acting ML anthelmintics resulted in the persistent transfer of active to the lambs via milk and in the case of moxidectin, this resulted in a significant reduction in the establishment of ML-susceptible, but not ML-resistant larvae.

Monday 17 August 2015

Concurrent Session 1.1

A: Drugs and drug resistance

Ref: O002/0409

Evaluation of worm replacement as a means to reverse the impact of multiple-anthelmintic resistant *Haemonchus contortus* on a sheep farm

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A flock of sheep infected with a highly benzimidazole (BZ) and avermectin/milbemycin resistant, and borderline levamisole (LEV) resistant population of *Haemonchus contortus* experienced severe clinical haemonchosis associated with anthelmintic treatment failure. Coinciding with relocation to a new farm, we replaced these multiple-resistant *H. contortus* with a fully susceptible laboratory isolate. Anthelmintic susceptibility and population genetics of the newly established worm population were subsequently evaluated for 2.5 years using *in vivo*, *in vitro*, and molecular methods. Prior to replacement, sheep were placed into a dry lot and treated with a multiple-day regimen of a LEV /albendazole (ABZ) combination, which reduced fecal egg counts by 99.0%. Individual sheep were then inoculated with 5,000 L3 of a drug-susceptible laboratory isolate, and upon infection patency moved to clean pastures on the new farm. Initially post-replacement, ABZ treatment yielded 98.5% fecal egg count reduction (FECR) and DrenchRite larval development assay (LDA) showed reversion to susceptibility to BZ, ivermectin (IVM), moxidectin (MOX), and LEV, confirming successful replacement. In an effort to maintain anthelmintic susceptibility, targeted selective treatment (TST) based on FAMACHA was implemented using ABZ. Surprisingly, within 1.5 years post-replacement, resistance to ABZ, IVM, and MOX was confirmed via FECRT (-55.6%, -200.0%, and 59.2% FECR, respectively) and LDA, while LEV remained effective (98.8% FECR). LEV was then used for all treatments using the same TST approach. However, within one year, resistance was detected to LEV by both FECR (40.7%) and LDA. Population genetic analysis of neutral microsatellite markers demonstrated the resistant post-replacement parasite population is more similar to the initial resistant population rather than the susceptible laboratory isolate used in the replacement. Pyrosequence genotyping of benzimidazole resistance-associated single nucleotide polymorphisms from samples collected across the 2.5-year study is currently in progress. These data should provide additional insight to the dynamics of change over time. Overall, these data suggest that the susceptible laboratory isolate possessed a fitness deficit, allowing the small number of residual resistant worms present post-replacement to outcompete the susceptible worms.

Monday 17 August 2015

Concurrent Session 1.1

A: Drugs and drug resistance

Ref: O003/0582

Development of anthelmintic resistance on commercial sheep farms in the UK

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In the UK a Technical Manual collating research based evidence for best practice for sustainable control of parasites in sheep (SCOPS) was produced in 2004. One of the main objectives, of the SCOPS initiative was to preserve the efficacy of the 3-ML anthelmintics and avert the situation where farms harboured populations of worms resistant to all available anthelmintics making effective worm control difficult or impossible. Data supporting the efficacy of the guidelines on the development of anthelmintic resistance has been largely from observations collected under experimental conditions in the laboratory or the field. There is little published data for the development of resistance in worm populations in a commercial setting and such data may increase our ability to develop more effective control guidelines. This report presents data for resistance to the 1-BZ, 2-LV and 3-ML drug classes from 16 farms between 2007 and 2012 half of which have been following SCOPS guidelines for control and half of which had not. The farms were selected based on a 2 cube factorial design with three factors known to affect worm epidemiology: control regimen (SCOPS and Traditional (CONTROL)); farm type (Upland and Lowland); and climatic region (Southwest and Northeast). A formalised plan for worm control using 7 potential resistance-delaying practices was prepared for each of the 8 SCOPS farms, in conjunction with the farms veterinarians. Treatment records for all of the farms were collected in the autumn of 3 study years between 2012 and 2014. Anthelmintic resistance (AR) was measured on each of the farms across the 3-year study period using a larval development test (LDT) to produce dose response, as well as discriminating dose (dd), data for the benzimidazoles (1-BZs) and imidazothiazoles (2-LVs). On farm Faecal Egg Count Reduction tests (FECRTs) were also carried out to measure resistance to the macrocyclic lactones (3-MLs). Comparisons were made using dd data generated in 2007 and 2014 and for dose response and FECRTs between 2012 and 2014. Data from this study suggests that 1-BZ and 3-ML resistance is present on a majority of the study farms and resistance levels are increasing. There is some indication that AR may be developing less rapidly on the SCOPS study farms compared to those carrying out more traditional control methods, and a significant effect was demonstrated in the case of 1-BZ resistance in the *Teladorsagia* populations between 2007 and 2014. Data from dose response lines generated for this study suggests that this may be a more sensitive method for measuring changes in resistance over time than the dd dose method. For example, the LV dd assays only detected resistance on one study farm in 2012 and three of the study farms in 2014 and the increase in resistance levels was minimal. However, using the dose response method, six of the farms had RF ≥ 2 in 2012 (i.e. more than 2 times the concentration of LV was required to kill 50 % of each of these population compared to the susceptible isolate). Furthermore, 15 of the isolates tested in 2014 had RF ≥ 2 . This suggests increased sensitivity, both to detect and to measure change, with this method. The data supports other studies that suggest that triple resistance is no longer a rare event in the UK. Results for the resistance assays, anthelmintic use and management practices on the farms allows us to better understand the process of selection on these commercial farms and the data underlines the importance of continued research to improve sustainable control strategies.

Monday 17 August 2015

Concurrent Session 1.1

A: Drugs and drug resistance

Ref: O004/0526

High prevalence of Fasciolosis and evaluation of drug efficacy against *Fasciola hepatica* in dairy cattle in the Maffra and Bairnsdale districts of Gippsland, Victoria, Australia

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Liver fluke (*Fasciola hepatica*) infection is common in grazing livestock in the south-eastern region of Australia and is responsible for significant production losses in the beef and dairy industries. Gippsland in Victoria is a major region for dairy production but no fluke prevalence data in livestock has been obtained in this region since 1980, prior to the introduction of Triclabendazole (TCBZ). TCBZ resistance is now widespread in cattle in south east Australia. Here, we evaluated the prevalence and intensity of liver fluke infections in dairy cattle in Gippsland and assessed the efficacy of TCBZ and other drenches against *F. hepatica* on one farm. We obtained 30 individual faecal samples from each of 15 different farms and, using the liver fluke coproantigen ELISA, tested bulk faecal samples pooled from each farm with any positive farm having all of the samples tested individually to assess the intra-herd prevalence. One farm in the Maffra district also had a coproantigen reduction test and faecal egg count reduction test to assess the efficacy of TCBZ, Clorsulon (CLOR) and Oxytoclozanide (OXY). The coproantigen ELISA proved to be a highly sensitive test for liver fluke with a high correlation ($R^2 = 0.8849$) observed between ELISA data from bulk samples and individual samples, suggesting that future larger scale screening on farms for fasciolosis could use the bulk analysis technique. The ELISA data revealed that animals on 6/15 farms were infected with *F. hepatica* with the herd prevalence ranging from 47-100% (mean 81%) which exceeds the prevalence value for production losses of 25%. The intensity of fluke infection in cattle varied considerably, both within and between herds, with a proportion of animals exhibiting a positive control value in the coproantigen ELISA of 50-88%. We also confirmed that TCBZ resistance was present on one farm but that CLOR or OXY can be used to remove the adult stage of the TCBZ-resistant parasites. We conclude that fasciolosis is a significant disease and a likely cause of production losses in dairy cattle in the irrigation zones of Gippsland and that TCBZ resistance is a serious threat to fluke control. More work needs to be performed in Gippsland to further define the full extent of fasciolosis and drug resistance and to ensure that effective chemical and non-chemical methods of fluke control are incorporated on farms in order to improve animal welfare and reduce financial impacts on producers.

Monday 17 August 2015

Concurrent Session 1.1

A: Drugs and drug resistance

Ref: O005/0667

A quantitative and qualitative analysis of uptake and attitudes of sustainable parasite control practices by UK sheep farmers

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Introduction: To facilitate the uptake of 'best practice' approaches to nematode management, a comprehensive understanding of the various factors such as farmer attitudes and farm structure that may influence stakeholder's engagement and adoption is required.

Materials and Methods: A retrospective analysis was conducted of historic questionnaire surveys of producers in 2000 (n= 97) and 2010 (n= 104 and 176) respectively. The objectives were to identify and compare trends in the uptake of nematode control practices and to evaluate factors associated with uptake advice, such as farming demographics and information sourcing. Logistic regression models were applied for Univariate and Multivariate analysis of variables. The output results (odds ratios) indicate the likelihood and strength of association between exploratory and response variables. Furthermore a telephone survey (n = 400) based on the theory of planned behaviour was conducted in 2015 to investigate the interaction between farmer attitudes and worm control behaviours.

Results: A shift towards the use of best practice advice is observed between time points, most notably a decline in the movement of treated animals to clean grazing as well as an overall reduction in treatment frequency. Statistical analysis identified statistically significant associations between farm characteristics and specific treatment strategies. Larger farms were more likely (p=0.036) to adopt a set treatment regime compared to smaller farm which were more likely (p=0.021) to treat based on clinical signs of infection. Sourcing of roundworm control information primarily from veterinarians was most associated with treatment timings. In 2015, >90% of respondents agreed/strongly agreed that worm control impacted on productivity and profitability of livestock but only 40% thought it was a threat to their business. 88% believed that a new anthelmintic class is crucial for the future of roundworm control.

Conclusions: Some evidence of changes in worm control practices between 2000 and 2010, which can help reduce the rate of resistance development. Furthermore, factors associated with high and low risk worm control practices were identified which can be considered when informing future recommendations.

Monday 17 August 2015

Concurrent Session 1.1

A: Drugs and drug resistance

Ref: O006/0427

Does refugia play a role in slowing down anthelmintic resistance?

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Maintaining a proportion of a population in refugia, i.e. not exposed to drug treatment, has long been acknowledged in entomology and helminthology as an important consideration for sustaining genes for drug susceptibility in a population. The practice of “dose and move” (D&M), i.e. administering anthelmintic to animals immediately prior to moving them onto pastures with low numbers of nematode larvae, is now generally accepted as being a ‘high’ risk practice for selecting anthelmintic resistance but, because of perceived production benefits, is still commonly used by UK sheep farmers.

This study aimed to examine the impact of refugia on both production and anthelmintic resistance indicators when using D&M or move and dose (M&D) strategies to control *Teladorsagia circumcincta* and *Haemonchus contortus* over a grazing season. A replicated field trial was set up with four groups; D&M or M&D, with either high (H) or low (L) refugia status. Eighty parasite-naïve lambs were infected with *T. circumcincta* and *H. contortus* with a resistance status estimated to be around 95% efficacy against fenbendazole (FBZ) and grazed on pasture seeded with a population of nematodes with a similar resistance profile. Several parameters were measured over the grazing season: lamb weight gain, faecal egg counts, pasture larval counts, pasture mass, tracer and permanent lamb worm burden, and FBZ resistance status, as measured by egg hatch tests. On day 0, lamb weights ranged between 11-30Kg with an average per group of between 24-24.7Kg. Low refugia groups had greater daily liveweight gain with D&M, averaging 180g per day over the course of the treatment trial. Lambs were treated twice with FBZ over the course of the grazing season. All groups showed a mean FBZ efficacy of 99% at first treatment and after the second treatment, all groups had reduced FBZ efficacy, with D&M-L showing a mean of 20% efficacy and D&M-H preserving the greatest mean efficacy at 77%. M&D groups both had mean efficacies around 38%. Egg hatch tests showed a shift in ED50 values in all groups post-treatment with FBZ. D&M-L showing the greatest shift in ED50 values between the first and second treatment (2.8 fold increase). To conclude, low refugia may be beneficial for lamb performance, however it does appear to contribute to selection for anthelmintic resistance in populations carrying a low percentage of resistance genes.

Monday 17 August 2015

Concurrent Session 1.2

B: Climate change, epidemiology and modelling

Ref: O007/0039

Endemic status of tick-borne infections and tick-species diversity amongst transhumant zebu cattle in Karamoja region, Uganda: implications for control approaches

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The tick-species diversity, seroprevalence of *Anaplasma marginale* and *Theileria parva*, and associated risk factors were investigated amongst cattle under a transhumant system in Karamoja region, Uganda. Serum was collected from cattle (n=300) of all ages selected by systematic sampling in 20 superherds. The MSP-5 competitive inhibition enzyme-linked immunosorbent assay and indirect fluorescent antibody test were used to detect antibodies against *A. marginale* and *T. parva*, respectively. Ticks were also collected and identified. Pastoralists were interviewed regarding herd characteristics, and a series of proportional piling were conducted with 20 focus groups to estimate the age-specific morbidity for tick-borne diseases (TBDs). The overall *A. marginale* and *T. parva* seroprevalences were high-89.7% (95% confidence interval (CI) 83.2%, 96.2%) and low-11.3% (95% CI: 1.8%, 20.9%), respectively. Cattle of more than two years of age were significantly ($p < 0.05$; OR=4.9) at a higher risk for *A. marginale* relative to those of 13-24 months and calves of 5-12 months. *Rhipicephalus appendiculatus* was the most abundant tick species (54.4%). Rare tick species to Uganda were collected: *Amblyomma lepidum*, *Hyalomma truncatum*, *Amblyomma gemma* and *Rhipicephalus pulchellus*. Two thirds of the cattle sampled had moderate (37.3%, 11-50 ticks) to abundant (28.6%, >50 ticks) ticks. The pastoralists indicated that TBDs affected all age groups of cattle. The present results may indicate that the region is endemically unstable for East Coast fever, probably due to limited exposure of calves below six months to *T. parva*, and endemically stable for anaplasmosis due to the broader transmission dynamics of anaplasmosis.

Monday 17 August 2015

Concurrent Session 1.2

B: Climate change, epidemiology and modelling

Ref: O008/0118

Molecular detection of tick and tsetse-borne bovine pathogens in Nigeria

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A total of 128 blood specimens were collected from apparently healthy cattle from north eastern and central Nigeria. All the specimens were initially screened for the presence of *Babesia*/*Theileria* and *Ehrlichia* / *Anaplasma* genomic DNA using PCR and Reverse line blot assays. The same specimens were further screened for trypanosome species using a single polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay, used to characterize all important trypanosome species. The RLB results showed that all 128(100%) of the cattle were positive for at least one or more parasites. The most common parasites were *Anaplasma marginale*, *Babesia bovis*, *Theileria mutans*, *Theileria velifera*, *Babesia bigemina* and *Theileria annulata*. The results of the PCR-RFLP showed that 23 % of the cattle were positive for trypanosomes. The only encountered species were *Trypanosoma theileri* 27(90%), *Trypanosoma brucei* 2(7%) and *Trypanosoma vivax* 1(3%). The results also show that the bovine population harbour varied haemoparasites, some of which are reported for the first time in Nigeria.

Monday 17 August 2015

Concurrent Session 1.2

B: Climate change, epidemiology and modelling

Ref: O009/0128

Bovine theileriosis in Australia: the state of play

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Bovine theileriosis is a tick-borne disease caused by protozoan parasites of the genus *Theileria*, particularly in subtropical parts of the world. In Australia, *Theileria* was first detected in cattle in the early 1900s, and infection has become endemic in some regions of the east of the country. The infection is thought to have been introduced from Japan into Australia via cattle infested with the ixodid tick *Haemaphysalis longicornis*. Traditionally, *Theileria* infection in cattle in Australia was usually asymptomatic and recognized to be associated with *Theileria buffeli*, although some clinical cases had been reported in Queensland in the 1960s. *T. buffeli* is now recognized as one of the members of the *Theileria orientalis* complex. Since 2006, there have been many outbreaks of theileriosis in dairy and beef cattle, mainly in New South Wales and Victoria, which have been linked mainly to two 'virulent' genotypes *ikedai* and/ or *chitose* of this complex. A recent study showed that bovine theileriosis can lead to significant milk production losses in dairy cattle. This paper reviews research on bovine theileriosis in Australia.

Monday 17 August 2015

Concurrent Session 1.2

B: Climate change, epidemiology and modelling

Ref: O010/0136

Molecular epidemiological investigation of equine piroplasmosis in Punjab, India

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Equine piroplasmosis (EP), caused by obligatory intracellular haemoprotozoan parasite *Babesia (Theileria) equi* is an unsystematically reported disease in India. The scarcity of information regarding the epidemiological status, risk factors and associated haematobiochemical effects of latent EP in Punjab (India) prompted authors to undertake this pioneer investigation. Out of 464 equines (248 male and 216 female) screened from five major agroclimatic zones of Punjab, 11.39 and 3.58 per cent revealed *T. equi* infection by molecular detection and microscopy, respectively. Donkeys/mules were at 2.87 times higher relative risk of infection (28.95%) than horses (10.09%). Female equine population (15.28%) was at about twice the risk of infection as compared to the male population (08.47%). Certain farm practices had marked impact on the incidence of *T. equi* such as organised farms (6.58%) as compared to unorganised farms (21.25%) and commercial farms (22.5%) as compared to recreational horse farms (5.92%). The farms with tick vectors (48.15%) had about 5 times higher propensity of *T. equi* infection as compared to those where ticks were absent (9.38%). *Hyalomma anatolicum anatolicum* (14.71%) ticks were having higher infection rate as compared to *Rhipicephalus microplus* (6.25%) ticks. Changes in the haemogram and biochemical parameters indicated pathological and pathophysiological situation in microscopic and PCR based positive cases, respectively. Nucleotide sequence analysis of PCR product showed similarities to isolates of *T. equi* from Brazil, Spain and South Africa. Present study revealing the endemicity of EP in Punjab, emphasizing the physiopathological manifestation associated with latent infection of *T. equi* detectable by PCR is the first report of its kind from India.

Monday 17 August 2015

Concurrent Session 1.2

B: Climate change, epidemiology and modelling

Ref: O011/0453

Corridor disease (*Theileria parva* buffalo-associated infection in cattle): Development of the carrier-state in naturally infected cattle in South Africa

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East Coast fever, a fatal disease of cattle caused by cattle-derived *Theileria parva* and transmitted between cattle by *Rhipicephalus appendiculatus*, was eradicated from South Africa by 1955. Corridor disease, another fatal form, associated with carrier buffaloes exists and outbreaks have increased in endemic areas. A buffalo-derived *T. parva* carrier state in cattle under natural situations has not been demonstrated, but remain a subject of controversy. The current study followed a severe outbreak that occurred in 2013 affecting cattle introduced onto a game park. Monitoring of the outbreak included clinical signs, mortality, the IFA test and real-time PCR, and tick pick-up and transmission attempts from recovered to susceptible cattle under controlled conditions. The cattle received block treatment using oxytetracyclines by the farmer during the period of outbreak. As control, ticks infected with a buffalo-derived stock of *T. parva* were fed on one bovine and simultaneously injected with oxytetracyclines simulating the infection and treatment method of vaccination. The bovine survived and its carrier state was confirmed by PCR. Clean nymphal ticks were applied onto this bovine and onto recovered cattle from the outbreak which were shown to be PCR positive for *T. parva*, one year after the outbreak. Adult ticks from both pickups transmitted fatal infections in susceptible cattle. This is the first demonstration of cattle to cattle transmission which is related to establishment of a Corridor-disease carrier state after using oxytetracyclines. Corridor disease should be considered a potential emerging disease requiring more stringent control methods, especially for therapeutic induced carrier-states.

Monday 17 August 2015

Concurrent Session 1.2

B: Climate change, epidemiology and modelling

Ref: O012/0265

***Angiostrongylus* hot spots in the UK – preliminary spatial epidemiological investigations**

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Angiostrongylus vasorum is a parasitic nematode that may cause cardiopulmonary disease, neurological signs and/or coagulopathies in the dog. Our recent nationwide survey established that the parasite was widely distributed in the UK, was being seen in areas where it had not been previously reported and that there were two endemic foci, or 'hotspots' of infection, in Greater London/south-east England and south Wales.

This study was designed to assess the extent to which the geographical distribution of hot spots of canine infection with *Angiostrongylus vasorum*, established in the UK on the basis of a postal survey of veterinary practices, could be explained by a number of environmental, host and bioclimatic factors. The investigation was conducted in three stages. Firstly, a number of key environmental, host and bioclimatic factors that might account for hot spots of canine infection were identified, including, although not necessarily confined to, host (dog and fox population density and distribution), climatic (temperature and rainfall) and environmental factors (land cover and vegetation). Secondly, climate forecasting was carried out and interpreted in light of the identified distribution of hot spots of infection. Finally, boosted regression trees (BRT) were used to model the probability of occurrence of *A. vasorum* cases as a function of the factors described above.

Dog population density, vegetation index (in December), land cover and average rainfall were the key risk factors identified using BRT, providing possible explanations for the previously-identified clusters of infection in south-east England and south Wales. Maps showing regions suitable for *Angiostrongylus* infection are presented and may be used to alert practitioners to a higher risk of local infection. Funding provided by Bayer Animal Health is gratefully acknowledged.

Monday 17 August 2015

Concurrent Session 1.2

B: Climate change, epidemiology and modelling

Ref: O013/0454

Patchy distribution of slug species and prevalence of infection with *Angiostrongylus vasorum* in two similar cities in the southern United Kingdom

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Angiostrongylus vasorum is an important metastrongylid nematode affecting dogs, and gastropod molluscs as intermediate hosts. It has been reported previously in Wales, and south-east and central England, and now is expanding to northern England and Scotland. The parasite distribution appears to be heterogeneous or patchy and little attention has been paid to the reasons behind this patchiness. A novel real time PCR assay to detect *An. vasorum* infection in slugs has the potential to improve our knowledge of the distribution of infection in different slug species, and its relation with the patchiness and emergence of the disease. The study was conducted in two nearby cities, Swansea in south Wales (a known endemic hotspot for *An. vasorum*) and Bristol in south-west England (where reported cases are rare). In each location, slugs were sampled from nine sites across three broad habitat types (urban, suburban and rural). A total of 526 slugs were collected in Swansea in different season which is autumn 2012, spring 2014 and summer 2014 while 338 slugs were collected in summer 2014 in Bristol. 10mg of slug foot tissue were tested for the presence of *An. vasorum* by amplification of the second internal transcribed spacer (ITS-2). There was a significant difference in the prevalence of *An. vasorum* in slugs between cities ($p < 0.01$): 29.4% in autumn 2012, 1.7% in spring 2014 and 3.6% in summer 2014 in Swansea and 0.3% in summer 2014 in Bristol. In autumn 2012 in Swansea, there was a substantially higher prevalence of *An. vasorum* in suburban areas compared to rural and urban areas ($p = 0.026$). By comparing slug fauna according to season, *Arion rufus* was significantly more common in Swansea ($N=70$) than Bristol ($N=29$), and in Swansea was more abundant in autumn ($N=68$) and less in summer ($N=2$) ($p < 0.05$). This could explain low infection prevalence in Bristol. In the absence of *Ar. rufus* as a preferred host for *An. vasorum*, *Ar. flagellus* and *Limacus maculatus* appear to act as versatile hosts that are present in both summer (prevalence in Swansea in *Ar. flagellus* 5.1%, $N=2/39$; *L. maculatus* 6.6%, $N=1/15$) and autumn (prevalence for *Ar. flagellus* 6%, $N=7/27$; *L. maculatus* 44%, $N=8/18$) ($p < 0.05$), and so provide *An. vasorum* with an alternative vehicle to reach the final host. We conclude that the composition of the slug fauna varies not only seasonally but also spatially, within and between cities, and that this could explain patchiness in the prevalence of *An. vasorum*.

Monday 17 August 2015

Concurrent Session 1.2

B: Climate change, epidemiology and modelling

Ref: O014/0368

***Toxocara* eggs in the environment: quantifying the contribution of different definitive hosts.**

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The relative contribution to the environmental contamination with *Toxocara* eggs by household dogs, household cats, stray cats, and foxes, all older than six months was quantified. Younger animals were not included because the currently advised deworming regimen for pet animals younger than six months should be propagated. To this goal, the model proposed by Morgan et al. (2013) was modified and informed with both original and published data on host density, prevalence and intensity of infection, proper disposal of faeces by dog owners, cats' access to outdoor areas, and coprophagic behaviour. Scenario analyses were performed to assess the expected reductions in the daily contribution of eggs produced by dogs according to different deworming regimens and faeces clean-up compliances. Estimates referred to the Netherlands.

Household dogs accounted for 39% of the overall daily egg output of >6 month-old hosts in whole of the Netherlands, followed by stray cats (27%), household cats (19%), and foxes (15%). However, cats as a whole were responsible for the largest contribution (46%) to the environmental contamination by *Toxocara* eggs. Focussing on urban areas, the overall daily egg output was dominated by stray cats (81%). Intervention scenarios were run for household dogs. Only with a high compliance (>50%) of dog owners to the advice to deworm four times a year the contribution of household dogs was estimated to be reduced to 28%. When 50% of the dog owners would be committed to always clean up their dogs' faeces, the expected contribution of dogs would drop to 20%.

We concluded that household dogs of six months of age and older are the main contributor to the overall contamination of the environment with *Toxocara* eggs, though cats as whole (i.e. both owned and stray) overcome the contribution of these dogs. To reduce the number of *Toxocara* eggs shed in the environment by household dogs older than six months by 50% or more, a higher compliance of dog owners to intervention advices is necessary than that reported in several studies. Moreover, actions focusing solely on household dogs and cats will never result in the elimination of *Toxocara* eggs from the environment, as stray cats and foxes are significant contributors as well, with stray cats being the most important contributor in urban areas.

Monday 17 August 2015

Concurrent Session 1.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O015/0218

Beneath the surface: Structure-activity relationships and the anthelmintic properties of ellagitannins and gallotannins.

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The use of synthetic drugs against gastrointestinal nematodes has led to a situation where resistance to anthelmintics is widespread and there is an urgent need for alternative solutions to control these ruminant parasites. One approach is to use bioactive plants and various *in vitro* results have been obtained on the anthelmintic properties of different plant extracts. Previous studies have shown the effect of tannin-rich forage to disturb the biology of intestinal nematodes of ruminants. However, most studies have focused on condensed tannins and no previous study has studied anthelmintic properties of ellagitannins or galloylglucoses/ gallotannins. Furthermore, it is essential to estimate which specific tannin structures are behind the positive effects of tannin-rich plants. In the present work, we studied the *in vitro* effect of series of 27 ellagitannins, 7 galloylglucoses/ gallotannins and their hydrolysis product, gallic acid, on egg hatching and motility of L1 and L2 stage larvae of the *Haemonchus contortus*. The effect of selected compounds on egg and larvae structure was further studied by scanning electron microscopy. The results indicated that the activity of these compounds depended on many structural features, including size, functional groups present in the structure, stereochemistry of certain functional groups, and the structural rigidity of the studied compounds. This better knowledge obtained on the activity of single ellagitannins and galloylglucoses/ gallotannins should promote the selection of most suitable plants to be tested for their anthelmintic properties.

Monday 17 August 2015

Concurrent Session 1.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O016/0299

2H-chromen-2-one an anthelmintic-like phytochemical isolated from *Gliricidia sepium* leaves.

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The emergence of anthelmintic resistance in cattle nematodes hardens to maintain worm burdens under optimal parameters, compelling novel approaches to be developed. *Gliricidia sepium* (GS) is a tropical forage legume with known anthelmintic-like properties; though, elucidation of the active molecule has not been performed. This study aimed to: 1) isolate anthelmintic-like phytochemicals from GS on basis of the ovicidal effect against *Cooperia* spp., and 2) assess ultrastructural damage engendered over *Cooperia* spp., eggs. Anthelmintic activity of GS was traced from an acetonic extract through the egg hatching inhibition assay (EHIA; 9.6 mg / ml). Phytochemicals were isolated through silica gel columns and elucidated through spectroscopic measurements (1H and 13C NMR). Final fraction was evaluated with EHIA using decreasing concentrations: 1.1; 0.50, 0.250, 0.125, 0.06 mg / ml. Scanning and Transmission electron microscopy (SEM and TEM) allowed the observation of ultrastructural changes engendered in *Cooperia* spp., eggshells. Kruskal-Wallis test was used to evaluate the anthelmintic effect among fractions. Egg hatching inhibition was calculated with the formula: $100 \times (1 - H_T/H_C)$. Lethal concentrations were calculated with a PROBIT analysis. Bio-guided isolation procedures lead to the elucidation of 2H-chromen-2-one which inhibited both hatching and embryo development of *Cooperia* spp., (LC₅₀ of 0.024±0.085 mg/ml) ($P < 0.05$). Electrodensity alterations and fractures were observed through the eggshell layers. After toxicity evaluations and *in vivo* assessment, 2H-chromen-2-one could be suggested as a novel AH-phytochemical for reducing larval density in pastures and worm burdens inside the host.

Monday 17 August 2015

Concurrent Session 1.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O017/0491

Insights into scabies mite's internal microbiome and identification of potential endosymbionts

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Scabies is a contagious skin disease caused by a parasitic mite, *Sarcoptes scabiei*. The mite burrows into dermis of the skin and causes intense itching. Scabies is often perceived as a simple itch, however it is an important risk factor for serious secondary infections with bacterial pathogens such as *Staphylococcus aureus* and *Streptococcus pyogenes*. Current treatment for scabies is limited to a number of acaricides and the mite's resistance to these drugs are emerging. Further there are no molecular tools for the diagnostic of scabies because the disease symptoms cannot be easily differentiated from other dermatological conditions. Relatively recently, many parasitic ticks and mites are reported to contain heritable endosymbionts that are essential for the arthropod host, and as such these bacteria have been targeted as a mean to control the parasites. Here we characterise the internal microbiome of scabies mites and investigate whether the mites carry endosymbiont. We used two parallel approaches; metagenome sequencing by Illumina HiSeq 2500 platform and 16S rDNA sequencing by 454 pyrosequencing. From a porcine model two different life stages of mites, specifically the adult females and eggs were collected. Single mites and egg samples were collected for the 16S rDNA sequencing and pools of adult female mites and eggs were collected for metagenome sequencing. Various washing techniques were used to remove external bacteria from samples, ie. 4% paraformaldehyde solution for 16S rDNA and 0.6% sodium hypochlorite solution for metagenome sequencings. Total DNA from samples were extracted and the microbita of each life stages were investigated. We identified a number of bacterial genera that may be important for the biology and pathogenicity of scabies mites, including potential endosymbionts. 16S rDNA analysis showed that *Corynebacterium*, *Pseudomonas* and *Klebsiella* were dominant in both life stages. High abundance of *Corynebacterium* was observed in both eggs and adults. Relative abundance of *Pseudomonas* was higher in eggs than in adult, while abundance of *Klebsiella* was higher in adults than in eggs. Metagenome analysis revealed that eggs contained significantly less bacteria than adults. Only 3% of contigs were identified as bacteria in eggs in contrast to 37% of contigs identified as bacteria in adults. Interestingly DNA sequences identified as potential *Streptomyces* and *Wolbachia*, which are known as endosymbionts of a number of arthropods were found in both eggs and adults. We are currently further investigating to confirm the presence of these potential endosymbionts in scabies mite by qPCR and FISH. Bacteria that are intrinsically linked to *S. scabiei* could not only be used to target scabies but also as biomarkers for diagnosis.

Monday 17 August 2015

Concurrent Session 1.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O018/0628

Anthelmintic effects of sainfoin against different cattle nematodes may be linked to concentration of condensed tannins in different gut compartments

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Objectives: We investigated the potency of a forage legume (sainfoin, *Onobrychis viciifolia*) against gastrointestinal nematodes in relation to concentration of condensed tannins (CT) along the digestive tract in cattle.

Methods: Fifteen 2-4 month-old Jersey calves were divided into two groups and fed either sainfoin pellets (SF, n=9) or starter feed pellets and ryegrass hay (CO, n=6). Diets were balanced for crude protein and net energy during the entire study. Then, calves were infected with 10,000 *Ostertagia ostertagi* and 66,000 *Cooperia oncophora* third-stage larvae (L3). The number of eggs per g of dried faeces (FECDM) was followed until 42 days post infection when the calves were euthanized for worm recovery and sampling of digesta for analysis of CT. The HCl-butanol-acetone and *in situ* thiolysis methods were used to assess the CT content and structural composition.

Results: The main finding was a 50 % reduction in adult worm counts for *O. ostertagi* in SF as compared to CO ($p < 0.05$). In contrast no difference was observed between groups for *C. oncophora*. Overall, FECDM was 40% lower in SF compared to CO ($p > 0.05$). The CT content was 6.5% of dry matter (DM) in sainfoin pellets and gradually increased along the digestive tract (5.9, 6.6 and 8.1% DM in the abomasum, small intestine and faeces, respectively). However, *in situ* thiolysis indicated more CT in the abomasum and less CT in other locations of the digestive tract. The CT contents for pellets, abomasum, small intestine and faeces were 1.9, 2.2, 0.01 and 0.5% DM, respectively. This suggests that CT become less accessible and reactive to the thiolysis reagent as the feed passes along the digestive tract.

Discussion: Feeding sainfoin significantly reduced the worm burden of *O. ostertagi* in the abomasum where CT are thought to be released from complexes with macromolecules due to acidic conditions. The lack of effect against *C. oncophora* in the small intestine could be related to an apparent unavailability of CT in this gut compartment.

Monday 17 August 2015

Concurrent Session 1.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O019/0353

Shaking up our approach to Gastrointestinal nematode control: Target fitness not faecal egg counts

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Ruminant health and production is severely limited by gastrointestinal nematode (GIN) infections. Efforts to develop effective and sustainable GIN control measures are ongoing. Currently, there appears to be an increasing trend in experimental research that relies upon the use of a single GIN life trait to evaluate the efficacy of a potential control: the capacity for the GIN to establish in their host. Establishment is predominantly measured using faecal egg counts (FEC) as a proxy, a measure whose accuracy has already been the cause of some debate. Reducing the number of GIN establishing in their host is indeed a positive step in GIN control, but it provides a very limited and misguided understanding of the consequent GIN infection dynamics. It tells us nothing of the following reproductive and survival capacity of the GIN thereafter. Here, we propose a more holistic and robust measure that we believe should form the basis of all GIN control in the long term: fitness. Fitness encapsulates all efforts by an organism to survive and reproduce throughout their life cycle. Reducing GIN fitness will theoretically reduce their success at several life traits and weaken consequent generations. This work provides several examples to demonstrate that while many of the most commonly used GIN control methods can reduce the success of a single life trait, these negations are followed by a 'compensation effect' whereby consequent life traits are augmented to maintain similar levels of fitness despite the challenge. The examples provided are relevant to controls using anthelmintic drugs, genetically resistant sheep and pasture management techniques. We conclude that by targeting GIN fitness, greater strides can be made in attaining effective and sustainable GIN control. An integrated parasite management approach to achieve this is discussed. Finally, we offer a method to measure GIN fitness that can be easily incorporated into experimental research without additional financial or labour costs.

Monday 17 August 2015

Concurrent Session 1.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O020/0699

Evaluation of entering cattle fecal egg counts and weights as indicators of response to deworming in a backgrounding operation

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Increasing levels of anthelmintic resistance in grazing animals worldwide have resulted in development of a number of strategies to reduce exposure of parasite populations to anthelmintics, including the use of targeted selective deworming strategies that concentrate anthelmintic use in the most heavily infected animals without substantially sacrificing economic productivity of the entire herd. This approach has been most successful with small ruminants. Devising a similar predictive system for selective deworming of beef cattle is more difficult. This study evaluated possible predictors of the benefit of deworming steers at the time of entry into a backgrounding period. Three hundred and thirty four fall born weaned steers from eight individual farms in Virginia that are part of the Virginia Department of Corrections farm system were entered into a backgrounding operation at the end of June and remained there for approximately six weeks. They were weighed at the beginning of the study and again at the end. Fecal samples were collected in June and August from all animals. At the time of entry into the backgrounding facility, 113 steers were treated with fenbendazole, 101 with doramectin and 120 were left as untreated controls. An additional fecal sample was collected from a subset of treated animals two weeks after deworming to confirm that each deworming treatment was effective. Data were analysed with linear mixed models accounting for farm variation as a random effect. Mean weight at the end of the study was significantly greater in treated than untreated cattle. Fecal egg count at the time of entry into backgrounding was weakly related to weight gain while starting weight was not related to final weight. The greatest variability was associated with farm of origin, which overwhelmed the impact of other factors. In situations such as this, where cattle are amassed from a variety of sources and mingled, application of targeted selective deworming programs may not be feasible.

Monday 17 August 2015

Concurrent Session 1.4

D: Vaccines and immunology

Ref: O021/0277

Identification and characterisation of novel vaccine targets from the salmon louse, *Lepeophtheirus salmonis*

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Sea lice are one of the most economically important parasites of farmed Atlantic salmon (*Salmo salar*), costing the Scottish aquaculture industry an estimated €33 million every year. In recent years, it has become increasingly apparent that the current control methods (usually including extensive use of chemotherapeutants) cannot provide sufficient control and so research has become increasingly focussed on alternative control methods, such as the development of a vaccine. Anti-parasitic vaccines require the identification of antigens which are essential to the survival of the target species, for example digestive proteases or proteins involved in reproduction or parasite establishment. Here, we have identified several potential antigens from the salmon louse, *Lepeophtheirus salmonis*, and used a variety of molecular techniques to investigate their role in this parasite.

The Halloween gene family encode a set of cytochrome P450 enzymes responsible for the production of ecdysone (moulting hormone) in arthropods. Two members of this family, shade and disembodied, were identified in *L. salmonis* and full length sequences were obtained using RACE PCR. Moulting is a potential vaccine target in sea lice as the parasites moult from early larval stages to adults whilst attached to the salmon host. Two cysteine proteases, cathepsin B and cathepsin L, were also investigated. Cysteine proteases have been studied as vaccine candidates in several parasite species with some success. These potential vaccine candidates were localised in *L. salmonis* using *in situ* hybridisation, and temporal expression patterns were characterised using qPCR. Candidates which were shown to be expressed in the sub-cuticular epithelium and gut (and are thus accessible to host antibodies) and which were expressed during the target lifecycle stages will be taken forward for future small-scale vaccine trials.

Monday 17 August 2015

Concurrent Session 1.4

D: Vaccines and immunology

Ref: O022/0282

Characterisation of host-parasite interactions between the salmon louse, *Lepeophtheirus salmonis*, and Atlantic salmon, *Salmo salar* using tissue culture systems

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The sea louse, *Lepeophtheirus salmonis* is economically detrimental to the salmon industry. The use of integrated pest management strategies has provided some control; but a commercially viable vaccine to reduce infection would be highly advantageous. Current approaches for vaccine development involve using large numbers of fish; however, development of *in vitro* Atlantic salmon tissue models would assist research on host-parasite interactions and reduce research animal use. Fish scale models could provide the structural support and tissue stability needed for maintenance of sea lice but there are limited studies concerning their use. Investigations into culturing scale-associated epithelial cells were undertaken, examining various parameters to optimise conditions needed. From initial experiments, caudal fish scales arranged in groups provided successful outgrowth of epithelial cells under standard tissue culture conditions, and extracellular matrix proteins were shown to enhance outgrowth. The potential of the developed culture system to support different sea louse larval stages was examined with regards to attachment of the motile infectious copepodid and survival of the sessile larval chalimus stage. Lice were successfully maintained for > 10 days. Toxicity studies to investigate the effect of cell culture products on sea lice highlighted difficulties in model development and use of anaesthetics in harvesting tissue were found to affect the viability of attached lice stages. Development of a culture technique that allows maintenance of sea lice larvae could provide a platform for investigation of localised host-parasite interactions and with further optimisation could limit the requirement for use of fish for sea lice and other aquatic ectoparasites.

Monday 17 August 2015

Concurrent Session 1.4

D: Vaccines and immunology

Ref: O023/0661

TLR2 and TLR4 transcriptions in fresh blood from dogs with canine leishmaniosis due to *Leishmania infantum*

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Innate immunity, in particular the role of toll like receptors (TLRs), has not been studied in canine *L. infantum* infection in detail. The correlation between TLR expression and clinical, immunological and parasitological parameters can help to better understand host-pathogen interactions in canine *L. infantum* infection and define the specific functions of certain TLRs in this disease. We hypothesize that TLR2 is upregulated while TLR4 might be down-regulated or unchanged in dogs with moderate disease. The main aim of this study was to determine the transcription of TLR2 and TLR4 in the blood of dogs with natural clinical leishmaniosis (Leishvet stage II, moderate disease) at the time of diagnosis and to compare it with a healthy control group. A second objective was to establish the best housekeeping transcriptional genes for canine blood samples. Twenty seropositive sick dogs with a high antibody level were studied and compared with 6 healthy seronegative dogs. Clinical status was defined by thorough physical examination, full CBC, biochemistry profile, serum electrophoresis, urinalysis and urinary protein creatinine ratio in all dogs. Fresh EDTA blood was stored in RNA later followed by RNA extraction and cDNA production. Six housekeeping genes (HMBS, SDHA, TBP, CG14980, HPRT1 and B2M) were assessed by real time PCR to establish the most stable genes as assessed by the Genorm program with 10 representative samples. Based on V and M values, the best housekeeping genes found were HPRT1, CG14980 and SDHA. TLR2, TLR4, HPRT1, CG14980 and SDHA were studied in each dog blood sample by real time PCR. Relative quantification of TLR2 was higher (mean 3.9) in sick dogs when compared with seronegative healthy dogs (mean 1.8; p=0.056) while relative quantification of TLR4 was similar between groups. In conclusion, these preliminary novel findings indicate an up-regulation of TLR2 in fresh non-stimulated blood in naturally infected sick dogs when compared to healthy dogs suggesting active innate immune and proinflammatory responses due to the presence of a high *Leishmania* parasite load or cellular damage as observed for other canine diseases. In contrast, TLR4 appears to be similar among groups and might indicate a mechanism of parasite immune system evasion or a less important role of this TLR in clinical canine leishmaniosis.

Monday 17 August 2015

Concurrent Session 1.4

D: Vaccines and immunology

Ref: O024/0035

***Eimeria bovis*-triggered neutrophil extracellular trap formation is CD11b-, CD18-, TLR4-, ERK 1/2-, p38MAP kinase- and SOCE- dependent**

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Eimeria bovis is an important coccidian parasite that causes high economic losses in the cattle industry. We recently showed that polymorphonuclear neutrophils (PMN) react upon *E. bovis* sporozoite exposure by neutrophil extracellular trap (NET) formation. So far, little is known on receptors or molecular mechanisms that are involved in parasite-triggered NETosis. Thus, we here focused on NET-related signal transduction and neutrophil receptors. PMN-sporozoite encounter led to an enhanced surface expression of neutrophil CD11b suggesting a potential role of this receptor in *E. bovis*-mediated NETosis. Antibody-mediated blockage of CD11b confirmed this assumption and led to a significantly decreased sporozoite-triggered NET. Furthermore, antibody-mediated blockage of TLR4 and CD18 led to Etosis inhibition adding these molecules to NET-related PMN receptors. In addition, *E. bovis*-induced NETosis was found to be Ca⁺²-dependent since the specific inhibition of store-operated calcium entry (SOCE) significantly diminished NETs. Furthermore, NADPH oxidase, neutrophil elastase (NE) and myeloperoxidase (MPO) were confirmed as key molecules in sporozoite-triggered NETosis, since inhibition thereof blocked parasite-triggered NETosis. PMN degranulation analyses additionally revealed a significant release of matrix metalloprotease-9 (MMP-9) containing granules upon sporozoite exposure. We further showed a significantly enhanced phosphorylation of ERK1/2 and p38 MAPK in sporozoite-exposed PMN indicating a key role of this signaling pathway in *E. bovis*-mediated NETosis. Accordingly, ERK 1/2 and p38 MAPK inhibition led to a significant decrease in NET formation. Finally, we demonstrate that sporozoite-induced NETosis is neither a stage-, species- nor host-specific process.

Monday 17 August 2015

Concurrent Session 1.4

D: Vaccines and immunology

Ref: O025/0068

Establishment of an *Eimeria arloingi* *in vitro* culture and first analyses on parasite-triggered host endothelial cell reactions

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Eimeria arloingi is an intestinal pathogenic coccidian species of goats, which frequently leads to significant economic losses in goat industry. However, the physiopathology of caprine coccidiosis is not yet fully understood. In recent studies, we succeeded to establish a pure *E. arloingi* strain for experimental goat infections and oocysts collection. Here we set up a suitable *in vitro* culture allowing for the intracellular macromeront (meronts I) formation in endothelial cells. Furthermore, we analyzed early *in vitro* responses of host endothelial cells to *E. arloingi* infections by determining mRNA levels of adhesion molecules (E-selectin, P-selectin, VCAM-1, ICAM-1), chemokines (CXCL8, CCL2, CCL5), GM-CSF and COX-2.

Freshly excysted *E. arloingi* sporozoites were used to infect different host cell types: primary bovine umbilical vein endothelial cells (BUVEC) and the permanent cell lines MDBK (Madin-Darby bovine kidney) and MARC-145 (Rhesus monkey foetal kidney). Although sporozoites were able to invade all types of host cells, full macromeront formation and the release of merozoites I was exclusively achieved in BUVEC. Differential supplementation of the cell culture medium with fatty acids, carbohydrates and FCS revealed enhanced glucose (10 mM) as a beneficial supplement for improved merozoite I production.

E. arloingi infection led to host endothelial cell activation since the gene transcription of all adhesion molecules investigated (E-selectin, P-selectin, VCAM-1, ICAM-1) was significantly enhanced within 12 hours of infection. In addition, BUVEC activation due to invasion was shown by significantly increased CXCL8 and CCL2, gene transcription indicating significant early innate reactions of the host cells themselves against the parasite.

Monday 17 August 2015

Concurrent Session 1.4

D: Vaccines and immunology

Ref: O026/0357

Development of a yeast surface display library cell line to examine tick:host interactions

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Tick paralysis is observed worldwide, however *I. holocyclus* in Australia, *Dermacentor andersoni* and *Dermacentor variabilis* in the United States and Canada, and *Rhipicephalus evertsi evertsi* in Africa are of particular importance due to observed toxicoses in humans, companion and livestock animals. *Ixodes holocyclus*, the Australian paralysis tick, is of veterinary and agricultural importance due to its ability to transmit infectious pathogens and the production of a potent neurotoxin, Holocyclotoxin-1 (HT-1). *I. holocyclus* is limited to Australia and has a three host life cycle. Its primary hosts are native bandicoot species with secondary hosts including companion animals and livestock, which often succumb to paralysis symptoms. Current strategies for treatment of companion animals involve either the avoidance of attachment by acaricides or the use of hyper-immune dog serum post infection. Cell surface display libraries (CSDLs) are a powerful tool for screening peptides, expressed single-chain variables or fragment antigens exposed upon the cell surface of *Escherichia coli* or yeast. This methodology allows for high throughput screening and selection for antibodies based on affinity, utilizing fluorescence activated cell sorting (FACS). Current technologies rely upon phage display libraries however significant limitations in affinity maturation and unpredictable expression bias are difficult to overcome. Previous attempts to express HT-1 using prokaryotic expression systems have been unsuccessful. Eukaryotic display libraries utilizing *Saccharomyces cerevisiae* possess protein folding and secretory machines homologous to mammalian cells. In this research, a novel *S. cerevisiae* cell line, IMTV014, was developed to extracellularly display HT-1. After galactose induction, FACS and fluorescence was used to detect the expression of HT-1 using Mouse HT1-specific antibodies. Additionally, HT-1 was purified from the cell wall of IMTV014/ht-1 and EBY100/ht-1 and successfully detected by SDS-PAGE, western blot and FACS. Not only are the IMTV014/EBY100 cell lines useful for CSDL, they can also be used to screen the proteins secreted by salivary glands of *I. holocyclus* during host-parasite interaction to identify proteins essential in this interaction, and highlight potential vaccine candidates and other molecules involved. This is the first display library report established using a tick parasite species.

Monday 17 August 2015

Concurrent Session 1.5

One health

Ref: O027/0120

Risk factors and spatial distribution of *Giardia* spp and *Toxoplasma gondii* in urban dwelling marsupials in the greater Perth region

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Wildlife living in urban areas are at risk of acquiring infections from humans and domestic pets. This is of concern regarding the impacts of such infections on wildlife population health, and the creation of wildlife reservoirs of infection. The marsupials quenda (or southern brown bandicoot, *Isodon obesulus*) and common brushtail possums (*Trichosurus vulpecula*) have remained in many areas of Perth despite urbanisation. We investigated the epidemiology of anthroponotic parasites in these marsupials in the greater Perth region, with a particular focus on *Giardia* spp. and *Toxoplasma gondii*. Primarily, we investigated the hypothesis that quenda and possums living in highly urbanised environments have an increased risk of infection with these parasites, compared to those living in urban remnant bush. We undertook a cross sectional study, sampling 280 quenda and 31 possums across 27 private residential properties, 7 private non-residential properties and 28 urban remnant bush sites in the greater Perth region. Immunofluorescence microscopy and PCR were used in the identification and differentiation of *Giardia* spp infections. The serum modified agglutination test was used to identify marsupials infected with *Toxoplasma gondii* (titre $\geq 1:40$). Results suggest that although *Giardia* spp. infection is common in Perth quenda, in both urbanised and non urbanised areas, it is almost invariably of the non zoonotic “quenda genotype” of *Giardia*. Apparent seroprevalence of *T. gondii* is low, though validation of this diagnostic test is pending. Full results, including statistical modelling and diagnostic test validation, will be presented.

Monday 17 August 2015

Concurrent Session 1.5

One health

0028/0217

Identification of *Cryptosporidium hominis* in Eastern Grey Kangaroo populations in Sydney catchments

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Cryptosporidium is considered as one of the most prevalent waterborne parasitic infections in the world, and represents the major public health concern of water utilities in developed nations. More than 27 species are regarded as valid with *C. parvum* and *C. hominis* the main species infecting humans. In Australia, marsupials (in particular kangaroos), cattle, sheep and rabbits are among the dominant animals inhabiting drinking water catchment and contribute large volumes of manure to water catchments. Therefore, it is important to determine the potential role that these species play in the dissemination of *Cryptosporidium* to drinking water sources and the associated human health risks. We are currently conducting a comprehensive quantitative survey of genotypes of *Cryptosporidium* in marsupials, rabbits, cattle and sheep and in sewage treatment plants (STP) across different catchments and states in Australia, over a three-year period. Data generated will be used to conduct modelling and quantitative microbial risk assessment (QMRA) for the various water catchments. Here, we report the identification of *C. hominis* in faecal samples from Eastern Grey Kangaroos (*Macropus giganteus*) inhabiting Sydney drinking water catchments. Of the 499 Kangaroo faecal samples collected regularly from the catchments area over 14 months, a total of 14 positives were detected by quantitative PCR (qPCR), a prevalence of 2.81% (1.5% - 4.7%, CI: 95%). Sequence analysis at the 18S locus identified *C. macropodum* in 4 isolates and *C. hominis* in 10 isolates. The original faecal samples were sent to the Australian Water Quality Centre (AWQC) for independent verification. The public health implications of the identification of *C. hominis* in kangaroos inhabiting Sydney's largest drinking water catchments will be discussed.

Key words: *Cryptosporidium*, Drinking water catchment, *C. hominis*, *C. macropodum*, Eastern Grey Kangaroo

Monday 17 August 2015

Concurrent Session 1.5

One health

Ref: O029/0234

Tick-borne pathogens carried by *Ixodes ricinus* of autochthonous and migratory birds captured in southern Italy

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The aim of this work was to determine the species of ticks and of tick-borne pathogens (TBPs) carried by autochthonous and migratory birds captured in one of the most important ecological reserves of southern Italy. From April 2010 to April 2011, 212 birds of 22 species (12 families) were captured and examined for ticks. A total of 75 (35.4 %) birds were found infested by ticks, which were all identified as *Ixodes ricinus*. Of the 451 ticks collected, 427 (204 larvae and 223 nymphs) from 70 birds were pooled (104 pools) and screened by PCR for *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Rickettsia* spp., *Babesia/Theileria* spp. and *Candidatus* Neoehrlichia mikurensis. Positive samples were sequenced and phylogenetic analysis performed. The mean number of ticks per bird was 6.1 (range 1-38). Five TBPs were identified, showing a minimum infection rate (MIR) of 17% (*R. monacensis*), 7% (*B. garinii*), 2% (*B. valaisiana*), 1% (*R. helvetica*) and 0.9% (*A. phagocytophilum*). Of the 70 birds with ticks, 54 (77%) harboured positive ticks for one or more TBP. In particular, *R. monacensis* was found in 70 (67.3%) pools of larvae and of nymphs from all the 12 species of birds; *B. garinii* in 27 pools (26%) of larvae and of nymphs from blackbird (*Turdus merula*), great tit (*Parus major*) and blue tit (*Parus caeruleus*); *B. valaisiana* in six pools (5.8%) of larvae and of nymphs from blackbird and mistle thrush (*T. viscivorus*); *R. helvetica* in three pools (2.9%) of nymphs from blackbird and *A. phagocytophilum* in four pools (3.8%) of nymphs from blackbird and house sparrow (*Passer domesticus*). Ticks from blackbirds were positive for all the five pathogens identified. The MIR was similar in nymphs and larvae, with the exception of *B. garinii* significantly more represented in nymphs. Migratory birds, i.e. blackbird, blackcap (*Sylvia atricapilla*) and chaffinch (*Fringilla coelebs*) were significantly more infected with *B. garinii* than local birds (45.6% vs 9.7%; $p < 0.01$), while no difference was found for *A. phagocytophilum* and rickettsiae. The sequences of all the TBPs identified were identical each other; conversely *B. garinii* showed high variability and clustered in six different groups. Migratory birds may introduce ticks and TBPs in new geographic areas, representing a potential source of infection for the local fauna.

Monday 17 August 2015

Concurrent Session 1.5

One health

Ref: O030/0354

What we do know, and what we need to know, about trypanosomes of Australian native mammals

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The indigenous mammalian fauna of Australia has long been recognised to be hosts of a diversity of *Trypanosoma* species largely considered to be host specific and with little impact on wildlife health. Despite their ubiquity and diversity, very little is known about how they are transmitted, their vectorial requirements, developmental biology and host parasite relationships. This lack of knowledge has been highlighted recently with research demonstrating an association between *Trypanosoma* spp. and the decline of the brush tailed bettong or woylie (*Bettongia penicillata*). We have found that the pathogenic potential of *Trypanosoma* infections is associated with the ability of certain species to invade cells and that this is exacerbated by polyparasitism, a phenomenon more frequently seen in woylies from the declining population than in woylies from a stable healthy population as well as in co-habiting species of marsupial. These results will be presented. In addition, the demonstration that marsupial trypanosomes are stercorarian, with some closely related to *T. cruzi*, has raised questions concerning biosecurity given the occurrence of human infections of *T. cruzi* in Australia. These aspects will also be discussed.

Monday 17 August 2015

Concurrent Session 1.5

One health

Ref: O031/0550

High prevalence of *Alaria alata* in farmed wild boars (*Sus scrofa*) in Denmark – preliminary results of ongoing surveillance of zoonotic parasites

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High prevalence of *Alaria alata* in red foxes and raccoon dogs in Denmark has been known for years, and new results have also demonstrated high prevalence in badgers. In contrast, the prevalence in wild boars in Scandinavia is unknown despite recent increasing prevalence of *A. alata* in wild boars from other European countries. At the same time the modern trend of organic pig production, consuming locally produced foods and raw or rare meat may favor emergence or reemergence of pork-borne parasites. Thus, this study was initiated to determine prevalence of important zoonotic parasites in wild boars in Denmark. The study was initiated in November 2014 and will continue throughout 2015. So far 58 wild boars from 5 different enclosures have been examined by the *A. alata* mesocercariae migration technique, the magnetic stirrer method for detection of *Trichinella* spp. and immunofluorescence microscopy and polymerase chain reaction for detection of *Cryptosporidium* spp. and *Giardia* spp. in faeces. In addition, presence of gastrointestinal parasites was analysed by a McMaster technique. Of 58 wild boars a total of 16 (27.6%) were positive for *A. alata*. Wild boars positive for *A. alata* originated from only two areas where prevalences above 50% were detected (10/19 and 6/11, respectively). Results from the McMaster analyses demonstrated *Eimeria* spp. in all animals (100%; 93.8-100%: 95% CI), 39 excreted Strongyle eggs (67.2%: 53.7-79%), seven excreted eggs of *Ascaris* spp. or *Trichuris* spp. (12.1%; 5-23.3%), and five wild boars were shedding Capillarid eggs (8.6%; 2.9-18.9%). Furthermore, five wild boars excreted *Cryptosporidium* spp. (200-1000 oocysts per gram), and *Giardia* sp. (200 cysts per gram) was detected in one animal; molecular analyses are ongoing. Based on these preliminary results we conclude that potentially zoonotic parasites are widespread among wild boars in Denmark. Detection of *A. alata* prevalences above 50% in certain areas where wild boars are hunted for human consumption is particularly disturbing since most meat is consumed locally by the hunters, and little is known about cooking methods that will effectively eradicate the potential human health risk. Thus, more studies are needed concerning infectivity to humans and points of control. Preventive educational campaigns may be necessary to avoid human cases of alariosis caused by insufficiently prepared meat or meat products.

Monday 17 August 2015

Concurrent Session 1.5

One health

Ref: O032/0123

Molecular detection of *Toxoplasma gondii* in water samples from Scotland using an optimised oocyst recovery method

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Waterborne transmission of *Toxoplasma gondii* is a serious public health threat worldwide and as yet there are no agreed optimised methods for the recovery, processing and detection of *T. gondii* oocysts in water samples. Using 1427 samples collected from 147 public water supplies throughout Scotland, modified methods of oocyst recovery and DNA extraction were developed and tested on the range of water sample types available. *T. gondii* DNA was detected using real time PCR (qPCR) targeting the 529bp repeat element and incorporating an internal amplification control. *T. gondii* DNA was detected in 8.79% of the samples analysed. One third of the water plants tested yielded at least one positive sample and these plants were widespread geographically. This is the first study reporting the detection of *T. gondii* DNA in water supplies in Scotland where environmental contamination with the parasite is known to be widespread.

The samples which were positive by qPCR, along with some of the negative samples, were reanalysed using the more established ITS1 nested PCR and results compared. The 529bp qPCR was found to be the more sensitive technique, therefore a full analysis of assay performance was carried out. This demonstrated the high efficacy of 529bp qPCR, when used in conjunction with the modified recovery, processing and DNA extraction methods described here, for the detection of *T. gondii* in water samples.

Monday 17 August 2015

Concurrent Session 2.1

A: Drugs and drug resistance

Ref: O033/0429

Global trends in antimicrobial use in food animals

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Antibiotics are widely used for disease prevention and growth promotion in food animals. In the United States, antibiotic use in livestock is estimated to account for approximately 80% of the annual antibiotic consumption; a significant fraction of which involves compounds that are also important in human medicine in the treatment of common infections and for surgical procedures. Variations in antimicrobial resistance levels across countries are attributable, in part, to differential patterns of antibiotic consumption but also to farming practices and intensification of the livestock production sector. Understanding these variations in consumption is important to monitor the rise of resistance levels in both animals and humans.

In this context, maps are essential tools for implementing rational-use policies of antibiotics, targeting surveillance efforts against antibiotic resistance and provide a baseline for the future assessment of antimicrobial reduction initiatives implemented by government, companies and international organizations. Here, using fine scale maps of the distribution of poultry, cattle and pigs combined with national reports of veterinary antibiotics sales we estimate the global burden of antibiotic use in livestock in 2010, predict its geographical distribution and identify regional hotspots of antibiotic consumption. We further project the potential consumption and geographic distribution of antibiotic use in Asia by 2030 as economic growth will drive an unprecedented development of the livestock production sector.

The cornerstone of our methodology is to estimate the consumption of antimicrobial in milligrams per population correction units (PCU): a standardized livestock weight unit that allows comparison of antimicrobial consumption between different types of livestock and countries. The number of milligram per PCU is derived from estimates of antimicrobial use in 32 member states of the Organisation for Economic Co-operation and Development using Bayesian regression methods.

Monday 17 August 2015

Concurrent Session 2.1

A: Drugs and drug resistance

Ref: O034/0244

Functional diversification of levamisole receptors in the trichostrongylid nematode *Haemonchus contortus*

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The cholinergic agonist Levamisole is widely used to cure parasitic nematode infections. This drug activates a class of acetylcholine receptors (L-AChRs) expressed at the neuromuscular junction. The L-AChR of the free-living nematode model *Caenorhabditis elegans*, is composed of five subunits encoded by *unc-63*, *unc-38*, *lev-8*, *lev-1* and *unc-29*. In contrast, the parasitic nematode of small ruminants, *Haemonchus contortus*, does not require *lev-1*, replaced *lev-8* by *acr-8* and contains four copies of *unc-29*. We examine the functional diversification of these subunits and aim to characterize novel, parasite specific, receptors produced. Reconstitution of the *H. contortus* L-AChR in *Xenopus* oocytes identified channels produced by UNC-29.1, UNC-29.3 and UNC-29.4 with identical affinity for acetylcholine but different sensitivities to levamisole. No responsive channel could be produced substituting UNC-29.2 for UNC-29.1 in *Xenopus*, although immunolocalization showed that the UNC-29.2 was present at the surface. Transfection of a levamisole resistant *C. elegans* *unc-29* mutant strain with each gene copy restores levamisole sensitivity showing these subunits, including UNC-29.2, are functional in *C. elegans*. Specific antibodies allowed us to observe identical expression of UNC-29.1 and UNC-29.2 in the body muscle of both sexes and uterine muscles in the female. UNC-29.4 expression is harder to detect and may be expressed in non-body muscle tissues. The anti-UNC-29.3 antibodies recognize the peptide antigen but fail to recognize the protein.

The *unc-29* copies of *H. contortus* are functionally divergent and produce receptors with distinct pharmacology. In addition, the unusual properties of UNC-29.2 suggest that this may provide an experimental platform to investigate the evolution of subunit interaction and assembly. Taken as a whole, this work establishes critically that evolution between nematode species must be taken into account when using *C. elegans* as a model and that it is essential to examine the molecular mechanisms of drug action in specific parasites in detail.

Monday 17 August 2015

Concurrent Session 2.1

A: Drugs and drug resistance

Ref: O035/0676

Moxidectin transfer via milk and sub-lethal dose of the active to suckling lambs

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Moxidectin is a highly lipophilic, broad-spectrum anthelmintic and when administered to lactating dairy sheep a portion of the dose has been shown to transfer to the mammary gland and eliminated in milk. The pharmacokinetic profile of Moxidectin (MOXD) in milk and plasma of ewes and lambs was evaluated following the administration of a persistent MOXD dose to single-bearing lactating Merino ewes.

At lamb marking, ewes were either treated with a long-acting subcutaneous Cydectin LA injection (1 mg/kg moxidectin) or untreated, while all lambs remained untreated. Blood and milk samples were collected from ewes and blood from lambs prior to treatment (day 0) and on days 1, 7, 21, 35, 49, 63, 77 and 91 days post treatment. Milk yield (mL/d) was calculated on days 0, 35 and 63. Gastrointestinal nematode infection (GI) for both ewes and lambs were monitored via faecal worm egg count (WEC) at the same time blood was collected. The natural infection was predominantly *Haemonchus contortus*. MOXD concentrations were measureable in plasma and milk of ewes to day 91 post treatment, using HPLC analysis. MOXD was detected in lamb plasma to day 35, with a reduction up to 50 % WEC in untreated lambs suckling treated ewes, indicating lambs received a sub-lethal dose of MOXD via milk. Bodyweight gain was higher in lambs suckling treated ewes showing that lambs benefited from partial removal of GI infection and also milk yield in MOXD treated ewes was higher than untreated lactating ewes post treatment. The conclusion made from this research is the milk residual concentration of MOXD in lactating ewes exposes lambs to a prolonged sub-lethal dose of the active which can hasten development of anthelmintic resistance.

Monday 17 August 2015

Concurrent Session 2.1

A: Drugs and drug resistance

Ref: O036/0615

Genetic evidence for the spread of a benzimidazole resistance mutation across southern India from a single origin in the parasitic nematode *Haemonchus contortus*

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It is important to understand how anthelmintic drug resistance mutations arise and spread in order to determine appropriate mitigation strategies. We hypothesized that a molecular genetic study of *Haemonchus contortus* in southern India, a region where resistance may be less advanced than in Western Europe and North America, might provide some important insights into the origin and spread of anthelmintic resistance. The F200Y (T**A**C) isotype-1 β -tubulin benzimidazole resistance mutation is common in *H. contortus* throughout the world and the F167Y (T**A**C) and E198A (G**C**A) mutations, although less common, have been reported in a number of different countries. We have investigated the haplotypic diversity and phylogenetic relationship of isotype-1 β -tubulin benzimidazole resistance alleles for twenty three *H. contortus* populations from small ruminants across southern India. The F200Y (T**A**C) mutation was most common, being detected in 18/23 populations at frequencies between 9 to 84% and the E198A (G**C**A) mutation was also detected in 8/23 populations at frequencies between 8 and 18%. The F167Y (T**A**C) mutation was not detected in any of the 23 populations. Phylogenetic haplotype network analysis suggested that the F200Y (T**A**C) mutation has arisen multiple independent times in the region with at least three independent origins of resistance alleles across the populations surveyed. In contrast, the E198A (G**C**A) was present on a single haplotype which, given the high level of haplotypic diversity of the susceptible alleles in the region, suggests this particular mutation has spread from a single origin; likely by anthropogenic animal movement. Population genetic analysis of twelve of the *H. contortus* populations using a panel of eight microsatellite markers revealed extremely low genetic differentiation between populations consistent with the hypothesis of high gene flow among sites. Additionally, there was no significant genetic differentiation between *H. contortus* taken from sheep and goats consistent with *H. contortus* populations being freely shared between these two different hosts. Overall, these results provide the first clear genetic evidence for the spread of an anthelmintic resistance mutation to multiple different locations from a single origin.

Monday 17 August 2015

Concurrent Session 2.1

A: Drugs and drug resistance

Ref: O037/0733

Anthelmintic resistance in local smallholder goat farms in Malaysia – an escalating problem

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Anthelmintic resistance in small ruminants in Malaysia has been reported since the early 1980's however, several strategies were employed to stem the rapid establishment of resistant worm populations in the small ruminant herds. Strongyle infections especially *Haemonchosis* is the main culprit with approximately 80-90% of small ruminant mortality attributed to this. Anthelmintic usage has been the main method of worm control and thus more than 75% of the farms surveyed showed worm populations resistant to 2 or more drug groups. In a recent survey, 8 goat farms complaining of severe helminthiasis showed that at least 2 groups of anthelmintics (levamisoles and closantel) were ineffective in 50% of the farms. This has led to the use of novel methods of worm control such as rotational grazing, cut and carry method or zero grazing as well as use of herbal remedies such as neem and various other local plants. In the tropics, helminthiasis is the major contributor to morbidity and mortality and there is an urgent need for sustainable control as this issue is related directly with food security for the nation and region.

Monday 17 August 2015

Concurrent Session 2.1

A: Drugs and drug resistance

Ref: O038/0335

A yeast growth assay to characterise interaction of different fungicides and macrocyclic lactones with P-glycoprotein 9 of *Cylicocylus elongatus*

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Macrocyclic lactones (MLs) are frequently used anthelmintics but ML resistance is widespread in parasites of horses and ruminants. P-glycoproteins (Pgps) have often been implicated in ML resistance. Transport of MLs was shown for mammalian Pgps, Pgps on nematode egg shells and very recently for Pgp-2 of *Haemonchus contortus*. To analyse its transporter function Pgp-9 of the equine parasite *Cylicocylus elongatus* was expressed in a *Saccharomyces cerevisiae* strain lacking seven endogenous efflux transporters. In a growth assay, Pgp-9 decreased susceptibility to the fungicides ketoconazole, actinomycin D, valinomycin and daunorubicin but not to thiabendazole. The interaction of MLs with Pgp-9 was investigated in an assay in the presence of the highest ketoconazole concentration not affecting growth. Yeasts were incubated with increasing ML concentrations to determine modulation of ketoconazole transport. Equimolar concentrations of ivermectin and eprinomectin with ketoconazole efficiently inhibited yeast growth and growth was abolished at fourfold higher ML concentrations. In contrast, selamectin and doramectin had no effect on susceptibility to ketoconazole. Moxidectin showed an intermediate interaction with ketoconazole. These results demonstrate direct effects of MLs on a recombinant nematode Pgp in an ML-specific manner. Active Pgp was detected on these yeasts using binding of UIC-2, which was increased in the presence of substrates such as ivermectin, moxidectin, daunorubicin and ketoconazole but not selamectin. This confirms that different MLs show quantitative differences in their interaction with a specific nematode Pgp.

Monday 17 August 2015

Concurrent Session 2.2

F: Genes and genomics

Ref: O039/0041

***Lucilia cuprina* genome unlocks parasitic fly biology to underpin future interventions**

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Lucilia cuprina is a parasitic blowfly that causes flystrike or myiasis. This disease is a serious problem in many countries around the world. In Australasia alone, ~ \$320 million are lost annually due to reduced wool and body growth in sheep as well as costs associated with treatment/control and animal morbidity. There has been a renewed focus on the development of new control strategies to combat the blowfly, including experimental vaccines, genetic transformation techniques and effective insecticides. However, the excessive use of insecticides to date has led to resistance in this insect against almost all currently used compounds. Here, we report the sequence and annotation of the 458 Mb draft genome of *L. cuprina*. Analyses of this genome and the 14,554 predicted protein-encoding genes provide unique insights into the fly's biology, host interactions and insecticide resistance. This genome and associated transcriptomes should assist investigations into many areas, including fly development and reproduction, parasitism, the pathogenesis of disease, population genetics and mechanisms of drug resistance, and are intended to support the development of new methods for the prevention/control of myiasis.

Monday 17 August 2015

Concurrent Session 2.2

F: Genes and genomics

Ref: O040/0252

The scabies mite genome – a targeted molecular approach to identify novel therapeutic targets to treat scabies and mange

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On a global scale, scabies is one of the three commonest dermatological conditions associated with overcrowded living conditions, imposing a considerable economic burden on individuals, families, communities and health systems. The scabies mite also causes significant disease in form of sarcoptic mange in companion, livestock and wild animals. A fundamental problem is that the scabies mite is poorly understood at the molecular level, compared with many other parasites.

We aim to assemble and annotate the scabies mite genome sequence and complement these data with transcriptomic and proteomic analyses. The obligate parasitic life cycle of scabies mites in intact skin cannot be maintained *in vitro*. Consequently, experimental studies are extremely challenging. We have developed a *natural* host experimental model using pigs. This model represents a nearly unlimited source of mites and provides us a major advantage in testing novel drugs and diagnostic methods.

Using a quantitative PCR-based method, we estimated the nuclear genome size of scabies mites to be 97 Mb. Using 360° rotational images of chromosomal spreads from scabies mite eggs, we have determined the chromosome number as 17 or 18 per diplodiploid (2n) nucleus. Using genomic DNA from scabies mites from our pig model and human clinical isolates, we prepared fragment libraries and sequenced them on the Illumina HiSeq 2500 platform. To date we have obtained a contig N50 of 45 kb. Next, we will undertake PacBio long read sequencing to scaffold these contigs. We performed RNA-sequencing and generated > 166 million paired end reads from which we obtained 112,989 contigs with an average length 2.6 kb which aligned to 9,012 unique proteins using the program BLASTX. Further analyses are underway. The transcriptome data will underpin the scabies mite genome annotation. We have generated a high-throughput proteomic database of the scabies mite, as an independent approach to accelerate genome annotation and curation.

Identification of scabies parasite-specific virulence factors, pathways important for host-parasite interactions and candidate therapeutic targets is a major, long-term outcome of this project.

Monday 17 August 2015

Concurrent Session 2.2

F: Genes and genomics

Ref: O041/0376

NGS-based resequencing and reannotation of the *Trypanosoma congolense* genome and transcriptome

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One of the most important protozoan parasites, *Trypanosoma congolense*, is the main causative factor of the Animal African trypanosomiasis. Unlike its closely related and most commonly used model species among the African trypanosomes, namely *Trypanosoma brucei brucei*, the IL3000 strain of *T. congolense* allows to cultivate all four life cycle stages in the laboratory conditions, i.e. the bloodstream, procyclic, epimastogote and metacyclic forms. Although the draft genome sequence of *T. congolense* has been published recently and undergoes systematic updates, the quality of this reference resource is still not sufficient for very detailed molecular analyzes. A big portion of the genomic contigs could not be placed successfully in one of the eleven chromosomes. Moreover, this big grouping of contigs (called BIN) holds a half of the currently annotated genes in *T. congolense*. Many of the predicted open reading frames lack start and/or stop codons, and there is basically no annotation of the untranslated regions (UTRs).

Here, using the next-generation sequencing, we have generated paired-end genomic reads with the Illumina HiSeq 2500 platform, reassembled the genomic sequence and subsequently reannotated the genes. Furthermore, using the high-throughput RNA sequencing (RNA-seq), we have obtained transcriptomic data of the four developmental stages of *T. congolense*. With that information, the stage-specific mRNA levels were determined and the differentially expressed genes between the stages were detected. Additionally, we were able to delineate the exact transcript borders, by precisely mapping the 5' splice-sites and 3' polyadenylation sites for numerous genes, many of which being stage-specific and alternatively spliced or polyadenylated.

The newly generated genomic and transcriptomic data provide a robust improvement to the genome annotation and a solid background for any future detailed molecular studies of *T. congolense*. An essential improvement reached with the use of RNA-seq data is the coverage of UTR information for many genes, since these sequences are known to harbor many cis-regulatory elements. This, together with the information on differential expression and stage-specific genes, can contribute to a better understanding of the complex life style and infectious cycle of this important parasite.

Monday 17 August 2015

Concurrent Session 2.2

F: Genes and genomics

Ref: O042/0507

***Ascaris suum*: A novel functional genomics platform for nematode parasites with potential for organism, tissue and cell-level drug target validation**

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Nematode parasites undermine animal health resulting in significant economic losses and reduced profitability in the agricultural industry. Unfortunately, as resistance pressures rise, effective treatment options for livestock parasites will become increasingly limited. Future control of animal parasitic nematodes (APNs) is reliant on the development of drug discovery platforms in key nematode pathogens to advance novel anthelmintic target identification and validation. RNA interference (RNAi) holds significant appeal in this respect but is hypervariable in veterinary APNs; challenges associated with APN RNAi include optimisation of *in vitro* parasite culture/maintenance conditions and appropriate trigger delivery approaches. *Ascaris suum* is an important veterinary nematode parasite of pigs, and an attractive APN model species due to its unrivaled genomic/transcriptomic resources, and experimental tractability. Here we report the development of a novel functional genomics platform in adult *A. suum* and highlight that: (i) adult *A. suum* are RNAi competent; (ii) adult *A. suum* gene silencing is robust, swift, consistent and persistent; (iii) multiple tissue-specific targets are RNAi susceptible; (iv) RNAi spreads in adult *A. suum*; (v) multiple target silencing is as efficient as single target silencing. The unique experimental tractability and robust gene silencing capabilities of *A. suum* provide a unique opportunity for a functional genomics platform incorporating whole organism-, tissue and cell-level functional analysis for the elucidation and validation of novel drug targets in veterinary parasitic nematodes.

Monday 17 August 2015

Concurrent Session 2.2

F: Genes and genomics

Ref: O043/0467

Intra-specific variation and errors in helminth genomes: a case study with *Haemonchus contortus*.

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The well-publicised advances in DNA sequencing technology are enabling many groups to sequence their favourite organism. Software to perform the assembly and annotation is also becoming more user-friendly. Once published, the community assumes a high level of accuracy. Our work in helminth and protozoan genomes has revealed cases where assembly and annotation errors can have a large and negative impact on analysis and biological interpretation.

Two genome assemblies for the parasitic nematode *Haemonchus contortus* are published. They were sequenced, assembled and annotated independently from different strains. We investigated the variation between the genomes with the aim of discriminating between legitimate biological differences and technical errors in one or both assemblies. We found important discrepancies. The genomes differed in their gene content. Specifically in genes that are known to be highly conserved across all eukaryotes, there was clear evidence for misassembly. Strikingly, only 45% of genes in one genome were orthologous in the other. One genome included many more paralogues than the other. Using the free-living and model organism *Caenorhabditis elegans* as a reference, we used gene location conservation to identify the likely better assembly and annotation.

The differences between the two assemblies far exceeded those expected as a consequence of biological differences between two *H. contortus* isolates. Read depth analysis showed that assumptions on genome size, heterogeneity and the structure are likely to have significantly affected both assemblies.

The findings of this work go beyond *H. contortus* genomics. The availability of two independent projects has allowed the first in depth comparison. For many species, one genome is available and we urge that researchers exercise caution when using such data.

Monday 17 August 2015

Concurrent Session 2.2

F: Genes and genomics

Ref: O044/0665

Exploring the role of microRNAs in *Haemonchus contortus* gene regulation

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The gastrointestinal nematode *Haemonchus contortus* poses major economic and welfare problems for small ruminant production across the world. With the rapid increase in anthelmintic resistance, novel targets for control are required. To this end, we have been studying the role of microRNAs (miRNAs). These short, non-coding RNAs modulate gene expression and are known to be vital in the development of the free-living nematode, *Caenorhabditis elegans*. We propose that miRNAs are also involved in regulating development in *H. contortus*.

Microarray analysis of 223 *H. contortus* miRNAs identified 61 with significant temporal variation across four life-cycle stages (L3, L4, adult males and adult females). 36 of these also demonstrated significant enrichment or depletion in gut tissue compared to adult female worms. Of these variant miRNAs, 39 are conserved in other species and three (miR-60, miR-228 and miR-235) were chosen for further study. miRNA target prediction was performed *in silico*, identifying binding sites across 8,072 genes, representing 33% of the predicted genes in *H. contortus*. Gene ontology was performed on putative homologues of genes with the strongest binding sites, however no enrichment was identified. Using transcriptomic data, the predicted target mRNAs are currently being examined for any relationships to miRNA expression profiles. When cultured *in vitro*, we have found that *H. contortus* L4, but not L3, absorb short, fluorescently-labelled RNA. We are studying the phenotypic effects in L4 of miRNA mimics (for miR-235) and inhibitors (for miR-60).

We have also compared the predicted targets and expression profiles of 34 miRNAs common to both *H. contortus* and *C. elegans*. Expression profiles were similar for 12 miRNAs although few targets were shared in both organisms. Further investigation of *H. contortus* miRNA targets and their expression patterns will identify key developmental pathways that can be used to design novel therapeutic agents.

Monday 17 August 2015

Concurrent Session 2.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O045/0428

The effect of monitoring weight gain in small (sentinel) groups of lambs to trigger whole group anthelmintic treatment

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One control method which has been used to slow the development of anthelmintic resistance in ovine gastrointestinal nematodes is to treat only individuals within the flock, termed targeted selective treatment (TST). A performance-based TST approach, where only individual lambs that do not meet pre-determined weight gain targets are treated, maintains growth rates and drench efficacy, whilst reducing drench usage. However, this approach may not be suitable for all farms due to the frequency of weighing required, and a less intensive regime may be more acceptable. Most farmers currently use strategically-timed whole-group treatments (ST) on predetermined dates; making no evaluation for the need for treatment. This study aimed to combine the knowledge gained from TST with the ease of ST by developing a method for evidence-based, pen-side anthelmintic treatment decisions by monitoring weight gain in a proportion of lambs (sentinel group) to determine the need for whole-group treatment. Field trials were conducted over 2 years to determine: 1. if performance of sentinel groups reflects that of the whole group, 2. optimal size of the sentinel group and 3. weight gain and anthelmintic use under different strategies. Two co-grazing groups of lambs were used: 'control' and 'flock'. Control lambs were treated following TST (year 1) or ST (year 2) (n=20). A proportion of 'flock' lambs were selected at the beginning of the trial and formed the sentinel group (SG). Liveweight gain was monitored in the SG and all 'flock' lambs were treated when 40% of lambs in the SG did not meet weight gain targets. The size of the SG varied from 45% (n=36, year 1) to 20% (n=12, year 2) of the flock. In both years, the performance of the SG reflected that of the 'flock'. When SG was 45%, cumulative weight gain and anthelmintic use was similar between groups ($14.9 \pm \text{SEM } 0.3 \text{ kg}$ vs $15.6 \pm 0.7 \text{ kg}$, mean 2.0 vs 1.5 drenches per lamb, in flock and TST, respectively). When SG was 20% 'flock' lambs received the same number of drenches (2 per lamb) as the ST group but were treated earlier and had a trend for slightly higher cumulative weight gains ($11.5 \pm 0.3 \text{ kg}$ versus $10.9 \pm 0.9 \text{ kg}$ in flock and ST, respectively). This study suggests that monitoring performance in 20% of a group of lambs can determine timings of whole-group drenching.

Monday 17 August 2015

Concurrent Session 2.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O046/0047

Diagnosis before treatment: Identifying dairy farmers' determinants for the adoption of sustainable practices in gastrointestinal nematode control

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Anthelmintic resistance is emerging in dairy cattle and this can result in a lack of effective control and production losses. Therefore, sustainable control strategies should be adopted by the industry such as the use of diagnostic methods to take informed treatment decisions. To understand the factors affecting the farmers' intention to adopt diagnostic methods before implementing anthelmintic drugs ('adoption intention'), a theoretical model was constructed. This framework was created to predict adoption intentions based on two fundamental theories in the field of behavioural psychology and health psychology: the Theory of Planned Behaviour and the Health Belief Model. The framework was validated through a cross-sectional survey carried out in dairy farms in Belgium (Flanders). In the tested model, adoption intentions were predicted based on attitudes towards anthelmintic drugs, attitudes towards diagnostic methods, subjective norms, behavioural control and perceived risk. Structural equation modelling was used for analyses. The model fitted the data well and explained 46% of the variance in adoption intention of diagnostics. The factors 'attitude towards diagnostic methods' and 'subjective norm'; i.e. the influence of significant others, had the strongest, positive influence on adoption intention of diagnostic methods. A multi-group analysis found no difference in the adoption of diagnostics for young stock vs. adult dairy cows. The results of this study can be used for the development of communication strategies to advertise sustainable nematode control on dairy farms.

Monday 17 August 2015

Concurrent Session 2.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O047/0051

Ewe body condition score is a useful trait for refugia-based treatment strategies

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Interest in animal traits that predict individual worm egg count (WEC) has increased as recognition of the importance of refugia for managing anthelmintic resistance has emerged. The traits need to be readily measured, allowing for real-time and within-yard decision making to determine which animals remain untreated. Body condition score (BCS) has been suggested as a possible trait as it is a subjective measure of whole body muscle mass which is known to confer significant advantages for resistance to gastrointestinal nematodes of sheep.

BCS was estimated (1–5 scale) and faeces collected, to determine worm egg counts (WEC), from 120 cross-bred (Border Leicester x Merino) ewes on each of 3, 5 and 5 properties during the years 2012, 2013, 2014 respectively in the Armidale region, NSW, Australia. Adult ewes were identified as twin-bearing and lambing occurred in September of each year. Measurements were timed to correspond with pregnancy scanning (July), prelambling (August), lamb marking (November), weaning (January) and mating (April). Ewe BCS was categorised into 0.5 unit bands and WEC was cube-root transformed. There were 12,111 observations with both BCS and WEC recorded and mean separation was performed using the multiple comparison non-parametric Wilcoxon method.

WEC was negatively ($p < 0.001$) associated with BCS in a manner best described by a negative exponential function with an approximate halving of mean WEC with each successive 0.5 unit increase in BCS. Back transformed mean WEC values for each BCS band were each significantly different from each other. The WEC values for each BCS band were: 1222^a epg for BCS band 2.0–2.5, 667^b epg for 2.5–3.0, 332^c epg for band 3.0–3.5, 161^d epg for band 3.5–4.0 and 115^e epg for band 4.0–4.5. The predominant parasites were *Haemonchus contortus* and *Trichostrongylus* spp.

These results suggest that ewe BCS is a useful trait for contemporaneously predicting WEC throughout the reproductive cycle and may be useful for refugia-based strategies.

Monday 17 August 2015

Concurrent Session 2.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O048/0288

New strategic control of gastrointestinal nematode parasites in beef cattle in Brazil: effects on weight gain

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This two-year, randomized, placebo study aimed to evaluate the effect of different anthelmintic treatment strategies on the performance of beef cattle in Brazil. Two cycles were performed from May 2013 to December 2014 and from May 2014 to December 2015, with 96 Nelore pre-weaned calves each. The selected animals were allocated in a randomized block design with time and area repetition, based on eggs per gram and body weight at day 0. All anthelmintics were administered subcutaneously in a single dose of 700µg/kg doramectin (DRM), 200µg/kg moxidectin (MOX) or 4,7mg/kg of levamisole phosphate (LEV) according to the following scheme: T1 (placebo); T2 (May – DRM/ November – DRM); T3 (May – doramectin/ July – LEV/ September – DRM); T4 (May – doramectin/ July – MOX/ September – DRM); T5 (May – DRM/ August – LEV/ November – DRM) and T6 (May – DRM/ August – MOX/ November – DRM). Cattle weighing was carried out at every 28 days with animals kept in feed and water restriction for 12 h. The untreated animals (T1) showed the lowest average weight gain ($P<0.05$): 55.90kg. The T2 resulted in weight gain of 69.25 kg; higher ($P<0.05$) than the T1 and similar ($P>0.05$) to T3 (77.31kg) and T4 (75.61kg). The highest weight gain was observed in T5 (80.83kg) and T6 (86.21kg). Although the T5 and T6 were similar to T3 and T4 ($P>0.05$), they were the only medicated groups which were superior to T2 ($P<0.05$). The anthelmintic treatment performed on May, August and November provided the highest weight gain of weaned calves in a beef cattle herd from Brazil.

Monday 17 August 2015

Concurrent Session 2.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O049/0297

Evaluation of indicators for targeted selective treatment programs to control type II haemonchosis in ewes in Ontario, Canada

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Type II haemonchosis is often associated with late gestation and parturition in ewes in Canada. Because of widespread anthelmintic resistance (AR), targeted selective treatment (TST), where individual animals are treated rather than the entire flock, is a possible strategy to control clinical signs in recently-lambled ewes while still maintaining refugia. A study was therefore conducted during the 2013 and 2014 lambing seasons (February-May) in Ontario to determine the ability of TST indicators to identify ewes with high fecal egg counts on three client-owned farms with documented anthelmintic resistance and problems with type II haemonchosis. Fecal samples were collected from all ewes within a few days of lambing, and were treated on the sampling day with oral closantel (10 mg/kg), a novel anthelmintic to Canada, if they met at least one of four criteria: a) the last grazing season was their first grazing season; b) body condition score (BCS) <2; c) FAMACHA score >3; and d) three or more lambs at foot. *Haemonchus* sp. percentages on each farm, determined by coproculture, ranged from 77% to 97% of total fecal trichostrongyle-type egg counts. Mean fecal egg counts (FEC-determined by modified McMaster testing) were significantly higher in treated ewes (n=138) compared with untreated ewes (n=104) for both years of the study (2013: p=0.002; 2014: p=0.014). Univariable analyses found that FAMACHA score (p<0.0001) and BCS (p=0.006) were significantly associated with FEC. A general linear mixed model was subsequently fit using the indicators as fixed effects, and year and farm as random effects. FAMACHA score was the sole indicator to remain significantly associated with FEC (p=0.001). These results suggest that FAMACHA score is the most useful predictor of FEC in ewes immediately post-partum on farms with type II haemonchosis and should therefore be included in TST programs to determine individuals requiring treatment.

Monday 17 August 2015

Concurrent Session 2.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O050/0301

Which is the best phenotypic trait for use in a targeted selective treatment strategy for sheep?

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Targeted selective treatment (TST) requires the ability to identify the animals for which anthelmintic treatment will result in the greatest benefit to the entire flock. A number of phenotypic traits have previously been suggested as determinant criteria (DC) for TST regimes; however, the weight gain benefit (and impact on anthelmintic efficacy) for each DC is expected to be dependent upon the level of nematode challenge and the timing of anthelmintic treatment. A mathematical model was used to simulate a population of 10,000 parasitologically naïve lambs (with heritable variation in host-parasite interactions) grazing on a medium-quality pasture (grazing density = 30 lambs/ha, crude protein = 140g/kg DM, metabolisable energy = 10MJ/kg DM) initially contaminated with 1000, 3000 or 5000 *Trichostrongylus circumcincta* larvae (IL₀, L₃/kg DM). Anthelmintic drenches were administered to 0, 50 or 100% of the population on a single occasion. The timing of treatment was modelled for every day within the 121 day simulation. Where TST scenarios were simulated (50% treated), lambs were either chosen by random selection (RS) or according to highest faecal egg count (FEC, eggs/g DM faeces), lowest live weight (LW, kg) or lowest growth rate (GR, kg/day). Average lamb LW and the resistance (R) allele frequency amongst the parasite population were recorded at day 121 (6 months of age) for each scenario. The average weight gain benefit (AWGB, kg) for each DC and treatment day were calculated by comparison to an untreated population. For all levels of IL₀, using FEC as the DC provided the largest AWGB (and increase in R allele frequency) for all treatment times. The AWGB when LW or GR were used as DC were less than RS for treatments occurring within the first 58, 32 and 30 days for IL₀ of 1000, 3000 and 5000L₃/kg DM, respectively. As time progressed, the AWGB for all DC became increasingly similar to the 100% treatment group. Similarly, the R allele frequencies when LW and GR were used as DC were less than RS for treatments occurring within the first 75, 50 and 45 days for IL₀ of 1000, 3000 and 5000L₃/kg DM, respectively. Whilst the R allele frequencies for all DC subsequently converge, they remained greatly reduced in comparison to the 100% treatment group. These model outputs are a consequence of the impact of immunity on both lamb growth rate and the distribution of parasitic burdens within the lamb population.

Monday 17 August 2015

Concurrent Session 2.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O051/0422

Targeted Selective Treatment (TST) on commercial farms in Scotland

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With resistance to anthelmintics in small ruminant gastrointestinal nematodes increasing globally, there is a growing need for methods of parasite control to slow the development of resistance. Targeted Selective Treatment (TST) based on Happy Factor calculation of weight gain targets for lamb production systems have proved to reduce anthelmintic usage and slow anthelmintic resistance without significant production costs in a research environment.

This study used two commercial and one research farm to explore the potential for this technique to be used in a commercial environment by treating half of the lamb flock under TST conditions, treating when failing to gain a predicted weight, and half using the normal drench regime (RT) employed by each farm (total number of lambs = 1640).

Weight and treatment data gathered during the normal grazing season was analysed to examine the effect of TST on the final bodyweight, liveweight gain and number of treatments administered.

TST animals received significantly ($P < 0.05$) fewer anthelmintic treatments on all three farms (1.17 vs 1.35 treatments per animal). However, there was no evidence that the difference in mean final bodyweight, daily liveweight gains and number of lambs reaching finishing weight between TST and RT groups were significant ($P > 0.05$) on any farm.

Weight-gain based TST, using the Happy Factor, can successfully be translated on-farm, and the adoption of EID and automated animal handling systems may make this method more practicable to the commercial user.

Monday 17 August 2015

Concurrent Session 2.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O052/0302

Internal parasite management in livestock requires no further research

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Internal parasitism of domestic livestock is the costliest endemic disease wherever it occurs. Parasite management continues to rely heavily on the use of chemicals, and the inevitable emergence of resistance is being countered by a steady stream of molecules with novel modes of action. Current research into internal parasites covers areas of molecular diagnostics (from estimating worm burdens to determining the chemical susceptibility/ resistance profile), alternative control methods, epidemiology, pathogenicity, genomics, mechanisms of resistance, etc. Targeted selected treatment is receiving attention as a method of reducing dependence on chemicals, with selection criteria ranging from anaemia for sanguivorous worms (e.g. FAMACHA), to body condition and diarrhoea for worms causing gastro-enteritis. Numerous field trials have demonstrated the efficacy of integrated parasite management (IPM) in containing economic losses due to internal parasites to acceptable levels. IPM requires decisions to be made repeatedly on selecting the most appropriate intervention. Those decisions need to be informed through the ongoing collection and interpretation of data on climatic and pasture conditions, herd/flock history, the husbandry calendar, parasite characteristics, etc. For the average livestock producer, this collection and interpretation of data represents an unacceptable impost and a serious barrier to the adoption of non-chemical means of parasite management. More effort should be expended on the most efficient way of persuading and assisting producers to adopt a holistic and integrated approach to parasite management, rather than generating more data on the importance of parasites.

Monday 17 August 2015

Concurrent Session 2.4

D: Vaccines and immunology

Ref: O053/0193

Ocular toxoplasmosis: a combination of assays to improve diagnosis and the use of pigs as a biological model

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Toxoplasma gondii is the most important agent of posterior uveitis in humans worldwide. In the present study we experimentally infected pigs with *T. gondii* tachyzoites, bradyzoites and oocysts in order to evaluate this species as a biological model for detection of human ocular toxoplasmosis. Herein, we used 18 pigs divided into five groups, G1 (infected with tissue cysts of M4 strain (type II) at day 28, n=5), G2 (infected with 1,000 oocyst of M4 strain at day 28, n=5), G3 (infected with tachyzoites of S48 strain at day 28, n=5), and finally G4 (uninfected unchallenged, control group n=3). At day 70 of the experiment all animals were culled, and serum and eye samples were collected to perform indirect ELISA, immunoblotting, and PCR (nPCR, and qPCR). All control pigs (G4) were serum negative throughout the experiment as well as ELISA and PCR from humors. The average of optical density (OD) from sera, in the cull day were OD_{G1}=1.21±0.38; OD_{G2}=1.44±0.27; and OD_{G3}=0.73±0.20. Nine pigs (60%) out of 15 were positive in vitreous humors (VH), and 7/15 (46%) were positive in aqueous humors (AH). Immunoblotting from humors showed a reaction against a major protein of approximately 29-30 kDa. Animals from G1 had more humors positive in both ELISA and PCR than the other groups. As a conclusion, we observed that pigs may be successful used as an animal model for human OT.

Monday 17 August 2015

Concurrent Session 2.4

D: Vaccines and immunology

Ref: O054/0012

Recombinant protein from *Trypanosoma congolense* a good diagnostic antigen in immunochromatographic test but fails to protect mice from lethal challenge

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Trypanosomes are hemo-flagellate protozoan parasites that cause disease in humans and livestock called sleeping sickness and nagana, respectively. So far, a vaccine has been elusive because of the immunodominant variant surface glycoprotein (VSG) in a phenomenon known as antigenic variation. According to amino acid domains structure, Tc38630 was assumed as a *Trypanosoma congolense* orthologue of the *T. brucei* invariant surface glycoprotein (ISG) therefore, a potential diagnostic antigen. Recombinant protein Tc38630 was successfully expressed, characterized and found to be antigenic. Among other serological tests, immunochromatographic test (ICT) has an advantage as a one-step rapid analysis, thus making it a convenient and sensitive diagnostic test. BALB/c mice were immunized with rTc38630 protein and serum was collected for IgG polyclonal antibodies purification for use as control line in ICT. The antigen-antibody reaction was detected by colloidal gold conjugated rTc38630 protein at the test line. The ICT result was found to be consistent with rTc38630 protein-based ELISA. For vaccine studies, five groups of BALB/c mice, were randomized and assigned as recombinant protein, recombinant GST, MCF homogenate, BSF homogenate and PBS and immunized respectively. They were challenged with a lethal dose of *T. congolense*. The recombinant protein did not protect the mice (survival analysis, P=0.09).

Monday 17 August 2015

Concurrent Session 2.4

D: Vaccines and immunology

Ref: O055/0659

Dietary protein supplementation regulates Type 2 immune response in parasitized lactating rats

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Background: Breakdown of immunity to gastrointestinal nematode (GIN) infection occurs during parturition, and this is believed to have nutritional basis; animals fed insufficient level of dietary protein showed greater penalties. Here, we tested the hypothesis that dietary protein has ability to modulate immune response in parasitized lactating rats.

Methods: Multiparous female Sprague Dawley rats were given primary infection of *N. brasiliensis* 3rd stage larvae (L3) prior to mating. Rats were fed either a High protein (H) or Low Protein (L) through the latter half of gestation and lactation. Half of the animals were administered secondary infection of L3 post parturition, whereas the remaining were sham-infected. Samples were collected 3 and 9 days post secondary infection (psi) for parasitological and immunological parameters.

Results: Expressions of Type 2 immunity related genes, known to have a role in parasite expulsion, including Arginase 1, PPAR- γ and 12/15 Lipoxygenase, in both intestine and spleen were significantly up-regulated in H fed animals ($P<0.05$) on day 3 psi. In addition, Type 2 cytokine IL-13 expression in the spleen on day 3 psi was significantly higher in H fed animals ($P<0.05$). Although expressions of intestinal epithelial cell genes (Relm β , AGR2) were not affected by the diet, worm burdens were significantly lower ($P<0.05$) in H fed animals on day 9 psi.

Conclusions: High protein intake had an effect on both local (intestine) and systemic (spleen) immune parameters. Up-regulation in Type 2 immunity related genes suggests that high dietary protein level may help enhancing effective nematode expulsion from the host and suppressing inflammatory responses. This shows that protein supplementation can regulate immune response at gene expression level, which can contribute to the development of sustainable parasite control strategies in mammals.

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Monday 17 August 2015

Concurrent Session 2.4

D: Vaccines and immunology

Ref: O056/0386

Immunization in heifers with an inactivated vaccine against bovine trichomonosis using different doses and administration routes

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The diagnosis and detection of bovine trichomonosis (BT) in Spanish beef cattle has significantly increased in the last years. The control of BT only relies on the identification of infected bulls followed by their removal from the herd. Vaccination has been proposed as an efficient control measure when risk factors are present. However, commercial vaccines are not available in Europe Union. The aim of the present work was the evaluation of different vaccine formulations for the prevention or reduction of *T. foetus* infection in the female reproductive tract. The efficacy of an inactivated vaccine based on total trophozoite antigens (TTA) of *T. foetus* in combination with saponins were evaluated in well-established bovine model of vaginal infection. Different doses of TTA and different administration routes (subcutaneous and intranasal) were tested (groups 1-3). Additionally, one group was immunized with live trophozoites by intranasal route (group 4) and another group received a commercial vaccine (group 5). Non-immunized/infected (group 6) and non-immunized/non-infected (group 7) groups were also included. Animals per group were twice immunized at 3-week intervals and challenged intravaginally with 10^6 *T. foetus* 3 weeks after the second immunization. For evaluation of the persistence of genital infection, cervico-vaginal mucus (CVM) was collected weekly from 0 to 112 days post-challenge (dpc). For antibody assays, CVM and sera were collected before challenge at days -42, -22 and -10 pi, and weekly from 0 to 112 dpc. In this study, all the immunized groups (groups 1-5) cleared the infection faster than the non-immunized/challenge group (33-46 versus 64 dpc). This protective results coincided with a significant increase in serum IgG and genital IgA responses after second immunization and after challenge, respectively. The subcutaneous immunization (groups 1-5) induced higher IgG antibody levels in serum than the combination of subcutaneous and intranasal administration routes (groups 2-4) ($P<0.05$). However, the IgA antibody levels in vaginal secretions were similar in all the immunized groups. These results indicate that the experimental immunization with inactivated antigens of *T. foetus* induced a protective immunity, which was able to clear the parasite from the reproductive tract of heifers. Further field trials in naturally infected herds with *T. foetus* are necessary to test the vaccine efficacy against reproductive failure.

Monday 17 August 2015

Concurrent Session 2.4

D: Vaccines and immunology

Ref: O057/0581

Challenges of diagnosis in the new era of canine *Leishmania infantum* vaccines

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The Leishvet group updates the current knowledge on diagnosis of canine leishmaniosis due to *Leishmania infantum* focusing on the challenges raised by the use of *Leishmania* vaccines. The purposes for which the diagnosis of *L. infantum* infection is carried out include: (1) to confirm clinical illness; and (2) to screen clinically healthy dogs for several reasons including vaccination, blood donation and breeding, to avoid the importation of infected dogs to non-endemic areas, and to monitor response to treatment. The clinical diagnosis of leishmaniosis is very difficult and complex because the spectrum of clinical and clinicopathologic abnormalities is broad and unspecific, the existence of subclinical infection and the use of vaccines. For these reasons, it is important to separate disease, infection and immunogenicity induced by vaccination and to apply different diagnostic techniques accordingly. Accurate diagnosis of canine leishmaniosis often requires an integrated approach consisting of a clinicopathological assessment and specific laboratory tests. The detection of *L. infantum* infection in dogs includes parasitological (cytology, histology, immunochemistry and culture), molecular (conventional, nested and real-time PCR), serological methods (qualitative and quantitative antibody tests) and methods for the evaluation of cellular immunity. Detection of specific serum antibodies should preferably be based on quantitative serological techniques, such as IFA and ELISA due to the variable diagnostic performance of rapid serological tests. The challenges of serology include cross-reactivity with other pathogens and antibodies elicited by vaccination against the disease. Considerations regarding immune response elicited by vaccination include antigen recognition, kinetics of antibody and cell mediated immune response, difference of assay diagnostic performance and individual variability among dogs. Antibodies reactive with *Leishmania* antigen have been reported to persist for 4-6 months in dogs vaccinated with a European vaccine for *L. infantum*. Techniques for the discrimination of immune responses due to vaccination and natural infection should be potentiated. It is essential to know details on vaccination of evaluated dogs in order to make a correct interpretation of the diagnostic test employed. All evaluated information should be combined together for a comprehensive assessment.

Monday 17 August 2015

Concurrent Session 2.4

D: Vaccines and immunology

Ref: O058/0585

Leishvet guidelines for the management of feline leishmaniosis in Europe

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In cats clinical illness due to *Leishmania infantum* seems to be rare in Europe, but subclinical infections may be more frequent in areas where canine leishmaniosis is endemic. Limited data is available and the Leishvet group presents a consensus of opinions and recommendations on the epidemiological role of cats in *L. infantum* infection, clinical manifestations, diagnosis, treatment and monitoring, prognosis and prevention of feline leishmaniosis (FeL) to promote a standardized management of this infection in cats. The consensus of opinion and recommendations were constructed by combining a comprehensive review of evidence-based studies and case reports, clinical experience and critical consensus discussions. Prevalence in endemic areas, based on serological or molecular techniques, range from 0% to 68%, and healthy cats should be tested in case of blood donation or importation to non-endemic areas. Sand flies acquire the infection from cats which, therefore, might act as a secondary reservoir. The most common clinical signs and clinicopathological abnormalities compatible with FeL include lymph node enlargement and ulcerative or nodular lesions of the skin (mainly on the head or distal limbs) or mucocutaneous junctions, ocular lesions (mainly uveitis), feline chronic gingivostomatitis, hypergammaglobulinaemia and normocytic normochromic anaemia. Clinical illness is often associated with impaired immunocompetence, as in the case of retroviral infections or immunosuppressive therapy. *Leishmania infantum* infection should therefore be evaluated and monitored in cats undergoing immunosuppressive treatments. Diagnosis is performed by serology, PCR, cytology, histology/immunohistochemistry or culture. Quantitative serology should be obtained with validated methods. When serological testing is negative or low-positive (IFA titer ≤ 80) in a cat with clinical signs compatible with FeL, the diagnosis should not be excluded and other direct diagnostic methods should be employed. Cats have been empirically treated and allopurinol is the most common used drug. Follow-up with routine laboratory tests is recommended for monitoring any potential drug side effects. Most treated cats clinically recover but they may relapse after discontinuation of therapy. Quantitative serology and PCR are afterwards essential for early diagnosis of clinical relapses. So far, preventative measures for this infection in cats have not been evaluated.

Monday 17 August 2015

Concurrent Session 2.4

D: Vaccines and immunology

Ref : O059/0589

LeishVet consensus recommendations on canine leishmaniosis control

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Canine Leishmaniosis (CanL) prevention requires an integrated approach to reduce the risk of infection, disease development, transmission and geographic spread. Use of insecticides with rapid and residual activity is essential to protect dogs. Several insecticide formulations have been demonstrated to be efficacious in reducing parasite infection to dogs in both laboratory and field conditions. In addition to insecticide use, vaccination can be considered for healthy dogs. Vaccination has been shown through recent published clinical trials to reduce the odds of evolution of infection to clinical disease. Vaccination should only be performed in seronegative dogs. Due to lower sensitivity of rapid serological test(s), these results must be confirmed by either IFAT or ELISA. Following the International guidelines of canine vaccination, *Leishmania* vaccine(s) are non-core vaccines. The decision to vaccinate, therefore, should be based upon multiple criteria. These include the individual benefit/risk, age, breed, life-style or use and reproductive status. When bringing a dog from a non-endemic area into an endemic area, topical insecticides may be best for protection. If travel is longer, prevention methods should be the same as advised for dogs in endemic areas. Every dog with diagnosed infection should be continuously treated with insecticides to prevent further transmission. A more recent publication has demonstrated that vertical transmission can maintain CanL within populations in non-endemic areas. Globalization of infectious diseases has greatly increased the risk of transfer of these diseases. Stronger regulatory measures are needed to prevent this. In the meanwhile, to avoid transmission of zoonotic CanL, infected dogs (traveling, rehoming, sale, transfer of dogs or reproductive materials for breeding) should not be imported into non-endemic areas. No control measure is an isolated action but should form part of a comprehensive prevention program for leishmaniosis in dogs living in or travelling to an endemic area, whether they are healthy, infected but clinically healthy, or infected and sick.

Monday 17 August 2015

Concurrent Session 2.4

D: Vaccines and immunology

Ref: O060/0627

A cluster of naturally occurring equine leishmaniosis with persistent progressive clinical manifestations, sub-clinical infection and evaluation of the horse as a reservoir host

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Equine leishmaniosis caused by *Leishmania infantum* has been rarely reported. Only a small number of cases have been reported so far from areas where the disease is endemic in dogs and humans, and it has usually been described as self-limiting cutaneous disease in which lesions heal spontaneously within 2-6 months. We describe a cluster of three horses from northern Israel with clinical leishmaniosis detected by cytology of lesions, histopathology, PCR with sequencing and culture, and additional sub-clinically infected horses. Two clinically-affected horses were from the same stable and another from a stable 20 km away. Two horses presented with nodular facial lesions in the upper eyelid which enlarged and ulcerated followed by the appearance of additional distant nodules, while the third horse had large ear pinnae ulcerated nodules. Histopathology from two horses indicated *Leishmania* infection and cytology from all three showed a high density of amastigotes. Lesions were positive by PCR and sequencing for *L. infantum* and parasites were cultured and characterized as *L. infantum* from two horses. All horses in the stable were tested by blood PCR for *Leishmania* and 13 of 30 were positive by kDNA PCR with apparent sub-clinical infection. Xenodiagnosis with *Phlebotomus perniciosus* sand flies was performed on two horses and 5 days post-feeding, alive sand flies were dissected and examined for the presence of promastigotes while those that died were tested by PCR for the presence of an equine blood meal and *Leishmania* DNA. To our best knowledge, this is the first attempt to perform xenodiagnosis for *L. infantum* in horses. None of the 73 female sandflies dissected were positive for promastigotes, however, of 36 sand flies positive for horse blood meal by PCR, two were positive for *L. infantum* DNA, one from each horse. While ear lesion healed spontaneously in one horse within 5 months, the two other horses showed progressive proliferative facial lesions for more than 8 months which worsened and were eventually treated with intralesional injections of meglumine antimoniate. This study indicates that *L. infantum* infection can cause progressive cutaneous disease in horses, and also sub-clinical infection with parasitemia in a large number of horses. Based on the presence of *L. infantum* DNA in sand flies that fed on two infected horses, it is speculated that horses may serve as secondary reservoirs for *L. infantum*.

Monday 17 August 2015

Concurrent Session 2.5

One health

Ref: O061/0439

First aberrant localization of *Onchocerca lupi* in a dog

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Onchocerca lupi (Spirurida, Onchocercidae) is an emergent vector-borne helminth, causing nodular lesions associated with acute or chronic ocular disease in dogs and cats. Since its first description in dogs in 1991, this filarioid has been increasingly reported in Europe and in the United States, with a proven zoonotic impact.

An 8 year-old, outdoor female mixed breed dog from Algarve region (southern Portugal) was presented with a history of severe dyspnoea and cyanosis, showing rattle sounds during pulmonary auscultation. Since cervical and thoracic radiography were unremarkable, an exploratory laryngoscopy was performed and moderate inflamed larynx, thyroid cartilage stenosis and purulent necrotic material was detected in the lumen of the trachea, along with filiform worms. A single inflammatory mass was identified in the dorsal wall of the larynx, protruding into the lumen, with a severe reduction of the glottis and trachea diameter. Four coiled threadlike nematodes were also found and collected for morphological identification. Nematodes had a thick and multilayer cuticle, bearing prominent annular ridges at the external layer and striations on the internal one, as well as, small and unsheathed microfilariae inside the uterus of female specimens. The molecular amplification and partial sequencing of mitochondrial cytochrome oxidase subunit 1 gene (cox1) was performed, showing 100% homology to *O. lupi* sequence deposited in GeneBank (AN KC686701). Histological examination was compatible with parasitic pneumonia and tracheal inflammation. During the laryngoscopy, a tracheostomy tube was inserted to relieve dyspnoea and prompt treatment with fenbendazole (25 mg/kg per os for 21 days) and corticosteroids was prescribed.

The life cycle, host range and pathology of canine onchocercosis are not fully known. Although *Onchocerca* sp. was already isolated from the trachea of a dog coming from an endemic area of *O. lupi* in Greece, to the best of the authors' knowledge, this is the first report of an erratic migration of *O. lupi*. This case highlights the medical community towards this newly recognized location of *O. lupi* and underline onchocercosis as a differential diagnosis of airway obstruction in dogs.

Monday 17 August 2015

Concurrent Session 2.5

One health

Ref: O062/0129

Isolation and characterization of paramyosin from *Onchocerca lupi*, a neglected zoonotic nematode

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Paramyosin, an invertebrate muscle protein, has been widely studied as a potential vaccine candidate against parasites. In addition, this protein has been used in a commercial kit, as an antigen to detect antibodies produced against the human filarial nematode *Wuchereria bancrofti*. The antibodies also recognize antigens from *Dirofilaria immitis* and other filarial nematodes. The aim of this study was to isolate and characterise the paramyosin gene of *Onchocerca lupi*, an increasingly important filarial nematode that causes acute and chronic ocular disease in dogs, cats and also humans. Current methods to detect microfilariae of *O. lupi* include skin snips, whereas adults may be removed surgically from ocular nodules in infested hosts. Both procedures are invasive, time consuming and laborious. No chemotherapeutic treatments are currently available. Therefore, a more rapid, less invasive technique is required to detect the parasite. Development of a serological diagnostic test, based on antibodies to *O. lupi* paramyosin, would assist in diagnosis of infestation and in the analysis of the prevalence and distribution of this zoonotic parasite. Adult and microfilarial nematodes were isolated from the eye and skin, respectively, of a dog from Portugal. RNA from an adult female was reverse transcribed into single stranded cDNA and a paramyosin transcript PCR amplified from this material. A 2.5kb paramyosin cDNA was isolated from *O. lupi*, BLASTn and BLASTx analysis indicating 98% nucleotide and 99% amino acid identity to paramyosin from *Onchocerca volvulus*. Phylogenetic analysis showed that *O. lupi* paramyosin protein clusters with *O. volvulus* and other filarial nematode paramyosin proteins. The predicted protein is 101kDa, has a conserved nematode-specific proton donor site, no signal peptide and no transmembrane spanning domains. Epitope mapping (including epitopes published in the literature) confirmed the presence of potential antigenic sites. The paramyosin molecule showed to be a suitable candidate for the development and testing of a serological diagnostic tool to detect the presence of *O. lupi*.

Monday 17 August 2015

Concurrent Session 2.5

One health

Ref: O063/0619

Canine ocular onchocerciasis in Western Canada: adoption-mediated introduction of a zoonotic parasite

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Onchocerca lupi is a zoonotic vector-borne helminth that has been emerging recently in the southwestern USA and areas of the Old World. The parasite causes ocular disease in dogs and cats, but in humans, aberrant infection may affect the central nervous system. Within Canada, there has been only one previous report in Prince Edward Island in a dog adopted from New Mexico. We report the second case of canine ocular onchocerciasis in the country, and first in Western Canada. A 7-years-old female Pit Bull mix, adopted from Kanab, Utah 2 years ago (previously lived in Maricopa County, Arizona 2 years prior to Utah), was presented with periodic orbital swelling for 1 year and a half. The dog was under corticosteroid therapy, and heartworm preventative (ivermectin + pyrantel for 2 years). During examination of the right eye, a 1mm conjunctival draining tract, overlying a lateral scleral thickening, from where two linear objects were projected. These were briefly assessed to be potential nematodes. The rest of the ocular exam for both eyes was normal. Under general anesthesia a 10 mmx 18 mm subconjunctival fibrous mass that was adherent to the sclera was excised and the material was sent for histopathology. Nematode-like structures were surgically removed within the mass during the excision. After surgery, ivermectin (0.1 mg/kg for 1 day, then 0.2 mg/kg for 1 day and then 0.3 mg/kg for 30 days) + doxycycline (20mg/kg for 28 days) treatment was implemented. Nematode fragments were identified as *O. lupi* based on cuticular morphology and PCR and sequencing of the ND5 region of the mitochondrial DNA. Histopathology demonstrated cross section of adult nematodes. Skin snips collected at 30 and 60 days post-surgery were negative for the presence of *O. lupi* microfilariae. We discuss the risk of *O. lupi* establishment in Alberta and Western Canada, in light of the knowledge of the distribution of the putative vector in California (*Simulium tribulatum*) and other black flies species, and demographic data of dogs adopted from *O. lupi* endemic areas.

Monday 17 August 2015

Concurrent Session 2.5

One health

Ref: O064/0349

Parasites, Plants and People

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Anthelmintic resistance is a fact of life in New Zealand as is the critical loss of biodiversity on productive land.

Concurrently there is an increasing interest in traditional medicine (rongoā), biological farming and natural health, both human and animal.

Rongoā is a holistic system of medicine that recognises a person's connection to the land and the importance of healthy land to the health of the population.

We worked with a Maori community to resolve the contradictions between intensive farming, parasite burdens (the prime animal health problem on the farm) and a desire for cultural revitalisation on their land.

Drawing on the literature and community discussions we identified rongoā species that would grow and which would promote local health. We then selected species with a reputation as anthelmintics and those which might act as tonics, improving animal health, thus helping reduce parasite burdens.

We identified areas of the farm that could be left in intensive agriculture and areas that could be planted with rongoā species for animal and human use. We devised anthelmintic browse bars and identified areas that should be withdrawn from grazing as the soils were damaged or watercourses imperilled but could usefully provide rongoā and other culturally important plants for the community.

This project is an example of One Health in action, working with local communities to promote animal and human health and protecting the soil and water upon which they depend.

Monday 17 August 2015

Concurrent Session 2.5

One health

Ref: O065/0735

Albendazole use for GI nematodes does not affect the susceptibility of sheep to infection with *Echinococcus granulosus*

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A decline in the fertility and number of *Echinococcus granulosus* cysts in sheep has been noted in Uruguay and Sardinia, and there has been speculation that this may be due to the use of albendazole drenches to control gastrointestinal nematodes. This hypothesis was tested in 56 Dorper-cross ewes experimentally infected with *E. granulosus* eggs. Lambs approximately 4 months of age were infected with 1000 eggs of *E. granulosus*. Groups of 10 animals were drenched with 4.75mg/kg body weight albendazole (Valbazen, Coopers 19mg/ml) from 3 months post infection at intervals of either 2 months, 4 months or 6 months, through to 31 months post infection. One group received albendazole at 9.5mg/kg body weight (double dose) at two monthly intervals and one group received no albendazole treatment. Sheep were necropsied at 31 months post infection and assessed for the number, size, viability and fertility of hydatid cysts. All sheep were found to be infected with *E. granulosus* at necropsy. Exposure to albendazole from 3 months post infection was found to have no effect on the number, size, viability or fertility of hydatid cysts, even in animals exposed at 2-monthly intervals to the drug at 9.5mg/kg body weight, compared with untreated controls. No evidence could be found to indicate that exposure of sheep to albendazole at dose rates and intervals that might be used to treat gastrointestinal nematode infections has any deleterious effect on *E. granulosus* infection in sheep.

Monday 17 August 2015

Concurrent Session 2.5

One health

Ref: O066/0262

New insight in lymnaeid snails (Mollusca, Gastropoda) as intermediate hosts of *Fasciola hepatica* (Trematoda, Digenea) in Ecuador

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Fasciolosis is a widely distributed disease in livestock in South America but knowledge about the epidemiology and the intermediate hosts are scarce in Ecuador. During 3 months, lymnaeid snails were sampled (n=1482) in Pichincha province in two sites located in a highly endemic area. The snails were identified (based on morphology and ITS2 sequences) and the infection status was established through microscopic dissection and a multiplex PCR-based technique. If morphologic-based techniques were not useful to accurately name the one species collected, alignment study ascribed it to *L. schirazensis*. Rediae were observed in 1.75 % (26/1482) and *Fasciola* sp. DNA was detected in 6% (89/1482) of the collected snails. The COX1 region permitted the parasite species identification: *F. hepatica*. The relative sensitivity and specificity of the microscope related to the PCR results was 25.84% and 99.78% respectively. The mean size of the snails recorded positive for *F. hepatica* through crushing and microscopy was significantly higher than the mean size of negative snails. There was not such difference in PCR positive snails. The role of *G. schirazensis* as an intermediate host of *F. hepatica* in Ecuador is discussed and a hypothesis of an adaptation of the snail to the trematoda is formulated. For the first time, an epidemiological survey, based on molecular biology-based techniques assessed the role of lymnaeid snail in the epidemiology of fasciolosis in Ecuador.

Tuesday 18 August 2015

Concurrent Session 3.1

A: Drugs and drug resistance

Ref: O067/0517

Investigating the acetylcholine receptor diversity in parasitic nematodes as potential targets for the development of anthelmintics

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Acetylcholine receptors (AChR) are pentameric ligand-gated ion channels involved in the excitatory neuro transmission in both vertebrate and invertebrate. In nematodes, they represent major targets for cholinergic agonist or antagonist drugs used as anthelmintic treatments. Despite a large diversity of AChR subunits present in nematode, only few AChR subtypes have been characterized so far. Interestingly, parasitic nematode species affecting human or animal health possess in their genome two closely related AChR subunit gene (*acr-26* and *acr-27*) that are essentially absent in free-living or plant parasitic species. In the present study we explored the ability to ACR-26 and ACR-27 subunit to form functional heteropentameric AChRs. Using the pathogenic parasitic nematode species *Haemonchus contortus* as a model, we found that *Hco-acr-26* and *Hco-acr-27* are co-expressed in muscular cells. We demonstrated that in *Xenopus laevis* oocyte, co-expression of Hco-ACR-26 and Hco-ACR-27 in combination with a set of ancillary protein led to the functional expression of an AChR highly sensitive to the anthelmintic morantel and pyrantel. Importantly we also reported that ACR-26 and ACR-27 from the distantly related parasitic nematode *Parascaris equorum* was also able to form a functional AChR (Peq-26/27) sharing essentially the same pharmacological properties. In accordance with these observations, when expressed in the nematode model *C. elegans* Hco-26/27 and Peq-26-27 were found to drastically increase morantel and pyrantel sensitivity of the worm, mirroring the pharmacological properties determined in *Xenopus* oocyte. Our results provide the first insight about the molecular composition of a novel class of nematode AChR and lay the basis for an optimized use of anthelmintics targeting parasitic nematode AChRs.

Tuesday 18 August 2015

Concurrent Session 3.1

A: Drugs and drug resistance

Ref: O068/0721

Functional characterization of the *Oesophagostomum dentatum* nicotine-sensitive AChR

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Background: The acetylcholine receptors (AChRs) are major targets for anti-nematodal drugs including levamisole, pyrantel, monepantel and derquantel. Two AChR subtypes have been described to mediate fast neurotransmission at the neuromuscular junction in the free-living model nematode *Caenorhabditis elegans*. The levamisole-sensitive AChR (L-AChR) which is a heteropentameric ion channel made of five different subunits and the prototypical nicotine-sensitive AChR (N-AChR) which is composed of five identical subunits encoded by the *Cel-acr-16* gene. *C. elegans* belongs to the clade V that also includes many parasite worm species having an important impact on animal health. In the context of growing concerns about anthelmintic resistance, the parasite N-AChR may be all the more of potential interest for the development of next generation nematocidal compounds. In the present study we have identified the N-AChR from the pig parasite *Oesophagostomum dentatum*. We have performed a detailed pharmacological characterization of the *O. dentatum* and *C. elegans* N-AChRs.

Methods: Here we have cloned the homologue of the *Cel-acr-16* gene in *O. dentatum*, synthesized the corresponding cRNA and injected it in combination with the *ric-3* ancillary protein cRNA in *Xenopus laevis* oocytes. Full dose-response experiments were carried out to explore the respective *O. dentatum* and *C. elegans* N-AChR sensitivities to acetylcholine (ACh) and analogues of nicotine using two-electrode voltage-clamp.

Results: First, we found that the *O. dentatum* ACR-16 sequence is highly similar to Cel-ACR-16 (80% identity, 89% amino acid similarity) therefore predicting that it would form an homopentameric channel having a similar pharmacological pattern. Interestingly, we found that injection of *Ode-acr-16* cRNAs gave rise to a functional homopentameric channel having an ACh EC₅₀ value very similar to that of the *C. elegans* N-AChR ($25.7 \pm 1.1 \mu\text{M}$ versus $21.4 \pm 1.1 \mu\text{M}$ respectively) but markedly higher than the ACh EC₅₀ of the *O. dentatum* levamisole receptors (Buxton, Charvet *et al.*, 2014). Surprisingly, nicotine was less potent than ACh in activating the *O. dentatum* N-AChR ($64.7 \pm 1.1 \mu\text{M}$), unlike *C. elegans* N-AChR ($13.8 \pm 1.1 \mu\text{M}$). Finally, the pharmacological analysis of nicotine derivatives reveals a potency series of ACh > nic > anabasine = cytosine > nornicotine for *O. dentatum* N-AChR different from that of *C. elegans* N-AChR nic > ACh > anabasine > cytosine > nornicotine. These results allowed us to refine a computer model of the N-AChR 3D structure able to predict ligand-receptor interactions *in silico* that will be used for drug optimization.

Conclusion: We have characterized for the first time the functional nicotine-sensitive AChR of a Clade V parasitic nematode and showed that its pharmacology differs from that of the *C. elegans* N-AChR. This work advances the understanding of N-AChR structure-function relationships in parasitic nematodes and opens the way for molecular screening of novel antiparasitic pharmaceuticals.

Tuesday 18 August 2015

Concurrent Session 3.1

A: Drugs and drug resistance

Ref: O069/0292

Closantel pharmacology: pattern of drug accumulation in adult *Fasciola hepatica* recovered from treated sheep

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Closantel (CLO) is a salicylanilide antiparasitic compound highly effective for the treatment of adult flukes, which may be formulated for oral or subcutaneous administration in ruminant species. CLO binds strongly (greater than 99.9%) and almost exclusively to plasma albumin, is poorly biotransformed and has limited distribution to tissues. Trans-tegumental diffusion and oral ingestion are the two potential routes available for drug entry into *F. hepatica*. Since adult liver flukes are blood-consuming parasites, plasma protein binding may have an important role in drug accumulation into the target parasite by oral ingestion. Therefore, the fluke could be potentially exposed to high drug levels in the bile (surrounding medium). The aim of the current work was to evaluate the *in vivo* time-course and patterns of CLO accumulation in adult *F. hepatica* specimens recovered from infected sheep over a 36-h post-treatment time period. Fourteen (14) adult sheep were infected with a susceptible isolate of *F. hepatica* metacercariae. Sixteen (16) weeks after infection, animals were treated with CLO by the oral (n=6, 10 mg/kg) or subcutaneous (n=6, 5 mg/kg) routes. At 12, 24 and 36 h post-treatment, animals were sacrificed (n= 2) and samples of blood, bile and adult *F. hepatica* specimens were collected. CLO concentrations were measured by HPLC. CLO peak plasma concentration after the oral (57.2 ± 4.1 µg/mL) and subcutaneous (40.3 ± 3.7 µg/mL) treatments were achieved at 36 h post-treatment. Similar CLO concentration vs time patterns were observed in the bloodstream and in the collected *F. hepatica*. Peak CLO concentrations within the adult flukes were 33.8 ± 11.8 (oral) and 22.8 ± 12.5 (subcutaneous) µg/g at 36 h post-treatment. Low CLO concentrations (≤ 2 µg/ml) were recovered in bile. Overall, the disposition kinetics and parasite accumulation data reported here confirm that the oral ingestion is a main route of drug entry into the flukes *in vivo* exposed to CLO.

Tuesday 18 August 2015

Concurrent Session 3.1

A: Drugs and drug resistance

Ref: O070/0238

Ivermectin and abamectin interaction: pharmacokinetic-pharmacodynamic assessment in *Cooperia* spp. infected calves

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Drug combined formulations are available as pharmacological tools designed to enhance anthelmintic efficacy and to delay the development of resistance. However, the *in vivo* pharmacological consequences emerging from the co-administration of anthelmintic molecules should be deeply evaluated in the early stages of combined formulations development. The current work investigated the pharmacokinetic-pharmacodynamic interaction occurring after the co-administration of ivermectin (IVM) and abamectin (ABM) to parasitised calves. The experimental trial was performed in two phases. In Phase I, twenty four (24) Aberdeen Angus-Hereford cross-breed calves experimentally infected with an IVM susceptible strain of *Cooperia* spp. were subcutaneously treated with IVM (0.1 mg/kg), ABM (0.1 mg/kg) or IVM+ABM (0.05 mg/kg of each compound). In Phase II, thirty two (32) calves naturally infected with an IVM resistant strain of *Cooperia* spp received the same treatments than in Phase I but at the therapeutic dose rate of 0.2 mg/kg. Blood samples were taken up to 21 days post-treatment to measure macrocyclic lactones (ML) concentrations by HPLC. The indirect estimation of efficacy was performed by faecal egg count reduction test (FECRT). In calves infected with resistant nematodes, a higher faecal eggs reduction was obtained after the IVM+ABM administration (89 %) compared to the ABM (76.9 %) and IVM (55 %) single treatments. Similar tendency was observed in calves infected with susceptible *Cooperia* spp. but treated with 50% of the therapeutic dose rate. The ML plasma concentrations were between 1.66 and 4.38- fold higher during the elimination phase (13-20 days post-administration) in the co-administered animals. The systemic exposure (measured as AUC) during this time period was 38 % (IVM) and 60 % (ABM) greater in the calves receiving the combined treatment. The improved performance observed after the co-administration of IVM and ABM may be based on drug to drug interactions at both pharmacodynamic (relative potency-related synergist effect) and pharmacokinetic (interaction on P-glycoprotein-mediated transport at host and parasite tissues) levels.

Tuesday 18 August 2015

Concurrent Session 3.2

G: Co-infection

Ref: O071/0253

The scabies mite associated microbiota

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On a global scale, scabies is one of the three commonest dermatological conditions associated with overcrowded living conditions, imposing a considerable economic burden on individuals, families, communities and health systems. The scabies mite also causes significant disease in form of sarcoptic mange in companion, livestock and wild animals. Nothing is known about the tripartite interactions among parasite, associated bacteria and patient. How do mites and bacteria affect host defences? Do they join forces against the host? Does the mite act as a carrier of bacterial pathogens? Does it rely on symbiotic bacteria that could be targeted therapeutically? Substantial epidemiological evidence has accumulated showing that scabies is linked to pyoderma. However, there is no information on the molecular mechanisms that underpin the link between scabies and bacterial pathogens. We propose that scabies mites play a role in the establishment, proliferation and transmission of opportunistic and potentially pathogenic bacteria. We have shown that scabies mites interfere with human complement protection, thereby promoting the growth of *S. pyogenes*, *S. aureus* and likely other opportunistic bacteria. The diversity and dynamics of microbes associated with scabies mites have not yet been investigated. It is also unknown whether scabies mites require endosymbionts for survival.

We aim here to investigate the impact of a scabies mite infection on the host skin microbiota and characterise the microbiome associated with scabies mites. Using a *natural* host scabies model in pigs we analysed experimentally in a longitudinal study the microbiota on the pig ear as primary site of mite infection. Scabies mites drastically altered the healthy skin microbiota. With the onset of mite infestation beneficial *Lactobacillus* species decreased and pathogenic *Staphylococcus* species increased. This dominance of pathogenic over benign species persisted beyond treatment for scabies and healing of the skin. Preliminary metagenome analysis of the mite internal microflora indicated that scabies mites harbour opportunistic pathogens and symbiotic bacteria. An understanding of the impact of mite infestation on the host skin microbiota and the identification of pathogenic and symbiotic bacteria carried by the scabies mite will initiate the development of treatments to specifically target mite-associated pathogens and mite symbionts.

Tuesday 18 August 2015

Concurrent Session 3.2

G: Co-infection

Ref: O072/0323

The hidden microbiome – the use of blocking primers to reveal zoonotic pathogens in ticks

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Ticks transmit a greater variety of pathogenic microorganisms than any other arthropod group and are among the most important vectors of diseases affecting humans, livestock and companion animals. *Ixodes holocyclus* and *Ixodes ricinus* are the most important tick species from both a medical and a veterinary perspective in Australia and Eurasia respectively. However, little is known about microbial populations associated with indigenous Australian tick species. In the present study, high throughput 16S rRNA metabarcoding (amplicon sequencing) of the Australian paralysis tick *I. holocyclus* and the European Sheep tick *I. ricinus* was conducted. Initial analysis identified that ~99% of 16S sequences from *I. holocyclus* and *I. ricinus* ticks belonged to the tick intracellular endosymbiont *Candidatus* Midichloria mitochondrii. Relative copy-number of the Midichloria masked other bacterial communities and was impervious to switching 16S primers, sequencing platform or sequencing at greater depths. The development and use of a specific *Candidatus* M. mitochondrii blocking primer decreased the number of endosymbiont sequences by 95.8% in *I. holocyclus* and 96% in *I. ricinus* samples. This allowed identification of 199 and 94 bacterial genera in *I. holocyclus* and *I. ricinus* ticks, respectively. Of particular interest, a novel *Candidatus* Neoehrlichia sp. was identified in *I. holocyclus*. Recently, *Candidatus* Neoehrlichia mikurensis was identified as an emerging tick-borne pathogen of animal and human health importance. Our preliminary data, with a 94-95% sequence homology to *Candidatus* Neoehrlichia spp., indicates an Australian variant found within *I. holocyclus*. Additionally, a relapsing fever *Borrelia* sp. was detected for the first time in Australia. These results show that without blocking primers and the use of NGS molecular advances, many bacteria of potential medical and veterinary interest could go undetected.

Tuesday 18 August 2015

Concurrent Session 3.2

G: Co-infection

Ref: O072/0145

Transcriptomic analysis of RNA interference in the sheep scab mite, *Psoroptes ovis*

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Next generation sequencing has paved the way for 'big data' studies of parasites and host parasite interactions. These studies have subsequently highlighted several possibilities for developing novel tools and strategies for achieving sustainable control of a variety of parasites. Recently, Roche 454 sequencing of the *Psoroptes ovis* transcriptome resulted in the identification of 12,160 putative gene-encoding isotigs, necessitating a method for selecting those suitable as potential vaccine candidates. RNA interference (RNAi) has been proposed as a high throughput approach for the screening of potential vaccine candidates with high specificity, speed and at relatively low cost compared to the existing methods whilst also reducing the number of animals required experimentally. This would enable assessment of a candidate gene's importance in mite survival and biology and therefore inform its potential as a vaccine candidate, whilst simultaneously improving the underlying knowledge of mite molecular biology. Evidence from transcriptomic analyses suggests there is good representation of the RNAi pathway in *P. ovis*; several of the systemic RNAi spreading components in addition to the key dsRNA processing components have been putatively identified. However, following non-invasive immersion in dsRNA overnight, as implemented successfully in the honey bee mite *Varroa destructor*, RNAi has yet to be demonstrated.

Tuesday 18 August 2015

Concurrent Session 3.2

G: Co-infection

Ref: O074/0419

Impact of a trypanosomosis-focused intervention on the kinetics of bovine babesiosis: a longitudinal field trial from Nigeria

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In 2012, a longitudinal trial was rolled out in the Plateau State, Nigeria, to control tsetse-borne African animal trypanosomosis (AAT) in indigenous cattle, through the use of 0.005% deltamethrin spray (Vectocid[®], CEVA Santé Animale) according to a restricted application protocol (RAP). The present study aimed to assess the impact of RAP on the kinetics of tick-transmitted bovine babesiosis.

In March 2012, 80 cattle older than 6 months were selected from two different herds (total=160) from one village and allocated to two different groups, namely RAP-treated (i.e. group A) and control animals (i.e. group B). In April 2012, all cattle were injected with diminazene aceturate (Veriben[®], CEVA SA). From April 2012 to January 2013, animals from group A were sprayed monthly according to RAP. From March 2012 to February 2013, animals from both group A and B were blood-sampled every three months. Collected blood samples were screened by PCRs and reverse line blotting targeting *Theileria/Babesia* spp. 18S rDNA. Throughout the sampling times, groups A and B were compared for prevalence and cumulative incidence of infection.

Both *Babesia bigemina* and *Babesia bovis* were recorded in group A and B at baseline (i.e. March 2012). The monthly RAP did not prevent the transmission of babesias to cattle. During the wet season, sprayed cattle were found significantly more infected than untreated ones by *B. bovis* and considerably, though not significantly, positive for *B. bigemina*. Possible causes of this discrepancy will be discussed (e.g. climatic and management factors, seasonality of boophilid ticks, residual activity of deltamethrin, etc.) to provide insights for the devising of future AAT control programmes in areas where bovine tick-borne infections also occur.

Tuesday 18 August 2015

Concurrent Session 3.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O075/0375

Gastrointestinal nematode infection of first grazing season dairy heifers: parasitological, clinical and grazing management indicators at individual and/or group levels for explaining daily weight gain

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Gastrointestinal nematode (GIN) infection is of concern for first grazing season (FGS) cattle and may be responsible for clinical signs or growth retardation. Intensity of infection is depending on grazing management factors but also on individual response. The objective of our study was to explain the variability of daily weight gain (DWG) due to GIN infection for 291 non treated heifers from 12 independent groups using parasitological, clinical and grazing management indicators at individual and/or group levels. Animals were followed up during their FGS at three periods (2-3 months after turn-out, 1-2 months after the first sampling, at housing) and were regularly weighed. At individual level, the parasitological indicators were faecal eggs count (FEC), pepsinogen value (PEP), anti *O. ostertagi* antibody level (ELISA) and the clinical indicators were the diarrhoea score (DS) and the breech soiling score (BSS). At group level, a combined indicator based on grazing management practices-GMP- (supplementation, month of turn-out and grazing season time) was used to determine the level of GIN exposure in three categories (low/medium/high). Over the FGS, FEC decreased from 2-3 months after turn-out, PEP and ELISA increased gradually until housing while clinical indicators did not show any seasonal variation. In bivariate analysis, FEC was negatively correlated with ELISA and PEP, but not with clinical indicators or DWG. PEP, ELISA as well as clinical indicators were positively correlated to each other and negatively with DWG. In the multivariate analysis, DWG was significantly explained by two individual indicators (ELISA, DS) and by the group indicator (GMP). DWG losses due to GIN exposure (ELISA) were estimated up to 39 kg per heifer for the overall grazing season. For groups with low GIN exposure level the difference between animals with low (<0.7 kg/day) and high (> 0.7 kg/day) DWG was explained by clinical indicator (DS) whereas for groups with medium and high GIN exposure levels the difference was explained by parasitological indicator (ELISA). This study highlighting the differences of the responses among heifers confirmed the possibility of targeting treatment on animals with the lowest DWG. Moreover, the association with a group indicator based on GMP could allow a better discrimination of animals to be treated.

Tuesday 18 August 2015

Concurrent Session 3.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O076/0463

Effect of anthelmintic treatment strategy on nematode species prevalence in grazing lambs in Scotland

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Refugia based anthelmintic protocols aim to reduce the rate of development of anthelmintic resistance in ovine gastrointestinal nematodes (GIN). Previous studies have illustrated the impact of different drenching regimes on drug efficacy and animal growth however, the impact on nematode populations has yet to be characterised. This study investigated the changes in species composition of GIN throughout the grazing season, following long-term (6 years) implementation of four different ivermectin drenching regimes: neo-suppressive monthly treatment (NST), targeted selective treatment (TST), strategic prophylactic treatment (SPT) and treatment upon observation of clinical signs of ill thrift (MT). Lambs were grazed on replicate paddocks each grazing season following treatment regimes assigned at the beginning of year 1. Faecal samples were collected fortnightly from all animals and allowed to hatch to first stage larvae (L₁). DNA was extracted from individual L₁ and a multiplex PCR assay targeting the ITS2 region of *Teladorsagia circumcincta*, *Trichostrongylus* species and *Haemonchus contortus* conducted. Other species were identified using species-specific PCR. Worm-naïve tracer lambs were grazed on the paddocks at the start and end of each grazing season. Adult worms were recovered from the abomasum, small and large intestines at post mortem to investigate the parasite population on pasture. Results showed an overall decrease in species diversity in egg output from the NST group within a single grazing season. Species diversity was protected over six years in groups implementing TST, SPT and MT treatment regimes, designed to maximise refugia. The expected shift in species prevalence throughout the season from *Teladorsagia* to *Trichostrongylus* was observed in all but the NST group where only *Teladorsagia* were recovered from trial lambs by the end of the experiment. Worm burdens indicated the presence of several species at very low prevalence on pasture in the NST group in 2011 however, these species were not represented in egg output from trial lambs, probably due to the frequent anthelmintic treatment administered throughout the grazing season. The comparable results of the three refugia-based treatment regimes suggest that nematode diversity can be maintained using part or whole group treatments if a rich supra-population of parasites are available to re-infect animals post treatment.

Tuesday 18 August 2015

Concurrent Session 3.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O077/0137

Managing resistance to long-acting injectable moxidectin in sheep nematodes through identifying relationships with the efficacy of oral moxidectin and co-administration of unrelated anthelmintic

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The value of concurrent administration of a short-acting but chemically-unrelated anthelmintic with long-acting moxidectin injectable (Cydectin LA) is being promulgated as a means to slow development of macrocyclic lactone resistance. While effects of concurrent administration on initial efficacy are readily estimated, it is not known if this practice will enhance the residual efficacy (i.e. persistency) through putative effects on larval establishment or parasite fecundity.

An experiment was conducted using Merino weaners on a property on the Northern Tablelands of NSW, Australia. The efficacy of a range of anthelmintics and anthelmintic combinations was determined over a 112 day period. Sample collection and measurements were designed to allow calculation of treatment efficacy, treatment persistency and growth rate. Initial worm egg counts (WEC) were high with mean WEC of the untreated control group being 3500 epG with approximately 70% identified as *H. contortus* and 30% as *Trichostrongylus* spp. There was severe moxidectin resistance detected in the *H. contortus* population. Initial mean treatment efficacy at Day 14 post treatment against *H. contortus* was 52% for moxidectin oral, 66% for Cydectin LA, and 100% for both Zolvix (monepantel) and Cydectin LA/Zolvix. Treatment with Cydectin LA resulted in mean WEC over the 112 day period being 7.2 fold higher than for animals treated with Zolvix at day 0, despite WEC following Cydectin LA treatment continuing to decline by 67% between Days 14 and 70. Treatment with Cydectin LA/Zolvix significantly reduced WEC, and in association with low pasture challenge, remained low. In contrast to effects on WEC, persistent anthelmintics supported higher growth for the first 49 days of the experiment with significant differences among treatments recorded (0.5kg, $P < 0.05$).

This study highlights both risks of ineffective treatments and benefits of long-acting moxidectin on production. Due to seasonal conditions during the experiment, questions remain about managing development of resistance to moxidectin with co-administration of an unrelated anthelmintic.

Tuesday 18 August 2015

Concurrent Session 3.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O078/0298

Investigating the feasibility of targeted selective treatment at lambing in controlling *Haemonchus* sp. while maintaining refugia on sheep farms in Ontario, Canada

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Type II haemonchosis is commonly associated with late gestation and parturition in ewes during the spring in Canada. Due to widespread anthelmintic resistance, targeted selective treatment (TST) of ewes with a novel anthelmintic at lambing was investigated as a potential strategy to manage haemonchosis in both ewes and lambs, while also maintaining refugia. In a pilot study in 2013 and 2014, six client-owned farms were randomly assigned as whole treatment (WT) farms, with all ewes (n=487) treated with oral closantel (10 mg/kg) at lambing, or TST farms, with individual ewes similarly treated at lambing only if they met predetermined criteria (n=293). Pasture samples to assess larval burdens and fecal samples from ewes (n=20) and lambs (n=20) were collected monthly during the grazing season (May-October). Additional anthelmintic treatments given by producers and deaths attributable to parasitism were recorded. Trichostrongyle-type fecal egg counts (FEC), as determined by modified McMaster testing, at the beginning of the study ranged from 200 to 4,539 epg (coproculture determined 13-94% *Haemonchus* sp.). TST reduced the number of animals treated by as much as 51%, and did not significantly affect the number of additional treatments given by producers (p=0.65) or the number of parasitism deaths reported (p=0.87). FECs were higher in lambs on TST than WT farms throughout both grazing seasons. In the first year, *Haemonchus* sp. larval burdens on pasture were generally higher on TST than WT farms at the beginning of the grazing season, but were not significantly different at the end of the grazing season. In the second year, *Haemonchus* sp. larval burdens on pasture were generally higher on TST than WT farms throughout the grazing season, especially at the end of the grazing season. This study suggests that TST of ewes at lambing appears to increase refugia while not significantly affecting the number of deaths or additional treatments given.

Tuesday 18 August 2015

Concurrent Session 3.4

H: Companion animal

Ref: O079/0471

The draft of the revised EMA/CVMP “Guideline for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats”

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The EMA/CVMP has decided to make a proposal for the 3rd revision of this guideline. As ectoparasitic products represent the commercially most interesting sub-market in animal health, there have been multiple new products developed in the previous years including generic and well established use products. A major difference for generics is the fact that one dose confirmation study is required per parasite, unless an approach using the least sensitive parasite species reflecting all aspects of the claim (e.g. immediate and persistent efficacy) is provided or bioequivalence can be shown. In-vivo efficacy evaluations between groups should provide for a statistical significance level of 5%.

Developing flea claims, dose determination and two dose confirmation studies are required followed by a field study in the case of new applications. Efficacy calculations applying Abbott's formula providing for 95% flea count reduction based on the arithmetic mean are mandatory for laboratory studies. Evaluation for immediate efficacy should be done within 0 to 24 hours post treatment. Short term and long term persistent efficacy claims are defined. Specific details are given to the requirements for the granting of claims related to the speed of kill, defining a threshold of 95% flea count reduction within 24 hours post treatment in animals treated with the minimum dose. Further details are given for IGRs and water stability.

There is further guidance on tick efficacy assessment, driven by a number of parenteral applied products being registered lately. The assessment of the acaricidal effect has been adapted. The engorgement status has been removed which facilitates the objective assessment and is seen as a positive step forward. The acaricidal effect is now based on the attachment status (free or attached) and the acaricidal effect (yes or no) only. The demonstration of immediate acaricidal effect is the precondition for the claim “treatment of existing tick infestations”. While locally acting products may generate both, a repellent and a killing persistent activity, systemically acting products can by the nature of its effect have only killing activity and a transmission of infectious diseases cannot be excluded.

This proposal for the revision of the guideline may be commented until end of September 2015. The final version of the guideline is expected early 2016

Tuesday 18 August 2015

Concurrent Session 3.4

H: Companion animal

Ref: O080/0295

False negative antigen tests in dogs infected with heartworm (*Dirofilaria immitis*)

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Dogs with inflammation may develop immune complexes blocking detection of *Dirofilaria immitis* antigen. To determine the prevalence of false negative antigen tests in heartworm infected dogs managed with macrocyclic lactones and doxycycline (slow kill, SK), serum was collected from 25 dogs previously heartworm antigen positive, placed on monthly macrocyclic lactones and doxycycline, and which later tested antigen negative. Serum was also collected from 34 dogs which were antigen negative in practice but the veterinarian still suspected a heartworm diagnosis due to history, clinical signs, or diagnostic imaging results. Samples were tested for *D. immitis* antigen before and after heat treatment to disrupt immune complexes. All samples were antigen negative on commercial assay (DiroCHEK®, Zoetis) by colorimetric detection and spectrophotometry prior to heating. After heating, antigen was detected in 13/25 (52.0%) samples from dogs on SK and in 22/34 (64.7%) samples from dogs for which the veterinarian suspected heartworm in spite of an initial negative test. Medical records indicated that following heartworm diagnosis only 11/25 (44.0%) dogs appeared to compliantly receive SK. Microfilariae of *D. immitis* were occasionally (n=8) detected from dogs with blocked antigen, including one dog managed with SK, but whole blood samples were not available for evaluation from most patients. These data suggest immune complexes can induce false negative antigen tests in many dogs, including those managed with SK, potentially misleading veterinarians and pet owners about infection status, and that compliance with SK appears poor. The presence of microfilaremia in heartworm antigen-negative dogs has implications for resistance selection.

Tuesday 18 August 2015

Concurrent Session 3.4

H: Companion animal

Ref: O081/0127

Release of lungworm larvae from snails in the environment: potential for alternative transmission pathways

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Gastropod-borne parasites may cause debilitating clinical conditions in animals and humans following the consumption of infected intermediate or paratenic hosts. However, the ingestion of fresh vegetables contaminated by snail mucus and/or water has also been proposed as a source of the infection for some zoonotic metastrongyloids. At the same time, the feline lungworms *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* are spreading rapidly among cat populations, along with their gastropod intermediate hosts. The aim of this study was to assess the potential of alternative transmission pathways for *A. abstrusus* and *T. brevior* L3 via the mucus of infected *Helix aspersa* snails and the water where gastropods died.

Twenty-four specimens of *H. aspersa* were infected with c.500 L1 of *A. abstrusus* and *T. brevior*, and assigned to six study groups. Snails were subjected to different mechanical and chemical stimuli, over a 20-day period, in order to elicit the production of mucus. At the end of the study, gastropods were submerged in tap water and the sediment observed for lungworm larvae for three consecutive days. Finally, snails were artificially digested and recovered larvae were counted, and morphologically and molecularly identified. The anatomical localization of *A. abstrusus* and *T. brevior* larvae within snail tissues was investigated by histology. L3 were detected in the snail mucus (i.e., 37 *A. abstrusus* and 19 *T. brevior*) and in the sediment of submerged specimens (172 *A. abstrusus* and 39 *T. brevior*). Following the artificial digestion of *H. aspersa* snails, a mean number of 127.8 *A. abstrusus* and 60.3 *T. brevior* larvae were recovered.

The results of this study indicate that *A. abstrusus* and *T. brevior* infective L3 are shed in the mucus of *H. aspersa* or in water when infected gastropods die submerged. Both elimination pathways may represent alternative route(s) of environmental contamination and sources of the infection for these nematodes under field conditions and may significantly affect the epidemiology of feline lungworm. Considering that snails may act as intermediate hosts for other metastrongyloid species, the environmental contamination by mucus-released larvae is discussed in a broader context.

Tuesday 18 August 2015

Concurrent Session 3.4

H: Companion animal

Ref: O082/0133

Feline lungworms unlock a novel mode of parasite transmission

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Snail-borne lungworms exert an enormous socio-economic impact on the health and welfare of animals and humans. Amongst these parasites, *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* affect the respiratory tract of felids. These lungworms share the same ecological niche and the species of snail (*Helix aspersa*) acting as intermediate host. Recently, the ability of *H. aspersa* to shed infective third-stage larvae (L3s) of *A. abstrusus* and *T. brevior* in the environment has been demonstrated, matching previous knowledge of mode of transmission of human lungworms. In this study, gastropods were experimentally infected with L3s of either *A. abstrusus* or *T. brevior*, or they were housed together with infected live (study 1) or dead snails (study 2). In addition, the survival time of *A. abstrusus* and *T. brevior* L3s was recorded at different temperatures (i.e., 4 and 26°C). Previously uninfected gastropods scored positive for *A. abstrusus* (n=168) and *T. brevior* (n=5). In addition, L3s of both lungworm species were detected in naïve snails co-housed with live or dead infected snails. The maximum survival time of L3s at 4 and 26°C was 35 and 13 days for *A. abstrusus* and 27 and 7 days for *T. brevior*, respectively. Here, we will describe, for the first time, the transmission of L3s from an infected to a naïve intermediate host and refer to this novel route of parasite transmission as intermediasis. The implications of snail-to-snail transmission in the epidemiology of snail-borne diseases will also be discussed.

Tuesday 18 August 2015

Concurrent Session 3.5

I: Novel diagnostics

Ref: O083/0544

Embryonation of *Ostertagia ostertagi* eggs affects the outcome of real-time quantitative PCR

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The aims of this study were to assess how the development of *Ostertagia ostertagi* eggs into first-stage larva (L1) affects the copy numbers of the Internal Transcribed Spacer region 2 (ITS2) of the ribosomal DNA; and based on these results, to suggest optimal storage conditions for faecal samples prior to detection and quantification by real-time quantitative polymerase chain reaction (qPCR). Fresh *O. ostertagi* eggs were isolated from cattle faeces and stored at 4°C or 25°C under aerobic or anaerobic conditions. Embryonation was monitored by microscopy and the ITS2 copies were determined by qPCR at predetermined intervals for up to 336 h. Under aerobic conditions, L1 was observed after 24 h at 25°C, while development to L1 took 336 h at 4°C. A corresponding significant increase of the ITS2 copies was also observed ($p < 0.0001$). However, anaerobic conditions inhibited embryonation at both temperatures and no significant effect of storage on ITS2 copies was noticed ($p = 0.8984$). ITS2 copies were significantly higher in L1 compared with copies in unembryonated eggs ($p < 0.0001$) and with lower coefficients of variation for L1 (33%) compared with unembryonated eggs (266%). In conclusion, storage conditions affect the outcome of qPCR analysis for the quantitative determination of *O. ostertagi* eggs in cattle faeces. Cold storage at 4°C for up to 3 days or anaerobic vacuum packing at 25°C for up to 336 h will entail no undesirable effects on ITS2 copies.

Tuesday 18 August 2015

Concurrent Session 3.5

I: Novel diagnostics

Ref: O084/0186

Evaluation and application of a semi-automated multiplexed-tandem PCR for the specific diagnosis of gastrointestinal nematode infections in cattle

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Parasite infections in livestock combined with problems of drug resistance remain a major economic burden to the industry on a global scale. Gastrointestinal parasitism in cattle is caused by a number of key nematode species that can differ in their pathogenicity and susceptibility to different anthelmintic drugs. The accurate diagnosis of infecting species is of central importance for the effective control but can be challenging and time-consuming by routine diagnostic methods. In particular, studies that investigate the efficacy of anthelmintic drugs on different nematode species, typically done as part of a faecal egg count reduction test, require a high diagnostic sensitivity and specificity of the laboratory test. Our aim was to develop a highly sensitive and specific molecular diagnostic test that can be applied to six common genera and species of gastrointestinal nematodes infecting cattle and to use this test to diagnose infections in cattle in Australia. Detailed assessment of this molecular diagnostic test demonstrated a high level of sensitivity and specificity as well as the ability to quantify the amount of parasite DNA in a faecal sample. Testing of faecal samples from the Australian winter-rainfall environment and direct comparison with standard egg counting procedure demonstrated a vastly improved sensitivity of the molecular test and determined prevalences of 100%, 66% and 7% for *Ostertagia ostertagi*, *Cooperia oncophora* and *Trichostrongylus* spp., respectively. Currently, test kits for gastrointestinal nematodes of sheep are commercially available. This test can be setup within any veterinary diagnostic laboratory and be used to achieve specific results within 24h. Development of a cattle version will be a welcome addition to the diagnostic toolkit required to ensure worm control programs are well-designed and monitored.

Tuesday 18 August 2015

Concurrent Session 3.5

I: Novel diagnostics

Ref: O085/0050

Semiquantitative multiplexed tandem PCR for the detection and differentiation of *Theileria orientalis* genotypes in Australia and New Zealand

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Oriental theileriosis is a tick-borne disease of bovines caused by *Theileria orientalis* complex. Current diagnostic methods to detect *T. orientalis* have limitations in specificity and sensitivity. This study aimed to establish and validate a multiplexed tandem PCR (MT PCR) assay for the simultaneous detection and semiquantification of four genotypes (*buffeli*, *chitose*, *ikedai* and *type 5*) of the *T. orientalis* complex and then apply it to assess prevalence and infection intensity of the genotypes. Analytical specificity, sensitivity and repeatability of the assay were assessed. Assay was then evaluated using DNA samples from herds on which oriental theileriosis had occurred ($n = 200$) and samples from a region where no outbreaks had occurred ($n = 110$). Subsequently, MT PCR assay was employed to test 448 and 154 blood samples from outbreaks in Australia and New Zealand, respectively. The results showed the MT PCR assay specifically and reproducibly detected the expected genotypes and reliably differentiated them, and was able to detect 1 fg of genomic DNA. The diagnostic specificity and sensitivity of the MT PCR were estimated at 94.0% and 98.8%, respectively. In Australia and New Zealand, genotype *buffeli* had the highest prevalence (80.5-87.6%), while *ikedai* had the highest infection intensity (5,528-349,271 DNA copies/ μ l). Genotype *ikedai* had significantly high infection intensity in symptomatic ($P = 0.004$) and severely anaemic cattle ($P = 0.009$) compared to asymptomatic and non-anaemic cattle, respectively. The MT-PCR assay is a practical and effective diagnostic tool and should assist studies of the epidemiology of oriental theileriosis in Asia-Pacific region.

Tuesday 18 August 2015

Concurrent Session 3.5

I: Novel diagnostics

Ref: O086/0350

A multiplexed hydrolysis probe quantitative PCR (qPCR) for diagnosis of bovine theileriosis caused by *Theileria orientalis*

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Theileria orientalis is an emerging pathogen of cattle in Asia, Australia and New Zealand. This organism is a vector-borne haemoprotozoan causing clinical disease characterised by anaemia, abortion and death, as well as persistent subclinical infections. Molecular methods of diagnosis are preferred due to their sensitivity and utility in differentiating between pathogenic and apathogenic genotypes. Conventional PCR (cPCR) assays for *T. orientalis* detection and typing are laborious and expensive. These assays do not provide an estimate of parasite load and therefore cannot be used to differentiate clinical and subclinical infections. Current real-time PCR assays cannot differentiate between clinically-relevant and benign genotypes or are only semi-quantitative without a defined clinical threshold. We have developed and validated a hydrolysis probe quantitative PCR (qPCR) assay which universally detects and quantifies *T. orientalis* and identifies the clinically-associated Ikeda and Chitose genotypes (UIC assay). Comparison of the UIC assay with previously validated universal and genotype-specific cPCRs demonstrated that qPCR detects and differentiates *T. orientalis* with high sensitivities and specificities. Comparison of quantitative results with percentage parasitaemia, determined via blood film analysis, and packed cell volume (PCV) revealed significant positive and negative correlations respectively. One way analysis of variance (ANOVA) indicated that blood samples from animals with clinical signs of disease contained statistically higher concentrations of *T. orientalis* DNA than animals with subclinical infections. We propose clinical thresholds to assist in classifying high, moderate and low-level infections and describe how parasite load and the presence of the Ikeda and Chitose genotypes relates to disease. The multiplex UIC assay has been used to diagnose bovine theileriosis caused by *T. orientalis* in new geographical regions and has also been applied to investigations of potential vectors for this disease in Australia.

Tuesday 18 August 2015

Concurrent Session 4.1

A: Drugs and drug resistance

Ref: O087/0140

Species composition of cultured larvae (L3) indicates reduced moxidectin susceptibility of immature *Cylicocycclus* species in horses

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Background: Shortening of the egg reappearance period (ERP) after ivermectin (IVM) or moxidectin (MOX) treatment is most likely caused either by a decreased susceptibility to treatment or by a faster development of the immature worms into egg producing adults. The species composition of the larval cultures obtained after treatment with IVM or MOX and pyrantel (PYR) can confirm the hypothesis of decreased susceptibility, as this is class-specific, whereas faster development can occur after treatment with anthelmintics with a different mode of action.

Method: Horses (n=24) from 3 farms were divided into 2 groups. The IVM-PYR-IVM group was treated twice with IVM (day 0 and 98) and once with PYR (day 56). The MOX-PYR-MOX group was treated twice with MOX (day 0 and 126) and once with PYR (day 84). Cultured cyathostomin L3s were counted and differentiated with a semi-quantitative reverse line blot using pooled L3, resulting in a larval count per cyathostomin species.

Results: The efficacy of IVM and MOX at day 14 was 100%. The ERP on all 3 farms was however, shorter than expected. The species composition of the L3 cultures obtained after MOX treatment, but not after IVM treatment, was different from that after PYR treatment. *Cylicocycclus* species were most abundant after MOX treatment, but *Cylicostephanus* was most abundant after PYR treatment. After MOX treatment, 3 cyathostomin genera were found on the 2 farms with the lowest efficacy, but only the *Cylicocycclus* genus was found on the farm with the highest efficacy.

Conclusion: The shortening of the ERP seems to be a gradual process in which an increasing number of developing worms from an increasing number of cyathostomin species are becoming less susceptible to MOX (and possibly IVM).

Tuesday 18 August 2015

Concurrent Session 4.1

A: Drugs and drug resistance

Ref: O088/0499

***In vitro* and gene expression studies evaluating the role of P-glycoproteins in resistance to macrocyclic lactones in cyathostomins**

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For decades, cyathostomin nematodes have remained highly sensitive to macrocyclic lactone (ML) anthelmintics, despite intensive selection pressure associated with high treatment frequency. Recent studies have reported a reduction in ML efficacy on stud farms that have a history of frequent ML treatments. Studies in other nematode species such as *Haemonchus contortus* suggest a role for P-glycoproteins (P-gps) in ML resistance. Here, we compared the effect of ivermectin (IVM) on a population of cyathostomins (R) that are phenotypically resistant to MLs (as measured by the faecal egg count reduction test) to an anthelmintic naive population (N) using two *in vitro* bioassays, the larval migration inhibition test (LMIT) and the larval development test (LDT). We consistently detected significant differences in IVM sensitivity between the two populations using the LMIT, but not the LDT. We subsequently evaluated the effect of P-gp inhibitors on populations R and N in the IVM-LMIT and the IVM-LDT using a general linear mixed model. We found that the P-gp inhibitors had a significant effect on IVM sensitivity in both populations in the LDT, but only in population R in the LMIT. This data indicates that the LMIT may be a more robust tool for detecting IVM resistance and evaluating its underlying mechanisms in cyathostomins. Finally, we demonstrated a significant increase in transcription of the gene, *Pgp-9*, in population R compared to N after exposure to IVM *in vitro*. This data suggests that P-gps may play a role in ML resistance in these nematodes. This discovery may lead to the development of an alternative therapeutic strategy, i.e. combining MLs with P-gp inhibitors, to increase ML effect against resistant cyathostomin populations. Such a strategy may be required as an option against triple-class resistant populations, because no new anthelmintics are currently under development for use in horses in the short to medium term.

Tuesday 18 August 2015

Concurrent Session 4.1

A: Drugs and drug resistance

Ref: O089/0494

Growth inhibitory effects of clofazimine on *Babesia* and *Theileria*

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Clofazimine (CF), currently used for treating leprosy, has antituberculosis, antimalarial, leishmanicidal, and antitrypanosomal activities. Objective of the present study is to evaluate the *in vitro* and *in vivo* inhibitory effects of CF on the growths of *B. bovis*, *B. bigemina*, *B. caballi*, *Theileria equi* and *B. microti* with comparison of diminazene aceturate (DA) that is most widely used for treatment of animal babesiosis. The IC₅₀ values of CF against the *in vitro* growth of *Babesia bovis*, *B. bigemina*, *B. caballi*, and *Theileria equi* were 4.5, 3, 4.3, and 0.29 µM, respectively. CF was then evaluated for its *in vivo* efficacy against *B. microti* in mice. The mice treated with oral administration of 20 mg/kg CF showed significant lower peak parasitemia of 5.3% than the control group (45.9%) indicating 96.5% reduction of parasitemia. In addition, we examined side effect of CF by monitoring haematocrit. Although slight anemia was observed in both CF- and DA- treated infected mice, level and duration of anemia was smaller and shorter than that of untreated infected mice. We also examined whether CF completely killed *B. microti* by PCR and blood transfer. On days 40 post-infection when the blood transfer was performed, parasites were not found in blood smears, but DNA of *B. microti* was detected by PCR in blood and several tissues of CF-treated mice. Regrow of parasites was observed in the mice after blood transfer from CF-treated mice. In conclusion, although CF showed the excellent inhibitory effect against *Babesia*, further study is required for the development of novel chemotherapy against piroplasmosis with CF such as combination with other chemicals in near future.

Tuesday 18 August 2015

Concurrent Session 4.1

A: Drugs and drug resistance

Ref: O090/0101

Discovery of Sarolaner: A novel, orally administered, broad spectrum, isoxazoline ectoparasiticide for dogs

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A novel isoxazoline, sarolaner, was identified during a lead optimization search for an orally-active compound with efficacy against fleas and ticks in dogs. The molecule has unique structural features important for its potency and pharmacokinetic (PK) properties, including spiroazetidine and sulfone moieties. The flea and tick activity resides in the chirally pure S-enantiomer. The mechanism of action was established in electrophysiology assays using CHO-K1 cell lines stably expressing flea (*Ctenocephalides felis*) and tick (*Rhipicephalus microplus*) GABA-gated chloride channels (GABACIs). Sarolaner potently inhibited flea and tick GABA-elicited currents, without blocking GABA-induced currents in human GABACIs ($\alpha 1\beta 2\gamma 2$ subtype). Initial whole organism screening was conducted *in vitro* using a blood feeding assay against the cat flea, *C. felis*. Active compounds were subsequently tested in an *in vitro* ingestion assay against the soft tick, *Ornithodoros turicata*. Efficacious compounds which passed safety screening in rodents were progressed to safety, PK and efficacy studies in dogs. *In vitro* sarolaner demonstrated an LD₈₀ of 0.3 µg/mL against *C. felis* and an LD₁₀₀ of 0.003 µg/mL against *O. turicata*. Sarolaner demonstrated safety in dogs, including avermectin-sensitive collies, at doses greater than those required for monthly residual flea and tick efficacy. When dosed orally to dogs, sarolaner had a $t_{1/2}$ of ~11-12 days and bioavailability of $\geq 85\%$. Doses ≤ 5 mg/kg provided efficacy for ≥ 35 days against both fleas (*C. felis*) and ticks (*R. sanguineus*, *Ixodes ricinus* and *Dermacentor reticulatus*) after oral administration to dogs.

Tuesday 18 August 2015

Concurrent Session 4.1

A: Drugs and drug resistance

Ref: O091/0325

Phenotypic and genotypic resistance to synthetic pyrethroids in *Rhipicephalus (Boophilus) microplus* populations from North and South America, South Africa and Australia

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Background: Resistance to acaricides, such as synthetic pyrethroids (SP), has become a widespread phenomenon in the cattle tick *Rhipicephalus (Boophilus) microplus* throughout its distribution area. Bioassays and molecular methods allow assessing the resistance level and pattern of resistance in field populations. In *R. (B.) microplus* four single nucleotide substitutions identified in Domains II and III of the sodium channel gene are known to be associated with target site SP resistance.

Methods: In the present study, we combined two approaches to assess the susceptibility to SP of *R. (B.) microplus* field populations originating from Brazil (17), Argentina (8), Mexico (3), South Africa (3), and Australia (2). We used the Larval Tarsal Test (LTT), a newly developed bioassay, to assess the susceptibility to flumethrin and cypermethrin of these field populations. In addition, we developed a multiplex PCR using allele-specific primers to amplify simultaneously wild type or mutated genotypes of three of the mutations conferring resistance to SP.

Results: The LTT confirmed the widespread resistance to SP in the Brazilian and Argentinean farms (94% and 88%, respectively), as well as in the samples from Mexico, South Africa and Australia.

One mutation conferring resistance to SP (L64I Domain II) was found in all the Brazilian, Argentinean and Australian populations and in one South African population, with resistant allele frequency between 38% and 100% in flumethrin and cypermethrin resistant populations. In contrast, this mutation was not found in samples from Mexico, while a Domain III mutation was found exclusively in this country. The homozygous resistant genotype frequency of the L64I Domain II mutation correlated significantly ($r^2=0.69$) with the survival rates of the ticks at discriminating doses of flumethrin and cypermethrin, as assessed with the LTT.

Conclusions: The molecular survey supported the phenotype assessment done with the LTT and demonstrated the widespread distribution of the L64I Domain II mutation and its presence in several continents.

Tuesday 18 August 2015

Concurrent Session 4.1

A: Drugs and drug resistance

Ref: O092/0424

Investigating the biological roles of scabies mite cysteine proteases and their potential as drug targets

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Cysteine proteases play an important role in many parasitic organisms including immune evasion, invasion and destruction of tissues and cells. The ESTs of *Sarcoptes scabiei* contained five papain-like cysteine proteases (termed Sar s 1a-e). Localisation studies have demonstrated that Sar s 1a-c are present in the mite gut and excreted faeces that are deposited in the mite burrows.

The cysteine protease activity was investigated using a range of synthetic fluorogenic substrates. As we have previously shown that scabies mites release several classes of proteins into the skin that block the host's complement system we tested Sar s 1c in degradation assays of the central complement factors C3 and C5. Consequently Sar s 1c disables complement activation by inhibiting the deposition of C3b, the formation of the C5 convertase complex and the release of the chemoattractant C5a. We propose that Sar s 1c, and possibly the remaining four homologous proteases Sar s 1a, b, d and e, contribute to the complement inhibition seen. This is likely to be only one of multiple roles these proteases play in pathogenicity, however, it already provides an indication that they are essential to the parasite and are therefore good drug target candidates.

As the mite's cysteine proteases are likely to have further functions (e.g. in digestion) we assessed whether the cysteine proteases cleave human epidermal or plasma proteins. We also screened against substrate libraries to define the specificities of the mite cysteine proteases to provide a basis for the design and synthesis of specific enzyme inhibitors. We propose to use the selective inhibitors to explore the proteolytic events involved.

Tuesday 18 August 2015

Concurrent Session 4.2

G: Co-infection

Ref: O093/0182

Bovine tuberculosis and fasciolosis co-infection in cattle populations in Cameroon, Central Africa

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The occurrence of bovine tuberculosis (bTB) and *Fasciola hepatica* infection appears to be negatively associated in the UK. Experimental studies show *F.hepatica* co-infection down-regulates the cell-mediated (CM) immune response to *Mycobacterium bovis* infection in cattle. Hence *F.hepatica* infection could decrease the sensitivity of CM diagnostics that are important in bTB surveillance and control. In Cameroon bTB is uncontrolled and its epidemiology poorly understood. Fasciolosis is suspected to be endemic but little is known about the species of *Fasciola* present. This study aimed to investigate the influence of *Fasciola* co-infection on bTB diagnosis in Cameroonian cattle.

In 2012-13 an abattoir study and a stratified cross-sectional study were conducted in two study sites. The abattoir study sampled 2,106 cattle and *Fasciola* parasites were also collected. The cross-sectional study randomly sampled 1,498 cattle from 100 herds. Individual cattle information was collected and 100 herdsmen were interviewed on husbandry practices. CM-tests and post mortem examinations (PME) were conducted for bTB diagnosis. Diagnosis of *Fasciola* infection was based on faecal egg counts (FEC), PME, a commercial antibody ELISA and development of a *F.gigantica* antibody ELISA.

F.gigantica parasites were identified by RAPD-PCR. No evidence of *F.hepatica* infection was seen. *F.gigantica* sero-prevalence ranged between 44.8-64.1% and bTB prevalence between 1.6-17.4%. Prevalence of both diseases varied within the study sites. 50% of bTB positive cattle were negative for *F.gigantica* infection. The potential influence of *Fasciola* infection on bTB diagnosis using multivariable logistic regression analysis, and the difficulties of using field data to investigate co-infection interaction are discussed.

Tuesday 18 August 2015

Concurrent Session 4.2

G: Co-infection

Ref: O094/0570

Effect of *Fasciola hepatica* on the Response of Bovine Macrophages to *Mycobacterium bovis*

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The liver fluke, *Fasciola hepatica*, affects a wide range of mammals, including man, with approximately 170 million of people at risk of infection. In the livestock industry worldwide, it is estimated to cause annual losses of about €2.5 billion annually. Our group have previously demonstrated that *F. hepatica* can alter the bovine immune response to concurrent *Mycobacterium bovis* and BCG infections by downregulating Th1 responses.

To further investigate the immunoregulatory potential of *F. hepatica*, monocyte derived macrophages (MDM) isolated from cattle prior to and after *F. hepatica* infection were infected *in vitro* with *M. bovis*. The response of the cells to infection was measured by examining nitric oxide (NO) and cytokine protein production as well as gene expression. The results show a significant reduction in pro-inflammatory cytokines IL-1 β , IL-6, TNF- α and NO produced by MDM stimulated with *M. bovis* after *F. hepatica* infection ($p < 0.05$ - $p < 0.001$). Additionally, *F. hepatica* infection reduced mycobacterial phagocytosis by MDM challenged with *M. bovis* or BCG, which correlated with lowered bacterial load in two separate *in vivo* studies when animals were co-infected with *F. hepatica* plus *M. bovis*. We hypothesise that this modification in the immune response of cattle could be the cause or the consequence of reduced mycobacterial phagocytosis and growth, promoted by a latency-like state generated by *F. hepatica*. Considering that liver fluke is endemic in many parts of the world, deciphering *F. hepatica* immune modulation provides an avenue for the understanding and management of infectious diseases where helminth co-infection may have a material effect on disease outcomes.

Tuesday 18 August 2015

Concurrent Session 4.2

G: Co-infection

Ref: O095/0037

Co-infections of *Neospora caninum* and BVDV in water buffalo (*Bubalus bubalis*) and cattle in the Northern Territory of Australia

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A total of 480 serum samples from water buffalo (*Bubalus bubalis*) and 192 samples from cattle, collected over a decade from 18 different survey sites throughout the Northern Territory of Australia, were tested by commercial ELISAs for anti-*N. caninum* and BVDV antibodies. Water buffalo sample testing demonstrated a seroprevalence of 88.3% (95% CI $\pm$ 2.9%) for *N. caninum* and 9.6% ($\pm$ 2.7%) for BVDV antibodies. In comparison, just 31.8% ($\pm$ 6.1%) but 73.4% ($\pm$ 6.4%) of the cattle sera tested positive for *N. caninum* and BVDV antibodies, respectively. This study presents evidence for the first time that *N. caninum* infection in water buffalo in the Northern Territory is highly endemic and infection rates much higher than those for cattle in the same locale. In comparison, this study also showed that although BVDV infection appears to occur in water buffalo in the Northern Territory, exposure to the virus appears low, contrary to the situation in cattle inhabiting the same area. The high prevalence of BVDV within cattle populations in the Northern Territory might suggest that there is a need for a BVDV control program to be developed in the North of Australia.

Tuesday 18 August 2015

Concurrent Session 4.2

G: Co-infection

Ref: O096/0403

A social-ecological approach to sustainable management of gastrointestinal nematodes at the wildlife-livestock interface in Botswana

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Gastrointestinal nematodes of small ruminants are a burden to smallholder subsistence farmers, causing reduced milk production, stunted growth, and mortality. In the Makgadikgadi Pans region of Botswana, farmers live on the border of a national park and grazing land is shared between wild and domestic ungulates. The area hosts one of Africa's largest seasonal migrations of wild herbivores, and the variation in host composition as well as a distinct annual rainy season lead to strong spatial and temporal patterns of transmission of both generalist and specialist parasitic gastrointestinal nematodes. In this project, we worked collaboratively with local farmers to develop sustainable, cost-effective management strategies for gastrointestinal nematodes of small ruminants, using targeted selective treatment methods adapted from Bath and van Wyk's Five-Point-Check©. Data from these farms, in combination with a previously validated climate-based model of nematode transmission, were used to assess seasonal patterns of nematode transmission in livestock in the villages. We extended the model to assess the role that wildlife could play in contributing to or mitigating nematode transmission within the ecosystem, and to identify optimal seasonal timing of treatment in each village. We aim to build resilience to attenuate human-wildlife conflict through a better understanding of the potential benefits of shared grazing land, and through improved livestock health by empowering farmers to treat their livestock in a low-cost, epidemiologically flexible and sustainable way.

Tuesday 18 August 2015

Concurrent Session 4.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O097/0657

Role of lactobacilli against *Giardia duodenalis* infection in suckling mice

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Giardia duodenalis is a protozoan responsible for giardiasis, a disease characterized by intestinal malabsorption, diarrhoea and abdominal pain in variety of mammals. Giardiasis is one of the most common intestinal parasitic diseases in the world making it a high veterinary and public health concern. It has been established that some probiotics confer protection against this parasite *in vitro* and *in vivo*. For instance, *Lactobacillus johnsonii* LA1 (LjLA1) plays a protective role against the growth of *G. duodenalis* WB6 in gerbil. The aim of this project is first to determine the mechanisms involved in this inhibitory effect and then to explore the capacity of other lactobacilli strains to control *G. duodenalis* growth.

Our group has recently identified several active metabolites, which are expressed by LjLA1 and are able to mediate this inhibitory effect. We have cloned lactobacterial enzymes yielding these metabolites as His-tag fusions, in heterologous systems, and purified them in order to study their enzymatic properties as well as their potency to mediate, alone, the anti-parasite activity against *G. duodenalis*. We were able to characterize their substrate specificities and to demonstrate that the resulting metabolites indeed prevented the growth of *G. duodenalis* trophozoites *in vitro*. *In vivo* experiments are currently ongoing. In parallel, a wide collection of diverse lactobacilli strains was screened to assay their potency to also display anti-giardia activity *in vitro*, which allowed identification of several strains having inhibitory effects comparable to those of LjLA1 on *G. duodenalis* growth *in vitro*. The most active of these strains and LjLA1 were then tested *in vivo* against *G. duodenalis* infection in a suckling mice model. Both strains were able to significantly reduce the parasite load in the small intestine, the newly identified *Lactobacillus* strains exhibiting significantly higher anti-Giardia effects than the LjLA1 strain, in newborns.

The output of this project is to propose new therapeutic strategies against *G. duodenalis*, based on the use of probiotics in order to counteract the emergence of drug resistant *G. duodenalis* strains both in human and veterinary applications.

Tuesday 18 August 2015

Concurrent Session 4.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O098/0274

***Eimeria bovis* modulates the LDL-mediated cholesterol up-take and cholesterol modification in infected host cells**

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During first merogony *Eimeria bovis* forms large macromeronts in endothelial host cells containing >120,000 merozoites I. Especially during multiplication, large amounts of cholesterol are indispensable for the enormous offspring membrane production. Cholesterol auxotrophy was proven for other apicomplexan parasites and consequently these parasites scavenge cholesterol from their host cell. We have recently shown that *E. bovis* exploits the host cellular *de novo* synthesis via up-regulating several molecules of the mevalonate biosynthesis pathway (MBP) and strengthened this finding by the fact that blockage of MBP-related molecules via specific inhibitors leads to a significant loss of parasite proliferation. Here, we focused on host cellular cholesterol processing and low density lipoprotein (LDL)-mediated cholesterol up-take. Via FACS analyses significantly increased levels of surface LDL receptor expression were detected in infected host cells. Furthermore, the binding and up-take of non-modified and acetylated LDL was significantly enhanced in *E. bovis*-infected cells and LDL medium enrichment boosted parasite replication. Furthermore, a significant increase of lipid droplet formation was observed in developing macromeronts. Consequently, cellular cholesterol processing was altered in infected cells as indicated by the up-regulation of sterol O-acyltransferase which promotes cholesteryl ester formation, the latter of which was found significantly enhanced. Furthermore, hydroxylation of cholesterol via cholesterol-25-hydroxylase was significantly increased in infected cells as proven by enhanced levels of CH25H gene transcription, protein expression and 25-hydroxy-cholesterol content. Overall, these results show that *E. bovis* considerably exploits the host cell cholesterol metabolism to guarantee its intracellular growth and replication.

Tuesday 18 August 2015

Concurrent Session 4.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O099/0255

Target validation of the inosine monophosphate dehydrogenase (IMPDH) gene in *Cryptosporidium* using Phylomer peptides

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Cryptosporidiosis, a gastroenteric disease characterized mainly by diarrheal illnesses in humans and mammals is caused by infection with the protozoan parasite *Cryptosporidium*. Treatment options for cryptosporidiosis are limited, with the current therapeutic nitazoxanide, only partly efficacious in immunocompetent individuals. The parasite lacks *de novo* purine synthesis, and is exclusively dependant on purine salvage from its host. Inhibition of the inosine 5' monophosphate dehydrogenase (IMPDH), a purine salvage enzyme that is essential for DNA synthesis, is an important drug target against this parasite. In the present study, we describe a unique integrated target validation platform using IMPDH as an example target. The platform, incorporating a forward yeast-two-hybrid system was used to identify Phylomer peptides (peptides derived from the genomes of 25 phylogenetically diverse bacteria) that targeted the IMPDH of *Cryptosporidium parvum* and *Cryptosporidium hominis*. We identified 38 unique interacting Phylomers, of which, 12 were synthesised and screened against *C. parvum* *in vitro*. Two Phylomers exhibited significant growth inhibition (81.2-83.8% inhibition; $P < 0.05$), one of which did not interact with human IMPDH proteins. The present study highlights the potential of Phylomer peptides as target validation tools for *Cryptosporidium* and other organisms and diseases because of their ability to bind with high affinity to target proteins and disrupt function.

Tuesday 18 August 2015

Concurrent Session 4.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O100/0336

Trypsin Activity in *Glossina morsitans morsitans* and some haematophagous insects (*Phlebotomus duboscqi*, *Aedes aegypti* and *Stomoxys calcitrans*) infected with *Trypanosoma brucei brucei*

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Trypsin is the most important factor responsible for the transformation of trypanosomes from the bloodstream forms to the procyclic forms. However, inhibition of trypsin activity using protease inhibitors *in vivo* reduces transformation rates. Trypsin activity was consistently low in teneral unfed *G. m. morsitans*, *P. duboscqi*, *A. aegypti* and *S. calcitrans*. Trypsin activity increased following a bloodmeal and reached a peak between 48-72 h in *G. m. morsitans*, and 24-36 h post-feeding in *P. duboscqi*, *A. aegypti* and *S. calcitrans*, respectively. After the peak, the trypsin levels dropped progressively and by 120 h post-feeding the trypsin level had returned to the same level like unfed flies. These results indicate that a bloodmeal is essential in the stimulation of trypsin synthesis. Midgut homogenates from unfed *G. m. morsitans*, *P. duboscqi*, *A. aegypti* and *S. calcitrans* and at 0 h post-feeding had little or no effect on the parasites, but following a bloodmeal midgut homogenates lysed bloodstream and procyclic *T. b. brucei* and *Leishmania major*. The activity of midgut trypsin of *Glossina m. morsitans* was significantly ($P < 0.0001$) inhibited by bloodstream forms of *T. b. brucei* ingested in a bloodmeal between 0 and 24-h postfeeding. The activities of midgut trypsin of *P. duboscqi*, *A. aegypti* and *S. calcitrans* fed on an infected bloodmeal were lower than the controls but not significantly. In all the insect species, the peak of trypsin was delayed for 6-12 hours. The activity of trypsin in midgut homogenates isolated from *G. m. morsitans*, *P. duboscqi*, *A. aegypti* and *S. calcitrans* midgut trypsin activity was significantly ($P < 0.0001$) inhibited strongly by D-mannose.

Tuesday 18 August 2015

Concurrent Session 4.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O101/0482

Comparison between strategic and TST deworming strategy against *Ascaridia galli* in commercial laying hens

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Ascaridia galli infection has increased markedly in recent years in Sweden due to an increase in non-caged housing of laying hens, which promotes transmission of this intestinal nematode of poultry. In Sweden many egg producers treat their flocks against *A. galli* on up to two occasions during the egg production period. Although we have shown that flubendazole is effective against all developmental stages of *A. galli*, hens seem to be susceptible to re-infection immediately post treatment. Thus contamination with parasite eggs often remains high in chicken barns.

The aim of this ongoing project is to compare the effect of strategic treatment (ST) with targeted selective treatment (TST) on the infection levels of *A. galli* in laying hens. Our hypothesis was that overall egg expulsion after early detection and deworming according to the TST strategy will remain low, which will prevent build-up of the infection and reduce the risks of re-infection.

The study farm encompasses six separate flocks, which were randomly allocated to three study groups in pairs of two; namely TST, ST and UT (untreated control). Pooled faecal samples are obtained every second week during the entire production cycle to calculate nematode eggs per gram faeces (epg). Until now 10 randomly selected hens from each flock have been sacrificed on two occasions (35 and 53 weeks post placement) and cloacal epg counts and worm burdens were assessed. In addition data on egg quality and production as well as body weight and condition are collected.

So far the results show that the overall epg levels from pooled faecal samples is lower in TST (149 ±65) than ST groups (1286 ±419), whereas in UT it is significantly higher (3503 ±694). Preliminary anthelmintic efficacy (E%) of fenbendazole based on epg is 98.9% and 99.4 % for TST and ST groups, respectively. The average worm burden for the first two sampling occasions are 19±18 (ST), 7 ±8 (TST) and 36 ±26 (UT). Cloacal epg counts are 885 ±554 (ST), 225 ±176 (TST) and 2239 ±2098 (UT).

Thus far, the TST programme succeeded at keeping both the number of worms and the overall egg expulsion low compared with the routine deworming strategy.

Tuesday 18 August 2015

Concurrent Session 4.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O102/0480

Purified extracts from chicory (*Cichorium intybus*) inhibit *Ascaris suum* glutathione-S-transferase activity and reduce survival of larvae *in vitro*

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New feed-based control options for parasitic nematodes are urgently needed due to the threat of drug resistance and consumer demand for organic animal products. Chicory is a nutritious bioactive forage that can be used for grazing outdoor-reared pigs and ruminants, and may have direct anthelmintic effects due to high concentrations of sesquiterpene lactones (SL). Here, we investigated direct anti-parasitic effects of extracts from two chicory cultivars on survival and glutathione-S-transferase (GST) activity of *Ascaris suum*, the most prevalent nematode parasite of pigs.

Fresh chicory leaves (cv. Spadona and Puna II) were collected and extracted with methanol. Extracts were then purified by solid-phase extraction for selective recovery of SL. Resulting SL-extracts were analysed by liquid chromatography-mass spectrometry (LC-MS). *A. suum* third-stage larvae (L3) were produced by *in vitro* hatching of eggs, whilst L4 were collected from the gut of experimentally infected pigs. Larval survival was assessed by motility for up to 36 hours after incubation in two-fold dilutions of SL-extracts. Native GST was obtained by homogenisation of adult *A. suum* and purification using a commercial GST isolation kit. GST activity was assessed by the CNDB assay.

Preliminary LC-MS analyses demonstrated distinct SL profiles between the two cultivars. Incubation with SL-extracts dramatically reduced the *in vitro* survival of both L3 and L4 stages of *A. suum*, however the effect was cultivar-dependent, with cv. Spadona significantly more potent than cv. Puna II. Spadona extract also strongly inhibited the activity of *A. suum* GST, suggesting that the anthelmintic mechanism may involve accumulation of toxic reactive oxygen species within the parasite.

In conclusion, if *in vivo* efficacy can be demonstrated, chicory has potential to be used as an alternative or complementary option to control *A. suum* in outdoor pigs. However, our results suggest that the selection of appropriate cultivars will be important. On-going experiments are further investigating the mechanism of anthelmintic action.

Tuesday 18 August 2015

Concurrent Session 4.4

H: Companion animal

Ref: O103/0240

Occurrence of *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* in cats from Italy

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The cat lungworm *Aelurostrongylus abstrusus* occurs throughout Europe while *Troglostrongylus brevior* has been recently recorded in cats from Spain, Italy and Greece. This survey investigated the occurrence of *A. abstrusus* and *T. brevior* in 835 cats (i.e. 97, 88, 134, 222, 182 and 112 from sites I-VI) from Northern (sites I and II) and Central (sites III-VI) Italy. Faecal samples were microscopically examined and their positivity was confirmed molecularly. Fifty-two (6.2%) cats were positive for *A. abstrusus*, 35 (4.2%) for *T. brevior*, while 15 (1.8%) showed a mixed infection. *Aelurostrongylus abstrusus* was found in 2 (2.1%), 11 (11.4%), 4 (3%), 7 (3.2%), 19 (10.4%) and 9 (8%) samples in sites I-VI, while *T. brevior* in 1 (1%), 13 (8.6%), 15 (8.2%), 6 (5.4%) in sites I, IV, V and VI. Mixed infections were recorded in sites IV (2/0.9%), V (11/6%) and VI (2/1.8%). Infected cats were classified based on their age in Groups A (0-6 month-old, 43 cats), B (6-24 month-old, 33 cats), C (older than two years, 26 cats). Cats with aelurostrongylosis, troglostrongylosis and mixed infections, belonged to Groups A (13/25%, 21/60% and 9/60%), B (19/36.5%, 11/31.4% and 3/20%) and C (20/38.5%, 3/8.6% and 3/20%), respectively. These results confirm the presence of *A. abstrusus* throughout Italy and a spreading of *T. brevior* in central regions, especially in kittens and young cats. These lungworms should be always considered in the differential diagnosis of cat respiratory diseases. Further studies are required on treatment and control of feline infections caused by lungworms.

Tuesday 18 August 2015

Concurrent Session 4.4

H: Companion animal

Ref: O104/0401

Efficacy of Broadline® spot-on against feline lungworms: a survey from Italy

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Domestic cats may be infected by several endoparasite species, which may cause a variety of clinical signs. Importantly, some of these pathogens represent a risk for human beings due to their zoonotic potential, whereas others, such as lungworms, may be highly pathogenic for cats. Consequently, the administration of an efficacious treatment against these pathogens, as well as the prevention of the infection, represents an act of responsibility towards animal welfare. In this frame, the increasing reports of feline lungworm infections by *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* spur the interest of the scientific community in pursuance of new pharmaceutical compounds. A novel topical combination of fipronil, (S)-methoprene, eprinomectin and praziquantel (Broadline®, Merial) has been released for the treatment of a variety of feline parasitic infections, but its efficacy has never been tested in cats exposed to feline lungworms under natural field conditions. In the present study, client owned animals (n=191) were enrolled from three geographical areas of Italy and faecal samples were examined by floatation and Baermann techniques. Twenty-three individuals were positive for L1 of *Aelurostrongylus abstrusus* (n=18) or *Troglostrongylus brevior* (n=3) or for both species (n=2) and they received a single dose of the product. In addition, seventeen of those cats were concomitantly infected by other gastrointestinal parasites. Four weeks after treatment, faecal samples were collected and examined. Based on larval counts, the efficacy of the treatment was 90.5% or 100% for *A. abstrusus* or *T. brevior*, respectively. Cats released significantly lower amounts of lungworm larvae after treatment compared to pre-treatment (p<0.0001). All but three cats were negative for other nematodes after treatment and all cats recovered from respiratory signs. Results of this study indicate that a single administration of the topical combination fipronil, (S)-methoprene, eprinomectin and praziquantel is effective and safe for the treatment of *A. abstrusus* and/or *T. brevior* infections in cats living under field conditions.

Tuesday 18 August 2015

Concurrent Session 4.4

H: Companion animal

Ref: O105/0436

Efficacy of emodepside/praziquantel spot-on (Profender®, Bayer) against adult *Aelurostrongylus abstrusus* nematodes in experimentally infected cats

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The adulticidal efficacy of a topical combination of emodepside 2.1% (w/v) plus praziquantel 8.6% (w/v) (Profender® spot-on for cats) against adult *Aelurostrongylus abstrusus* nematodes was evaluated in two randomized, placebo-controlled laboratory efficacy studies. Each study involved 16 cats experimentally inoculated with L3 (800 and 600 each in studies no. 1 and 2, respectively) and randomized into two study groups of 8 cats each after onset of patency. While cats in the treatment group in study no. 1 received a single spot-on application at the minimum therapeutic dose (3 mg/kg emodepside and 12 mg/kg praziquantel), cats in study no. 2 were treated twice with an interval of 14 days. The faecal output of first stage larvae was monitored throughout the study. Necropsy was conducted 4 or 5 weeks after the (first) treatment and the worm counts were used for efficacy calculations. The control groups showed a geometric mean of the total worm count (live and dead worms) of 28.8 (study no. 1) and 17.6 (study no. 2), respectively. All control animals were infected. While the single treatment in study no. 1 resulted in a reduction of the total worm burden by 73.0% ($p = 0.0070$), the treatment protocol in study no. 2 was 99.2% effective ($p = 0.0035$). Based on live worm counts, the efficacy in study no. 2 was 100% ($p = 0.0030$). It is concluded that two applications of Profender® spot-on given two weeks apart represent a safe and highly efficacious treatment regime against feline aelurostrongylosis.

Tuesday 18 August 2015

Concurrent Session 4.4

H: Companion animal

Ref: O106/0636

Northward spread of the nematode *Angiostrongylus vasorum* in the United Kingdom

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The cardiopulmonary nematode *Angiostrongylus vasorum* causes potentially severe disease in dogs, and appears to have spread in recent years outside known endemic foci. Several countries reported their first cases in dogs in the past five years, although increased awareness and prospective sampling no doubt explain some of this apparent expansion. In the UK, the perception of northward spread is similarly challenged by the lack of objective data. The hearts and lungs of 442 foxes of known geographic origin throughout Great Britain were flushed and dissected to recover *An. vasorum* adult worms, following the methods of an earlier survey in 2005 to ensure comparability. Prevalence was 18.3 % compared with 7.3 % previously (n = 546), and had increased in all regions, roughly doubling in those previously endemic (maximum 50.8 %, in the south-east), and appearing frequently in foxes from the north of England and Scotland (prevalence 7.4 %, previously zero). Occurrence in the north was investigated by parallel investigations in dogs and in gastropod intermediate hosts. While none of 60 dogs presenting to veterinary clinics with signs compatible with angiostrongylosis tested positive using molecular, serological or coprological examination, gastropods in three parks in western Scotland were found to be infected by real-time PCR. Prevalence in molluscs was 6.7 % (2.2 – 11.0), and parasite DNA was found in the slugs *Arion ater* and *Ar. hortensis* and the snail *Cornu aspersum*. Results confirm unequivocally the northward spread of *An. vasorum* in Great Britain. Furthermore, this study demonstrates the value of wildlife surveillance and prospective molecular sampling of intermediate hosts in providing objective information on parasite distribution, in a system in which data from the domestic host is highly susceptible to sampling bias.

Tuesday 18 August 2015

Concurrent Session 4.4

H: Companion animal

Ref: O107/0649

Seroprevalence of *Angiostrongylus vasorum* in Swedish dogs: a nationwide study

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Angiostrongylus vasorum, the French heartworm, is a parasite of dogs described in several parts of the world, including continental Europe and the British Isles. Regarding parasite occurrence in Scandinavia, endemic foci are widely present in Denmark, and single endemic cases of *A. vasorum* infections have been identified in dogs and foxes in Sweden, where the parasite was first identified in 2003 on the island of Sydöster (Västra Götaland County). Since then Swedish endemic cases of *A. vasorum* were diagnosed through positive canine faecal samples once in 2011 and 2012 each, while it was found 4, 5 and 4 times in 2013, 2014 and during the first three months of 2015, respectively. A progressively increasing number of faecal samples has been submitted to SVA (Swedish National Veterinary Institute, Uppsala), however the prevalence in dogs appears to be quite low. A large-scale collection of canine serum samples was planned in order to identify the presence and distribution of *A. vasorum* in Sweden using more sensitive methods, i.e. serological methods able to detect parasite antigens and antibodies developed against the parasite. In this first large scale survey, 3886 sera from pet dogs were collected from the Clinical Chemistry Laboratory of the University Animal Hospital (UDS-SLU, Uppsala) as well as from SVA and 544 (14% of 3886) have been tested until now by an ELISA for the detection of circulating antigen of *A. vasorum* and by a separate ELISA detecting specific antibodies against the parasite. Among the analysed samples a total of 0.55% (n=3, 95% Confidence Intervals, CI: 0.11-1.60%) of the animals were positive in both ELISAs, while 2.2% (n=12, CI: 1.1-3.8%) of the tested dogs were antigen-positive only and 2.9% (n=16, CI: 1.7-4.7%) were positive for specific antibodies only. These preliminary results confirm that *A. vasorum* is established in Sweden with a prevalence comparable to other European countries. Definitive results from ongoing analyses will provide a deeper insight on the dissemination of the parasite over the country.

Tuesday 18 August 2015

Concurrent Session 4.4

H: Companion animal

Ref: O108/0710

Prevalence of gastrointestinal and lung parasites in Swiss cats and first evaluations of ELISAs to detect antibodies against *Aelurostrongylus abstrusus*

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From 2013 to February 2015 a total of 405 faecal samples of cats were collected from shelter cats (45.4%), privately owned cats (37.8%) and stray cats (16.8%) in Switzerland. All samples were analysed by sedimentation/flotation method with zinc chloride solution (specific gravity 1.45) and 331 also by the Baermann-Wetzel technique for the detection of first larval stages of lung worms. *Toxocara cati* was the most common detected parasite (n=49, 12.1%), followed by the protozoan parasite *Isospora* sp. (n=33, 8.1%), by *Taeniidae* (n=23, 5.7%) and *Capillaria* sp. (n=18, 4.4%) eggs, and first stage larvae of the lung worm *Aelurostrongylus abstrusus* (n=10, 2.5%). These parasites were particularly common among stray cats (44.1%, 20.6%, 22.1%, 8.9% and 10.3%, respectively), followed by shelter cats (8.7%, 8.2%, 2.7%, 3.3% and 1.1%, respectively) and the privately owned cats (2%, 2.6%, 2%, 3.9% and 0.7%, respectively). Single cats were positive for *Sarcocystis* sp. (n=1), *Toxoplasma gondii* (n=1), *Dipylidium caninum* (n=2) and hookworms (n=3) and *Giardia* sp. (n=4).

Appropriate collection of faecal samples from cats for routine analyses, i.e. collection of faecal material over 3 days, as recommended to increase sensitivity of the diagnostic methods, is often difficult, since free roaming animals typically defecate outdoors. In addition, particularly for the detection of lung worm larvae, fresh faeces are needed. In this view and considering that the course of chronic lungworm infected cats is often subtle and absent of evident clinical signs, serological methods for the detection of *A. abstrusus* would be of great help. First evaluations of different ELISA settings will be presented.

Tuesday 18 August 2015

Concurrent Session 4.5

A: Drugs and drug resistance

Ref: O109/0527

Up-regulation of MHC class II receptors in *Leishmania infantum* infected DH82 cells following TLR2 stimulation

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Canine leishmaniosis (CanL) caused by the protozoan parasite *Leishmania infantum* is a chronic systemic disease resulting from complex interactions between the parasite and the host immune system. Toll-like receptors (TLRs) are essential components of the innate immune system and facilitate the early detection of many infections.

The objectives of this study were (1) to evaluate whether *in vitro* infection of DH82 cells with *L. infantum* promastigotes alters the expression of major histocompatibility molecule (MHC) types I and II with and without stimulation using commercial ligands Pam3CSK4 (TLR2 agonist) and Poly (A:U) (TLR3 agonist) by flow cytometry and (2) to assess and correlate IL-6, IL-10, IL-12 and TNF α production in supernatants. The impact of these ligands on MHC types I and II were determined for *L. infantum* macrophage invasion and replication at 12, 24 and 48 hours post infection. Infection rate and parasite load were calculated by counting the number of infected cells per 100 macrophages and the number of amastigotes per infected cell. Flow cytometry data was compared with the percentage of cells infected at each time point and this correlated with IL-10, IL-12, IL-6 and TNF- α production by ELISA.

No differences in mean fluorescence intensity (MFI) were seen at any time-point for either MHC or in the number of cells expressing either MHC molecule in both unstimulated infected and non-infected cells. Stimulation of DH82 cells with Pam3CSK4 resulted in a lower rate of infection compared with unstimulated and Poly (A:U) stimulated cells. Stimulation of DH82 cells with either ligand had no effect on the percentage of cells expressing MHC class I. However, the percentage of cells expressing MHC class II was significantly higher in Pam3CSK4 stimulated infected cells at every time point observed compared to unstimulated and Poly(A:U) stimulated infected cells. In addition, TNF- α and IL-6 production were significantly higher in Pam3CSK4 stimulated cells compared with both other groups. The results from this study demonstrate an immune evasion mechanism induced by the parasite. In contrast, a significant association between TLR2 agonist and conferring protection against *L. infantum* infection was found highlighting the potential for Pam3CSK4 to be applied as an adjuvant for vaccine or immunotherapy.

Tuesday 18 August 2015

Concurrent Session 4.5

A: Drugs and drug resistance

Ref: O110/0510

Efficacy of orally administered fluralaner (Bravecto™ chewable tablets) and topically applied imidacloprid / moxidectin (Advocate®) against generalized demodicosis in dogs

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A laboratory study compared the efficacy of fluralaner (Bravecto™), formulated as chewable tablets, with the efficacy of imidacloprid / moxidectin (Advocate®), formulated for topical administration, against naturally acquired generalized demodicosis in dogs. Sixteen dogs diagnosed with generalized demodectic mange were randomly allocated to two equal groups consisting of 8 dogs each. Fluralaner was administered orally at a minimum dose of 25 mg/kg body weight on a single occasion to one group of dogs, while the second group was treated topically on three occasions at 28-day intervals with imidacloprid / moxidectin according to label instructions. Mites were counted in skin scrapings and demodectic lesions were evaluated on each dog before treatment and at 28-day intervals thereafter over a 12 week study period. After the single oral administration of fluralaner, mite numbers in skin scrapings were reduced by 99.8% on Day 28 and by 100% on Day 56 and Day 84. Mite numbers in dogs treated topically on three occasions at 28-day intervals with imidacloprid / moxidectin were reduced by 98% on Day 28, by 96.5% on Day 56 and by 94.7% on Day 84. Statistically significantly ($P \leq 0.05$) fewer mites were found on Days 56 and 84 on the fluralaner treated dogs. Both treatments provided a marked reduction in the clinically observed skin lesions on enrolled dogs.

Tuesday 18 August 2015

Concurrent Session 4.5

A: Drugs and drug resistance

Ref: O111/0351

A platform for anthelmintic drug discovery using genome-modified *C. elegans*

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Drug resistance now occurs to all anthelmintics at market, yet recent development is only introducing new molecules to existing targets. As a result, drug resistance quick jumps to these new drugs and they become ineffective shortly after market introduction. Drug development needs to focus on finding drugs with activity on alternative targets. The daf-12 signaling pathway is a promising new target for drug development and may contribute in part to the complex therapeutic effects of Ivermectin. The natural ligand of the daf-12 nuclear hormone receptor is dafachronic acid, which promotes entry into vegetative growth in both nematodes and parasites. Agonist and antagonists of daf-12 hold promise as a novel class of therapeutics for controlling parasite infections in livestock and companion animals. AxumBio is refining high throughput approach for discovery of daf-12 therapeutics. A platform for detecting parasite-specific activity is created in *C. elegans* by inserting a parasite ligand-binding domain (LBD) in place of the native LBD for daf-12. Multiple biomarkers of daf-12 signaling activity have been confirmed by qPCR. Biomarkers are currently being converted to fluorescent reporters of gene activation to enable high-throughput plate reader assays. Ligand libraries are being screened at biomarker loci to find compounds with nanomolar effectiveness. By inserting parasite genes into the *C. elegans* genome, a high-throughput drug screen is created for detecting activity in a whole animal model. The drug-screening platform is being adapted to a wide variety of parasites. The lead platform uses the *L. loa* LBD in replacement of the native daf-12 LBD (paraDAF12-Lloa). Activity data demonstrates the paraDAF12-Lloa chimera rescues dafachronic acid signaling by promoting exit from dauer (parasitic larval) life stage. To battle the growing incidence of drug resistance in companion animal therapeutics, *D. immitis* (paraDAF12-Dimm) and *T. Canis* (paraDAF-12-Tcan) platforms are in production. Platforms to 20 parasites of various social and economic importance are also under development. Because the platform are tailored to detect specific activity to a wide variety of parasites, the method provides a rapid path to discovery and eventual introduction of a novel class of anti-parasite drugs with both specific and broad-spectrum activity.

Tuesday 18 August 2015

Concurrent Session 4.5

A: Drugs and drug resistance

Ref: O112/0344

Unexpected drop in milk production after fenbendazole treatment during early grazing season in dairy cows

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Gastrointestinal nematodes (GIN) infection can impair milk production (MP) in dairy cows. The effect on MP of an anthelmintic treatment applied during autumn-winter has been broadly studied. The MP response after spring anthelmintic treatment would deserve a similar examination as such a targeted treatment could enable to preserve a larger population of parasites in *refugia* (free-living stages on pasture). To investigate whether MP would be optimized by spring targeted selective treatment in grazing cows, we assessed (1) the effect on MP of an anthelmintic treatment applied 1.5 to 2 months after turn-out, and (2) herd and individual indicators associated with the post-treatment MP response. A randomized controlled clinical trial was conducted in 13 dairy farms (578 cows) in western France in spring 2012. In each herd, lactating cows of the treatment group received fenbendazole orally, control cows remained untreated. Daily cow MP was recorded from 2 weeks before until 15 weeks after treatment. Individual serum pepsinogen and anti-*Ostertagia* antibody levels (expressed as ODR), faecal egg counts and bulk tank milk (BTM) ODR were measured at treatment time. Anthelmintic treatment applied during the previous housing period was recorded for each cow. In each herd, information regarding heifers' grazing and treatment history were collected to assess the Time of Effective Contact (TEC, in months) with GIN infective larvae before the first calving. The effect of treatment on weekly MP averages and its relationships with herd and individual indicators were studied using linear mixed models with two nested random effects (cow within herd). Unexpectedly, spring treatment had a significant prolonged detrimental effect on MP ($p < 0.0001$): -0.92 Kg/cow/day on average for the whole period of follow up, down to -1.8 Kg/cow/day on week₉ after treatment. This negative MP response was particularly marked in high producing cows, in cows not treated during the previous housing period or with high pepsinogen levels, and in cows from high-TEC herds or with a high BTM ODR. This post-treatment decrease in MP could be associated with immuno-inflammatory mechanisms, potentially exacerbated by the use of fenbendazole. Until further studies can assess whether this unexpected result can be generalized, non-persistent treatment of immunized adult dairy cows against GIN should be targeted in autumn rather than in early grazing season.

Tuesday 18 August 2015

Concurrent Session 5.1

A: Drugs and drug resistance

Ref: O113/0489

microRNAs and drug resistance in parasitic nematodes

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microRNAs are small non-coding RNAs that play a role in most biological systems by modulating levels of gene expression. We have studied their potential role in anthelmintic resistance using the model nematode *Caenorhabditis elegans* and the parasitic species, *Haemonchus contortus*. In *C. elegans* that have been selected to grow on increasing levels of ivermectin, the expression of two miRNAs is altered significantly compared to N2 (wild type) worms. *mir-85* is significantly up-regulated while *mir-788* levels are reduced. Additionally, in well-characterised *C. elegans* strains that carry known mutations conferring resistance to ivermectin, such as DA1316 and CB4856, the expression of these two microRNAs is altered in the same manner. However, construction of a *C. elegans* line that mimics the expression of these microRNAs (greatly increased *mir-85* in a *mir-788* null background) had no effect on drug resistance. This suggests that while expression of these miRNAs is altered in worms able to tolerate ivermectin, their effects are likely to be indirect.

To identify mRNAs that differ in expression between ivermectin-resistant worms, the *mir-85* transgenic worms and wild type *C. elegans*, we undertook RNAseq to identify possible targets of *mir-85* and *mir-788*. This analysis demonstrated that several *cyp* genes were down-regulated in IVM-resistant worms while in the transgenic lines, the expression of a number of DAF-16 regulated genes was altered.

Finally, a similar analysis in a variety of *H. contortus* isolates yielded a single miRNA that was up-regulated in different isolates of ivermectin-resistant worms. A number of bioinformatics programmes were used to identify possible mRNA targets of this miRNA. We are currently using transcriptomic data from resistant versus susceptible *H. contortus* to narrow down the list of potential target mRNAs.

Tuesday 18 August 2015

Concurrent Session 5.1

A: Drugs and drug resistance

Ref: O114/0208

Expression of nicotinic acetylcholine receptors from parasitic nematodes in *Caenorhabditis elegans*

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Studies on parasitic nematodes are complicated and expensive, even when technically feasible, due to the need for passage through infected animals. The development of a system for expressing important parasite genes in *C. elegans* could make the study of parasitic nematodes much easier and cheaper, allowing us to utilize the powerful genetic resources already available for this model organism. We have shown that we can express ivermectin target genes, such as *avr-14*, from the parasite *Haemonchus contortus* in drug-resistant strains and cause them to revert to drug susceptibility, and also rescue behavioral phenotypes. We have now extended these studies to genes encoding subunits of the levamisole-sensitive nicotinic acetylcholine receptor (nAChR) located at the neuromuscular junction which are well conserved in parasitic nematodes, focussing on *unc-29* and *unc-38*. We have cloned cDNAs of the *unc-29* and *unc-38* orthologs from the clade V parasite, *H. contortus*, and the clade III species, *Ascaris suum*, downstream of the relevant *C. elegans* promoters. These constructs were expressed in *unc-29* (*ok2450*) and *unc-38* (*ok2896*) knock-out mutants of *C. elegans* and co-transformed with a *pmyo-2::GFP* marker plasmid to aid detection of transformed worms. We obtained transgenic lines expressing all of the parasite cDNAs. The *unc-38* (*ok2896*) strain of *C. elegans* has severely defective locomotion and growth and it was immediately apparent that expression of the *H. contortus* UNC-38 subunit rescued these defects. Multiple independent transgenic lines expressing this subunit showed increased sensitivity to levamisole, with IC₅₀ values very similar to those of wild-type *C. elegans*. This rescue was not observed in lines expressing the *A. suum* UNC-38 subunit, which remained uncoordinated and slow-growing. The *unc-29* (*ok2450*) mutants were less severely affected; a partial rescue of the levamisole resistance was observed in some line expressing UNC-29 from *H. contortus*, but very little effect was observed following expression of the *A. suum* UNC-29. The mixed success we have obtained is likely to be due to the complex multimeric nature of the nicotinic receptor and the need for five different gene products to assemble into a functional channel.

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Tuesday 18 August 2015

Concurrent Session 5.1

A: Drugs and drug resistance

Ref: O115/0346

Biotransformation of benzimidazole anthelmintics in *Caenorhabditis elegans* and *Haemonchus contortus*

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Understanding the processes by which parasites metabolize and eliminate anthelmintics is an important goal both for drug discovery and anthelmintic resistance research. We are studying the metabolism of benzimidazole drugs in order to begin to explore these processes.

We have shown that BZs are metabolized to a much greater extent than the other important drug classes; Levamisole, Ivermectin or Moxidectin. We have used a bespoke Larval Motility Digital Analysis (LaMDA) system to measure the effects of drug exposure on worm motility in *C.elegans* and found that metabolism of BZs reduces the potency of the drug on the worm. HPLC and LC/MS analysis of the metabolites produced by *C. elegans* and *H. contortus*, has revealed that, each of five different members of the benzimidazole (BZ) family were modified by the addition of a glucose (+other) moiety, and that *C. elegans* and *H. contortus* produce similar metabolites, suggesting they may share similar detoxification pathways. Biotransformation of drugs by the addition of glucose appears to be nematode specific as it is rare in mammals and had not been previously reported for the benzimidazoles. Glucosidation is catalysed by the mammalian UDP-glucuronosyl/glucosyl transferase (UGT) enzymes which may also be involved in the biotransformation of benzimidazoles in nematodes as we show that Chrysin, a pharmacological inhibitor of UGT, decreases the amount of metabolite produced. Using RNA-Seq to examine the global transcriptomic response to drug exposure has identified candidate enzymes that may be responsible for the metabolism of benzimidazoles and we are now undertaking functional studies to explore this further. These results provide some fundamental insights into how nematodes metabolize an important class of anthelmintic drugs and suggest that *C. elegans* is a valid model in which to study these processes for stronglyid parasites.

Tuesday 18 August 2015

Concurrent Session 5.1

A: Drugs and drug resistance

Ref: O116/0086

Comparison of ivermectin and moxidectin resistance profiles in the nematode *Caenorhabditis elegans*

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The anthelmintic macrocyclic lactones ivermectin (IVM) and moxidectin (MOX) are the most widely administrated drugs to treat nematode infections. Their widespread use has led to the emergence of resistance to these two drugs. Although they have identical modes of action, differences in terms of pharmacokinetics, spectrum and toxicity have been reported between MOX and IVM. Moreover, they do not have the same ability to select for drug resistance. The objective of this study was to investigate and compare the mechanisms underlying the development of MOX and IVM resistance in the nematode model *Caenorhabditis elegans*. For this purpose, we have evaluated the capacity of *C. elegans* to become resistant to MOX through stepwise exposure. Sensitivity to IVM and MOX of the MOX-selected worms was determined and compared with wild-type N2B and IVM-resistant strains with a larval development assay. *C. elegans* developed resistance to MOX but, after 40 weeks, worms were able to grow only on 5.7 nM of MOX, which was twice lower than the IVM concentration on which IVM-selected worms were able to survive. While, in wild-type strain, IVM was more effective than MOX (IC50 of 1.55 ± 0.09 and 2.21 ± 0.41 nM, for IVM and MOX, respectively), MOX had higher efficiency in both IVM-selected (12.57 ± 1.34 and 2.82 ± 0.20 nM, for IVM and MOX, respectively) and MOX-selected (12.76 ± 0.87 and 4.12 ± 0.34 nM, for IVM and MOX, respectively) strains. The effect of co-administration of MDR-reversing agents on drug sensitivity was evaluated in the different strains and discussed regarding the transcriptional profiles of *C. elegans* detoxification system.

We have, for the first time, selected and characterized a MOX-resistant *C. elegans* strain. We show that there are significant differences in the development of resistance to IVM and MOX in *C. elegans*. This may help to understand the differences in IVM and MOX resistance development observed in parasitic nematodes.

Tuesday 18 August 2015

Concurrent Session 5.1

A: Drugs and drug resistance

Ref: O117/0724

A novel pharyngeal nicotinic acetylcholine receptor formed by EAT-2 and EAT-18 as a potential drug target

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Anthelmintic resistance is a major cause of concern in terms of both prevalence and severity. The emergence of resistant strains of parasites has provided the spur for research to develop new anthelmintic targets and drugs. Among the various classes of drugs available, nicotinic agonists are effective and widely used agents. The nicotinic agonists target nicotinic acetylcholine receptors (nAChRs) found at the nematode neuromuscular junction, causing depolarisation and hyper contraction of the parasite. Unfortunately, extensive and uncontrolled use of anthelmintics has led to the emergence of resistance in almost all drug classes including the nicotinic agonists. To address the issue it has become imperative to develop novel antinematodal targets and agents. The aim of our study is to identify the potential of EAT-2, a pharyngeal nAChR subunit and EAT-18, a small transmembrane protein, as chemotherapeutic targets. EAT-2 and EAT-18 have been reported to be involved in pharyngeal pumping in *C. elegans*, hence affecting feeding behaviour. Using molecular techniques, we expressed EAT-2 and EAT-18 from *C. elegans* in *Xenopus laevis* oocytes. To study the pharmacology of the receptor we used the two electrode voltage-clamp technique. Expression of either EAT-2 or EAT-18 alone did not produce a functional nicotinic ion channel. When expressed in combination, EAT-2 and EAT-18 produced a functional receptor that responded robustly to acetylcholine in a concentration-dependent manner. The rank order potency of agonists on this receptor was acetylcholine $\approx$ nicotine > methacholine > carbachol $\approx$ butyryl choline > epibatidine > oxantel. However, there was no response to levamisole, cytisine, pyrantel, and tribendimidine. Various nicotine analogues including alpha-cotinine, nornicotine, anabasine, altinicline failed to produce any agonistic activity. Also, alpha-bungarotoxin did not show any significant antagonistic effect on the expressed nAChR. It is worthy of note that EAT-2 is not an nAChR alpha subunit. In conclusion, we report the ability of a non-alpha subunit to express as a functional homomer in oocytes for the first time. The pharmacology of this previously uncharacterised receptor appears to be different from other nAChR's characterized in nematodes. Thus, this subunit offers a possibility for being used as a potential target site for developing new anthelmintic compounds.

Tuesday 18 August 2015

Concurrent Session 5.1

A: Drugs and drug resistance

Ref: O118/0115

Molecular and genetic basis of drug resistance in *Trypanosoma congolense*

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Isometamidium Chloride (ISM) is one of the principal drugs used to counteract *Trypanosoma congolense* infection in livestock, both as a prophylactic as well as a curative treatment. Numerous cases of ISM resistance have been reported in different African regions, representing a serious problem in the battle against animal African Trypanosomiasis (AAT). To identify genetic signatures associated with ISM resistance in *T. congolense*, the sensitive strain KTT/MSORO M7 has been selected to induce resistance in the murine host. The induction experiment started with the very low dose of 0.005 mg/kg of ISM. After an induction of 4 months, the strain became fully resistant to ISM 1mg/kg, the maximal dose used in livestock. 3 independent biological replicates of the ISM-resistant KTT/MSORO M7 strain have been generated. Differential ISM uptake has been observed by flow cytometry between the sensitive strain and the induced clones, and the drug compartmentalization and accumulation are currently being investigated by confocal microscopy. Full genomes of the sensitive strains and the induced drug resistance strains will be compared in order to identify the putative genomic changes associated with the acquisition of drug resistance. These will be validated using 54 ISM-resistant and -sensitive field-isolated strains that were recently sequenced for phylogenetic and population genetic analysis. The ultimate aim of this study is to characterize the main driving genetic variations responsible for the observed differential ISM uptake and the acquisition of drug resistance.

Tuesday 18 August 2015

Concurrent Session 5.1

A: Drugs and drug resistance

Ref: O119/0723

Asu-ACR-16: a homomeric nAChR drug target widely distributed in *Ascaris* tissues

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Control of nematode parasite infections relies on the use of anthelmintic drugs, several of which act on nicotinic acetylcholine receptors (nAChRs). Widespread use of anthelmintics has given rise to concerns of resistance development in many species. There is an urgent need for development for both new resistance-busting drugs, which require characterization, and validation of novel anthelmintic drug targets. We report functional expression and pharmacological characterization of the homomeric nAChR subunit gene, *acr-16*, from a nematode parasite. Using molecular cloning, PCR and two-electrode voltage-clamp electrophysiology, we have cloned and expressed *acr-16* from *Ascaris suum* in *Xenopus* oocytes. Sequence comparison with vertebrate nAChR subunits revealed *Asu-acr-16* to be most closely related to $\alpha 7$, but with some striking distinctions that may account for their pharmacological differences. RT-PCR showed wide distributions for *Asu-acr-16* mRNA in *A. suum* somatic muscle, ovijector, head and intestine, suggesting it is involved in different functions including digestion, reproduction and movement. In electrophysiological experiments, *Asu-ACR-16* did not respond to cholinergic anthelmintic agonists with the exception of oxantel which produced small current responses. However, other nicotinic agonists: nicotine, acetylcholine, cytosine, 3-bromocytosine and epibatidine produced robust current responses. Predicted structures of the *Asu-ACR-16* extracellular domain were homologically modeled based on the crystal structures of the human $\alpha 7$ nAChR chimeras (PDB ID: 3SQ9, 3SQ6, 4HQP). Modelling inferred the possible binding modes and corresponding binding affinities of nicotinic agonists in *Asu-ACR-16*. Using nicotine as a pharmacophore and the predicted structures of orthosteric ligand-binding sites of *Asu-ACR-16*, the relationship between the chemical structures of nicotine analogs and the electrophysiological responses they elicited in *Asu-ACR-16* were investigated. The nicotine analogs: SIB 1508Y (EC_{50} $0.4 \pm 0.2 \mu M$) and anabasine (EC_{50} $2.0 \pm 0.2 \mu M$) were more potent than nicotine (EC_{50} $4.3 \pm 0.4 \mu M$) on *Asu-ACR-16*, while nornicotine (EC_{50} $22.2 \pm 8.0 \mu M$) was less potent. Interestingly, unlike $\alpha 7$, *Asu-ACR-16* was insensitive to α -bungarotoxin, and did not respond to genistein or other $\alpha 7$ positive allosteric modulators. We hypothesize that *Asu-ACR-16* presents an attractive target for new anthelmintic drugs.

Also, *Asu-ACR-16* had a lower calcium permeability than $\alpha 7$. We hypothesize that *ACR-16* has diverse tissue-dependent functions and presents an attractive target for new anthelmintic compounds.

Tuesday 18 August 2015

Concurrent Session 5.1

A: Drugs and drug resistance

Ref: O120/0726

Effects of cholinergic anthelmintics in a filarial parasite, *Brugia malayi*

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Filarial nematodes (*Dirofilaria immitis* and *Brugia malayi*) cause serious human and animal health problems in tropical and subtropical environments. Treatment of the adult stages of these infections is limited because there are few effective macrofilaricide drugs available. Current drug treatments (microfilaricides) are directed at the larval stages. The *Brugia malayi* genome sequence has allowed us to identify nicotinic acetylcholine receptors (nAChRs) that are predicted to be targeted by existing cholinergic anthelmintics suggesting that some of the classic and newer anthelmintics may be active. We performed single muscle cell PCR assays to identify the nAChR subunits present and whole cell patch clamp recordings on *B. malayi* to characterize filarial muscle nAChR current responses to different cholinergic anthelmintics. The most potent compound was pyrantel which generated the largest current responses (EC50 63.5nM) but acetylcholine (EC50, 2.36 μ M), levamisole (EC50, 3.35 μ M), nicotine (EC50, 30.5 μ M) and buphenium (EC50, 4.24 μ M), also produced currents. We have also been able to test the effects of newer cholinergic anthelmintics like derquantel which caused competitive inhibition of acetylcholine and pyrantel responses. Our results show that the body muscle nAChR receptor of the adult filaria, *B. malayi*, has similarities to other gastro-intestinal nematode parasites. Characterizing the pharmacological properties of the nicotinic receptors of these parasites properties will help to identify novel drug targets as well as it will open up the possibility of designing logical combination therapies with other anthelmintics like ivermectin and emodepside.

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Tuesday 18 August 2015

Concurrent Session 5.2

B: Climate change, epidemiology and modelling

Ref: O121/0222

Spatial distribution of *Fasciola hepatica* in Swedish beef cattle herds and assessment of risk factors

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The liver fluke *Fasciola hepatica* is the causative agent of fasciolosis, which is a production limiting parasitic disease affecting livestock worldwide. In the European Union, financial losses due to *F. hepatica* in ruminant production were recently estimated to € 1.1-2.0 billion per annum.

Spatial models based on Geographic Information Systems (GIS) can be used to develop risk maps and to identify predictors for fasciolosis. In the present study, a serological survey of *F. hepatica* in Swedish beef cattle was performed to determine *F. hepatica* seroprevalence and high-risk areas in Sweden and to characterize potential risk factors for fasciolosis in beef cattle.

A total of 2767 serum samples were collected from first season grazing beef cattle on 2135 Swedish herds between November 2006 and May 2007. The *F. hepatica* antibody levels in serum samples were determined using an in-house *F. hepatica* ELISA. To localize hot spots with *F. hepatica*, spatial clustering of positive and negative herds was tested using a spatial scan statistic. Identification of clusters was performed by Bernoulli model using the SaTScan software. A logistic regression models using climate, environmental data and GIS were developed to evaluate risk factors.

The national seroprevalence of *F. hepatica* on a herd level in Swedish beef cattle was 10%. An irregular spatial distribution of *F. hepatica*, with two main clusters, was observed in south-west Sweden. Beef cattle herd density, dystic leptosol and Fe concentration were positive predictors. Absence of coniferous forest was the environmental variable with highest predictive value. Precipitation in July-September, dystic cambisol, dystic regosol, as well as P and Cu soil concentrations were negative predictors.

Our data suggests that risk for *F. hepatica* infection on spatial level in Sweden is affected by environmental factors rather than climate.

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Tuesday 18 August 2015

Concurrent Session 5.2

B: Climate change, epidemiology and modelling

Ref: O122/0269

Risk factors for lungworm infections in Dutch dairy herds

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Lungworm infections in cattle, caused by *Dictyocaulus viviparus*, are a permanent risk for the dairy livestock industry, due to respiratory symptoms (e.g. coughing), weight loss and reduced milk production. Cattle lungworm infections occur during the grazing season among young stock, but may also affect heifers and adult cows that have not been previously exposed to a sufficient infection level of lungworm larvae to develop an adequate immunity. Lungworm infections can be diagnosed by fecal examination and by detecting *Dictyocaulus viviparus*-specific antibodies in serum or (bulk-)milk. The relative amount of antibodies in bulk milk samples, as detected by an ELISA, is related to the seroprevalence in the herd and the occurrence of clinical lungworm infections, during the current or recently finished grazing season. Bulk milk ELISA results of GD Animal Health (The Netherlands) revealed that on Dutch farms, lungworm infections had occurred less frequently in 2012 as compared to 2011.

The present study was performed to determine which farm management practices, carried out either intentionally or unintentionally, were associated with a negative bulk milk test result in 2012, after positive result in the previous year. Therefore, a questionnaire survey was conducted among dairy farmers in a case-control study. Case farms had positive bulk milk ELISA results in both years, while control farms tested positive in 2011 and negative in 2012. Questionnaires included questions about the general farm characteristics, the occurrence and severity of lungworm infections and on farm management factors regarding animal health, pasture, grazing and anthelmintic treatment.

Exact logistic regression analysis showed that management practices that were associated with an increased odds to retain a positive bulk milk test result in 2012, were a duration of the grazing season longer than 180 days, the presence of other grazing animal species on the farm (e.g. sheep and horses) and housing of dairy cattle to prevent (further) damage due to lungworm infections.

Tuesday 18 August 2015

Concurrent Session 5.2

B: Climate change, epidemiology and modelling

Ref: O123/0651

Quantifying liver fluke (*Fasciola hepatica*) prevalence in wild red deer (*Cervus elaphus*) and associations between infection risk and habitat characteristics

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Wild red deer (*Cervus elaphus*) are hosts of the common livestock parasite, liver fluke (*Fasciola hepatica*). However, the prevalence of *F. hepatica* infection in wild deer and, therefore, their potential to act as reservoirs of infection for other species, is uncertain. Furthermore, the inherently unrestricted movement of wildlife hosts makes identifying habitat characteristics associated with fluke infection risk challenging. In light of well-established parasite-limiting climatological factors, and recently expanding distribution of *F. hepatica*, it is desirable to identify habitat characteristics of wild hosts that may associate with increased future infection risk to sympatric wild and domestic herbivores. This study has two main aims: (i) identification of a suitable diagnostic method to facilitate quantification of *F. hepatica* prevalence in wild deer; (ii) quantification of *F. hepatica* prevalence in wild deer and identification of habitat characteristics, such as land cover, that associate with probability of infection.

Firstly, we quantified the statistical agreement between the widely-used faecal egg count (FEC) method and a commercially available coproantigen ELISA (cELISA) method using wild red deer samples; and in so doing, determine whether diagnosis of liver fluke infection in this species by cELISA is practical. During our study, diagnoses of liver fluke infection in 353 individual red deer by FEC and cELISA were compared. Our results show a high proportion of observed diagnostic agreement between the cELISA and FEC data, which is in part the result of the apparent low prevalence of infection in our study animals. Nevertheless, the cELISA holds at least as much potential as FEC as a practical diagnostic tool for *F. hepatica* infection in red deer.

Following the diagnostic method comparisons, prevalence of *F. hepatica* infection in wild deer was quantified, by cELISA, for nine open-hill sporting estates in the Scottish Highlands. Overall (n = 959), 31% of stags and 18.5% of hinds were infected. Moreover, by using infection status and estimated home range of 886 wild deer, where cull locations were precisely known, we identify habitat characteristics that significantly associate with the probability of infection by *F. hepatica*.

Tuesday 18 August 2015

Concurrent Session 5.2

B: Climate change, epidemiology and modelling

Ref: O124/0476

A decade with *Haemonchus contortus* in Swedish sheep flocks

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The blood sucking abomasal nematode *Haemonchus contortus* is a pathogenic pasture borne parasite of small ruminants. In this study we describe long-term changes in the prevalence and distribution of this parasite in Swedish sheep flocks. Diagnostic records collected between 2005 and 2014 were retrieved from a commercial laboratory (Vidilab), which receives faecal samples from sheep farmers affiliated to the Farm and Animal Health organisation. These farmers are advised to submit faeces from six individual sheep on two occasions annually. The first sampling occasion should ideally target ewes before turn-out and the second should target lambs more than three weeks post weaning. In the laboratory, faeces from groups of three sheep were pooled and nematode eggs were counted with a modified McMaster method. Presence of *H. contortus* was determined mainly by egg morphology but, in some cases, larvae were cultured for confirmation. During the decade of investigation a total of 16,468 faecal samples were analysed from 2,961 herds each submitting samples on an average of 5.6 occasions. There was a gradual annual increase in the number of investigated farms, which increased from 543 in 2005 to 1,711 in 2014. Although only a few of these flocks (0.8%) were examined twice yearly during the whole study period, 19% were investigated more than ten times. In those flocks that were examined more than 10 times and confirmed *H. contortus* positive, parasite eggs were found on an average in 32% of the sampling occasions. Annual prevalence of *H. contortus* positive farms varied between 33% and 42% but did not show any specific trend. On the other hand, the distribution of positive farms increased in mainland Sweden, and *H. contortus* is today found up to the Arctic circle. Furthermore, infected flocks were rarely found on the islands of Öland and Gotland in the Baltic before 2006 but has since then been more frequently diagnosed each year. In conclusion, *H. contortus* is today widespread in sheep throughout Sweden of which some flocks are infected with worms that are resistant to both ivermectin and albendazole.

Tuesday 18 August 2015

Concurrent Session 5.2

B: Climate change, epidemiology and modelling

Ref: O125/0700

Naturally acquired helminth infections in organic pigs

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Introduction: Surveys using faecal egg counts have shown that helminths are common in Danish organic production systems where pigs spend the first 7-9 weeks of life on pastures before transfer to indoor pens with outdoor concrete runs. However, worm burdens have never been directly assessed.

Methodology: Fifteen pigs from the same cohort of litters were selected randomly from each of two Danish organic farms and necropsied at weaning (7-9 weeks old), transfer from the weaning unit to the fattening unit (11-13 weeks old) and at normal slaughter weight (21-23 weeks old). The gastrointestinal tract was examined for nematodes and faecal egg counts enumerated. Soil and bedding material was examined for helminth eggs.

Results and discussion: Infective *A. suum* eggs were detected on pastures and in stables. The overall *A. suum* prevalence across age groups was very high (85%). Liver white spots and recovery of immature *A. suum* (lungs and intestine) peaked in weaned pigs with the lowest levels in the oldest pigs which in contrast had the highest adult worm burdens and egg counts, thus supporting experimental studies showing that pigs may over time develop partial immunity against migrating larvae (prehepatic barrier) while adult *A. suum* may persist. However, in one herd the adult *A. suum* population appeared to decrease following weaning where after it increased once more in the fattening pigs. *Oesophagostomum* spp. burdens were very low in one herd, but all pigs in the second herd were infected and infection levels increased with time, indicating that transmission took place in the stable. Occurrence of *T. suis* was sporadic in both herds.

Conclusions: Piglets were generally exposed early in life on farrowing pastures and were infected when introduced to the in-door pens, where they continued to be exposed. The necropsy results confirm previous results inferred from faecal egg counts and experimental infection studies although there is a large variation between herds. The implications are that cleaning of in-door facilities needs to be combined with deworming at housing if weaners experience clinical problems or poor growth.

Tuesday 18 August 2015

Concurrent Session 5.2

B: Climate change, epidemiology and modelling

Ref: O126/0705

Periparturient parasitism increases the environmental footprint of lamb production

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Background: Ewe parasitism reduces lamb weight gain and increases maternal weight loss. Consequently, this may increase resource need to produce similar lamb weaning weights and restore body weight relative to uninfected ewes, and thus impacting on environmental footprint. Here, we used ewe productivity, feed digestibility and methane yield data to calculate ewe parasitism impacts on methane intensity of lamb production.

Methods: Twin-rearing ewes were fed pelleted lucerne *ad libitum* and infected with *Teladorsagia circumcincta* (PAR, n=16) or sham-infected (CON; n=16) from day₋₃₉ to day₃₆ (day₀ is parturition). A third group were as CON during pregnancy but fed at 80% of CON intake during lactation (RES, n=16). Daily dry matter intake (DMI) was measured throughout, ewes and lambs were weighed weekly, and apparent total tract organic matter (OM) digestibility and methane yield in respiration chambers were determined around day₂₉ and day₃₅, respectively. Methane yield was calculate as methane production per kg DMI, digestible OM intake (dOMI) and lamb body weight gain until weaning at 25 kg. The latter used total predicted DMI, i.e. for lactation and to restore initial ewe body weight. Feed conversion ratio (FCR) was calculated as predicted DMI divided over lamb live weight gain.

Results: Methane yield per kg DMI averaged 10.4±0.56 g and was similar for CON, PAR and RES ewes. However, methane yield per kg dOMI for CON, PAR and RES ewes was 22.0, 25.2 and 20.9 g (s.e.d. 1.54 g; P<0.05). Methane intensity per kg lamb weight gain for CON, PAR and RES ewes was 71.5, 82.7 and 72.1 g (s.e.d. 2.63 g; P<0.05). FCR for CON, PAR and RES ewes was 6.65, 7.77 and 6.97, (s.e.d. 0.46; P<0.05).

Conclusions: Our results support the view that periparturient parasitism increases ewe methane intensity for lamb production, effectively arising from increased overall FCR. Extrapolation of the latter to lambs would predict that methane costs of parasitism in lambs could be considerably greater due to relatively greater impact of parasitism on FCR.

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Tuesday 18 August 2015

Concurrent Session 5.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O127/0725

Epidemiology of gastrointestinal nematode infection in exotic artiodactylids at Disney's Animal Kingdom and efficacy of *Duddingtonia flagrans* on infective larvae in feces

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Gastrointestinal nematodes (GIN) are parasites of major concern for domestic and exotic artiodactylids. Seasonal infection and forage larval availability were assessed at Disney's Animal Kingdom (Florida, USA). Infection peaked during the summer and winter months and the peak forage larval availability was during winter months. The artiodactylids most affected were giraffe, roan/sable antelope and gerenuk. Zoological facilities have used anthelmintics as their primary control method, and challenges in accurate dosing and administration contributed to the development of resistant GIN populations in those settings. The historic dependency on anthelmintics for control is no longer a reliable option. Alternatives are urgently needed and one such alternative is the use of the nematophagous fungus, *Duddingtonia flagrans*. This study evaluated the efficacy of *D. flagrans* (chlamydospores (CS, International Animal Health Products, Australia) administered as a powdered mixture incorporated into feed, in reducing GIN infective larvae (L3) in feces at a dose of 30,000 CS per kg/BW. The CS/feed mixture was fed daily over an 8 week period to giraffe, antelope and gerenuk. Fecal samples were collected on a weekly basis for fecal egg count and then cultured to recover and count GIN L3. Results indicated that *D. flagrans* effectively reduced GIN L3 in the feces during the period of feeding by over 80% in all 3 exotic artiodactylid species. The results from this study demonstrated that the use of *D. flagrans* in exotic artiodactylids infected with GIN could be a long term prophylactic tool to reduce larval availability on forage. Used in conjunction with other control methods, *D. flagrans* could be part of an integrated GIN parasite control program for exotic artiodactylids in zoological facilities.

Tuesday 18 August 2015

Concurrent Session 5.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O128/0709

Vernonia amygdalina: An answer to ivermectin resistance in goat helminths?

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Helminth diseases have been known to lower livestock productivity and cause mortality and ivermectin is a drug commonly used in Nigeria because of it is effective against endoparasites as well as ectoparasites. However, the recognition of chemical residues effect and drug resistance problems in the livestock industry has led to the search for alternatives to treating helminth diseases which are not only environmentally friendly but equally or more effective in controlling helminth infections.

Thirty West African Dwarf goats with no history of anthelmintic treatment were obtained from households and stabilized together. The goats were divided equally into three groups. A was treated with ivermectin injection subcutaneously, B with aqueous extract of *Vernonia amygdalina* and C was control. Faecal samples were collected pre-treatment from each animal and larval culture was carried out. Faecal egg count reduction (FECR) test was carried out for each group and the data analysed using T test at $p < 0.05$.

Predominant helminth infections from larval culture were *Haemonchus contortus* (70%), *Trichostrongylus* spp (61%) and *Oesophagostomum* spp (56%). Mixed infection was present in all the animals. From the FECR test *Vernonia amygdalina* extract was significantly more effective against helminths (100%), compared to ivermectin (78.15%). The result showed that *V. amygdalina* has great potentials that could be explored as for the treatment of helminth diseases in goats. Thus for the rural farmer with little resources to invest in chemical control of goat helminths, planting of *V. amygdalina* in the surroundings for the animals to graze is recommended to help in helminth control.

Tuesday 18 August 2015

Concurrent Session 5.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O129/0053

***In vitro* and *In vivo* effects of *Aloe ferox* plant on gastrointestinal nematodes and live weight gain of growing sheep**

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Producing a cheap and safe anthelmintic drug is the aspiration of many involved in this field because of the negative impact of nematodes on small ruminant production globally. This study evaluated the *in vitro* and *in vivo* effects of medicinal plant *Aloe ferox* Mill on gastrointestinal nematodes in sheep. Ethanolic extracts of different *A. ferox* fractions (dried leaves, pulp & cuticle, and gel of fresh leaves) were tested *in vitro* at concentrations of 5, 10 and 20%. Dried leaves had the highest effect ($P < 0.001$) on nematode larvae. A further *in vivo* investigation was done. Twenty four lambs (aged 3-4 months; initial body weights of 22.1 ± 4.3 kg) were used. Gender, initial eggs per gram (EPG) of faeces and initial body weight were used to place lambs into 4 groups of 6 each. Groups were then assigned to 4 treatments randomly. Four level of dry leaves 0, 50g, 100g and 250g per lamb were given daily for 10 weeks after mixed it with a standard diet. Lambs were weighed weekly. Rectal faecal samples were taken every 7 days up to day 70 and EPG were counted on individual samples. Average daily gain (ADG) increased with *A. ferox* treatments and EPG decreased ($P < 0.001$) with time. A dosage of 250g of dried *A. ferox* leaves caused the highest ADG and maximum effect on EPG. These findings suggest that *A. ferox* has the potential to improve animal weight gain and may be used in conjunction with other anthelmintics to control nematodes.

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Concurrent Session 5.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O130/0134

Effect of *Gliricidia sepium* leaves intake on larval establishment of *Cooperia* spp., in calves

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The use of bioactive plants has been suggested as an alternative for the control of gastrointestinal nematodes. Recent *in vitro* studies have demonstrated the anthelmintic (AH) effect of *Gliricidia sepium* (GS) extracts against cattle nematodes. This study aimed to: 1) assess the *in vivo* anthelmintic effect of GS on the establishment of *Cooperia* spp., third-stage larvae in calves, 2) assess morphological features of established worms, and 3) isolate an AH-like phytochemical from leaves offered to animals. Twelve ¾ Holstein x Zebu calves were divided in two experimental groups: control and treatment (T1 and T2; n=6). The 17-day trial was divided in three periods: i) adaptation (7-d), ii) experimental (6-d), and iii) post-treatment (4-d). After adaptation, each calf was infected with an oral dose of 400 L₃ *Cooperia* spp/kg LW. During the trial, the basal diet consisted of *Digitaria decumbens* hay (6.27%CP) and commercial concentrate (12%CP). In addition, during the experimental period T2 received fresh GS leaves (26.88%CP) *ad libitum*, while T1 remained on the basal diet with the amount of concentrate adjusted to obtain isoproteic diets. Six calves were randomly selected for slaughter 9-d post-infection, and worm counts and measurements performed. Larval establishment rates were: 13.44±0.13% and 3.1±1.42% for T1 and T2, respectively ($P<0.05$). The reduction of larval establishment was 76.9%. Differences were found for dry matter intake and worm length ($P<0.05$). Bio-guided fractionation lead to the isolation of a flavonol with anti-exsheathment activity. Results confirm that the ingestion of GS leaves reduced larval establishment of *Cooperia* spp., in cattle.

Tuesday 18 August 2015

Concurrent Session 5.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O131/0318

Can chitinolytic microfungi be used to reduce the number of chicken ascarid eggs in soil?

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Parasitic ascarids (*Ascaridia galli* and *Heterakis* spp.) are highly prevalent in laying hens kept in organic and free-range systems. This problem is mainly associated with continued access of hens to the outdoor facilities where ascarid eggs may accumulate, survive and remain infective in the soil for many months. In this study, we investigated the ability of three species of naturally occurring chitinolytic microfungi (*Pochonia chlamydosporia* Biotype 10, *Metarhizium brunneum* KVL04-57 and *Trichoderma harzianum* T22) to degrade ascarid eggs dispersed in soil. Fresh unembryonated eggs of chicken ascarids (mainly *A. galli*) were added to sterilized or unsterilized soil in Petri dishes without (Control) or with conidia/chlamydospores of each of the three fungal isolates and incubated in darkness at 22°C. The eggs were isolated from the soil by a sieving and flotation technique, and counted using McMaster slides at day 0 and 30 post treatment. For the sterilized soil, the mean egg count reductions from day 0 to 30 in the *P. chlamydosporia* Biotype 10, *M. brunneum* KVL04-57, *T. harzianum* T22 and the Control treatments were 45.7%, 30.2%, 4.7% and 4.6%, respectively. In contrast, incubation in unsterilized soil showed reductions of 68.5%, 76.7% and 68.1% by *P. chlamydosporia* Biotype 10, *M. brunneum* KVL04-57 and Control, respectively. The contrasting results between the sterilized and unsterilized soil indicate that the survival of ascarid eggs and the effectiveness of fungal treatment may be influenced strongly by biotic factors of the soil. Before assessing the applicability of this new approach, further investigations using several soil types with different microbial activity may elucidate the influence of these factors on ascarid egg survival.

Tuesday 18 August 2015

Concurrent Session 5.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O132/0643

Effects of carob pod (*Ceratonia siliqua*) meal fed in sheep against gastrointestinal nematodes

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The present study aimed to examine the potential effect of condensed tannins in carob (*Ceratonia siliqua*) pods incorporated in sheep rations against *Haemonchus contortus* and *Trichostrongylus colubiformis*. This, was studied in 3 trials testing the anthelmintic effect of: i) carob (CAR) pods meal incorporated in different concentrations in sheep ration ii) CAR with or without a tannin inhibitor (PEG) and iii) CAR meal fed alone or in combination with sainfoin (*Onobrychis viciifolia*/S) fed as pellets. Animals enrolled in all trials were 3-month old lambs, experimentally infected with both parasites. CAR rations were administered from two weeks before infection and controls were fed a diet based on lucerne. Faecal egg counts (FEC) were performed at regular intervals and at the end all animals were sacrificed for worm burdens and female fertility. In trial 1, 35 lambs (5 groups of 7 lambs each) were fed 5 different concentrations of CAR varying from 0% to 100% of the maximum possible concentration in feed. The results showed a reduction in FECs, up to 35.3%, only in the group fed with the maximum concentration of carob in the ration. The latter was the CAR concentration in feed used for trials 2 and 3. In trial 2, 4 groups of 6 lambs were enrolled from which, 2 groups were fed with a CAR or CAR+PEG diet and two further receiving a tannin-free diet, served as Controls (C), (C & C+PEG). PEG was administered orally on a daily basis for 2 weeks and FEC were recorded on days 4, 8, 12, 16. Lambs were sacrificed a week later. The results showed a reduced FEC, *H. contortus* adult worm counts and female fertility in CAR group and not in CAR+PEG group. The effect of PEG to reverse this effect indicates a potential condensed tannins action. In trial 3, 4 groups of 6 lambs received a diet based on CAR, S or a combination of both compared to a Control diet. FECs were recorded for 6 times on a weekly basis and then lambs were sacrificed. Results showed reduced FEC values in both CAR+S and S groups over time (up to 80%) together with *H. contortus* reduced worm numbers (>50% reduction) compared to the controls. Moreover, a reduced *T. colubiformis* female fertility values (46%) was recorded in the CAR+S group suggesting a possible synergism. Overall, the results confirm the potential effect of carob meal to disturb the biology of GINs. However the variability in the results suggests a strong need for further studies.

Tuesday 18 August 2015

Concurrent Session 5.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O133/0664

Targeted use of bioactive forages as a curative treatment for organic lambs

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In developing a practical control program for organic lambs, the ability to identify individual animals suffering from parasitism and subsequently graze them on bioactive forages (chicory, plantain) should provide a chemical free method of reducing the impact of parasitism. Trial area consisted of two 2 ha blocks, each block had 1.5 ha sown in ryegrass (AR1) and 0.5 ha in a mixed sward lay of chicory/plantain and white cover. Blocks were either seeded with *Teladorsagia circumcincta* or *Trichostrongylus colubriformis* eggs. In year one 64 weaned lambs were allocated on liveweight to one of two block (n=32), and were rotational grazed within each block on the ryegrass sward, from November 2012. On each block 8 animals were drenched monthly and grazed on pasture only. Animals were monitored at two weekly intervals for liveweight, to identify those animals whose performance was below their target weight, and these animals were then drafted off and allowed to graze the bioactive swards. Individual faecal samples (FEC) were taken fortnightly; pasture production measured weekly and was used to set liveweight targets determined by 'happy factor' algorithm (Greer et al 2010). Overall growth rates on the lambs grazing on the *T.circumcincta* and *T.colubriformis* infected blocks were 200 g/d and 160 g/d respectively. For both pasture suites there was a significant paddock time (p<0.05) early on that reflected a benefit of hospitalisation on LWG early in the season, with 80% of lambs responding to treatment. Drenching improved growth rates by 32% and 39% in comparison to TST animals for *T. circumcincta* and *T. colubriformis* blocks, respectively. Mean FEC were high (>1000 epg) on both blocks and there were no significant differences in FEC between animals grazing ryegrass or hospital paddocks. In year two any bioactive effect of the plantain /chicory sward was investigated. After 4 weeks grazing, half of the lambs allocated to ryegrass or bioactive sward were drenched. Lamb LWG and FEC were monitored weekly for a further 4 weeks. Drenching of lambs onto bioactive sward was more beneficial than drenching back onto pasture however LWG were reduced by 80% in untreated lambs grazing on either ryegrass or plantain /chicory sward.

Tuesday 18 August 2015

Concurrent Session 5.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O134/0682

Bioassay-guided purification of anti-parasitic compounds from indigenous Ethiopian medicinal plants

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Background: Smallholder farmers and pastoralists in developing countries often rely on ethno-veterinary medicinal plants to control livestock parasitism. Here, we used bioassay-guided fractionation and purification of active constituents from *Cissus ruspalii* and *Adenia* sp., two Ethiopian medicinal plants with purported anthelmintic activity.

Methods: Powdered plant root samples (50 g each) were macerated in 1 l of de-ionized water containing 0.05% chlorobutanol through continuously stirring at room temperature for 72 h. Macerated samples were filtered through Miracloth and centrifuged. Clarified extracts (6.6 ml) were passed through glass columns containing 132 ml bed volume of Bio-Gel P-2, eluted with de-ionized water. Fifty 4-ml fractions were collected. Nematode egg hatch inhibition (EHI) was quantified over 48 h by incubating *Teladorsagia circumcincta* eggs with the crude extracts and collected fractions. The active fractions were further separated into distinct bands by preparative layer plates, purified and again bio-assayed.

Results: Crude extracts of both plants showed 100% EHI, compared to the <1% for water with 0.05% chlorobutanol. Furthermore, six *C. Ruspalii* and seven *Adenia* sp. fractions showed remarkably strong, dose-dependent EHI activities, with several undiluted fractions showing similar EHI as the crude extracts. Some of the purified compounds from these fractions showed 83% of EHI and larvicidal effect against the first stage larvae.

Conclusions: Our bioassay-guided fractionation and purification of compounds with demonstrated anthelmintic properties assist to validate the ethno-medical use of the two plants studied. The purified active compounds have been submitted for mass spectrophotometry and proton & carbon-13 nuclear magnetic resonance analysis so that compound structure can be elucidated.

Acknowledgements: The authors gratefully acknowledge funding from CIDLID, supported by DFID, BBSRC and SG (BB/H009299/1) and SRUC International Engagement Strategy.

Tuesday 18 August 2015

Concurrent Session 5.4

H: Companion animal

Ref: O135/0015

In vitro synergistic effect of permethrin combined with dinotefuran against the cat flea (*Ctenocephalides felis felis*)

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Introduction: Neonicotinoids and pyrethroids are two classes of insecticides which are combined (dinotefuran:permethrin, 1:7.3) in an ectoparasiticide (Vectra®3D) which is currently used as an effective treatment against ticks and fleas in dogs. This study was designed to assess the susceptibility of fleas (*Ctenocephalides felis felis*) to permethrin, dinotefuran and combination.

Methods: Insecticidal efficacy of dinotefuran, permethrin and a dinotefuran/permethrin combination (1:7.3 ratio) was evaluated using an in vitro coated-vial bioassay. Serial dilutions of each compound were conducted with an acetone/triton solution. Vials were treated with dinotefuran, permethrin, dinotefuran/permethrin (1:7.3 ratio) or solvent alone. Vials were capped and allowed to dry for at least 4 hours before the addition of 10 newly emerged (0-7 days old) unfed adult fleas. Flea susceptibility was assessed at 48 hours post-exposure by evaluating mortality. EC₉₀ values and a combination index (CI) values were calculated to assess the potential for synergistic activity.

Results: No significant mortality was observed in the control treatments (solvent only and untreated vials). Dinotefuran (EC₉₀ = 0.76 µg/cm²) was more potent than permethrin (EC₉₀ = 1.34 µg/cm²) against fleas. The combination of the two active ingredients was more potent (EC₉₀ = 0.28 µg/cm²) than any individual active. Moreover, the CI (0.41) was below 1, indicating a strong synergistic effect between the two active ingredients at the studies ratio.

Conclusion: This study contributes to demonstrate that the combination of dinotefuran and permethrin at the ratio 1:7.3 is synergistic and more potent against fleas than each individual active.

Tuesday 18 August 2015

Concurrent Session 5.4

H: Companion animal

Ref: O136/0029

Efficacy of fipronil combined with permethrin commercial spot-on (Effitix®) preventing *Phlebotomus perniciosus* from feeding on dogs

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A clinical trial was carried out to assess the anti-feeding and adulticidal effect of a spot-on combining fipronil and permethrin (Effitix®, Virbac, Carros, France) against *Phlebotomus perniciosus* in dogs. Twelve dogs selected out of 14, based on pre-treatment engorged sandfly counts were included in this single-center, randomized, blinded and controlled trial, performed in accordance with the Animal Welfare, Good Clinical Practices and the Ethics Committee. They were randomly allocated into two groups of six dogs: an untreated control group and a group treated with Effitix® at the recommended dosage (minimum dose of 60.00 mg.kg⁻¹ for permethrin and 6.70 mg.kg⁻¹ for fipronil). All dogs were challenged with 80 female sandflies for 60 ± 5 minutes on Days -7, 1, 7, 14, 21 and 28 (Day 0 being treatment day). The number of engorged, dead and live sandflies was determined 1 and 24 hours after each exposure to treated and untreated dogs. The anti-feeding effect of the spot-on formulation was 94.1, 97.8, 96.3, 90.8 and 87.0% on Days 1, 7, 14, 21 and 28 respectively. The mortality effect at 24 hours was 98.9, 99.3, 99.8, 97.6 and 91.1% on Days 2, 8, 15, 22 and 29 respectively. At each challenge point, the mortality and anti-feeding effects on sandflies were significantly different between the control and treatment group ($p < 0.05$). The results indicate that the combination of permethrin and fipronil could be used as an effective sandfly control strategy in dogs and is therefore recommended to be integrated in a leishmaniosis prevention program.

Tuesday 18 August 2015

Concurrent Session 5.4

H: Companion animal

Ref: O137/0083

Rapid onset of pulicidal activity of spinosad compared to afoxolaner

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Advances in the development of monthly pulicides have seen movement away from topical application to more reliable oral treatment. The first monthly oral pulicide, spinosad, has demonstrated rapid onset of flea knockdown and a full month of activity. Recently, afoxolaner has also been developed as a monthly oral pulicide. To compare the speed of flea knockdown of these oral products, two parallel assessor-blinded studies were initiated in two different laboratories, using different dogs and flea sources. Infestations were completed with approximately 100 fleas per dog on Days -5 (for randomization), -1 and 7. In each study, 6 groups of 6 dogs per group were randomized to treatment with either spinosad or afoxolaner—each administered according to label—or to a negative control group, and then to flea counts at either 1 or 3 hours post treatment (infestations present at treatment), and 1 or 3 hours after infestations on Day 7. In both studies on both assessment days, arithmetic mean live flea count reductions were significantly greater in the spinosad than in the afoxolaner group at both 3-hour assessments ($p < 0.0001$ to $p = 0.0013$), and in one study at 1 hour post-treatment ($p = 0.0263$) and 1 hour post infestation on Day 7 ($p < 0.0001$). Between-treatment differences remained significant if moribund fleas were included as dead fleas. In conclusion, orally administered spinosad provided faster flea knockdown than afoxolaner against existing and subsequent infestations for at least one week after treatment.

Tuesday 18 August 2015

Concurrent Session 5.4

H: Companion animal

Ref: O138/0456

Evaluation of a collar device based on Deltamethrin and Propoxur in controlling sand flies on dogs

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Some strategies of control such as the use of collars impregnated with insecticides are considered important tools to control the infection by *Leishmania infantum* in dogs. In this study the efficacy of a collar device impregnated with Deltamethrin (4g) and Propoxur (12g) in dogs experimentally exposed to *Lutzomyia longipalpis* was assessed. Healthy dogs (n = 16), with different breeds and ages were used in this study. The animals were allocated in two groups containing eight dogs each. The study was conducted during a period of 182 days, and eight experimental infestations were performed for each animal. Initially, the animals were sedated and then put individually in an experimental cage (glass box) closed in some points with a net. Sand flies (60 females and 20 males) were released inside the box and maintained for a period of 50 minutes. The results showed in the control group an average of 95% of the insects remained alive during the experimental infestation. On the other hand, in the treated group the mortality rate was higher (94.91%) and this collar device provide 89.93% of protection against sand flies bites. In conclusion, the collar herein studied played an important role in the control of sand flies, promoting the protection of the animal during six months. Therefore, the risk of infection by *Leishmania infantum* in dogs and consequently in humans, may be reduced through the use of this collar device.

Tuesday 18 August 2015

Concurrent Session 5.4

H: Companion animal

Ref: O139/0487

Rapid and complete flea control in dogs using a Dinotefuran-based flea spray

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Rapid and complete treatment of existing flea infestations and prevention of new infestations is very important in canine boarding or hospitalization situations in order to prevent environmental infestation by incoming animals and subsequent infestation of other pets sharing that environment. The objective of this work was to demonstrate the rapidity and adulticidal efficacy against fleas (*Ctenocephalides felis*) of a dinotefuran spray (Vectra[®] Pro Flea Spray) in dogs.

Sixteen beagle dogs were used in this randomized, negative control study. Dogs were allocated to two groups of eight animals based upon flea carrying capacity demonstrated by infestation and 24 hour flea counts conducted prior to the study. Dogs were infested with 100 (± 3) viable unfed adult fleas on Day -1. Vectra[®] Pro Flea Spray was applied on Day 0 at a rate of 100 mg/m² and control dogs were sham treated. The time after treatment that the first dead flea fell off each animal and the number of dead fleas recovered from a catch pan under each cage at 15, 30, 120, and 240 minutes after treatment were recorded. Live fleas were counted and removed at 4 hours after treatment. Dogs were re-infested with 100 (± 3) viable unfed adult fleas on Day 3 and live fleas were counted and removed 48 hours after infestation on Day 5.

No dead fleas dropped from any of the control animals during the 4-hour observation period whereas dead fleas began to drop of the dogs treated with Vectra[®] Pro Flea Spray within 15 minutes and the numbers of dead fleas recovered increased progressively. The percent efficacy at four hours (calculated on the live flea counts using Abbots formula and geometric means) was 96.3% and the percent efficacy on Day 5 was 100%. All results were significantly different than controls. There were no abnormalities or adverse events noted in any of the dogs.

These results demonstrate that Vectra[®] Pro Flea Spray is safe for use in dogs and it is rapidly efficacious against fleas: it starts killing fleas within 15 minutes of application, is effective for treatment of existing infestations by four hours after treatment, and is effective at prevention of re-infestation for at least 5 days after treatment.

Tuesday 18 August 2015

Concurrent Session 5.4

H: Companion animal

Ref: O140/0583

A combination of imidacloprid and flumethrin (Seresto® Collar) prevents transmission of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* from naturally infected *Ixodes scapularis* ticks to dogs

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Vector borne diseases in dogs are of growing concern for pet owners and veterinarians. Protecting dogs against vectors, e.g. ticks, represents a significant challenge. To determine the effectiveness of the Seresto® Collar (imidacloprid/flumethrin, Bayer Health Care, Animal Health) in the prevention of transmission of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* from naturally infected *Ixodes scapularis* to dogs, thirty, male or female beagle dogs, greater than 1 year-old were allocated to 3 groups of 10 dogs each. Treatment groups were: Group 1: non-treated control dogs; Group 2: dogs with collars placed 7 months prior to tick infestation; Group 3: dogs with collars placed 1 month prior to tick infestation.

Ixodes scapularis were obtained from infested environments in Rhode Island, USA (in a representative sample 54% were found to be infected with *B. burgdorferi*; 12% with *A. phagocytophilum*). Dogs in all groups were infested by placing ticks (approximately 30 males plus 50 females per dog) in the hair coat along the dorsal midline distant from the collar. Each dog was tested for *B. burgdorferi*-specific antibodies prior to infestation and on days 17, 31, 45, 65, and 86 after infestation using the Canine Lyme Multiplex Assay and the Snap® 4DX Plus test (IDEXX Laboratories). Three dogs were removed from the study (1 in group 1; 2 in group 3) due to discrepancies in the Canine Lyme Multiplex Assay results.

Efficacy was based on day 86 post infestation results: 7 of 9 group 1 dogs, 0 of 10 group 2 dogs and 1 of 8 group 3 dogs were positive for *B. burgdorferi* antibodies (Osp A, C, or F) using the Canine Lyme Multiplex Assay. When serum samples were examined with Snap® 4 DX Plus, 8 of 9 group 1 dogs, 0 of 10 group 2 dogs, and 0 of 8 group 3 dogs were positive for *B. burgdorferi* antibodies. All 9 dogs in group 1 were positive for *A. phagocytophilum*, while none of the dogs in groups 2 or 3 (n=8) were positive for *A. phagocytophilum* (Snap® 4DX Plus test).

We conclude that the Seresto® Collar was effective for at least 7 months in the prevention of transmission of *B. burgdorferi* and *A. phagocytophilum* from infected ticks to dogs under the conditions of this study.

Tuesday 18 August 2015

Concurrent Session 5.4

H: Companion animal

Ref: O141/0630

Comparative efficacy of topically applied permethrin/ imidacloprid and oral administration of afoxolaner or fluralaner against transmission of *Ehrlichia canis* by infected *Rhipicephalus sanguineus* ticks to dogs

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The ability of the commercial topical spot-on combination of permethrin/imidacloprid (Advantix®) to prevent transmission of *Ehrlichia canis* by infected *Rhipicephalus sanguineus* ticks to dogs has been previously reported. The recent market introduction of chewable tablets containing the novel compounds, afoxolaner (NexGard™) and fluralaner (Bravecto™) enabled us to conduct a comparative efficacy study with respect to the ability of these three products to prevent transmission of *Ehrlichia canis* by ticks to dogs.

The study was a blinded parallel group design, wherein 32 dogs were randomised into 4 different groups of 8 dogs, dosed on day 0 with fluralaner, respectively days 0, 28 and 56 with permethrin/imidacloprid, afoxolaner or with a placebo (negative control). Each dog was artificially challenged by releasing 80 adult *E.canis*-infected *Rhipicephalus sanguineus* ticks into their sleeping kennels on day 31, 38, 45 and finally on day 52.

The animals were monitored for clinical signs of monocytic ehrlichiosis (pyrexia and thrombocytopenia) and were tested for the presence of *E.canis* DNA by qPCR and for specific antibodies using IFA. A dog was considered infected with *E.canis* if both PCR and IFA yielded positive test results up to day 84.

Six out of eight control dogs became infected with *E.canis*, as demonstrated by the detection of *E.canis* DNA and the presence of specific *E.canis* antibodies in blood samples.

Two out of eight dogs treated with Bravecto™ and four out of eight dogs treated with NexGard™ acquired the infection, whereas none of the dogs treated with Advantix® became infected with *E.canis*.

It is concluded that only the monthly treatments of Advantix® completely blocked the transmission of *E.canis* and thus provided adequate protection against monocytic ehrlichiosis.

Tuesday 18 August 2015

Concurrent Session 5.4

H: Companion animal

Ref: O142/0662

Prognostic markers for the outcome of acute *Babesia canis* infections

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The tick-borne haemoprotzoan parasite *Babesia canis* is a prevalent and clinically relevant canine *Babesia* species in Europe characterised by haemolytic anaemia. The disorder manifests as uncomplicated or severe forms. The complicated form is connected to severe clinical signs and high mortality. The severity of acute babesiosis is not always proportional to the degree of anaemia, and it's not correlated to the level of parasitaemia. Hence, we evaluated routine laboratory and fast in-clinic parameters to predict the infection outcome in acutely infected dogs. We compared the standard haematological and blood biochemical profile, hand-held lactate and glucose analysers, and an acute phase response in two groups of naturally infected dogs (7 survivors and 8 non-survivors). Samples were collected at the time of first admission and before any treatment. We found that non-survivors showed significantly higher levels in lactate- ($p < 0.001$), triglycerides- ($p < 0.01$) and phosphate levels ($p < 0.02$) and a significantly lower haematocrit ($p < 0.05$), leukocyte counts ($p < 0.01$), total protein levels ($p < 0.03$), and thrombocyte counts ($p < 0.03$) than survivors. Next, prognostic cut-off values of these seven initial identified factors were determined by a ROC analysis: the best prognostic sensitivity and specificity (both 100%) to differentiate survivors and non-survivors were found for lactate levels and leukocyte counts. All non-survivors showed hyperlactataemia (mean: 8.15 mmol/L; 95%CI: 6.32 – 9.98), whereas most survivors (6/7) had values within the reference range (mean: 1.77 mmol/L; 95%CI: 0.78 – 2.75). The leukocyte counts for all of the survivors were located within the reference range (mean: $7.07 \times 10^3/\mu\text{L}$; 95%CI: 5.40 – 8.73), unlike the group of non-survivors, which showed leukopenia (mean: $2.77 \times 10^3/\mu\text{L}$; 95%CI: 1.50 – 4.03). By following the course of the prognostic parameters in three dogs experimentally inoculated with *B. canis*, the lactate-, triglyceride-, and phosphate levels, and the thrombocyte counts exceeded the prognostic cut-off towards poor signs of an acute breakdown. Taken together, our data provide predictors associated with poor prognosis.

Tuesday 18 August 2015

Concurrent Session 5.5

J: Genetics of host resistance

Ref: O143/0647

Mathematical modelling of nematode infection and the development of novel diagnostic tests

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Nematode infections of sheep are now one of the best understood of all parasitic infections. There are a number of counter-intuitive regulatory loops that make predictions of infection outcomes difficult. Therefore we have created a mechanistic, data-driven, immunologically and genetically explicit, dynamic model of the infection process. This model used data from several flocks of naturally infected sheep as well as deliberate infections. The model was extensively validated against population means variances and distributions. In particular, immune parameters derived from deliberate infections accurately predicted the distribution of parasites in naturally infected sheep. One of the purposes of this model was to compare methods of parasite control. Modelling the response to selection suggested that IgA is a better marker of parasite resistance than faecal egg counts. We have therefore developed a diagnostic test that measures salivary IgA activity against a mixture of larvally-derived parasite molecules. Initial trials with 2700 Lleyn sheep indicated that salivary IgA has a similar heritability to faecal egg count and the traits are favourably genetically correlated. IgA activity is associated with increased muscle depth. As IgA is cheaper and safer to measure than faecal egg counts, it appears to be a better way to identify sheep with increased resistance to nematode infection.

Tuesday 18 August 2015

Concurrent Session 5.5

J: Genetics of host resistance

Ref: O144/0484

Host response and differential gene expression in Scottish Blackface lambs with divergent phenotypes for resistance to gastrointestinal nematodes

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Gastrointestinal nematodes (GIN) are a major threat to the health, welfare and productivity of sheep populations worldwide. Natural variation exists among lambs in their ability to control GIN infection. GIN resistance is moderately heritable and highly polygenic with many genes implicated in expressing a resistant phenotype. In order to identify genes and biological processes associated with GIN resistance, Scottish Blackface lambs at the extremes of the distribution of resistance to GIN were selected and their response to challenge with *Teladorsagia circumcincta* was characterised. Resistant lambs displayed consistently lower faecal egg count throughout the course of infection, which was primarily due to decreased worm fecundity in these animals. The anti-nematode immune response was also mediated, at least in part, by IgA with resistant animals having significantly higher levels of serum anti-*T. circumcincta* IgA throughout the course of infection and significantly higher abomasal mucosa anti-carbohydrate larval antigen IgA at slaughter. Gene expression in the abomasal lymph node of resistant and susceptible lambs was examined using next-generation sequencing technology. In total, 194 genes were identified as differentially expressed (DE) between resistant and susceptible lambs at 7 days post-infection (dpi) and 144 genes were identified as DE between resistant and susceptible lambs at 14 dpi. Genes relating to the inflammatory response, attraction of T lymphocytes and binding of leukocytes were more highly expressed in the abomasal lymph node of resistant animals at 7 dpi. In contrast, genes relating to these processes were more highly expressed in susceptible animals at 14 dpi. This suggests that resistant Scottish Blackface lambs mount an immune response to GIN challenge earlier than susceptible animals. Single nucleotide polymorphisms (SNP) in DE genes along with SNP in genomic regions previously associated with GIN resistance were tested for association with nematode resistance in a flock of Scottish Blackface lambs (n = 253). A number of SNP were suggestively associated with nematode resistance but no individual marker was significant at the genome-wide level. Therefore a panel of SNP may be more useful than individual markers in predicting GIN resistance.

Tuesday 18 August 2015

Concurrent Session 5.5

J: Genetics of host resistance

Ref: O145/0492

Relative maturity and immunity to parasites: comparison of resistant and resilient Romney lambs

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Selection of Romney sheep that are resistant or resilient to gastrointestinal nematodes has resulted in populations that appear to vary in both their immunological capability and production potential. Resistant animals from these lines are smaller in frame size and appear to have a reduced growth potential compared with their resilient counterparts. In this study the timing of immune development was investigated by measuring the live weight, faecal egg count (FEC) and antibody profiles every 10 days from weaning (90 days-of-age) in lambs from resistant (n=30) or resilient (n=30) Romney lines when exposed to a natural gastrointestinal nematode infection from pasture. At weaning, resistant lambs were lighter, viz, 18.4 ± 0.77 kg than their resilient counterparts, viz, 23.0 ± 0.57 kg ($P < 0.01$) and remained lighter throughout. Peak FEC of 380 eggs per g (epg) occurred at 175 days-of-age at a weight of 23.1 ± 0.84 kg for the resistant lines, with FEC peaking at 580 epg at 214 days-of-age and a weight of 32.8 ± 0.87 kg for the resilient lines. Although chronologically resistant lambs appeared to develop their immunity earlier than resilient lambs, the timing of immune development was not different ($P > 0.05$) when expressed as either a proportion of relative maturity (0.46 for resistant v 0.48 for resilient) or as a function of metabolic age (114 d for resistant v 115 d for resilient). These results indicate that development of immunity to gastro-intestinal nematodes may, at least in part, be dependent on relative maturity. The implications of this when selecting animals that are more resistant to gastrointestinal nematodes to minimise any negative influence in production potential or even when comparisons are made between lines of animals of differing immune status appear to be worthy of consideration.

Tuesday 18 August 2015

Concurrent Session 5.5

J: Genetics of host resistance

Ref: O146/0530

Selection of sheep for resistance to gastro-intestinal nematodes in France: where are we and where are we going?

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Genetic selection of sheep for resistance to gastro-intestinal nematodes (GIN) is one alternative method to anthelmintic treatments. In France, this method has been experimented in two ovine breeds: a meat sheep breed (Romane) and a dairy sheep breed (Manech Tête Rousse). Current French breeding schemes are pyramidal: at bottom, a large number of sheep breeders are only users of genetic progress, above, a selection nucleus is composed of sheep breeders where individual performances of animals are recorded and pedigrees are fully known and on top, young rams, coming from the selection nucleus, are tested in individual control stations (ICS), later, a sub-group of them (= elite rams) are used for artificial insemination. These elite rams are bred together in ram centres (RC). The originality of the French breeding scheme is to collect phenotypes for resistance to GIN after *Haemonchus contortus* experimental infections of rams in ICS and RC. This protocol includes two one-month periods of infection separated by two weeks of recovery. Faecal egg counts are performed at the end of both infection periods. In the Manech Tête Rousse breed, estimates of the heritability of egg excretions were 0.06 in the first infection and 0.24 in the second one. Preliminary estimated breeding values (EBVs) were established in this breed and their relationships with production traits EBVs regarding milk production or fat and protein milk contents were neutral. Phenotypic measures are in progress in the Romane breed. In parallel to this program, a genome scan was carried out using Romane x Martinik Black Belly lambs. The Illumina OvineSNP50 Bead Chip was used for QTL detection. Four QTL regions associated with faecal egg counts were identified (OAR5, 12, 13, 21) on this population. A customized assay including 1000 SNP was created to increase the density of marker coverage in these regions. The interest of these QTL regions was recently confirmed in a Romane pure breed population challenged with *H. contortus* and genotyped with the dedicated 1000 SNP Bead Chip.

Tuesday 18 August 2015

Concurrent Session 5.5

J: Genetics of host resistance

Ref: O147/0468

Resist measuring resilience: Recording high performers in their parasitised environment is simpler and as good

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Although discussed for at least 25 years, there appears to be renewed interest in measuring resilience of sheep to gastro-intestinal nematodes. While not novel, increasing resilience to the effects of infection remains an appealing approach to parasite management in the face of multi-drug resistance. We highlight that the complications associated with measuring resilience can be avoided by using simpler measures of productivity, thus highlighting that resilience is indeed an artless measure of an animal's ability to perform when challenged by infection. To measure resilience, a cross-over experiment was conducted to compare phenotypic measures of resilience to gastro-intestinal nematodes (predominantly *Haemonchus contortus*) in Merino sheep and their association with resistance and production. Ewes were serially treated with long-acting anthelmintics (suppressed; n=360) to suppress infection, or periodically treated according to management practices of the farm (infected; n=360). After one year, sheep had their anthelmintic treatments switched for a further year. Measures of apparent resilience were greasy fleece weight (GFW), live weight gain (LWG) and haematocrit (HCT) when infected and measures of true resilience were the difference in these variables within an animal when infected and suppressed. Measures of resilience based on GFW, LWG and HCT were only moderately correlated suggesting they represent different traits. Correlations between apparent and true resilience were higher indicating that measurement during infection is an adequate measure of resilience. These results demonstrate that simple measurement of performance traits while infected (i.e. receiving periodic treatments associated with typical farm management) is a reasonable approximation of true resilience. They also highlight that resilience to gastro-intestinal nematode infection is not a single trait, but rather a suite of moderately correlated traits. Given that genetic selection of production traits occurs in a parasitised environment, the marginal benefit to be gained from additional genetic indicators of resilience remains of questionable value.

Tuesday 18 August 2015

Concurrent Session 5.5

J: Genetics of host resistance

Ref: O148/0308

Toxoplasma gondii infection in mouse impairs long-term fear memory consolidation and downregulates Arc expression

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Chronic *T. gondii* infection becomes established in tissues of the central nervous system, where parasites may directly or indirectly modulate neuronal function. However, the mechanisms underlying parasite-induced neuronal dysfunction in the brain remain unclear. Here, we examined long-term memory associated with conditioned fear in mice, and found that *T. gondii* infection impairs consolidation of fear memory. To examine brain pathology induced by *T. gondii* infection, we analyzed parasite load and histopathological changes. *Toxoplasma gondii* expands throughout the brain, yet the cortex exhibits more severe tissue damage than other regions. Next, we analyzed neurotransmitter and immediate early gene levels in brain regions related to fear memory. Glutamate, a major excitatory transmitter, was decreased in the cortex and thalamus of infected mice. Moreover, the homovanillic acid/dopamine ratio was increased in infected mice in all brain regions examined. Interestingly, *Arc* expression levels, which are associated with fear memory, were downregulated by *T. gondii* infection. These results suggest that *T. gondii* infection affects fear memory via downregulation of Arc function.

Wednesday 19 August 2015

Concurrent Session 6.1

A: Drugs and drug resistance

Ref: O149/0256

Resilience to worm infections in lambs may modify anthelmintic treatment protocols

Brown Besier, Daniel Roberts

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A key recommendation for the management of anthelmintic resistance in sheep nematodes in Australia is the periodic monitoring of worm egg counts, to avoid unnecessary anthelmintic treatments. The action appropriate for different mean flock counts is based on factors affecting the impact of parasites (such as sheep ages, reproductive status and nutritional situation), with treatment recommended above threshold values. However, investigations in lamb-producing flocks in Western Australia suggest that threshold worm egg counts cannot be simply applied to indicate the requirement for treatment. As expected, observations on 14 farms over 2 years showed no consistent relationship between the worm egg counts of individual meat-breed lambs and their growth rates. However, there was also great variation in the relationship between mean flock counts and mean lamb weight gains. In some cases, flocks with mean counts generally considered indicative of significant parasitism were associated with serious production loss, but in other flocks, identical or greater counts resulted in no adverse growth effect. In most cases, lamb flocks with the demonstrated potential to grow rapidly (indicated by control groups of worm-suppressed lambs) were more tolerant of worm burdens, and grew at acceptable rates despite higher worm egg counts than poor-performing flocks. This indication of resilience (but not necessarily resistance) to nematodes suggests the need for a revision of the recommendations for anthelmintic treatment in this environment. Modified guidelines could include: different worm egg count treatment thresholds according to a growth-rate index; extended monitoring intervals for fast-growing lambs; or a targeted selective treatment approach by which only the lightest lambs in fast-growing flocks are treated at certain worm egg count values. Incorporating resilience to worm infections as a factor in treatment decisions could reduce exposure to anthelmintics and therefore the rate of resistance development. Further, identifying the factors limiting lamb growth rates in specific poor-performing flocks (presumed to be mostly nutrition or genetics) will allow management changes that reduce the dependence on anthelmintics.

Wednesday 19 August 2015

Concurrent Session 6.1

A: Drugs and drug resistance

Ref: O150/0554

From Genome and transcriptome revealing the true detoxome of *Fasciola hepatica*

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Fasciolosis is a global welfare issue to the livestock industry and a threat to food security. At present there are no commercial vaccines to underpin control programmes. Therefore, the reported treatment failures and resistance to Triclabendazole (TCBZ) is of serious concern as TCBZ is the only anthelmintic with activity against both mature and pathogenic immature fluke. Uncovering the potential detoxification capabilities of liver fluke is of paramount importance in order to sustain future anthelmintic control of Fasciolosis. Currently, our understanding of Phase I detoxification capabilities in parasitic Platyhelminthes is fragmented. To this end, we are testing the hypothesis that liver fluke have a broad based Phase I and Phase II capacity to neutralise anthelmintics, despite previous reductionist biochemistry predicting limited detoxification potential and the reliance on members of the abundant soluble glutathione transferase (GST) Phase II superfamily to simply passively bind toxins. The recently completed genome sequencing of liver fluke and new RNA-seq approaches provide comprehensive new tools to systematically reveal the parasite's detoxification capacity (the detoxome). To date, our gene mining has highlighted some key findings, most interestingly the presence of the Phase I detoxification Cytochrome P450 (CYP), which has previously failed to be directly biochemically detected in liver fluke. Two liver fluke CYPs have been identified, termed FhCYP1A1 and a potentially novel FhCYP1B. The detection of the Phase I CYP transcript expression in adult liver fluke starts to readdress the previously perceived importance of Phase II level detoxification in parasitic flatworms. Our recently completed transcriptome of defined liver fluke isolates under anthelmintic stress provides a complementary route to delineate the parasite's detoxome.

Wednesday 19 August 2015

Concurrent Session 6.1

A: Drugs and drug resistance

Ref: O151/0383

Computer processed high definition video imaging of *Dirofilaria immitis* larvae; can motility be used to determine macrocyclic lactone resistance status?

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Motility remains the phenotype of choice for measuring the effects of candidate anthelmintic drugs on parasitic nematodes. Compared to alternatives, assessing motility is relatively rapid and straightforward, and is amenable to some degree of automation. Recently we have described a modification of WormAssay, developed at the University of California, San Francisco, to permit objective measurements of the motility of microscopic larval stages of filarial nematodes and we have applied this method to study the effects of ivermectin on various strains of the canine heartworm, *Dirofilaria immitis*. Recently, it has been proven that macrocyclic lactone-resistant heartworms are circulating in parts of the United States, and we wished to assess the ability of motility assays on the microfilariae (Mf) and L3 stages to distinguish between susceptible and resistant strains. Four strains maintained in our laboratory were used in this study; Missouri-2000, a fully susceptible strain, MP3 (2005), a susceptible strain that displays a “less sensitive” in vivo phenotype as compared to other earlier lab isolates tested, JYD-27 (2010), a resistant strain that has been confirmed as highly resistant in multiple in vivo studies, and Yazoo-2013, a resistant strain recently isolated by our laboratory and which established a patent infection in two dogs in the laboratory, despite administration of Heartgard Plus every 30 days for 6 months. Microfilariae and L3 of all strains were incubated without drug, and with ivermectin (0, 10^{-3} , 10^{-2} , 0.1, 1, 2, 6, 10, 20, 50 μ M) in vitro at 37 °C in 5% CO₂ and 24 hrs later their motility was assessed using the ‘Worminator’ application. As previously reported, very high concentrations of ivermectin (3-4 orders of magnitude higher than in vivo concentrations) are required to inhibit the movement of *D. immitis* Mf, and the level of inhibition never exceeded 90%. The preliminary IC₅₀ value for Missouri was 5.2 μ M and for MP3, 5.4 μ M. The maximum inhibition of JYD-27 or Yazoo-2013 motility measured was ~60% with an apparent IC₅₀ of ~5 μ M. A second passage of Yazoo-2013 yielded Mf that showed no significant inhibition at 50 μ M ivermectin. This suggests that under laboratory conditions quantitative measurements of Mf motility can be used to assess the ML-resistance status of a *D. immitis* strain. However, quantification of this phenotype using typical dose-response criteria remains a challenge. Similar studies on the L3 stage are in progress.

Wednesday 19 August 2015

Concurrent Session 6.1

A: Drugs and drug resistance

Ref: O152/0694

Interaction of macrocyclic lactones with a *Dirofilaria immitis* P-glycoprotein

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Dirofilaria immitis is a filarial nematode which causes dirofilariasis (heartworm disease) in dogs, cats. Effective prevention of the disease is mainly by use of macrocyclic lactone (ML) drugs as heartworm preventives. MLs have been used for more than 25 years in this way. However, prevention has been compromised by the proven development of resistance in recent years in USA. The mechanism of resistance in *D. immitis* has yet to be established but preliminary evidence correlated the presence of two polymorphic loci in the P-glycoprotein 12 (Pgp-12) gene with loss of efficacy of ML anthelmintics. We have identified the full length of *D. immitis* Pgp-12, expressed it in mammalian cells, and studied the functional activity of the expressed protein. We have characterized its interaction with the four ML preventives, ivermectin, selamectin, moxidectin and milbemycin oxime using different fluorescence substrates. The avermectins, ivermectin and selamectin markedly inhibited rhodamine 123 (Rho123) transport in a concentration-dependent and saturable manner, whereas the milbemycins, moxidectin and milbemycin oxime were found to have different inhibition profiles with Rho123 transport. However, both avermectin and milbemycin preventives inhibited the transport of another fluorescent substrate, Hoechst 33342 (H33342) by *D. immitis* Pgp-12 in a concentration-dependent and saturable manner, although differences existed in terms of efficacy and potency of inhibition between the two sub-families of MLs. It is postulated that *D. immitis* Pgp-12 may have two to three functional drug binding/transport sites, as in mammalian Pgp; including the 'R' site for Rho123 and the 'H' site for H33342. The avermectins appear to bind the 'R' binding site, competing strongly with Rho123, whereas competition for the Rho123 site by the milbemycins was weaker. However, both sub-classes of MLs appeared to interact well with the 'H' site of *D. immitis* Pgp-12. Differences in the interaction of different heartworm preventives with the parasite Pgp could have implications for the expression of ML-resistance to heartworm preventives.

Wednesday 19 August 2015

Concurrent Session 6.2

B: Climate change, epidemiology and modelling

Ref: O153/0079

A simulation model to investigate host-parasite interactions between first season grazing calves and *O. ostertagi*

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Gastrointestinal (GI) parasitism of calves is ubiquitous and its control is under review due to the threat of anthelmintic resistance. *Ostertagia ostertagi* is the most prevalent and economically important of the GI parasite species. Hence a deterministic, dynamic model was developed to investigate the interactions, in terms of performance and level of parasitism between first season calves and *O.ostertagi*. The key model inputs included animal growth and immune characteristics, grass quality and larval intake. The inputs were defined to reflect a range of given situations and key outputs, the latter were; A) worm burden, total egg output and faecal egg counts and B) performance measures of feed intake and bodyweight. Model parametrisation was based on published data from controlled infections with different challenge protocols and a sensitivity analysis was conducted to test the uncertainty of the parameters. The model was validated and was effective in simulating reasonable predictions for parasitological outputs for varied levels of infection compared to published literature. From this, inference could be made on calf performance outputs by predicting the effect of parasite-induced anorexia and subsequent effects of infection on calf bodyweight. The model predicts disproportionately greater body weight losses with increasing daily infection levels. Simulations for calves infected on a weekly basis suggest that these experimental infections can provide a close proxy to daily infections. Future work will consider natural infections, and hence the epidemiology of the infection and variation between individual calves, to provide a basis for modelling possible control strategies for GI parasitism.

Wednesday 19 August 2015

Concurrent Session 6.2

B: Climate change, epidemiology and modelling

Ref: O154/0189

Bayesian modelling of factors potentially influencing the spatial distribution of *Echinococcus multilocularis* in foxes

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Alveolar echinococcosis is considered one of the most dangerous autochthonous parasitic zoonoses in central Europe. The red fox (*Vulpes vulpes*) represents the main definitive host of *Echinococcus multilocularis* in Europe.

To investigate the potential influence of environmental factors on the spatial epidemiology of *E. multilocularis*, 38,446 foxes were sampled in two German Federal States (Brandenburg and Thuringia) and the results of the parasitological examination linked to a geographic information system. The landscape composition per spatial unit was derived from a high-resolution land-survey vector database and supplemented by a digital elevation model. Data were analyzed using a hierarchical Bayesian model.

On the municipality level and with environmental data at higher resolution, the study confirmed results of previous work, in which we had utilized exact locations of foxes and micro-habitat data. Furthermore, the preference of infected foxes for open landscapes with pasture was demonstrated in both regions despite the different landscape characteristics. However, prediction of endemic areas was not possible alone on the basis of land-use classes in the study areas, while neighbourhood to an area with *E. multilocularis* infections in foxes was a good predictor for the occurrence of the parasite.

We conclude that *E. multilocularis* is likely to spread to neighbouring areas more or less regardless of environmental factors that can influence the spatial distribution of the parasite, if the parasite is present in a region in Germany.

Wednesday 19 August 2015

Concurrent Session 6.2

B: Climate change, epidemiology and modelling

Ref: O155/0194

GLOWORM-PARA: Development and validation of a generic model framework for the parasitic phase of gastro-intestinal nematode parasites in ruminants

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Gastro-intestinal nematodes (GIN) remain an important threat to livestock farming. Parasite transmission models could be useful decision-support tools to develop or to validate sustainable worm control strategies. Recently, a general model framework for the free-living phase of GIN in ruminants was created (GLOWORM-FL). However, to explore the effect of different worm control approaches, a complementary model for the parasitic phase is needed. The GLOWORM-PARA model provides a conceptual model framework for the parasitic phase that can be applied to a range of GIN species in ruminants. The model outline is consciously kept as straightforward as needed to avoid needless parameter estimation or parameter fitting and tracks the mean number of nematodes and worm egg production in a group of hosts. To demonstrate its use, the model was parameterised and validated for two GIN species of major importance in cattle, i.e. *Ostertagia ostertagi* and *Cooperia oncophora*. Model parameterisation was realised using estimates obtained by meta-analysis and recent field data. Model predictions of the egg excretion in the faeces were validated using observations of 7 dairy farms in Belgium. Linear regression of predictions against observations showed in most cases a good fit for the *O. ostertagi* model. For the *C. oncophora* model, the slope was marginally different from 1 in several cases indicating some bias. In further research, the integration of GLOWORM-PARA with GLOWORM-FL should lead to a full life cycle based model to understand gastro-intestinal nematode epidemiology under changing climate and management scenarios.

Wednesday 19 August 2015

Concurrent Session 6.2

B: Climate change, epidemiology and modelling

Ref: O156/0330

Predicting the impact of climate change on gastrointestinal nematodes

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Gastrointestinal nematodes are a major constraint on ruminant livestock production worldwide, affecting welfare and productivity. Changes in the phenology of GINs and an increase in the incidence of disease have been observed in some regions in association with climate warming. Such changes, combined with the development of anthelmintic resistance, threaten the future sustainability of the ruminant livestock sector, undermining food security and the potential for sustainable intensification. An improved understanding and awareness of climate-driven changes in the epidemiology of GIN infections is therefore necessary to ensure appropriate mitigation of changes and the long-term sustainability of intervention strategies. A number of models aiming to predict the impact of climate change on GIN infections have been developed in recent years, aided by recent advances in our understanding of the ecology and behaviour of GINs. Key outputs of two such models are presented here. The first simulates the climate-dependent population dynamics of three key GINs infecting sheep (*Haemonchus contortus* & *Teladorsagia circumcincta*) and cattle (*Ostertagia ostertagi*) while the second provides an instantaneous estimate of environmental suitability and propensity for population growth in *H. contortus*. Despite the contrasting approaches, similar patterns of change are predicted between models and predictions are consistent with other models of parasite responses to climate change. Predictions are characterised by non-linear interactions and trade-offs between temperature-dependent development and mortality, and moisture-limitations on nematode behaviour, emphasising the value of mechanistic modelling approaches. The impact of predicted changes in climatic conditions, interactions with farm management and the potential for mitigation of changes are discussed.

Wednesday 19 August 2015

Concurrent Session 6.2

B: Climate change, epidemiology and modelling

Ref: O157/0345

Development of a simulation model of the parasitic risk related to gastrointestinal nematode infection in grazing heifers

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Control of gastrointestinal nematode (GIN) infections in heifers is often based on systematic treatments, applied at standard periods, without adapting to the specific parasitic risk in each herd. This parasitic risk depends on the pasture infectivity level (GIN larval challenge which increases from turn out onwards) and on the immunological status against GIN. A model based on these 2 key factors has been developed to assess the parasitic risk periods in field conditions. The increasing pasture infectivity during the grazing season is modeled by calculating the numbers of parasitic cycles realized since turn out, the duration of one cycle being the addition of the prepatent period in the host and the development time from egg to infective larvae on pastures. As this development time depends on temperature, it is calculated by using a previous model developed by Grenfell et al. (1987) based on daily average temperatures. The grazing management practices (one *versus* several pastures, time spent on each pasture) are also taken into account because they can broadly influence the rate of increase of the pasture infectivity level. To model the development of immunity, data regarding heifers' grazing and treatment history are used: the Time of Effective Contact (TEC, in months) with GIN larvae is calculated, heifers being considered as non-immune as long as TEC < 8 months.

The model's output is based on the number of larval generations present on the pastures grazed by non-immune heifers. To determine the number of larval generations beyond which a parasitic risk can be predicted, model's outputs and field data (larval counts in grass samples) were compared. We concluded that when the third infective larval generation was predicted by the model, the pasture infectivity level could be high enough to cause weight gain losses or clinical signs. As long as non-immune heifers remain on such a pasture at risk, different therapeutic or agronomic control strategies can then be tested and recommended. It has been shown that this model is a useful tool to optimize the use of anthelmintics in the control of GIN on the field, through a better definition of optimal period for targeted treatment. The use of such a model should facilitate the development of evidence-based parasite control strategies and potentially halve the number of treatments used in standard strategies.

Wednesday 19 August 2015

Concurrent Session 6.2

B: Climate change, epidemiology and modelling

Ref: O158/0464

Estimation of the effect of *F. hepatica* infection on beef carcass performance using Bayesian statistics, adjusting for abattoir liver inspection imperfectness

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Liver fluke, caused by *F. hepatica* is a multi-host parasitic disease affecting many countries worldwide. The infection in cattle mainly manifests as a sub-clinical disease, resulting in indirect production losses, which are difficult to estimate. The lack of obvious clinical signs results in these losses commonly being attributed to other causes such as poor weather conditions or bad quality forage. Moreover, due to the endemic nature of the infection in many countries, affected animals commonly have low levels of infection. To date it remains unclear at what levels parasite burden leads to production losses, or how the risk of losses can be measured using diagnostic tests.

This study aims to fill these gaps by estimating the effect of infection on beef production and investigating the use of diagnostic tests in a quantitative manner to aid control. We used a Bayesian approach to build a regression model for the time taken to reach recorded slaughter weight, based on age, weight and the liver fluke status, taking breed and the random effect of farm into account. The model included sensitivity and specificity estimates of abattoir liver inspection for liver fluke. Data for this model were routinely collected at Scotland's leading red meat abattoir between 2012 and 2015. The posterior estimates indicated that cattle with liver fluke took 6.9% [1.2-12.7 95% credible interval] longer to reach slaughter weight, compared with healthy animals. Using a subset of the data, we included the fibrosis score (0-3) as a proxy for severity of fluke infection. This indicated that cattle with liver fluke took 7.1% [3.9-10.6%] longer for each unit increase of fibrosis score. Further preliminary results suggest that a serum antibody ELISA can be used to estimate the effect on growth rate, while faecal egg counts and a copro-antigen ELISA can be used to estimate parasite burden.

Overall, improved knowledge on the effect of *F. hepatica* infection on beef production in conjunction with the availability of diagnostic tests able to estimate production loss and parasite burden can inform targeted treatment management strategies and encourage more producers to adopt control measures, while reducing the establishment of anthelmintic resistance.

Wednesday 19 August 2015

Concurrent Session 6.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O159/0683

In vitro and *in vivo* validation of bioactive plants for parasite control

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Background: Scientific evidence on plant efficacy and safety is needed to promote phytomedicine as a reliable parasite control strategy. Here, we present the *in vitro* and *in vivo* approach followed to evaluate anti-parasitic activity and detrimental side effects of purported Ethiopian and UK medicinal plants.

Methods: Anthelmintic activity of water, ethanol and hydro-ethanol extracts of plants, selected via an ethnomedicinal survey (Ethiopia) and chicory (UK), were evaluated *in vitro* against *Teladorsagia circumcincta* using a larval motility assay and *Haemonchus contortus* using egg hatch inhibition assay. A subset of plants that showed high activity *in vitro* was tested for anthelmintic activity and toxicity in mice, infected or not with *Heligmosomoides bakeri*. Finally, the anthelmintic activity and side effects of two plant extracts was tested in sheep, infected or not with either *T. circumcincta* or *H. contortus*.

Results: Anthelmintic activity was validated through *in vitro* larval motility for 23 of 25 Ethiopian plants selected, and for UK's chicory, whilst all showed significant egg hatch inhibition. Twelve plants were tested in mice, and whilst none resulted in a significant reduction in parasite load, one of the extracts reduced feed intake. Oral dosing of two selected plants significantly reduced worm egg excretion in *T. circumcincta* infected lambs, though to a lesser extent in *H. contortus* infected lambs. In agreement with the mouse studies, one of the two plants tested resulted in temporarily reduced feed intake.

Conclusions: Our data validated that many purported medicinal plants have demonstrable anthelmintic activity, supporting the use of phytomedicine in parasite control strategies. Our use of the mouse *H. bakeri* model would indicate its value may be greater to evaluate plant anti-nutritional rather than plant anti-parasitic effects.

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Wednesday 19 August 2015

Concurrent Session 6.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O160/0109

Body condition score as a practical selection tool for targeted selective treatment-based nematode control strategies in adult Merino sheep

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Investigations in Western Australia have demonstrated that mature sheep in the lowest body condition score (BCS) in flocks can show a significantly greater response to treatment than their higher BCS counterparts, providing a simple index for targeted selective treatment (TST) decisions. A more recent investigation aimed to clarify the production effects of BCS-based selection compared to random selection of sheep left untreated in a flock, in situations where non-haematophagous worm species dominate, and adult sheep carry worm burdens typically associated with sub-clinical parasitism. Two flocks at two locations in south west Western Australia were measured for body condition score, body weight and worm egg counts, where half of the sheep in each flock received an anthelmintic treatment and half remained untreated. Comparisons of sheep production (liveweight and BCS changes) and pasture contamination effects (worm egg counts) were performed by creating "virtual flocks" of varying proportions of untreated sheep by drawing animal numbers as appropriate from the worm-suppressed and non-worm suppressed groups, either at random or by choosing those in highest BCS. Results suggest that selecting sheep for treatment randomly results in no greater loss in production at the flock level than selecting sheep based on low body condition score. Although sheep in low BCS should not be left untreated, this finding confirms that TST can be easily implemented as part of an anthelmintic resistance management strategy.

Wednesday 19 August 2015

Concurrent Session 6.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O161/0493

The effect of acid fertilisers on parasite larval development and viability

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The acidic nature of many common fertilisers allows the possibility for them to disrupt parasite larval development and survival, possibly leading to a novel method of reducing gastrointestinal parasite loading on pastures for grazing livestock. Here we investigated the impact of three commercially available acid fertilisers; effective microorganism mixture (EM), liquid Urea (20%N) and superphosphate on parasite development. Egg hatch assays demonstrated a dose dependent inhibition on egg hatching for *Trichostrongylus colubriformis* and *Teladorsagia circumcincta*. For EM, egg hatching was reduced from $92.6 \pm 2.7\%$ in the controls to $93.3 \pm 1.33\%$ at 0.01% dilution ($P > 0.05$), $6.34 \pm 1.32\%$ at a concentration of 1% ($P < 0.01$) and $5.11 \pm 0.72\%$ at 10% ($P < 0.01$). For liquid urea, egg hatching was reduced to $94 \pm 0.56\%$ at 1% dilution, $74 \pm 1.18\%$ at 10% dilution ($P < 0.05$) and $0.83 \pm 0.28\%$ at 50% dilution ($P < 0.001$). Superphosphate solution reduced egg hatching to $72.6 \pm 6.24\%$ at 0.01 % dilution, $24.2 \pm 2.03\%$ at 0.1% dilution and completely prevented all eggs from hatching at 1% dilution. Further investigations revealed these effects were pH dependent with a pH less than 5.0 preventing egg hatching. Larval migration assays revealed that larval migration was also reduced with the addition of EM, Urea or superphosphate from $92.5 \pm 4.61\%$ in the controls to $57.8 \pm 3.76\%$. Finally, the impact of topical application of either EM, Urea or superphosphate at commercially recommended rates onto faeces at day 8 of incubation, to coincide with expected larval migration from faecal mass, revealed a reduction in larval recovery rates (compared with numbers of eggs present) from $46.1 \pm 2.70\%$ to $27.2 \pm 2.60\%$ ($P = 0.04$). These results indicate the application of these fertilisers has the potential to disrupt parasite larval development and viability, the effects of which may, at least in part, be pH dependent. Further investigations on the ability of these treatments to reduce the parasite loading on grazing livestock or to clean up populations of parasites *in situ* are currently underway and have the potential to offer a novel and alternative method of parasite control.

Wednesday 19 August 2015

Concurrent Session 6.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O162/O180

Economical optimization of targeted selective drenching in horses

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Numerous cases of reduced efficacy of anthelmintic treatment have been recently reported worldwide urging the need to alleviate the selection pressure put on parasitic population. Targeted selective treatment (TST) strategy based on Faecal Egg Count (FEC) has been proposed as an alternative strategy but associated costs may hamper this strategy. Our study aims at an economical optimization of the FEC-based TST. FEC have been determined in a Welsh pony herd thrice a year from 2010 to 2014. This database was exploited to explore costs and benefits associated with various FEC-based strategies as follows. The optimal FEC price for a cost-efficient individual based strategy was determined *in silico*, by randomly sampling 1000 virtual pony herds of various sizes (1 to 100 ponies) from the available database and estimating the associated costs (FEC price ranging from 1 to 10€, anthelmintic costs and labor-associated costs). Following the strategy proposed by Eysker et al. (2008), we also investigated the costs/benefits of the pooling of several individuals for FEC analyses. This is thought to reduce FEC-based costs but may leave highly infected individuals undrenched as a result of FEC overdispersion. For various pool-size (1 to 20 ponies) and various cut-offs (50 to 200 eggs/g), we sampled 1000 pony herds to estimate the associated costs and to determine the number of positive ponies within a negative pool. Following these simulations, pool-based FEC of various sizes were performed on 40 ponies to confront predictions to real data. Within 4 years, anthelmintics costs have been cut by 80% but with free FEC. *In silico* estimations, suggested that individual FEC-based TST would not be cost-efficient in this context for a FEC price above 5€. Additional analyses performed for a pool-FEC strategy, suggested the proportion of false negative horses never exceeded 15% of the pool size. In addition, an optimal combination of 14-ponies pool associated to a cut-off of 150 eggs/g would minimize both total costs and the number of positive horses left undrenched. Real data obtained from infected ponies however, suggested that pool size should not exceed 10 individuals, as unhomogeneous mixing of faecal material seemed to reduce the correlation between average pool-FEC and the mean of individual-FEC. Our study provides clear guidelines that should be valuable for emphasizing FEC-based approaches in the field.

Wednesday 19 August 2015

Concurrent Session 6.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O163/0362

Partitioning production loss due to *Trichostrongylus colubriformis* into direct and immune-mediated components in grazing meat-breed lambs

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This experiment tested the hypothesis that greater production loss is associated with immune-mediated responses of grazing meat-breed lambs to *Trichostrongylus colubriformis* infection than the direct effects of infection. The experiment was a 3x2 factorial design using 6-7 month old meat-breed lambs which remained uninfected or received 2,000 or 4,000 *T. colubriformis* L3/week for 84 days (IF) and were either immunosuppressed using methylprednisolone acetate once weekly or non-immunosuppressed. Lamb bodyweights and worm egg counts were assessed weekly and data were analysed in three distinct periods corresponding to host stages in response to infection (P1; days 0-35, P2; days 35-56 and P3; days 56-84). Production losses were partitioned into direct and immune-mediated components by calculating the difference in growth rates of uninfected and infected groups within level of immunosuppression.

Worm egg counts were increased ($p < 0.05$) by immunosuppression (P3 mean epg; 2210 v's 1220 for non-IS). There was an interaction ($p = 0.04$) between IF and immunosuppression in P1. IF reduced the growth rate of non-immunosuppressed lambs (36 g/d) but not of the immunosuppressed (14 g/d) lambs. Thereafter interactions were not significant and main effects are reported. IS reduced ($p < 0.0001$) growth rate of lambs in P3 (53 v's 93 g/d for non-IS). IF reduced ($p = 0.0001$) growth rate during P3 (54 v's 92 g/d for non-IF). Immune-mediated responses accounted for 75% (2.3 kg) of the overall production loss attributable to *T. colubriformis*, with the majority occurring during P1 (1.6 kg). In contrast, most of the production loss associated with the direct effect of *T. colubriformis* occurred in P3 (0.9 kg).

These results support the hypothesis that greater production loss occurs due to immune-mediated responses of grazing meat-breed lambs to *T. colubriformis* infection than to the direct effects of infection. These findings are supportive of previous studies with this parasite in housed sheep.

Wednesday 19 August 2015

Concurrent Session 6.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O164/0179

Knockdown of ferritin genes increases the susceptibility of the hard tick *Haemaphysalis longicornis* to bacterial infection

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Ticks are vectors of multiple pathogens and may be exposed to bacterial pathogens when they feed on a septicemic host. Therefore, tick innate immunity is crucial in limiting pathogen multiplication to ensure tick survival. Iron-withholding through iron-binding proteins, such as transferrin and lactoferrin, is a known component of innate immunity of most organisms. This mechanism limits the access of bacteria to iron, suppressing their multiplication. However, this function of the iron-binding protein ferritin is still unknown. We have previously characterized two types of ferritin (HIFER) in *Haemaphysalis longicornis*, known to be a vector of virus, bacteria and parasite etc., and showed their antioxidant function. Here we examined the role of HIFERs in immunity against bacterial infection. After *Hlfer* silencing, adult ticks were injected with live or heat-killed enhanced green fluorescence protein (EGFP) expressing-*Escherichia coli*, and then monitored for survival rate. Hemolymph including hemocytes was collected for microscopic examination and *E. coli* culture to demonstrate bacterial viability. *Hlfer*-silenced ticks had significantly lower survival rate after live *E. coli* injection compared to control. Microscopic examination of hemocytes showed that plasmatocytes from *Hlfer*-silenced ticks had greater number of bacteria in the cytoplasm, particularly at 72 hours after bacterial injection, as compared to that of control ticks. Furthermore, culture of hemolymph from *Hlfer*-silenced ticks yielded more bacterial colonies than control ticks. These results indicate that the iron storage function of HIFERs is important in limiting bacterial multiplication, and is therefore crucial to tick immunity.

Wednesday 19 August 2015

Concurrent Session 6.4

D: Vaccines and Immunology

Ref: O165/0474

Relationship between humoral immunity and endoparasite burden in cattle

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Intensive pasture grazing systems commonly seen in New Zealand dairy farms provide favourable conditions for the development of gastrointestinal (GI) parasitism.

The objectives of this study were to seek associations between levels of serum or milk antibody to gastrointestinal nematode parasites and faecal egg counts (FEC), milk yield, worm burden and levels of infective L3 larvae ingested from pasture. In one experiment, a group of lactating dairy cows grazing pasture at a high stocking rate (HSE, n=31) and fed supplements were compared with a low stocking rate group (LSE, n=30) without supplements, both on helminth-infested pastures. Monthly measurements were performed for faecal egg counts (FEC), pasture L3 larvae level and anti-Ostertagia antibody level (OD) in pooled milk or serum with daily milk yields. A significant positive relationship was observed between daily milk yield and serum OD and 'corrected' milk OD in both the HSE ($r=0.9$, $p=0.002$; $r=0.9$, $p=0.9$) and the LSE ($r=0.9$, $p=0.002$; $r=0.9$, $p=0.006$) groups. The correlation between anti-Ostertagia antibody levels (OD) in serum and final worm burdens in the slaughtered animals was negative ($r=-0.8$; $p=0.05$). There were no significant differences in the milk OD ($p=0.13$) or serum OD ($p=0.5$) between the HSE and LSE groups. Similarly, there was no significant difference between the HSE and LSE groups with regard to final worm burden or sequential FEC, but there was a trend ($p=0.06$) for a greater percentage of younger (cluster of 2–4 years) cows to have positive FECs. There was a positive correlation ($r=0.87$, $p=0.05$) between FEC and pasture L3 levels in the HSE group. There were higher levels of infective L3 in the old versus new pasture ($p=0.009$) and a trend for higher levels ($p=0.09$) in pastures grazed by the HSE versus the LSE groups. In another experiment, lactating dairy cows (n=60) were treated with anthelmintic and infected (truncated) orally (or not) with infective larvae (oral dose of 50×10^3 mixed L3/cow). Artificial infection did not affect mean worm burden or anti-Ostertagia antibody levels (OD) in serum but the latter reflected percentage of FEC+ cows ($r=0.5$, $p=0.3$).

In summary, levels of anti-Ostertagia antibody in the serum (or milk) do not reflect worm burden or L3 ingestion but are associated with percentage of cows with positive FEC. Stocking density did not influence gastrointestinal worm infections in adult dairy cows.

Wednesday 19 August 2015

Concurrent Session 6.4

D: Vaccines and Immunology

Ref: O166/0495

Immune responses of dairy cattle naturally exposed to *Fasciola hepatica* in the UK

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Fasciola hepatica is a common parasite of livestock causing clinical disease and economic loss. In the UK, its control is becoming increasingly difficult as a result of drug licensing and resistance issues. Vaccines may prove helpful in the management of fasciolosis, but before these can be applied, more information is needed about host immune responses in animals naturally exposed to the parasite.

To assess immune responses to primary infection, cohorts of heifer calves, previously unexposed to *F. hepatica* (n=42), were sampled monthly over a grazing season on three commercial dairy farms. To examine chronic infection, adult dairy cows (n=27) from a single commercial herd were sampled at the end of a lactation; animals that tested positive for fluke infection then received triclabendazole treatment and a second sample was collected 10-16 weeks later, shortly after calving.

Exposure to fluke infection was determined using an antibody ELISA, and whole blood was collected for haematological analysis. Peripheral blood mononuclear cells (PBMCs) were isolated for phenotypic analysis, parasite specific proliferation and cytokine responses.

During the primary infection, increases in CD4+ T-cells, CD4:CD8 ratio and IL-4 production were detected together with an eosinophilia and an increase in IL-5 transcription. These results suggest a type 2 T helper cell response develops in naturally infected calves and is consistent with results from experimental infections.

In chronically infected cattle, following treatment, there was an increase in parasite specific proliferation, supporting the hypothesis that infection with *F. hepatica* has an immunosuppressive effect, although no changes in the regulatory cytokines IL-10 and TGF-beta, were detected.

These results suggest *F. hepatica* causes modulation of the immune responses of cattle naturally exposed to the parasite, which may have implications for a vaccine programme that depends on stimulation of a type 1 immune response.

Wednesday 19 August 2015

Concurrent Session 6.4

D: Vaccines and Immunology

Ref: O167/0666

Analysis of antibody affinity maturation following vaccination with recombinant *Fasciola hepatica* antigens

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Vaccination of cattle against *Fasciola hepatica* using recombinant Cathepsin L proteases has resulted in varying levels of protection. Antigen specific IgG production is often measured following vaccination. While antibody titres and isotype profile are important parameters to examine, antibody avidity may be an important consideration in the optimisation of the immune response following vaccination. Antibody affinity can be defined as the strength of the interaction between antigen and antibody, with avidity being the average affinity of a polyclonal antibody population. In this study, potassium thiocyanate (KSCN) elution was used to estimate the avidity of specific IgG1 and IgG2 antibodies generated in cattle sera in response to *F. hepatica* infection and to *F. hepatica* recombinant CL1 (rmFhCL1) vaccination in two separate trials. [KSCN]₅₀ values, (the molarity of KSCN required to reduce the optical density of a serum diluted to titre by 50%), were measured as a proxy for antibody avidity. In the first trial, cattle received two vaccinations of rmCL1 in Montanide ISA 70 VG, three weeks apart, followed by a booster vaccination one year later. The cattle were infected with low fluke burdens by natural exposure from one month after 3rd vaccination. The avidity of both IgG1 and IgG2 significantly increased with time after vaccination. The avidity of the IgG1 antibodies remained high even one year after the 3rd vaccination, although titres had decreased. At each time point examined, the avidity of the IgG1 antibodies was significantly higher than that of IgG2. In the other trial, cattle received two vaccinations (three weeks apart) of rmFhCL1 in Montanide ISA 70 VG or Montanide ISA 206 VG. These cattle were also naturally infected from 3 weeks post 2nd vaccination and fluke burdens were moderate-high. Fluke burdens were reduced by 42.7% and 49.2% in the ISA 70 VG and ISA 206 VG group, respectively. IgG1 avidity was higher than the controls for both vaccinated groups at the time point examined. IgG1 avidity was significantly higher than IgG2 avidity. At similar time points, when avidity values from the two trials were compared, there was no significant difference between avidity of IgG2 antibodies, whereas the IgG1 avidity was significantly higher in the second trial. The results suggest that the antibody avidity is an important parameter to consider for liver fluke vaccine development and increasing IgG2 avidity could potentially increase protection.

Wednesday 19 August 2015

Concurrent Session 6.4

D: Vaccines and Immunology

Ref: O168/0685

Stage-specific immunity against *Haemonchus contortus* in sheep

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The third stage larvae of *Haemonchus contortus*, has been considered a major target of effective immunity in sheep. Two major mechanisms of immune rejection have been proposed against these third stage larvae. One, an antibody-dependent cell cytotoxicity mediated by eosinophils and specific immunoglobulins and; a second, rapid rejection of establishing larvae in the mucosa involving globular leucocytes. Our recent studies suggest poorly defined effective immune responses directed at other larval and adult stages of this parasite may be equally important. Galectin-11 has been found to preferentially bind the L4 and adult stages, not the L3 stage. Galectin-11 inhibits the *in vitro* development and growth of L4 in vitro, and expressed at high levels in immune sheep. Immunity against adult stages has been established in comparative studies between a relative susceptible Canaria Sheep (CS) breed and resistant Canaria Hair Breed (CHB) sheep. Although, no between breed differences in terms of larval establishment/length in the first 7 dpi occurred; in the resistant CHB sheep, significant differences in worm burden, length and fecundity were found. This mechanism is likely to involve gammadelta T cells, eosinophils and mucosal IgA in this poorly defined novel mechanism of resistance. In this presentation, potential mechanisms of immunity to multiple developmental stages of the parasite will be presented and the implications for future *H. contortus* multi-stage vaccination discussed.

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Wednesday 19 August 2015

Concurrent Session 6.5

I: Novel diagnostics

Ref: O169/0359

Development of a novel 'Nemabiome' sequencing assay for the relative quantitation of parasitic nematode species in cattle fecal samples

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Parasitic nematode infections are estimated to cost the US cattle industry \$2 billion/year in treatment costs and lost production. Nematodes often occur as mixed species infections, which vary in pathogenicity and drug sensitivity. Fecal Egg Counts (FEC) are used to assess parasite burdens, however, larvae must be inspected for morphological differences to determine species, which is labour intensive. Detection of resistance involves conducting FECs pre- and post-treatment and determining decreases in egg counts. This procedure is not very sensitive and does not yield information of species present before and after treatment. Molecular markers are faster, more sensitive and more accurate than traditional methods. The rDNA ITS-2 locus is the favoured region due to its appropriate level of species-specific variation. Previous approaches such as qPCR are semi-quantitative, can only detect pre-defined species, and have repeatability and scalability problems. We have developed an approach, akin to 'microbiome' sequencing, in which the rDNA ITS-2 region is simultaneously sequenced from thousands of parasites per sample at once. The assay provides digital data, allowing accurate assessment of relative species quantities per sample. Multiple samples can be sequenced simultaneously in a single Illumina MiSeq run. We have established the protocol from sample preparation to data analysis and have verified the sensitivity, specificity and quantitative accuracy using artificially created larval pools from the major cattle nematodes. This assay will provide a powerful tool to conduct epidemiological studies, surveillance and improve drug efficacy assessments in cattle parasites. We have applied the assay to investigate parasite species prevalence in Canadian cattle. We have observed regional differences in the distribution of nematodes. *Cooperia oncophora* and *Ostertagia ostertagi* are the most prevalent species across Canada, while *Cooperia punctata* is present in the eastern provinces. We have observed distinct shifts in parasite proportions following anthelmintic treatment, implicating potential resistance in some species.

Wednesday 19 August 2015

Concurrent Session 6.5

I: Novel diagnostics

Ref: O170/0316

Non-destructive sampling and Next Generation Sequencing reveal zoonotic pathogens in archived ticks

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Implicated in transmitting a greater variety of pathogenic microorganisms than any other arthropod group, ticks (Acari: Ixodida) are the latest ectoparasites to have their viral and bacterial communities characterised through the use of deep sequencing techniques (next generation sequencing NGS). Ticks and other micropredators archived in museum collections are a previously untapped source of investigating infectious diseases, and could provide valuable data from such molecular advances. Access to archived specimens is often challenging as the retrieval of nucleic acids often destroys the specimen. However, previous success of retrieving archival DNA (arDNA) from various archived specimens without altering morphology provides the necessary advantage required to allow researchers to access such specimens. The aims of this study were to 1) isolate arDNA using a non-destructive technique from archived ticks and to 2) to identify the presence or emergence of pathogenic bacteria in ticks over the past Century using NGS. To demonstrate the viability of the bacterial profiling approach, arDNA successfully isolated from 19 ticks (*Haemaphysalis longicornis*, n = 2; *Ixodes cornuatus*, n = 2; *I. holocyclus*, n = 10; *I. tasmani*, n = 5) acquired from the Australian National Insect Collection, CSIRO Canberra, were amplified using the universal bacterial 16S rRNA gene (V1-2) and sequenced using the Ion Torrent semiconductor NGS platform. Preliminary results revealed both environmental and pathogenic bacteria preserved in the archived ticks. Bacterial genera of particular animal and human health interest were *Francisella* sp., *Rickettsiella* sp. and *Rickettsia* sp. This pilot study suggests that archived ticks provide a means to document the emergence of infectious diseases in Australia, which has potentially significant contemporary implications for human and animal diagnoses.

Wednesday 19 August 2015

Concurrent Session 6.5

I: Novel diagnostics

Ref: O171/0650

***Dirofilaria immitis* derived microRNAs represent a species-specific biomarker for infection**

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Heartworm infections of companion animals are endemic in regions of North America and Southern Europe, with potential to be zoonotic. The mosquito-borne filarial nematode *Dirofilaria immitis* is an important parasite of domestic dogs and wild canids. Current diagnostics can detect adult female-derived antigens, while microfilariae, can be detected microscopically: there is currently no method for the diagnosis of pre-patent heartworm infections. This study focuses on host- and parasite-derived micro (mi)RNAs as markers of heartworm infection. In addition to their intracellular roles in transcriptional regulation, extracellular miRNAs have been demonstrated in blood and other bio-fluids and they are validated biomarkers for several human diseases. This study aimed to establish if parasite-specific 'miRNA fingerprints' occur in sera from parasitized hosts. We found that: (1) *D. immitis*-derived miRNAs are diagnostic of heartworm infection and can differentiate heartworm-infected sera from hosts carrying other filarial infections; (2) Host-derived miRNAs, possibly linked to parasite-induced tissue damage, are elevated in infected vs uninfected sera; and, (3) Small RNA sequencing demonstrates, for the first time, the presence of miRNAs in heartworm L3-derived exosomes. The relative abundance of individual miRNAs in these exosomes are similar to those of L3 tissue extracts, suggesting that miRNA loading into exosomes may not be a specific process as proposed by studies on exosomal miRNAs from other parasites. This work has direct applications in the development of helminth parasite diagnostics, and in developing understanding of the host-parasite interface. This work was supported by a Gates Foundation grant OPP1083083 to PM.

Wednesday 19 August 2015

Concurrent Session 6.5

I: Novel diagnostics

Ref: O172/0714

A novel snapback primer assay for the detection and discrimination of *Haemonchus* species using DNA melting analysis

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Haemonchus parasites are gastro-intestinal nematodes of ruminants with great socio-economic impact across the world. The present study introduces a novel qPCR assay for the detection and simultaneous discrimination of ovine *Haemonchus* species.

A total of 162 samples of adult *Haemonchus contortus*, *H. placei*, *H. longistipes* and hybrids from countries of four continents (Argentina, Pakistan, Nigeria and Austria) were collected. Alongside 3rd stage larval samples from *H. contortus* and related nematodes (e.g. *Teladorsagia circumcincta*, *Cooperia curticei*, *Trichostrongylus axei*, *Trichostrongylus colubriformis* and others) were collected, DNA extracted and cloned for sensitivity and specificity analysis. Species verification was done by conventional PCR of nematode conserved regions of the ITS2, COI-, β -tubulin and ND4-genes. A novel snapback primer with unlabelled probe assay targeting a 142 bp long fragment of the ITS2-gene was designed. Distinct melting temperatures depending on the species were produced ranging from 52.3 – 66.0 °C. The results were validated against different platforms including Roche LC480, Biorad CFX and Applied Biosystems QuantStudio 6 Flex. Similar melting temperature profiles were received in all qPCR cyclers included, highlighting the applicability of the novel assay across various laboratory settings.

We conclude that the novel snapback primer assay is capable of detecting the three ovine adult and larval *Haemonchus* species as well as hybrids. By using the novel qPCR assay, the samples can now be differentiated simultaneously to species level by DNA melting analysis. This unlabelled probe approach is a simple, valuable and economical tool. It can be used in molecular genetic and epidemiological studies for shedding more light on the serious problem of increasing anthelmintic resistance.

Thursday 20 August 2015

Concurrent Session 7.1

F: Genes and genomics

Ref: O173/0114

Genetic diversity in the natural *trypanosoma congolense* population

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Trypanosoma congolense is one of the major parasites responsible for Animal African Trypanosomiasis (AAT), a disease affecting about 10 million km² of the sub-Saharan region and considered as one of the principal causes of hunger and poverty in those countries. A key step in the fight against AAT is to monitor the level and distribution of genetic diversity in *T. congolense* populations, to reveal the biologically relevant structural variations present in their genomes that influence pathogenesis, virulence, and mechanisms leading to drug resistance. Using Next-Generation Sequencing technology, we sequenced 50 Savannah and 4 Forest strains sampled between 1971 and 2010 in 9 countries where the disease is endemic. A total of 614859 SNPs were identified and long deletions were detected in both coding and non-coding regions, revealing a high genetic diversity. Interestingly, analyses of linkage disequilibrium decay indicated that genetic recombination and complex genetic exchanges are frequent between *T. congolense* parasites. Additional phylogenetic and structure analysis highlighted a geographically asymmetric distribution of genetic diversity across Africa. Parasites collected within a short period of time in Zambia showed a greater diversity compared to the strains sampled over a period of 30 years in the other countries. The factors resulting in this asymmetric diversity remain speculative but we suggest that the close proximity to wildlife observed in this region and the particular tsetse fly distribution are playing a key role. This last hypothesis is currently being tested by studying the capability of several tsetse species to develop a mature *T. congolense* infection.

Thursday 20 August 2015

Concurrent Session 7.1

F: Genes and genomics

Ref: O174/0187

A novel phylogeny for the piroplasms

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Piroplasms are tick-transmitted apicomplexan parasites including the genera *Theileria*, *Babesia* and *Cytauxzoon*. The order Piroplasmida comprises species of significant global socio-economic importance, some of which are lethal to companion- and production-animals or humans. Piroplasms show a puzzling systematics characterized by multiple clades, poorly resolved taxa and paraphyletic or polyphyletic genera. In the present study, screening of Tasmanian platypuses (*Ornithorhynchus anatinus*), was performed to investigate the parasite molecular phylogeny, prevalence and pathogenicity. DNA was extracted from blood (n=28), ectoparasites (n=13) and tick eggs. Phylogenetic reconstructions were based on the 18S rRNA- and hsp70-genes. All blood samples and four adult ticks were positive by PCR, but no particular clinical signs were observed. In blood smears, highly pleomorphic intra-erythrocytic organisms, also forming tetrads and extra-erythrocytic schizonts, were morphologically consistent with previously described *Theileria ornithorhynchi* Mackerras, 1959. The phylogenetic reconstruction produced for this parasite for the first time reveals a novel monophyletic species complex, basal to most known clades. Compared to previous studies, the topology of the tree obtained appears as one of the most strongly supported and complete, produced up to now for the order Piroplasmida.

Thursday 20 August 2015

Concurrent Session 7.1

F: Genes and genomics

Ref: O175/0447

Mitochondrial genome analyses suggest multiple *Trichuris* species in humans, baboons, and pigs from different geographical regions

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The whipworms *Trichuris trichiura* and *T. suis* are two parasitic nematodes of humans and pigs, respectively. Although whipworms in human and non-human primates historically have been designated *T. trichiura*, recent reports suggest that several *Trichuris* spp. are found in primates. Parasites with wide global occurrence might comprise several cryptic species. Complete mitochondrial genomes of *Trichuris* recovered from a human in Uganda, two Olive baboons held in captivity (US and DK), and two pigs from Denmark and Uganda were sequenced and annotated. Comparative analyses with other published mitochondrial genomes of *Trichuris* recovered from a human and porcine host in China and from François' leaf-monkey (China) were performed, including phylogenetic analyses and pairwise genetic and amino acid distances. Genetic and protein distances between human *Trichuris* in Uganda and China were high (~19% and 15%, respectively) suggesting that they represented different species. One baboon *Trichuris* was genetically related to human *Trichuris* in China while the other was nearly identical to human *Trichuris* from Uganda. Baboon-derived *Trichuris* were genetically distinct from *Trichuris* from François' leaf-monkey suggesting multiple whipworm species circulating among non-human primates. The genetic and protein distances between pig *Trichuris* from Denmark and other regions were roughly 9% and 6%, respectively, while Chinese and Ugandan whipworms were closely related. Our results suggested that *Trichuris* species infecting humans and pigs differed across geographical regions, which might have important implications on implementing suitable and effective control strategies in different regions. Moreover, we found further support that *Trichuris* infecting primates comprises a cryptic species complex and may transmit to humans. The evolutionary and demographic history of *Trichuris* in primates and pigs will be discussed.

Thursday 20 August 2015

Concurrent Session 7.1

F: Genes and genomics

Ref: O176/0498

The mitochondrial genome of *Dirofilaria repens*: organization and identification of potential markers for population genetics

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Over the past years, there has been considerable effort devoted to sequencing the mitochondrial genomes of parasitic nematodes. Genetic markers, located in the mitochondrial genome are very well suited for population genetics and phylogenetic studies. Currently, mitochondrial genomes of several parasitic nematodes have been sequenced, including some of filarial worms relevant to humans and animals, such as *Onchocerca volvulus* and *Dirofilaria immitis*. In this study, the complete mitochondrial genome of an individual *Dirofilaria repens* worm from Italy was sequenced. *D. repens* has been associated with autochthonous infections of dogs and most recently also humans in Germany and is considered to be an emerging pathogen in northern areas of Europe. As shown for other filarial mitochondrial genomes, *D. repens* encodes 12 proteins involved in the electron transport chain or ATP synthesis and 2 rRNA genes but lacks the ATP synthetase subunit 8 gene present in most animal mitochondrial genomes. The organization of the genome is essentially the same as that of *O. volvulus* and *D. immitis*. The protein-coding and rRNA genes display a moderate identity with those of *D. immitis* and identities range from 84% to 92% at the nucleic acid level, with the small subunit ribosomal RNA gene showing the highest and nadh2 the lowest conservation. The AT-rich control region of the mitochondrial genome, located between the genes *coiii* and *nadl4*, shows the weakest match (71%) with the respective region of the *D. immitis* genome and the alignment is disrupted by numerous gaps (17%) and no significant similarity for the first 21 bp. The apparently very low selection pressure at this region implies a good suitability for intra-specific comparisons and a reasonable likelihood that markers for population genetic studies can be identified. Currently ongoing investigations on material from other regions of Europe aim to identify genetic structures in European *D. repens* populations, which should allow the identification of the geographic origin of populations that will be introduced in new endemic areas by traveling or import of dogs from Mediterranean and Eastern European areas.

Thursday 20 August 2015

Concurrent Session 7.2

B: Climate change, epidemiology and modelling

Ref: O177/0553

Impact of climate change and subtherapeutic treatment on the development of anthelmintic resistance in nematode parasites of cattle

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Gastrointestinal nematodes (GIN) affect the health and productivity of calves and cattle and even cause occasionally fatal infections. Reports of anthelmintic resistance (AR) in cattle parasites are increasing. Additionally, influences of predicted climate changes on the survival of free-living parasite stages are most likely. A greenhouse and a climate chamber experiment verified that predicted future drought stress and higher temperatures will reduce the refugium for larvae on pasture and therefore increase selection pressure. In a field experiment the effect of subtherapeutic benzimidazole (BZ) treatments on the development of AR was evaluated. Parasite naïve calves were infected with an *O. ostertagi* isolate selected for BZ-resistance. The isolate had 50% of resistance-associated alleles in Codon 200 of β -Tubulin Isotyp 1 (tbb-1). This single nucleotide polymorphism (SNP) causes the expression of tyrosine instead of phenylalanine, which prevents the binding of BZ. During the 32 weeks lasting field experiment, each group of calves was replaced by naïve calves, which acquired natural infections by grazing the contaminated pasture. After 3-4 weeks on pasture, calves were treated with a subtherapeutic albendazole dose (ranging from 35 to 65%). Regularly EPG counts and species specific PCRs confirmed the presence of *O. ostertagi* surviving treatment. Pyrosequencing showed the percentage of the resistance-associated SNP increased rapidly (from 55% to 89%), highlighting the problem of frequent and subtherapeutic treatments.

Since subtherapeutic treatments can be expected to occur frequently in the field and the effects of climate change can accelerate selection for AR, both factors have to be considered as challenges for sustainable GIN control.

Thursday 20 August 2015

Concurrent Session 7.2

B: Climate change, epidemiology and modelling

Ref: O178/0394

Moisture requirements for the horizontal migration of infective stage larvae of gastrointestinal nematodes

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Specific knowledge of the effects of climate on the epidemiology of gastrointestinal nematodes is needed to effectively apply sustainable control strategies. Although the effects of temperature and rainfall on larval development have been well characterized for gastrointestinal nematodes of ruminants, much less is known about migration out of faeces and onto herbage. This was addressed for *Haemonchus contortus* and *Teladorsagia circumcincta* in a series of modified-environment experiments. Methods were firstly developed to investigate the pattern of third stage larvae (L3) migration out of faeces in the laboratory. Then further experiments were conducted to determine the migration rate of L3 under simulated rainfall, so that the parameter could be integrated into a mathematical model which predicted pasture infectivity of GIN. The results showed that free water was needed for the migration of larvae out of faeces. However, a single rainfall event was not sufficient for migration from faeces of which the crust has hardened after having been kept in dry conditions. Light and regular rainfall resulted in rapid emergence from moist faeces kept in humid conditions, but much slower emergence from dry faeces in dry conditions. Ambient relative humidity therefore appears to act through faecal moisture content (FMC) to modify the effect of rainfall on larval migration. Further experiments indicated that the FMC threshold for larvae migration appeared to be around 40%. The timing and nature, as well as total amount of rain, could therefore be important to whether or not substantial numbers of larvae are released after rainfall.

Thursday 20 August 2015

Concurrent Session 7.2

B: Climate change, epidemiology and modelling

Ref: O179/0449

Consistency of strongyle egg shedding in horses

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Recently, studies have aimed to quantify the consistency of strongyle egg shedding in individual horses over time, with the rationale being that if horses generally stay within the same shedding category (if not treated with an anthelmintic), they can be assigned a status of high or low egg shedders, reducing the need for frequent faecal egg count (FEC) testing. This could act as an incentive to horse owners to adopt targeted deworming programmes as it may be associated with reduced costs and effort. Equine FEC data used in this analysis were collected from 26 horse yards across England and Scotland between 2011 and 2012. All faecal samples were analysed using a centrifugal-flotation method with an egg detection limit down to 1 egg per gram (EPG). In total, 573 horses were included in the analysis. Only horses that had an initial screening sample collected on more than one occasion were included. Each FEC from each horse on each screening occasion (S1 = first screen, S2 = second screen, S3 = third screen and S4 = fourth screen) was assigned to an egg shedding category ranging from 1 (0-49 EPG) to 7 (>500 EPG). In addition, each sample was assigned a treatment category; <200 EPG (no treatment) or ≥200 EPG (treatment). The rank change in shedding category and treatment category between S1 and S2, S1 and S3 and S1 and S4 samples was calculated. The same analysis was performed on 304 horses that had not received any anthelmintic during the entire course of the study to assess if withholding treatment was associated with rising egg counts in individuals over time. The effect of age, last anthelmintic administered and the number of weeks after the expected egg reappearance period (ERP) of each anthelmintic on the likelihood of a horse changing shedding or treatment category was assessed using multivariate binary logistic regression. In total, 73% of all horses tested remained in the same egg shedding category and 81.8% in the same treatment category. In the untreated group, 82.7% remained in the same egg shedding category and 95% in the same treatment category. Horses <5 years were 3.3 and 2.8 times more likely to change egg shedding and treatment category compared to other age groups, respectively. Knowledge that horses consistently shed the similar levels of strongyle eggs over time can help to establish evidence-based control strategies.

Thursday 20 August 2015

Concurrent Session 7.2

B: Climate change, epidemiology and modelling

Ref: O180/0364

Meta-analysis of cyathostomins community structure and diversity

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Cyathostomins are parasitic nematodes of veterinary importance in horses, because of their pathogenic effects and their increasing level of resistance to current anthelmintic compounds. Empirical findings about their ecology and community structures after horse necropsy tended to show that few species were consistently dominant across studies although no quantitative assessment is available. Through an exhaustive literature meta-analysis of 41 papers, we evaluated cyathostomin community structures, investigated inter-specific relationships and quantified the impact of environmental factors (continent, climate, horse management) on species richness, prevalence and relative abundance. Across studies of the 36 cyathostomin species, *C. nassatus*, *C. longibursatus* and *C. catinatum* were consistent key players (prevalence and relative abundance above 70% and 15% respectively). A nestedness pattern was found ($p = 0.01$) highlighting low- and high-disperser species and suggesting that lower diverse communities are subset or species-richer communities. Prevalence was negatively correlated to species fecundity ($r = -85\%$) but not to species relative abundance ($p = 0.2$). Species ranks based on prevalence were highly correlated ($r = 81-87\%$) across continents, climates or management of horses but this correlation was weaker for a ranking based on relative abundance, especially between records from Africa and from Oceania ($r = 67\%$) suggesting species turnovers. Cyathostomins were more prevalent (+28%) but not more abundant in unmanaged horse populations. In parallel, significant increased prevalences and abundances were also found in European areas under cold climatic conditions (+9% in prevalence and +26% in abundance) or American tropics (+18% increase in prevalence) or temperate areas (+26% in abundance). However, none of these factors significantly impacted species richness even if unmanaged horses counterintuitively showed a tendency toward reduced biodiversity ($p = 0.06$). These findings contrast with the more climate-dependent pattern observed in ruminants and militates for a unique management framework of cyathostomins and limited influence of climate.

Thursday 20 August 2015

Concurrent Session 7.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O181/0010

Tick-repellent and acaricidal properties of plant-derived extracts: a review of current *in vitro* bioassays

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Ticks are haematophagous arthropods ranked close to mosquitoes in their capacity to transmit disease agents of importance. Current tick control is based on routine use of synthetic chemicals on or in animals and their environment. However, years of overuse of these chemicals have resulted in development of resistance in these parasites and negative environmental impacts hence the need for cheaper, safer and more environmentally friendly alternatives such as the development of medicinal plants. Peer-reviewed articles on medicinal plants with acaricidal or tick-repellent activities against immature and adult stages of ticks were collected from ten scientific databases with the aim of comparing and evaluating the bioassays employed. Search words include “acaricidal”, “tick-repellent”, “anti-tick assays”, “medicinal plants” and “phytomedicine”. Methods and analytic parameters were inconsistent across repellency and acaricidal tests. These include but not limited to petri dish, tick climbing, olfactometer, larvae packet and immersion bioassays of which tick-climbing repellency and adult immersion tests appeared the most used. Ethanol was the most used solvent and *Rhipicephalus (Boophilus) microplus* was the most commonly studied tick across all the reviewed papers. However, since this species is a one-host tick, it is unclear whether the outcome of this analysis can be applied to other species of ticks that infest humans and animals. Also, most of the assays did not discriminate between olfaction and tactile chemoreception-based repellency and though, some of the observed methods were similar, results differ significantly, and these aspects will need further evaluations. A standardized laboratory method is critically needed.

Thursday 20 August 2015

Concurrent Session 7.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O182/0195

Evaluation of Herbal complex against *Haemonchus contortus* and *Rhipicephalus microplus*

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A herbal complex (HC), based on aqueous extracts of *Azadirachta indica* leaves, *Nicotiana tabacum* leaves, *Calotropis procera* flowers and *Trachyspermum ammi* seeds were evaluated against *Haemonchus contortus* and *Rhipicephalus microplus* in sheep and cattle, respectively. Egg hatch test (EHT), adult motility assay (AMA) and fecal egg count reduction test (FECRT) were performed against *H. contortus* and larval packet test (LPT), adult immersion test (AIT) and ear bag method (EBM) were determined against *R. microplus*, following their standard procedures. In EHT, LC50 values of HC and oxfendazole (reference drug) were 275.1 and 0.016 $\mu\text{g ml}^{-1}$, respectively. In AMA, 100% mortality of *H. contortus* was observed 6 hr post-exposure to 3.125-200 mg ml^{-1} concentrations of HC and 2 hr post-exposure to levamisole. In FECRT, maximum (96.2%) fecal egg count (EPG) reduction was recorded in sheep treated with HC @ 4 mg kg^{-1} body weight; whereas, 89.3% reduction in EPG was recorded in sheep treated with levamisole @ 7.5 mg kg^{-1} body weight. The HC extract exhibited lethal effects on egg laying (Index of egg laying = 0.371404 ± 0.00435), hatching (22.35%) and total larval mortality at 50 mg/ml and reduced tick intensity on the infested calves (18 detached out of 35 at 45% w/w suspension, topically applied). A graded dose response was noted in all the tests used in the present study to evaluate the anthelmintic and anti-tick activities of HC, thus may be recommended as suitable alternatives. Large-scale trials on efficacy and safety, however, are recommended before the HC is considered for commercialization in crude form.

Thursday 20 August 2015

Concurrent Session 7.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O183/0229

Essential oils in the control of the donkey chewing louse, *Bovicola ocellatus*

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Chewing lice are widespread and clinically compromising parasites of livestock and equines. Their management is complicated by growing levels of resistance to commonly applied insecticides. Hence, the development of novel approaches to their control are required. Here we discuss the results of a field trial in which essential oils were incorporated into a grooming programme for populations of donkeys with natural infestations of *Bovicola ocellatus*, in the UK and Ireland. After two applications, the groups groomed with lavender or tea tree oil emulsions showed a significant reduction in louse intensity, with a mean decline in louse abundance of 78% (95% CI 76-80%). Louse numbers in the groups groomed with excipient only either did not change or increased significantly. The implications of this research and the feasibility of using natural products for the control of ectoparasites are discussed.

Thursday 20 August 2015

Concurrent Session 7.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O184/0250

Acaricidal activity against *Rhipicephalus microplus* resistant to acaricides of extracts from *Capraria biflora* collected in different seasons

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Chemical acaricides have played a pivotal role in the control of *R. 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. As a result new strategies for tick's control which are less hazardous and more environmentally friendly are searched for; such as the use of plant extracts. This study evaluated the acaricidal activity of methanolic extracts from leaves of *Capraria biflora* against larvae and adults of *R. microplus* resistant to acaricides collected in dry and rainy seasons. Methanolic extracts of leaves of *C. biflora* were obtained in dry and rainy seasons in Yucatan, Mexico. From a farm previously diagnosed as resistant to organophosphates, pyrethroids and amitraz adult ticks were collected. Acaricidal activity of methanolic extracts was tested using the larval immersion test (LIT) and adult immersion test (AIT). Concentrations of 20%, 10%, 5.0%, and 2.5% were used to determine lethal concentration at 50% and 99% (LC₅₀, LC₉₉) against larvae, and reproductive index against adults of *R. microplus*. In LIT *C. biflora* extracts of dry and rainy seasons showed LC₅₀ of 2.7% and 7.7%, respectively, and LC₉₉ of 11.7% and 68.6%, respectively. Moreover, extracts at 20% concentration of dry and rainy seasons in AIT showed 66.7% and 16.6% of mortality, respectively; egg laying inhibition of 81.1% and 32.0%, respectively; and hatchability inhibition of 34.3% and 86.3%, respectively. The methanolic extracts of *C. biflora* collected in dry season are promising candidates to control larvae and adults of *R. microplus* resistant to acaricides. Further studies are required to identify the active fractions and compound of these crude extracts. We are very grateful to CONACYT for supporting this project (CB-2012-01-178216).

Thursday 20 August 2015

Concurrent Session 7.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O185/0653

Artificial feeding of *Ixodes ricinus* and *Dermacentor reticulatus* ticks

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Research on ticks and tick-borne diseases may benefit from the possibility to feed ticks *in vitro*, as this could facilitate applications such as the *in vitro* infection of ticks with pathogens, the *in vitro* screening of acaricides and studies on pathogen transmission dynamics, eventually leading to a reduction in the use of laboratory animals. The development of artificial feeding techniques for ixodid ticks is impeded by the complexity of tick feeding behaviour and the long duration of blood feeding. All methods developed thus far result in poor feeding- and reproduction ratios in comparison to ticks fed on animals. They are also laborious since regular blood changes are typically required, which has hampered the adaptation of these techniques. In the 'Optimization and Automation of Artificial Tick Feeding' (OAKS) project, efforts are undertaken to optimize the *in vitro* feeding for five selected ixodid tick species in order to develop a technique comparable to *in vivo* feeding concerning effort and rearing success. The selected tick species differ in their mouthpart length, life cycle, host finding strategy, reproduction strategy or host specificity, which should make the outcomes of this study applicable to a broad range of other tick species. The effect of parameters such as blood storage temperature, blood supplements, environmental conditions and mechanical and olfactory attachment stimuli are being studied using a modified version of the silicone membrane feeding system which was developed at the Institute of Biology, University of Neuchâtel, Switzerland. Using this system, the life cycle of *Ixodes ricinus* could be completed *in vitro*. A second feeding method was developed in which the artificial tick feeding process is partially automated, making the technique less laborious. In this semi-automated system, glass tick chambers are fitted in a flow-through chamber through which a defined amount of blood can be pumped. Preliminary results showed that the average weight of engorged *Dermacentor reticulatus* females fed using this system did not significantly differ from that of *D. reticulatus* females fed *in vivo*. Further investigations on critical factors for each of the five selected tick species and methods to further simplify and automatize the artificial feeding will be pursued in the remaining period of the OAKS project. The OAKS project was funded by grant 031A228 of the German Federal Ministry of Education and Research (BMBF).

Thursday 20 August 2015

Concurrent Session 7.3

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: O186/0728

Use of vegetation index to predict the distribution of ticks in pastures

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Among domestic animal who suffer on tick infestations and tick-borne diseases worldwide, cattle grazing outside are of great concern. They can harbour massive tick infestations as well as some important tick-borne diseases as anaplasmosis, ehrlichiosis, babesiosis, cowdriosis and theileriosis. To elaborate local strategies against them, veterinarian and farmers should know precisely the risk at the scale of each pasture, or even better, in different part of a pasture. A recent paper working on dairy cattle herd in Brittany, defines a Vegetation Index (VI) they shown to be correlated to the presence of ticks *Ixodes ricinus* in different areas of pastures.

The aim on our study was two-fold: 1) to test the hypothesis than Vegetation Index (VI) could be a way to assume the presence of tick in a specific spot; and 2) to study the best way to localize the pastures at risk in a farm.

The study was carried out in 4 dairy cattle farms in in France during 2012. A total of 80 transects were drag sampled during spring and autumn following the selection of transects in pastures. On these 80 transects, 37 were low prediction (LPR) rate areas and 43 high prediction (HPR) rate areas using the Vegetation Index (VI). Considering the VI as a diagnosis test, the drag sampling as the gold standard and the VI as a test, we obtained a sensitivity of 90%, a specificity of 59%, a PPV (Positive Predictive Value) of 45% and a NPV (Negative Predictive Value) of 94%. Given that, the Vegetation Index is a steady indicator of the potential tick density in a pasture.

Thursday 20 August 2015

Concurrent Session 7.4

D: Vaccines and immunology

Ref: O187/0310

First season performance of Barbervax, a novel vaccine for *Haemonchus contortus*, now commercially available in Australia

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Barbervax, the first commercially available defined antigen vaccine for any parasitic nematode, was registered in Australia in October 2014. The entire first batch of vaccine was sold out within a few days and was used to immunise approximately 50,000 lambs in the New England district of North Eastern New South Wales over the 2014-2015 summer season.

Haemonchus is endemic in New England and the most common cause of death and disease in the several million fine-wool Merinos which are farmed there. The disease is seasonal because the parasite prefers warm, wet conditions, the winters are frosty and most of the rain falls in the summer months. *Haemonchus* is also the dominant gastrointestinal nematode genus in the region and resistance to all but the two newest broad spectrum anthelmintic drugs is common place.

This background made New England a challenging large-scale test bed to evaluate Barbervax under commercial conditions. Farmers who bought the product were supplied with two egg counting kits so that some monitoring of the effect of the vaccine could be made. These results were compared to contemporary egg count data obtained from unvaccinated lambs on anonymous farms in the region.

Good rains in January ensured the presence of a strong parasite challenge by mid March 2015, just as this abstract was being prepared. The performance of Barbervax under the conditions that prevailed will be reported.

Thursday 20 August 2015

Concurrent Session 7.4

D: Vaccines and immunology

Ref: O188/0381

Cysteine proteases and superoxide dismutase as vaccine antigens against the bovine lungworm *Dictyocaulus viviparus*

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Cysteine proteases are important for tissue migration, immune evasion and nutrition of parasitic helminths, whereas superoxide dismutase (SOD) of *Dictyocaulus viviparus* is described to be highly immunogenic. Therefore, these proteins were selected as vaccine candidates to evaluate their protective potential against the bovine lungworm in the EU-FP7-project PARAVAC. In clinical trials, animals were given three vaccinations in three week intervals prior to challenge infection with 2000 infective third stage larvae. Recombinant vaccine antigens [cathepsins (CP) L, CPB-1, CPB-2, CPB-3, SOD, legumain-1 and -2] were tested individually using Matrix-Q™ as adjuvant and in different antigen combinations with Quil A. Statistically significant differences in weight gains were solely observed following immunization with antigen combination legumain-1+legumain-2: vaccinated cattle gained 81.9% more weight ($p=0.002$) compared to the control group. Based on geometric means, reductions of worm burden were observed for CPL (9.8%), CPB-2 (32.4%), CPB-3 (63.8%) and antigen combination CPB-group+CPL (48.1%). Geometric mean reduction of cumulative faecal larvae counts were detected for legumain-2 (45.7%), CPB-1 (33.7%), CPB-2 (29.6%) and CPB-3 (16.3%) as well as antigen combination CPB-group+CPL (52.8%). Significantly reduced worm lengths were observed following immunization with CPB-1, CPB-2, legumain-2 as well as antigen combinations legumain-1+legumain-2 and CPB-group. To determine antibody development, serum immunoglobulin levels of IgG, IgG1, IgG2, IgA, IgM and IgE were investigated weekly during the vaccine trials. Antibody responses were mainly observed for IgG and IgG1. For IgE, serum antibody response was completely lacking. A vaccine trial using SOD as vaccine antigen is currently in progress and results will be presented.

Thursday 20 August 2015

Concurrent Session 7.4

D: Vaccines and immunology

Ref: O189/0405

Characterization of exosomes in the ovine nematode *Teladorsagia circumcincta*

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Teladorsagia circumcincta is a major cause of ovine parasitic gastroenteritis in temperate climatic regions. The development of anthelmintic resistance by the parasite challenges its future control. Recent research indicates that many parasite species release exosomes into their environment. These vesicles are considered to play an important role in the intercellular communication between parasites and their hosts, and thus represent potentially useful vaccine targets. Here, we wanted to examine whether *T. circumcincta* produces exosomes and if so, what proteins they contain. Exosomes were purified from the Excretory/Secretory (ES) products of *T. circumcincta*'s fourth larval stage. Transmission electron microscopy (TEM) and proteomic analysis were used to confirm the presence of the vesicles and to identify the proteins they contain, respectively. The TEM confirmed the presence of exosomes and the proteomic analysis revealed several types of proteins within the vesicles including: Activation-associated secreted proteins, Actins, Metalloproteinases, and RAB proteins. A comparative analysis of exosomes, exosome-free and total ES products showed that approximately 35% of the proteins found in the vesicles could also be identified in the exosome-free ES products, whilst the remaining 65% were present only in the exosome-enriched fraction. Finally, contents of the exosomes were found to be immunogenic, since they were bound strongly by IgG and IgA present in sera obtained from *T. circumcincta* infected sheep but not from the animals prior to infection. Thus, some of the proteins that were identified here are targets of the host immune response and could potentially be targeted in future vaccine intervention studies aimed to control this important parasite.

Thursday 20 August 2015

Concurrent Session 7.4

D: Vaccines and immunology

Ref: O190/0518

First evidences of N-glycosylation of the cathepsin clade in the Newly Excysted Juvenile (NEJ) *Fasciola hepatica*

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In recent years, the prevalence of *Fasciola hepatica* infection in livestock has increased considerably, and there has also been a rise in the occurrence of resistance to flukicidal drugs. The availability of a vaccine to protect livestock from infection would be a valuable tool for the control of fasciolosis. To date, there has been considerable effort to identify potential vaccine candidates based on proteomic and transcriptomic approaches. However, there has been less work on analysis of the parasite glycome. Glycans are likely to be important in mediating immune responsiveness and immunoregulation in animals infected with *F. hepatica*, and differences in glycosylation between native and recombinant antigens may limit the protective capacity of some putative vaccines. The Newly Excysted Juvenile (NEJ) is the first parasite stage to come in contact with the definitive host. Hence, we carried out a study to identify the glycoproteins located on the NEJ tegument (NEJTeg). NEJTeg was isolated after *in vitro* excystation of *F. hepatica* metacercariae. The glycoproteins were chemically biotinylated, separated after avidin-affinity chromatography and identified with liquid chromatography and tandem mass spectrometry (LC-MS/MS). From the set of proteins identified in NEJTeg, eight were also detected in the glycosylated fraction. In addition, all the proteins detected in this fraction are predicted to contain at least one potential N- and/or O- glycosylation sites in their peptide back-bones. *F. hepatica* Cathepsin B3 (FhCB3) was one of the proteins detected within the glycosylated fraction, containing 1 potential N-glycosite in the seq 194-CHAGYNQ-202. In order to confirm glycosylation of cathepsins and the glycan(s) attached to them, NEJTeg was run in SDS-PAGE and the bands containing the cathepsin clade proteins were excised, digested by trypsin and analysed by LC-MS (MS) and ETD. Our results demonstrate that the predicted N-glycosites for cathepsin L3 (CL3), cathepsin B1 (CB1) and procathespin B2 (proCB2) are fully occupied, in all cases, by either Man α 1-3/6Man α 1-4GlcNac β 1-4GlcNac β 1-Asn or its fucosylated version. To our knowledge, this is the first report of N-glycosylation findings in *F. hepatica* cathepsins.

Thursday 20 August 2015

Concurrent Session 7.5

I: Novel diagnostics

Ref: O191/0332

DNA-based testing for liver fluke infection in sheep, an evaluation of Loop-mediated Isothermal Amplification

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The diagnosis of liver fluke infection remains challenging in the live animal. Several of the current tests are invasive e.g. blood-testing, and hence not attractive to sheep farmers. Non-invasive methods would be preferred but have their limitations. For example, faecal egg counting (FEC) can only detect patent infections (>8 weeks post-infection) and not the immature fluke responsible for acute disease in sheep. A commercial coproantigen ELISA (cELISA, BioX Diagnostics, Jemelle, Belgium) showed early promise as an alternative test, being capable of detecting fluke infection 3-4 weeks earlier than the standard FEC in challenge experiments. However, several recent studies have shown that the cELISA lacks sensitivity when applied in the field. There is emerging evidence that liver fluke DNA can be detected in faecal samples by PCR, using ITS-2 and mtDNA targets, and as early as 2 weeks post-infection. A new DNA-based detection method, Loop-mediated isothermal Amplification (or LAMP), shows considerable promise for this application. In the present study, we evaluated the analytical specificity, analytical sensitivity and clinical sensitivity of LAMP, compared to PCR. We also compared the dynamics of detection of the existing FEC, cELISA and PCR tests with LAMP, using our in-house triclabendazole-resistant experimental challenge model in sheep. The *Fasciola hepatica* LAMP proved to be at least as sensitive as the equivalent PCR and was less prone to inhibition from faecal contaminants. In the experimental challenge model, LAMP was able to detect infection significantly earlier (from 3 weeks post-infection) and more consistently than PCR (from 13-14 weeks post-infection). The cELISA became positive from 7 weeks and FEC positive from 10 weeks post-infection. However, the LAMP assay proved to cross-react with DNA from the rumen fluke, *Calicophoron daubneyi*, a common co-infection in both sheep and cattle in the UK and Europe. This necessitated the development of a new *F. hepatica* LAMP assay, which did not cross-react with *C. daubneyi*, although it did cross-react with DNA from other rarer fluke species of lesser diagnostic importance. Recent improvements in LAMP chemistry and assay design are being applied to improve the speed, sensitivity and specificity of the current *F. hepatica* LAMP assay and the assay is being evaluated on field samples and also in cattle.

Thursday 20 August 2015

Concurrent Session 7.5

I: Novel diagnostics

Ref: O192/0365

Loop-mediated isothermal amplification (LAMP) for rapid identification of invasive *Aedes* mosquito species

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Invasive aedine mosquito species are of increasing concern in Europe as most of them are effective or putative vectors of pathogens. Surveillance of these mosquitoes is mainly done by collecting eggs with ovitraps and their identification by using MALDI-TOF mass spectrometry or morphological identification of larvae hatched from these eggs. A rapid molecular egg identification method, preferably applicable in the field, would greatly facilitate surveillance and control. Loop-mediated isothermal amplification (LAMP) is a rapid method to amplify DNA with high specificity and efficiency under isothermal conditions. Over the last 10 years, LAMP has been used in clinical diagnosis of infectious diseases as a valuable tool to detect a variety of parasites, microorganisms and viruses. The aim of this work was to develop LAMP assays for the rapid identification of eggs of container-breeding aedine species, including the invasive *Aedes aegypti*, *Ae. albopictus*, *Ae. atropalpus*, *Ae. japonicus*, *Ae. koreicus*, *Ae. triseriatus* as well as the indigenous *Ae. geniculatus*. rDNA internal transcribed spacer 1 and 2 sequences were determined for each species from several specimens from three different geographic regions/populations. LAMP primers targeting the conserved regions of the consensus sequences were designed for all species, and their specificities were confirmed with DNA from all other species. All assays were optimized with regard to betaine and MgSO₄ concentrations. LAMP products were detected by a color change of hydroxy naphthol blue from violet to sky blue visible by the naked eye. Unexpectedly, colour change already occurred in assays containing homogenates of eggs (but not adults) before adding the DNA polymerase. This challenge was overcome with a preceding incubation at 92 °C for 5 min, which likely denatured egg proteins that interfere with the reaction. Thus, LAMP and mass spectrometry are established for rapid and cost-efficient identification of *Aedes* eggs gathered in surveillance programmes for invasive species.

Thursday 20 August 2015

Concurrent Session 7.5

I: Novel diagnostics

Ref: O193/0722

FECPAK^{G2} is a novel online faecal egg counting system for remote location parasite monitoring

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FECPAK^{G2} is a revolutionary new approach for remote location parasite monitoring that reduces the need for highly specialised parasitology expertise in the field. Faecal samples are processed by a simple manual method and digital photomicrographs are acquired in the field and submitted on-line for analysis. The system utilises novel fluidics to quantitatively accumulate buoyant parasite ova into a single viewing area. A specialised imaging unit eliminates microscope sample translation and simplifies digital image acquisition. Recorded images are automatically linked to accompanying sample and location data and uploaded to secure cloud-based storage facilities. Remotely located experts perform analysis on the online images using custom software applications to simplify faecal egg counting (FEC). Nematode ova are identified and digitally marked on the images, which provides a fully auditable electronic record for each specimen and its analysis. The validated FEC results can be linked instantly to related parties via email and/or web portal viewing, improving interpretation and technical support. Results can then be consolidated and reported by region, user, host species etc, enabling live parasite surveillance.

Thursday 20 August 2015

Concurrent Session 7.5

I: Novel diagnostics

Ref: O194/0389

Pooling sheep faecal samples for the assessment of gastrointestinal strongyle infection intensity and anthelmintic drug efficacy using McMaster and Mini-FLOTAC

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In small ruminants, faecal egg counts (FECs) and reduction in faecal egg count (FECR) are the most common methods for the assessment of gastrointestinal (GI) strongyle infection intensity and anthelmintic drug efficacy, respectively. The main limitation of these methods is the time and cost to conduct FECs on a representative number of individual animals. An alternative is to examine pooled faecal samples. In the present study, the impact of pool size and analytical sensitivity on pooled FEC/FECR results was evaluated. Surveys were carried out on 10 Italian sheep farms where anthelmintic resistance is rare and on 5 Scottish sheep farms where anthelmintic resistance is widespread. In the Italian farms, ewes were treated with albendazole (10 groups) whereas in Scotland, lambs were treated with fenbendazole (4 groups), levamisole (3 groups), ivermectin (3 groups), or moxidectin (1 group). For each group, individual faecal samples were collected from 20 animals (when possible) at (day 0) and after (day 14) anthelmintic drug administration. Faecal samples were analysed as pools of 5, 10, and 20 individual samples. Both individual and pooled samples were screened using two FEC techniques with three different analytic sensitivity, including Mini-FLOTAC (analytic sensitivity of 10 eggs per gram of faeces (EPG)) and McMaster (analytic sensitivity of 15 and 50 EPG) for GI strongyle and *Nematodirus* eggs. FECs based on a pooled examination strategy correlated positively with mean FECs of the corresponding individual samples (ranging from 0.86 to 0.99), regardless of pool sizes and analytic sensitivities. There was no significant difference in FECs between a pooled and an individual examination strategy. For both Mini-FLOTAC and McMaster (analytic sensitivity of 15 EPG), there was a perfect agreement in classifying the drug efficacy as either 'normal', 'doubtful' or 'reduced', and this regardless of pool size. When using the McMaster method (analytic sensitivity of 50 EPG) drugs were often falsely classified as 'normal' or did not allow for the assessment of the efficacy at all (zero FEC at baseline), and this became more pronounced when the pool size increased. In conclusion, pooling ovine faecal samples holds promise as a valid strategy for assessing GIN FEC and FECR. However, for the assessment of FECR it is important to consider the baseline FEC, pool size and analytic sensitivity of the method.

Thursday 20 August 2015

Concurrent Session 7.5

I: Novel diagnostics

Ref: O195/0257

Characterisation and detection of *Cryptosporidium* using platform technologies

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Cryptosporidium is an enteric parasite that infects a wide range of humans and animals and is considered the second greatest cause of diarrhoea and death in children after rotavirus. With treatment options limited, control relies on improved detection and characterisation of the parasite and knowledge of the biology, biochemistry and transmission dynamics of *Cryptosporidium* species. Comparisons between droplet digital PCR and quantitative PCR-based analyses and between Sanger sequencing and high-throughput sequencing (HTS) on an Ion Torrent platform, were conducted and analysed for their sensitivity and accuracy in detecting and characterising *Cryptosporidium* isolates at multiple loci. An alternative technology, metabolomics, was also analysed. Metabolomics may be useful for the diagnosis of *Cryptosporidium* infections, as it allows for detection based on metabolite differences caused by the infection rather than detecting oocysts in faeces by PCR, where sensitivity is limited by both the numbers of oocysts present and the intermittent shedding of oocysts. Metabolomics analysis of human and mice-infected *Cryptosporidium*-positive faecal samples revealed that despite differences in faecal metabolite profiles between these two hosts, metabolomic analysis in both studies was still able to clearly differentiate between infected and uninfected hosts, as well as providing information on the metabolic activity of the parasite during the infection based on faecal metabolite profiles. The application of platform technologies to elucidating current knowledge gaps in *Cryptosporidium* will be discussed.

Thursday 20 August 2015

Concurrent Session 7.5

I: Novel diagnostics

Ref: O196/0717

Identification of phlebotomine sand fly blood meals by real-time PCR

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Introduction

Phlebotomine sand flies are vectors of protozoa of the genus *Leishmania*, the causative agents of leishmaniasis. The female sand flies are haematophagous and feed on various vertebrate species, including humans. Different methodologies have been applied to assess the dietary habits of phlebotomine sand flies, but in most cases, they are laborious or lack sensitivity. In this context, the objective of this study was to standardize and evaluate the usefulness of a quantitative PCR (qPCR) assay to characterize the blood sources of field-collected female sand flies. Six pairs of primers were designed based on *cytochrome b* gene sequences available in GenBank from the following potential hosts: dog, cat, horse, chicken, black rat, and human. Firstly, SYBR Green-based real time PCR assays were conducted using a standard curve with eight different concentrations (i.e., 10 ng, 1 ng, 100 pg, 10 pg, 1 pg, 100 fg, 10 fg and 1 fg per 2 µl) of DNA samples extracted from EDTA blood samples from each target animal. Then, DNA samples extracted from 100 field-collected engorged female sand flies, belonging to three species (i.e., *Lutzomyia longipalpis*, *L. migonei* and *L. lenti*), were tested by the protocols standardized herein. Additionally, female sand flies were experimentally fed on a black rat (*Rattus rattus*) and used for evaluating the time course of the detection of the protocol targeting this species.

The protocols performed well with detection limits of 10 pg to 100 fg. Field-collected female sand flies were fed on blood from humans (73%), chickens (23%), dogs (22%), horses (15%), black rats (11%) and cats (2%). Interestingly, 76.1% of the *L. longipalpis* females were positive for human blood. In total, 48% of the tested females were fed on a single source, 31% on two and 12% on three. The analysis of the time course showed that the real time PCR protocol targeting the black rat DNA was able to detect small amounts of the host DNA up to 5 days after the blood meal. The real time PCR assays standardized herein successfully detected small amounts of host DNA in female sand flies fed on different vertebrate species and, specifically for the black rats, up to 5 days after the blood meal. These assays represent promising tools for blood meal identification in field-collected female sand flies.

Thursday 20 August 2015

Concurrent Session 8.1

A: Drugs and drug resistance

Ref: O197/0617

Inhibition of motility in the L3 stage is a poor phenotype for detecting and measuring resistance to avermectin/milbemycin drugs in gastrointestinal nematodes

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The inhibition of nematode motility is a commonly used phenotype for assessing the anthelmintic activity of candidate compounds, and for detecting resistance to anthelmintic drugs. In an effort to further refine and automate assays based on the measurement of motility, we recently developed a modified version of the WormAssay system to quantitatively measure the motility of microscopic stages of parasitic nematodes. This new platform, called the "Worminator" uses computer processing of high definition video images to quantitatively and simultaneously measure motility of all parasites in an assay well. Because the L3 stage is easily obtained, and can remain viable in storage for extended periods, this stage is commonly used in motility-based assays, such as the larval migration inhibition assay (LMIA). Using the Worminator, we performed a study comparing the dose-response characteristics for multiple avermectin/milbemycin (A/M) drugs, using L3 stage parasites from both A/M-susceptible (S) and A/M-resistant (R) *Cooperia* spp. (abamectin, doramectin, eprinomectin [EPR], ivermectin [IVM], and moxidectin [MOX]) and *Haemonchus contortus* (EPR, IVM, MOX). Concentrations tested ranged from 0.156 to 40 μ M for all drugs. For all compounds and for both species, differences in EC_{50} between the S and R isolates were small and not statistically different, with resistance ratios (RR) ranging from 0.98 – 1.55 for *H. contortus*, and from 1.0 – 1.28 for *Cooperia* spp. These results contrast greatly with LDA data we obtained using the same *H. contortus* isolates, where the RR was 12.7. Furthermore, EC_{50} were very high, ranging from 2.6 to 5.5 μ M; these concentrations are >1000X higher than tissue levels achieved in vivo following treatment. Interestingly, even at the highest concentration tested (40 μ M), 100% inhibition of motility was never achieved, and differences in maximum inhibition between the R and S isolates were not significant. These data strongly demonstrate that dose-response characteristics for inhibition of motility in the L3 stage of GI nematodes do not differ significantly for S and R nematode isolates. This is an interesting finding that raises important questions as to the appropriateness of using motility inhibition of L3 as a phenotype for detecting and measuring A/M resistance in GI nematodes. Additional research is needed to determine if other life stages might provide greater discrimination of R and S isolates.

Thursday 20 August 2015

Concurrent Session 8.1

A: Drugs and drug resistance

Ref: O198/0569

Monepantel resistance of *Haemonchus contortus* in a Dutch sheep flock urges the application of preventive management measurements

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On a sheep farm in the Netherlands with suspected monepantel (MPL, Zolvix, Novartis Animal Health) resistance, a faecal egg count reduction test was carried out according to the method of the World Association for the Advancement of Veterinary Parasitology, in two groups of lambs. Group 1 was the untreated control group, and group 2 was treated with MPL at the manufacturer's recommended dose rate. Efficacy of treatment with MPL was 0 per cent.

Larval identification of pre-treatment coprocultures and of coprocultures after treatment with MPL revealed 100 per cent *Haemonchus contortus* larvae.

On this farm, after a suspected lack of efficacy of ivermectin and doramectin, the sheep farmer started using MPL in 2012, and since then, treatment with MPL was performed as the sole anthelmintic at least twice a year in the breeding sheep. The lambs were treated with MPL at least four times a year. Despite a relatively high frequency of treatment that may have established a high selection pressure for resistance, and possibly provided a competitive advantage for resistant *H. contortus*, it is remarkable that resistance to MPL was established in such a very short notice.

This study confirms, to the best of our knowledge, the first case of *H. contortus* resistance to MPL. In an earlier study in New Zealand, resistance to *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* was confirmed in sheep and goats, also after only using this anthelmintic for a period of two years. Preventive management measures such as limited use of anthelmintics and correct dose rates, combined with targeted grazing management are important features of parasite control, especially because recent Dutch surveillance data show increasing occurrence of multiple anthelmintic resistance, and reversion to susceptibility is unlikely to occur.

Thursday 20 August 2015

Concurrent Session 8.1

A: Drugs and drug resistance

Ref: O199/0445

Next-generation deep sequencing approaches to assess distribution of gastro-intestinal nematode species and benzimidazole resistance in Western Canadian sheep flocks

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Clinical Haemonchosis is of increasing concern to Western Canadian sheep producers and our preliminary survey has detected moderate to high infection intensities of trichostrongylid nematodes in the majority of flocks we have examined in Alberta to date. Preliminary fecal egg count reduction test data suggest that benzimidazole and ivermectin resistance is common in *H. contortus* in the province. We are developing novel next-generation sequencing approaches to improve the diagnosis and surveillance of parasite infections and of anthelmintic resistance. In this presentation, we will discuss the development of an assay, based on “microbiome-style” next-generation deep sequencing of the ITS-2 rDNA locus, to quantitatively determine the proportions of the different parasite species in sheep fecal samples. Preliminary application of this assay suggests that *H. contortus* is the predominant parasite on many Western Canadian sheep farms but that some farms have mixed infections of species associated with parasitic gastroenteritis. We will also present the results of a deep sequencing assay we are developing to detect and quantify benzimidazole associated mutations in the isotype-1 β -tubulin gene and to investigate the haplotypic diversity and phylogenetic relationships of resistance alleles in the field.

Thursday 20 August 2015

Concurrent Session 8.1

A: Drugs and drug resistance

Ref: O200/0260

Does *in vitro* egg hatch test predict the clinical failure of benzimidazole treatment in *Haemonchus contortus*?

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A variety of *in vivo* and *in vitro* methods are available for monitoring anthelmintic resistance. Considerable research efforts have been directed towards defining *in vitro* tests that may diagnose resistance in pre-parasitic stages of goat parasites. The objective of the study was to compare *in vivo* the faecal egg count reduction (FECR) test, the *in vitro* Egg Hatch Test and the molecular determination of the beta-tubulin isotype 1 codon 200 BZ-resistance associated allele frequencies in *H. contortus* stages obtained from infected goats. In this study 54 goat kids were each infected with mixture of 2500 third stage larvae of resistant (benzimidazole, ivermectin) and susceptible *H. contortus* isolates. Composite infection doses consisted of 10, 20, 30, 40, 60 and 80 % of the respective resistant isolate. Each infection dose was employed in xx lambs. Faecal samples for *in vitro* test were collected on separate days. The results of the *in vivo* FECR test showed that albendazole treatment reduced infections by 91.3, 78.0, 63.3, 48.4, 36.5 and 41.4 %, respectively at the above listed infection groups. The results of ED₅₀ values in the *in vitro* EHA varied from 0.09±0.01 to 15.63±12.10 µg/ml TBZ. The results of PyrosequencingTM method revealed that the frequency of the susceptible (TTC) and resistant (TAC) beta-tubulin codon 200 alleles of *H. contortus* in larvae derived from different groups of goats were closely related with the respective composition of larvae which have been used during infection. The results of the *in vitro* tests show solid base for an estimation of clinical resistance *in vivo*. By using of egg hatch delineation dose test (0.3 and 0.5 µg/ml TBZ) we can precisely estimate the efficacy of drug *in vivo*.

The study was supported by Grant Agency VEGA, Grant No. 2/0151/13 of the Scientific Agency of the Slovak Academy of Sciences and Slovak Research and Development Agency Project.

Thursday 20 August 2015

Concurrent Session 8.1

A: Drugs and drug resistance

Ref: O201/0644

Field application of pyrosequencing assays to survey benzimidazole resistance in trichostrongylid nematodes in three key European countries

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BZ resistance (BZ-R) in most/all gastrointestinal nematodes (GIN) is determined by the presence of specific mutations (SNPs) in the parasites' β -tubulin gene, specifically at codons 167, 198 and 200. Species-specific pyrosequencing assays have been developed for the key GIN species, namely *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus* sp., the latter assays being developed specifically for this purpose. The respective single species assays were applied individually to perform a BZ resistance survey across key European regions. Field samples (faeces) from sheep were collected according to a spatial sampling strategy on ovine farms in Ireland, Italy and Switzerland. For each farm, faecal samples were analysed by FLOTAC for presence of nematode eggs and extracted eggs coprocultured to the third larval stage (L3). A sub-sample of L3 was subsequently identified morphologically to species/genus level for reference and the remainder archived as a biobank in ethanol for molecular genotyping studies. Representative samples were collected in both 2012 and 2013 and from the same farms where possible. In regards to the β -tubulin isotype 1 gene, key findings from the BZ-R survey were: (i) for sheep, the SNP in codon 200 was the most common determinant of BZ-R across all 3 countries and in all 3 species, reaching allele frequencies of 90-100% in some cases e.g. *Haemonchus* & *Trichostrongylus* in Switzerland. The codon 200 SNP is also the dominant feature of BZ-R *T. circumcincta* in Ireland. (ii) In directly comparable farms, there was an increase in codon 200 BZ-R allele frequency from 2012-2013, with a concomitant reduction in 167 & 198 e.g. *Teladorsagia* in Italy. (iii) The Swiss samples had the highest BZ-R allele frequencies, followed by Ireland and Italy. This highlights the overall common problem of BZ-resistance in the different countries as well as the difference in prevalence. In a few farms increased SNP frequencies were observed, which occurred either in codon 167, 198 or 200 of the β -tubulin isotype 2 gene of *Trichostrongylus*. Functional analysis will be required to how, whether these genotypes are able to confer phenotypical BZ-resistance.

Thursday 20 August 2015

Concurrent Session 8.1

A: Drugs and drug resistance

Ref: O202/0321

Detection of anthelmintic resistance in gastrointestinal nematodes from spanish sheep flocks comparing the egg hatch assay and the presence of resistant alleles by pyrosequencing

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Anthelmintic resistance (AR) in ovine gastrointestinal nematodes (GIN) to benzimidazole (BZs) is associated with single nucleotide polymorphisms (SNPs) in the isotype 1 of β -tubulin, at codon 200 principally, but also at codons 167 and 198. In this study we compared the levels of AR of GIN field strains by the Egg Hatch Assay (EHA) and the percentage of BZ resistance alleles obtained after pyrosequencing. Throughout the years 2012 and 2013, variation in resistance was checked by EHA in two sheep flocks (A and B). Resistance was measured by the proportion of eggs hatched at discriminating dose of 0.1 g/ml thiabendazole. DNA extraction was carried out from a pool of larvae and eggs from faecal samples. Prior to pyrosequencing, the fragments including the SNPs198/200 were amplified for *Trichostrongylus* spp, *Teladorsagia circumcincta* and *Haemonchus contortus*, independently for each species; a fragment for the SNP167 in *T. circumcincta* was also amplified. Flock A had a high level of hatching throughout the study, with a mean of 77.74% (57.21-90.64%); in flock B, the resistance level increased over time, with values of 33.25% in 2012 (15.81-57.46%) and 52.02% in 2013 (46.06-55.19%). Pyrosequencing analysis detected the presence of the BZ resistance allele at codon 200 in *Trichostrongylus* spp in both flocks; the percentage of the resistant allele was 81.28% (19.53-100%) in flock A and 21.37% (1.57-51.48%) in B. However, for codon 198, the resistant allele in this species was at a low level in both flocks, 2.74% in A and 5.04% in B, respectively. In *T. circumcincta*, the resistant allele incorporating SNP200 was not present in any flock, with a mean of 0.32%; these low levels were very similar at codons 198 and 167, with values of 0.87 and 1.61%, respectively. In *H. contortus*, pyrosequencing was only carried out in flock B, where this species was detected after morphological identification; the proportion of the BZ resistance allele was 6.33% (0-22.77%) at codon 200 and 1.35% (0-4.93%) at codon 198. When comparing the results of EHA and the frequency of BZ resistance alleles at codon 200 in *Trichostrongylus* spp, in both flocks and throughout the experiment, a significant correlation between the two techniques ($r=0.6$; $p<0.01$) was found. These results suggest that *Trichostrongylus* spp is the main contributor to the development of AR in these Spanish GIN field strains. Study funded by Spanish INIA(RTA2013-00064-C02-02)

Thursday 20 August 2015

Concurrent Session 8.2

B: Climate change, epidemiology and modelling

Ref: O203/0669

High prevalence of *T. foetus* 'bovine genotype' in faecal samples from domestic pigs in a mixed farming system with *T. foetus* negative cattle

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Bovine reproductive tract disease caused by the flagellate *Trichomonas foetus* is a notifiable disease in Australia. Recently, *T. foetus* has extended its host range and been implicated in chronic large bowel diarrhea in domestic cats world-wide. While *T. foetus* is pathogenic in both cattle and cats, it has long been established that the same *T. foetus* also colonizes the stomach, caecum and nasal cavity of pigs without apparent clinical significance. Previous multi-locus genotyping has grouped the non-pathogenic porcine *T. foetus* with the pathogenic 'bovine genotype', rather than with the 'feline genotype' *T. foetus*. Indeed, it was shown that the porcine *T. foetus* is experimentally capable of causing disease in cattle. While bovine trichomoniasis is now uncommon due to wide-spread use of artificial insemination, whether *T. foetus* remains prevalent in pigs where bovine trichomoniasis has been eradicated remains unknown. We surveyed fecal samples from 31 pigs farmed in close proximity with *T. foetus*-negative cattle and found 64.50% were *T. foetus*-positive based on real-time PCR. We report the first firm evidence of *T. foetus* 'bovine genotype' present in Australian domestic pigs and established a reference strain of *T. foetus* PIG30/1. Multi-locus genotyping at 10 loci match PIG30/1 identically to the 'bovine genotype' *T. foetus*. We sequenced the transcriptome of PIG30/1 and show that this isolate has, at a cell-wide level, a closer relationship with the bovine *T. foetus*. Furthermore, we compare virulence factors between the porcine, bovine and feline isolates and identify candidates that may determine parasitic potential in *T. foetus*.

Thursday 20 August 2015

Concurrent Session 8.2

B: Climate change, epidemiology and modelling

Ref: O204/0732

Prevalence of *Eimeria* spp. in dairy cattle in northern and central Italy and correlation between species and oocyst excretion

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Bovine coccidiosis is one of the most common parasitic diseases of cattle. Twelve different bovine species of *Eimeria* have been identified worldwide. Coccidiosis is mainly a subclinical disease and results in economical losses due to reduced growth, production loss and management issues. *E.bovis*, *E.alabamensis* and *E.zuernii* are highly pathogenic species causing mortality and morbidity specially in young animals. This study is part of the ElanCOX Program, developed in collaboration with Eli Lilly Italia, and carried out to determine the prevalence of coccidiosis in Intensive Dairy Production Systems in Northern and Central Italy. Despite the importance of Dairy cattle farming in Italy, little is known about the prevalence of bovine *Eimeria* spp. in this country. The aim of the program is also to define the correlation between pathogenic *Eimeria* species and oocyst excretion. From May 2013 we collected 403 pools of fecal samples from 1939 calves (one to six month of age) in 110 milk farms; we tested all the samples with Flotac® dual technique to evaluate the oocyst excretion per single pool; the fecal samples were also processed to identify the *Eimeria* species in every farm. Coccidiosis was present in all farms and in 341 of 403 fecal pools (84,6%). We observed ten *Eimeria* species; multiple infection with different species were found in 83,6% of samples. The main species detected were *E.ellipsoidalis*, *E.bovis* (both with a prevalence of 61,5%) and *E.zuernii* (45,9%). The ElanCOX Program is still ongoing, so the authors hope to be able to collect a greater amount of data to enlarge the knowledge about the prevalence and distribution of *Eimeria* spp. in Italy.

Thursday 20 August 2015

Concurrent Session 8.2

B: Climate change, epidemiology and modelling

Ref: O205/0080

Prevalence of pathogenic *Eimeria* spp. in Dutch dairy replacement calves and associated risk factors

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A field study in the Netherlands was conducted to determine the prevalence of pathogenic *Eimeria* spp in healthy dairy calves. On 66 participating farms, three to five rectally taken fecal samples were collected in two different age groups. Group I, consisting of calves from 21 days until weaning and group II from weaning until 164 days old. A questionnaire on calf husbandry was filled in by the visiting veterinarian. Per farm, fecal samples were pooled per age group and analysed through microscopy and standard McMaster flotation technique with a saturated salt solution. *Eimeria* oocysts were typed based on morphology.

Average within-farm prevalence of pathogenic *Eimeria* spp. was 87.9%, with a prevalence in group I 48.5% and in group II 65.2%. Within the pathogenic *Eimeria* species, within-farm prevalence was 69.7% for *E.alabamensis* (group I =34.9%; group II = 45.5%), 53% for *E.bovis* (group I= 25.8%; group II = 34.9%) and 25.8% for *E.zuernii* (group I = 10.6%; group II = 18.2%). Overall prevalence of pathogenic *Eimeria* spp. was in group II higher than in group I.

No risk factors could be identified for calves in group I. In group II, age at weaning and the presence of pathogenic *Eimeria* in group I calves were negatively associated with the prevalence of pathogenic *Eimeria* spp., where as relocation at weaning was positively associated with the prevalence.

Thursday 20 August 2015

Concurrent Session 8.2

B: Climate change, epidemiology and modelling

Ref: O206/0273

Encephalitozoonosis- more than a rabbit's parasitosis

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Encephalitozoon cuniculi is the causative agent of central nervous dysfunctions and other clinical signs such as phacoclastic uveitis in rabbits. It is known to be an opportunistic pathogen for humans with immune deficiencies and a cause of cataract and uveitis in cats. We assume that the impact of *E. cuniculi* on a broad variety of animals might be higher than previously thought and that the infection is frequently underdiagnosed. From 2004 to 2014 we could detect antibody titres against *E. cuniculi* in 61% of 360 rabbits with suspected encephalitozoonosis with overt clinical signs. Also, lenses of 20 cats with cataract tested positive by PCR and all these cats had positive antibody titres. Four dogs with lens cataracts had a positive antibody titre and two of these tested positive by PCR after phacoemulsification. Furthermore, the eye of one snow leopard with phacoclastic uveitis was positive by PCR and positive antibody titres could also be detected. Finally, we could detect *E. cuniculi* in the eye of two out of 20 birds of prey by PCR. In one case this could be linked to a cataractous lens and might be an explanation for bird window or tree strike or any other impairment birds may have through a loss of vision. *E. cuniculi* may be more important as a pathogen and infect a wider range of domestic and wild animals with clinical outcome than previously assumed. Improved diagnostic tests will shed more light on this issue.

Thursday 20 August 2015

Concurrent Session 8.2

B: Climate change, epidemiology and modelling

Ref: O207/0272

Endoparasite fauna of whales (*Balaenoptera musculus*, *B. physalus*, *B. borealis* and *Physeter macrocephalus*) in the North Atlantic

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Members of the whale family Balaenopteridae and Physeteridae are the largest and heaviest mammals in the world. Like terrestrial mammals whales can be affected by a wide range of protozoan and metazoan parasites. We here report on the gastrointestinal endoparasite fauna of different whale species, e. g. blue whales (*Balaenoptera musculus*), fin whales (*B. physalus*), sei whales (*B. borealis*) and sperm whales (*Physeter macrocephalus*) from the Azores Islands, Portugal. In total, 17 individual whale faecal samples were collected from free-living animals in 2014. Parasitic stages were identified by using standard flotation technique as well as *Giardia*/*Cryptosporidium* copro-ELISAs. Overall, more than 7 taxa were detected in the whale samples. Within protozoan parasites, cysts of *Entamoeba* spp. occurred most frequently (64%), followed by *Balantidium* spp. and *Giardia* spp. (5.8%). Most prevalent metazoan parasite eggs found in whale samples originated from *Anisakis* spp. (41.1%), followed by Acanthocephala spp. (11.7%), strongyles (11.7%), trematodes (11.7%), cestodes (5.8%) and spirurids (5.8%). These metazoan eggs were mainly found in the toothed predator whales (sperm whales), whilst protozoan parasites were exclusively detected in rorqual whales (blue, fin and sei whales), which might be related to the respective diets of these species. The here identified parasite fauna of whales from the North Atlantic does not only bear a pathogenic potential for the whales themselves but also has an anthroponozoonotic potential. To our best knowledge, we here report for the first time of *Balantidium*-, *Entamoeba*- and *Giardia*-infections in sei whales, fin whales and blue whales.

Thursday 20 August 2015

Concurrent Session 8.2

B: Climate change, epidemiology and modelling

Ref: O208/0431

Effects of *Teladorsagia circumcincta* infection and subsequent anthelmintic administration on the ovine gastro-intestinal microbiome

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The recent plethora of human studies has highlighted the contribution that the microbiota makes to mammalian health, body function and, in some cases, disease. However, an appreciation of how parasitic infections impact on the microbiota in commercially important production animals lags behind *Homo sapiens*. Interactions between parasitic nematodes and mammalian intestinal microbiomes may form an important component of the disease phenotypes and aetiology. Previous studies have detected effects of parasitic nematode infections on the colonic microbiota of macaque monkeys and pigs, but the ovine intestinal microbiome and the effect of endemic parasites on its composition have been poorly studied.

Here we investigate the effect of artificial infection of housed sheep by the parasitic nematode *Teladorsagia circumcincta* on the ileal and rectal faecal microbiota using 16S DNA DGGE analysis and whole genome shot gun high through put sequencing. We initially examined the inherent variability of the gut microbiome from two different areas of the intestinal tract in *T. circumcincta* infected lambs and then subsequently the effect of infection and treatment of susceptible and resistant strains of *T. circumcincta* on the microbiome.

Rectal faecal samples from both housed and farm-reared sheep contain relatively conserved, diverse microbial communities dominated by Bacteroidetes and Firmicutes, while ileal microbial communities are less diverse, depleted in Bacteroidetes and exhibit greater inter-individual variation. Fine-scale analysis of community structure in sheep with or without *T. circumcincta* infection reveals specific changes in the rectal faecal microbiota associated with infection, and subtle changes due to infecting parasite strain and drug treatment (monepantel). Similar patterns are observed in ileal epithelial microbiota from the same animals. LEfSe analysis reveals a specific reduction in Bifidobacteriales abundance in worm-infected sheep. Understanding the dynamic mechanisms required to sustain and control the balance between pathogen/commensal microbial communities within the gastrointestinal tract will enable progress in developing new treatments for diseases involving the gastro-intestinal microbiome.

Thursday 20 August 2015

Concurrent Session 8.3

F: Genes and genomics

Ref: O209/0625

The *Fasciola hepatica* genome: gene duplication and polymorphism reveals adaptation to the host environment and the capacity for rapid evolution

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The liver fluke *Fasciola hepatica* is a major pathogen of livestock worldwide, causing huge economic losses to agriculture, as well as 2.4 million human infections annually. Here we provide a draft genome for *F. hepatica*, which we find to be amongst the largest known pathogen genomes at 1.3Gb. This size cannot be explained by genome duplication or expansion of a single repeat element, and remains a paradox given the burden it may impose on egg production necessary to transmit infection. Despite the potential for inbreeding by facultative self-fertilization, substantial levels of polymorphism were found, which highlights the evolutionary potential for rapid adaptation to changes in host availability, climate change or to drug or vaccine interventions. Non-synonymous polymorphisms were elevated in genes shared with parasitic taxa, which may be particularly relevant for the ability of the parasite to adapt to a broad range of definitive mammalian and intermediate molluscan hosts. Large-scale transcriptional changes, particularly within expanded protease and tubulin families, were found as the parasite migrated from the gut, across the peritoneum and through the liver to mature in the bile ducts. We identify novel members of anti-oxidant and detoxification pathways and defined their differential expression through infection, which may explain the stage-specific efficacy of different anthelmintic drugs. The genome analysis described here provides new insights into the evolution of this important pathogen, its adaptation to the host environment and external selection pressures. This analysis also provides a platform for research into novel drugs and vaccines.

Thursday 20 August 2015

Concurrent Session 8.3

F: Genes and genomics

Ref: O210/0596

An *in vitro* platform for the interrogation of *Fasciola hepatica* development

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Fasciola hepatica liver fluke causes considerable economic losses to the agriculture industry of at least US\$3.2 billion annually and poses a significant human health burden with over 17 million people infected. The infective juvenile stage of this neglected tropical disease causing parasite has long been the focus for research into novel drug and vaccine targets as it causes the acute form of the disease and is a key drug/vaccine target for effective liver fluke control. With rapidly improving genomic/transcriptomic data for fluke there is a pressing need for effective functional genomics tools that facilitate research on liver fluke biology. A hurdle to gene function studies is the inability to maintain developing liver fluke *in vitro* for extended periods. Here we report the development of a culture system that facilitates juvenile fluke growth and development; supporting 80% survival for more than 200 days (more than two fold longer than in any previous study). These juveniles display developmental changes in the tegument, reproductive system and gut that are consistent with those reported from flukes recovered from hosts *in vivo*. We have found that juvenile fluke growth and development is supported by the proliferation of neoblast-like cells (putative totipotent stem cells), which originate throughout the parenchyma. Once formed, these cells migrate, differentiating into mature cells in distinct tissues. These advances in monitoring *in vitro* development are now facilitating the evaluation of genes thought to be involved in growth and development and have the potential to enable their control target candidature to be evaluated using RNAi-based gene silencing. Here we examine the utility of RNAi as a method to probe gene function in developing fluke and optimize methods for sustained gene knockdown and functional studies on aspects of worm development. We have found that a single exposure to RNAi triggers enables effective but transient knockdown in fluke that are developing rapidly. However, multiple RNAi trigger exposure protocols enable prolonged gene silencing studies that facilitate the study of fluke developmental biology. Here we report the use of this platform to probe the function of genes associated with development.

This work was supported by a Glover Memorial Scholarship grant to PM^cC and BBSRC-Link grant BB/K009583/1.

Thursday 20 August 2015

Concurrent Session 8.3

F: Genes and genomics

Ref: O211/0500

What does the population structure of *Fasciola hepatica* in British sheep and cattle tell us about parasite transmission?

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Fasciola hepatica is a trematode parasite of economic and welfare importance in livestock. Little is known about the genetic structure of *F. hepatica* populations, nor how clonal expansion in the snail intermediate host and aggregation of metacercariae on pasture affect genetic diversity and gene flow.

Using our panel of eight polymorphic microsatellites we:

- a) produced a multilocus genotype (MLG) for 1579 adult British *F. hepatica* recovered from the livers of 44 lambs and 31 cattle (aged 2.0 to 16.6 years)
- b) determined the genotype of infections within *Galba truncatula* that had been experimentally infected with multiple miracidia of known MLGs.

The parasite genotypic diversity, measured by F_{ST} , was high: 0.890 in sheep and 0.919 in cattle, and, given that the sheep were in their first grazing season, also reflects the diversity of metacercariae on pasture. F_{ST} across all loci was 0.0143 and 0.0286 in parasites from sheep and cattle, respectively; indicating high gene flow and low population structure. A proportion of parasites were genotypically identical: 0.179 in sheep and 0.129 in cattle, with 96 MLGs observed multiple times. Forty-nine snails were experimentally infected with multiple miracidia, 87.8% survived to six weeks post infection and of those 4.1% shed cercariae. Genotyping of infected snails showed that miracidia of different genotypes all underwent clonal expansion.

In conclusion, genotypically identical parasites are present in naturally infected sheep and cattle, probably due to genetic clonal expansion in the snail and aggregation of metacercariae on pasture. Despite this *F. hepatica* populations demonstrate high diversity, high gene flow and low population structure, one explanation for which is the expansion of multiple genotypes in the snail.

Thursday 20 August 2015

Concurrent Session 8.3

F: Genes and genomics

Ref: O212/0588

***Fasciola hepatica* GPCRs - candidate flukicide targets?**

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G protein coupled receptors (GPCRs) are established drug targets in human medicine. In helminths, GPCRs are receptors for both classical and peptidergic neurotransmitters, where they are involved in core neuromuscular functions that have led to their adoption as promising targets for new anthelmintics. Most flatworm GPCR data are derived from *Schistosoma* spp. blood fluke, while GPCRs have also been mined from cestode and planarian genomes. Several GPCRs have been orphaned, again primarily in schistosomes. No GPCRs have yet been described in the liver fluke *Fasciola hepatica*. This study employs Hidden Markov Models (HMMs) based around the GRAFS GPCR classification scheme to annotate at least 95 GPCRs from a draft *F. hepatica* genome. These comprise frizzled-, glutamate-, rhodopsin-, and secretin-like sequences; notably, adhesion-like GPCRs appear absent. Homology analyses divide the rhodopsin family into aminergic GPCRs (including putative adrenergic, cholinergic, dopaminergic and serotonergic receptors), peptidergic GPCRs (including receptors for allatostatin-, angiotensin-, capa-, neuropeptide F/Y-, and tachykinin-like peptides), and orphan receptors including putative representatives of the flatworm-specific PROF1 family, previously described in schistosomes, cestodes and planaria. Notably, the *Fasciola* PROF1 complement appears contracted relative to schistosomes. Our dataset also includes 11 putative flatworm-specific 7 transmembrane receptors without homologues in other genera. This work represents the first description of GPCRs in liver fluke. These are druggable targets of value for discovery screens, and will also enable analyses of fluke neuromuscular functions. We are currently engaged in efforts to map the temporal and spatial expression patterns of these receptors, alongside functional/validation experiments using gene-silencing methods. Funded by BBSRC grant BB/K009583/1.

Thursday 20 August 2015

Concurrent Session 8.3

F: Genes and genomics

Ref: O213/0200

Integrating genomics, transcriptomics and proteomics to investigate *Fasciola hepatica* virulence and invasion

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Infection of the mammalian host with the trematode parasite *Fasciola hepatica* involves parasite activation from a dormant stage followed by penetration and migration through the intestine to the liver. As the parasite migrates it is continually developing and growing while encountering different host molecules, tissues and micro-environments that it must contend with, requiring strict gene regulation. Here we report the integration of the draft *F. hepatica* genome with the generation of extensive transcriptome datasets for the lifecycle stages present in the mammalian host, involved in the first 24 hours of invasion/infection; namely metacercariae, newly excysted juveniles (NEJ), 1hr, 3hr and 24 hr post excystment. In particular our analysis has revealed that even within this short period of 24 hours remarkable differential gene expression is occurring within the parasite. Investigation into the gene transcription occurring within the infective stage, the metacercariae, has also revealed that several genes are 'switched on' contrary to the view of these stages being dormant.

By integrating this data with the newly available *F. hepatica* draft genome, gene expression of multi-copy gene families such as the cathepsin cysteine proteases can be investigated. Preliminary analysis of parasite gene transcript and protein turn-over/storage was carried out by comparing the gene expression data with extensive proteomics data of the excreted/secreted proteins of the NEJ and the somatic proteome of the metacercariae. To exploit fluke biology for control strategies it is vital that we understand how the parasite interacts with its host to ensure survival. This study provides information on how the parasite prepares for infection and the changes that it must undergo once within the mammalian host, which can be exploited for control strategies to target this early stage of infection.

Thursday 20 August 2015

Concurrent Session 8.3

F: Genes and genomics

Ref: O214/O221

Transcriptomic analyses of haematophagous parasitic lifestyle adaptations in the poultry red mite (*Dermanyssus gallinae*)

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Infestation with the poultry red mite, *Dermanyssus gallinae* De Geer, is a major economic and welfare issue for hen health worldwide and requires novel methods of control. A more comprehensive understanding of the biology of the mite is required to achieve this so, here, we have used comparative genomic and transcriptomic analyses to inform the critical parasitological components of the biology of *D. gallinae*. From transcriptome sequencing 13,330 contigs were analysed and BLASTx analysis identified 3,808 (29%) with significant ($<1e^{-02}$) homology to sequences deposited in the NCBI nr database. A comparative transcriptomic/genomic analysis with three other mite species with differing lifestyles and biology identified mechanisms enriched in, or uniquely associated with, *D. gallinae*. Sequences which were common to all species represented 16% of the *D. gallinae* contigs and those that were unique to *D. gallinae* represented 67% of the total contigs examined. In *D. gallinae*, several feeding-induced transcripts associated with food digestion, mite development and reproductive processes were identified, along with components of multidrug resistance mechanisms and the arachidonic acid pathway. Amongst the sequences associated uniquely with *D. gallinae* in these analyses were contigs representing four arthropod-specific viruses, three of which are insect-specific viruses previously only isolated from haematophagous insects.

Thursday 20 August 2015

Concurrent Session 8.4

K: Lifelong education (UG and PG)

Ref: O215/0441

The III ParSCo (Parasitology Summer Course): a FantasTick parasitological experience in Southern Italy

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The Parasitology Summer Course (ParSCo) is an intensive weeklong course on advanced Veterinary Parasitology organized by the Parasitology of the Department of Veterinary Medicine, University of Bari (Italy), with the support of the European Veterinary Parasitology College and the journal *Parasites & Vectors*. This course traditionally takes place in Basilicata, Southern Italy, in the heart of the Mediterranean. This region is fairly suitable for an optimal development of arthropods and thus for the life cycles of many parasites causing arthropod-borne diseases. In the third edition (2014), oral lectures (36%) covered various aspects of both vectors (e.g., ticks, phlebotomine sand flies, *Phortica variegata*) and transmitted pathogens (e.g., various haemoparasites, *Leishmania infantum*, *Cercopithifilaria*, *Thelazia callipaeda*, *Onchocerca lupi*) and lungworms of carnivores (*Troglostrongylus* spp., *Aelurostrongylus abstrusus*, and *Eucoleus aerophilus*). The remaining 64% of the course was devoted to practical activities conducted within and around the Parco Regionale di Gallipoli Cognato. These activities included: the collection of the above cited arthropods (tick flagging and dragging, light traps and sticky traps), necropsy of wild felids and canids for lungworm collections, taxonomic identification of collected parasites and diagnostic techniques, and a visit to a traditional local farm. The III ParSCo counted with a diverse group of 13 attendees, of 9 nationalities, with institutional links with academia or industry in 11 countries in Europe, South and North America, Oceania and the Middle East. Here, an overview of the activities experienced during this dynamic course is presented along with a discussion on the importance of conducting such practical programs for training in our discipline and for creating research-networks based on a strict cohabitation among scientists and on collaborative field and laboratory work.

Acknowledgments: ParSCo is sponsored by Merial and Bayer HealthCare - Animal Health, with the participation of SOIPA (Società Italiana di Parassitologia) and of the journal *Parasites & Vectors*.

Thursday 20 August 2015

Concurrent Session 8.4

K: Lifelong education (UG and PG)

Ref: O216/0608

Parasitology for digital natives

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Teaching veterinary parasitology to undergraduate students has traditionally relied upon standard printed material in the form of study guides, and tools like dichotomous keys to assist with parasite identification during practical sessions. Providing a sufficient number of knowledgeable and experienced tutors to assist groups of 120 students or more with these resources can be a limiting factor, highlighting the need for better resources which enable self-directed learning.

We report on, and demonstrate, the progress of a new parasitology eBook designed to be a comprehensive teaching aid for our tech-savvy Veterinary Parasitology students as well as a useful reference guide to the graduated Veterinarian or postgraduate student.

The main eBook chapters, organised by parasite class, are compilations of interactive lists, charts and keys embedded with high resolution images illustrating morphological features essential for parasite identification. A chapter on diagnostic parasitology contains protocols and demonstrational videos for commonly used laboratory procedures, and an image library allows quick links to all parasite images indexed by genus and species name.

Overwhelmingly positive feedback has been received following preliminary student trials and we anticipate that this modern learning format will enhance the overall learning experience of students and translate into improved performance in practical exams.

Thursday 20 August 2015

Concurrent Session 8.4

K: Lifelong education (UG and PG)

Ref: O217/0708

Defining North American Veterinary Parasitology Competencies by the AAVP

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In recent years there has been a trend towards competency based training in medical schools and in allied health professions. Veterinary medical education in North America is following that trend. As part of the American Veterinary Medical Association (AVMA) accreditation/reaccreditation process for college/schools of veterinary medicine (CVM/SVMs), the AVMA Council on Education (AVMA COE) has broadly defined nine areas of competency for graduating veterinarians that must be documented at the individual student level. Therefore, AVMA accredited CVM/SVMs must develop a system of documenting student mastery of skills/knowledge/competencies beyond typical written exams to meet AVMA COE requirements. The American Association of Veterinary Parasitologists (AAVP) has been playing an important role in defining core competencies in the area of veterinary parasitology. Through the AAVP/Companion Animal Parasite Council sponsored Educator Symposia (2011 and 2013), a group of educators/instructors from 27 CVM/SVMs have developed a detailed list of competencies based on the AVMA COE accreditation requirements. These guidelines include both in depth knowledge of common parasites of domestic animals and skills in completing and interpreting the results of common parasitologic diagnostic methods. The guidelines can be used by parasitology instructors in several ways including: 1) defining parasitology competencies in the overall professional curricula of CVM/SVMs, 2) structuring the course content of professional courses in veterinary parasitology, 3) integrating parasitologic topics, knowledge and skills into other courses in professional curricula. This proactive approach by the AAVP to define core competency standards in veterinary parasitology establishes a benchmark for other veterinary specialty groups. The parasitology core competency guidelines should serve as a useful tool for maintaining the importance and relevance of the subject in veterinary medical education.

Thursday 20 August 2015

Concurrent Session 8.5

I: Novel diagnostics

Ref: O218/0315

Monitoring of *Dictyocaulus viviparus* clinical infection in adult dairy cows through primiparous sera and tank milk MSP-ELISA

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A Major Sperm Protein (MSP) ELISA on bulk tank milk (BTM) has been previously developed as an epidemiological tool for monitoring infection with *Dictyocaulus viviparus* adult worms. In this study, MSP-ELISA on BTM or sera from primiparous cows (n=6) was evaluated for the diagnosis and surveillance of herd-level clinical infection (husk) in dairy cattle farms in western France. Fifteen dairy cattle herds were followed regularly for one year whereas 142 additional herds were visited twice, in June and October-November. Among the 15 dairy herds, 6 showed coughing as clinical symptom at pasture (irrespective of the number of cows involved, the duration and intensity of coughing and the possible other causes than husk) and were considered as having a positive clinical status (CS+) whereas the remaining 9 herds were negative (CS-). At the time of coughing, all the CS+ herds were coprologically positive for lungworm larvae using 30 g pooled faeces from 10 primiparous cows. An additional herd was coprologically positive despite the absence of clinical signs. With the BTM ELISA (cut-off: 0.41 ODR), 4 CS+ herds out of 6 were positive at the time or just before the clinical symptoms occurred. In contrast, using primiparous sera (cut-off: 0.50 ODR), 11 herds were positive (6 CS+ and 5 CS-). ROC analysis showed that the cut-off value for the BTM ELISA based on the occurrence of clinical symptoms within the herd might be adjusted to 0.335 with respective values of sensitivity and specificity of 83 and 91%. With this cut-off, 5 CS+ herds out of 6 were positive as well as 2 CS-. In contrast, ROC analysis did not allow to define a satisfactory cut-off for the primiparous sera ELISA based on the CS+ or CS- status. Information on the occurrence of husk in western part of France was available from the survey on 142 herds: 44% of herds reported clinical cases (80% on adult cows). 50% of these records were made between June and August and 90% between June and October. The results of the BTM ELISA at two blocked sampling dates, i.e. June and October-November were not correlated to the occurrence of clinical cases during the grazing season.

Thursday 20 August 2015

Concurrent Session 8.5

I: Novel diagnostics

Ref: O219/0138

Coproantigen ELISA – can treatment failure in *Fasciola hepatica* be reliably detected?

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The coproantigen ELISA (cELISA) has been reported previously as an appropriate tool to detect treatment failure, and potentially detect drug resistance, against *Fasciola hepatica* by 7–14 days post-treatment in the field (Flanagan *et al* 2011, Brockwell *et al* 2013). This was following the initial assessment of the cELISA as a specific and sensitive diagnostic tool, regardless of burden present (Mezo *et al* 2004, Flanagan *et al* 2011). Gordon *et al* (2013) challenged the reliability of the commercialised cELISA against pre-patent and low level infections following on-farm trials. Controlled experimental multi-age infection studies are currently lacking in the literature. Therefore, sheep were infected orally with 50 *F. hepatica* metacercariae on three occasions, twelve, six and two weeks prior to a single flukicide treatment with triclabendazole, closantel or albendazole. Sheep were sampled for a further seven weeks prior to necropsy. Following effective treatment, faecal antigen was negative within 7 days. When immature stages survive treatment, coproantigen reappears from 6 weeks post-infection. Therefore, sampling 7-28 days after treatment exposes obvious treatment failure in adult *F. hepatica*, but is not sufficiently sensitive to detect the survival of resistant immature parasites until these mature and produce higher levels of coproantigen.

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Thursday 20 August 2015

Concurrent Session 8.5

I: Novel diagnostics

Ref: O220/0228

Evaluation of the performance of five diagnostic tests for *Fasciola hepatica* infection in naturally infected cattle using a Bayesian no gold standard approach

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The clinical and economic importance of fasciolosis has been recognised for centuries. Tests available for the diagnosis of the infection in cattle are far from perfect. The evaluation of diagnostic tests has mainly been carried out using gold standard approaches or under experimental settings, the limitations of which are well known.

In this study, a Bayesian no gold standard approach was used to estimate the diagnostic sensitivity and specificity of five diagnostic tests for fasciolosis in cattle. The tests evaluated were detailed liver necropsy including gall bladder egg count, faecal egg counting, a commercially available copro-antigen ELISA, an in-house serum excretory/secretory antibody ELISA and liver inspection routinely carried out at the abattoir.

In total 619 cattle slaughtered at one of Scotland's biggest abattoirs were sampled, during 3 different sampling periods; summer 2013, winter 2014 and autumn 2014. The sensitivities and specificities of the aforementioned tests were estimated. These were allowed to vary between seasons if tests were *a priori* believed to perform differently for any reason. Additionally, correlation coefficients between parasite burden, liver fibrosis scores and the continuous measures from the ELISA based tests and FECs were estimated.

The results of this analysis provide novel information on the performance of these tests in a naturally infected cattle population and at different times of the year where different levels of acute versus chronic infection are expected. With good estimates of its sensitivity and specificity, routine abattoir liver inspection can be used as a tool for monitoring the epidemiology of *F. hepatica* as well as evaluating herd health planning. Furthermore, the results provide evidence to suggest that the copro-antigen ELISA does not cross-react with *Calicophoron daubneyi* rumen fluke parasites, while the serum antibody ELISA does.

Finally, good correlations of FEC and copro-antigen ELISA with parasite burden show that these tests might be used to estimate the level of infection, while the serum antibody ELISA could be used to estimate extent of fibrosis, which in turn could be a measure of the infection's effect on production.

Thursday 20 August 2015

Concurrent Session 8.5

I: Novel diagnostics

Ref: O221/0522

Luminex technology in the diagnostics of *C. oncophora*, *D. viviparus* and *F. hepatica* in bovine serum and milk samples

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A major constraint for the effective control and management of helminth parasites is the lack of rapid, high-throughput, routine diagnostic tests to assess the health status of individual animals and herds and to identify the parasite species responsible for the disease. Based on existing immunological assays, a versatile bead-based assay using fluorescence detection (Luminex[®] xMAP technology) was developed aiming the simultaneous detection of antibodies against *Cooperia oncophora*, *Dictyocaulus viviparus* and *Fasciola hepatica* in cattle serum and milk samples. Covalent coupling of the recombinant antigens onto magnetic carboxylated beads was performed using a *C. oncophora* 14.2 kDa ES protein, the major sperm protein for lungworm and the Cathepsin L1 for liver fluke. Initially antigens, secondary antibodies, streptavidin as well as samples were tested in different concentrations in order to determine the optimal assay conditions. Importantly, no or only minimal cross-reactivity of coupled beads was detected between target species and with sera from calves specifically infected with *Ostertagia ostertagi*, *Trichostrongylus colubriformis* or *Haemonchus contortus*. Examination of negative serum samples was characterised by low median fluorescence intensity (MFI). Bovine sera of experimentally infected calves showed high specificity and sensitivity for the established serum cut-off values. Examination of paired serum and milk samples determined suitable cut-off values for the milk assay suggesting a generally higher sensitivity and specificity of the Luminex[®] technology. Comparison with existing immunological assays indicated good strength of agreement, especially when the same recombinant antigens were used. However, detection of false positive results, occurred particularly in ELISA using excretory/secretory antigens, led to decreased agreement between the assays. In order to evaluate the field applicability of the new assay, an epidemiological survey was performed in several European countries and the collected samples were analysed with the standardised Luminex[®] technology assays.

Thursday 20 August 2015

Concurrent Session 9.1

A: Drugs and drug resistance

Ref: O222/0213

Anthelmintic efficacy against cattle gastrointestinal nematodes: Results of multi-site faecal egg count reduction study in England

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The anthelmintic resistance (AR) situation in UK cattle is unclear; existing reports differ in dosing accuracy, sample handling, egg-counting technique sensitivity and statistical analysis, preventing clear conclusions being drawn. A three grazing season investigation was conducted to evaluate effective protocols for the early detection of anthelmintic resistance in cattle. One-hundred and forty-two groups of co-grazing first-season animals in geographically distinct English regions had their faecal egg counts (FEC) monitored post turn-out, 88 groups (2,259 animals in total) were subjected to a Faecal Egg Count Reduction Test (FECRT) once a pre-defined composite FEC threshold (≥ 150 epg) was reached, with the remainder either housed without reaching threshold (33 groups), or withdrawn for other reasons (36 groups). Macrocyclic Lactone (ML; ivermectin (IVM) and doramectin (DOR) pour-on (PO) & injectable (INJ)) and fenbendazole (FBZ; drench) products were used; two products were used simultaneously on 15 groups. Treatment was administered by vets or technicians based on individual bodyweights (estimated by weighband or scales), following licensed dose rates. FEC were performed using a combination of the McMaster (McM) method (15 egg per gram (epg) sensitivity) on all samples and the Sensitive Centrifugal Salt Flootation (SCSF - 1epg sensitivity) on samples measuring ≤ 60 epg (≤ 120 epg in 2012) by McM. Larval speciation was conducted on cultured pre- and post-treatment samples (bulkied by treatment). Percentage reductions in egg counts were calculated, by group, using pre- and post-treatment group means (1), and 95% confidence limits (CL) constructed. Thresholds of $>95\%$ efficacy and $>90\%$ lower 95% CL were used to indicate efficacy (2). Overall, 28.4% (25/88) FECRTs demonstrated efficacy (9/19 DOR INJ, 4/8 DOR PO, 10/18 FBZ, 1/27 IVM INJ, 1/16 IVM PO) and 71.6% (63/88) demonstrated a lack of efficacy (10/19 DOR INJ, 4/8 DOR PO, 8/18 FBZ, 26/27 IVM INJ, 15/16 IVM PO) according to adapted WAAVP interpretation criteria. *Cooperia oncophora* larvae predominated post- ML treatment. These results from the first large-scale multi-site study to be conducted in cattle across England and provide valuable insight into the current status of anthelmintic performance in cattle gastrointestinal nematodes.

Thursday 20 August 2015

Concurrent Session 9.1

A: Drugs and drug resistance

Ref: O223/0216

Zolvix® Plus Cattle – Efficacy against gastro-intestinal nematodes infecting cattle

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Zolvix (25 g/L monepantel) is the first product containing an amino-acetonitrile derivative to be commercialized for treatment of gastro-intestinal nematodes. The first marketing authorization was for use in sheep in 2009 (New Zealand) and this was followed by approvals elsewhere in the world. In sheep, monepantel shows a wide spectrum of activity against gastro-intestinal nematodes including those resistant to other drug classes. Subsequent to the development of Zolvix in sheep further work has been completed to demonstrate efficacy in cattle when used in a combination formulation with 2 g/L abamectin, viz. 'Zolvix Plus'. Dose determination/confirmation studies have been completed in Australia, Brazil and the UK, and these are supported by field studies from Australia, Brazil and New Zealand. The effective oral dose for monepantel was confirmed to be 2.5 mg/kg, the same as for sheep. Efficacy of Zolvix Plus (from a pooled analysis) in the pen-based slaughter studies against *Ostertagia*, *Haemonchus*, *Trichostrongylus*, *Nematodirus*, *Cooperia*, *Bunostomum*, *Oesophagostomum* and *Trichuris* was >99.9% (fourth-stage larvae; *Bunostomum* and *Trichuris* not tested) and 98.3 to >99.9% (adults). These studies included some isolates that are resistant to the older anthelmintic classes. In the field studies, efficacy measured by fecal egg count reduction ranged 98.3 to >99.9% (pooled sites within country) in the 2-week period after treatment. In conclusion, Zolvix Plus was demonstrated to be effective against a wide selection of gastro-intestinal nematode species infecting cattle. The treatment was considered safe to the animals treated.

Thursday 20 August 2015

Concurrent Session 9.1

A: Drugs and drug resistance

Ref: O224/0289

Assessment of the ricobendazole plus levamisole nematocidal combination in cattle

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Mixtures of drugs with different mechanisms of action have been proposed as a useful tool to delay the development of resistance. In theory, this is a valid strategy if the efficacy of each of the anthelmintic molecules approaches 100%. This situation is more feasible to be applied in cattle production, where the development of anthelmintic resistance is less widespread than in sheep farms. The main goal of the current trial was to study the clinical efficacy and the potential pharmacokinetic (PK) interactions occurring after the subcutaneous (SC) administration of ricobendazole (RBZ) and levamisole (LEV) given either separately or co-administered to parasitized calves in two seasons, winter and spring, with predominance of different nematode population. Sixty (60) male calves naturally infected with gastrointestinal nematodes susceptible to both drugs were randomly allocated into four groups (n= 15): Control group: animals did not receive anthelmintic treatment; RBZ group: treated with RBZ by the SC route (3.75 mg/kg); LEV group: treated with LEV by the SC route (8 mg/kg); RBZ+LEV group: simultaneously treated by the SC route with RBZ and LEV (at the same dose rates). Eight (8) animals from each treated group were randomly selected to perform the PK study. Drug concentrations were measured by HPLC. The clinical efficacy was determined at 14 days after treatment by the Faecal Egg Count Reduction Test (FECRT). No pharmacokinetic interactions were observed after the combined treatment, with similar PK parameters ($P>0.05$) obtained between the single-drug and combination-based strategy. In winter time (June/July in the southern hemisphere) with only 10% of *Ostertagia* spp. observed in the faecal cultures (control group), the FECRT at 14 days post-treatment were 96.1% (RBZ), 99.8% (LEV) and 100% (RBZ+LEV). However, in spring (October) where *Ostertagia* spp. represent 28% of the total L3 composition, the FECRT were 95.1(RBZ), 93.1(LEV) and 96.1% (RBZ+LEV). The increased presence of *Ostertagia* spp. in spring determined a tendency to reduced clinical efficacies compared to winter time, even for the combined nematocidal treatment. Overall, the potential therapeutic advantages of combined anthelmintic treatment should be cautiously assessed before implementing under field conditions.

Thursday 20 August 2015

Concurrent Session 9.1

A: Drugs and drug resistance

Ref: O225/0639

Reduced field efficacy of ivermectin against *Ostertagia ostertagi* and *Cooperia oncophora* in Danish cattle

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Field reports of anthelmintic resistance against the widely-used macrocyclic lactones (ML) in gastrointestinal nematodes (GIN) of cattle have appeared in NW-Europe in recent years.

Objective: The aim of this study was to assess the efficacy of ivermectin (IVM) against field infections with GIN in Danish cattle. In addition, we evaluated a novel quantitative (q) real-time PCR assay for accurate identification of surviving nematode species after treatment.

Methods: Six farms were selected based on mean faecal egg counts (FEC) of ≥ 100 nematode eggs per gram in first-season grazing heifers. All selected farms had a history of use of ML. In each farm, 20 heifers were selected for faecal egg count reduction test (FECRT) and individual weights were measured using a girth-tape. Ten animals were treated (s.c.) with 0.2 mg IVM/kg body weight and ten animals were left untreated as controls. FEC were investigated in all animals at the day of treatment (0) and at days 14 and 21 post-treatment (p.t). In parallel, L3 were cultured from pooled faeces from all animals in each group. DNA isolated from pooled L3 was analysed by a qPCR quantifying copies of the second internal transcribed spacer (ITS2) specific for *Ostertagia ostertagi* and *Cooperia oncophora*. FEC reduction percentages (FECR%) in IVM-treated groups were calculated following WAAVP guidelines including FECs of the control group. Reduced efficacy was defined when $\text{FECR}\% \leq 95\%$ and lower confidence interval [CI] $\leq 90\%$.

Results: At day 14 p.t., FECR% of IVM treatments in all six farms varied from 75% to 96% (lower CI ranging from 39% to 91%). At day 21 p.t., IVM treatments showed a reduced efficacy in all farms, with FECR% varying from 45% to 91% (lower CI ranging from -34% to 65%). At day 0, ITS2 copies from *O. ostertagi* and *C. oncophora* were identified in larval cultures from treated groups in all six farms. At day 14 p.t. in treated animals, *O. ostertagi* L3 were found in 1 farm and *C. oncophora* L3 in 3 farms. On day 21 p.t., *O. ostertagi* L3 and *C. oncophora* L3 were detected in treated animals of 2 and 4 farms, respectively.

Discussion: Reduced field efficacy of IVM was confirmed by FECRT in 5 out of 6 farms at day 14 p.t. and in all farms at day 21 p.t. The qPCR was able to identify *O. ostertagi* and *C. oncophora* populations surviving IVM treatment. This emphasizes the need of monitoring the efficacy of anthelmintic treatments in cattle using sensitive methods.

Thursday 20 August 2015

Concurrent Session 9.1

A: Drugs and drug resistance

Ref: O226/0019

Vaccination against coccidiosis ameliorates drug resistance in commercial poultry production

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Drug resistance is a problem wherever livestock are raised under intensive conditions and drugs are used to combat parasitic infections. This is particularly true for the anticoccidial agents used for the prevention of coccidiosis caused by protozoa of the apicomplexan genus *Eimeria* in poultry. Resistance has been documented for all the dozen or so drugs approved for use in chickens and varying levels of resistance is present for those currently employed. A possible solution may be the introduction of drug-sensitive parasites into the houses where poultry are raised so that they may replace such drug-resistant organisms. This can be achieved by utilizing live vaccines that contain strains of *Eimeria* that were isolated before most anticoccidial compounds were introduced. Such strains are inherently drug-sensitive. In this report we provide evidence to support this contention. Five successive flocks of broiler were reared in floor-pens and given various drug programs followed by vaccination. Parasites were isolated following the fifth flock and their sensitivity to a widely used drug (salinomycin) investigated. Results showed that sensitivity to this drug had been restored. We conclude that alternation of drug use with vaccination in the field could result in restoration of drug sensitivity. A proposal for a yearly broiler production cycle involving chemotherapy and vaccination is presented. There are few, if any, examples in veterinary parasitology where it has proved possible to restore sensitivity to drugs used to control a widespread parasite.

Thursday 20 August 2015

Concurrent Session 9.1

A: Drugs and drug resistance

Ref: O227/0227

Characterisation of *Dermanyssus gallinae* glutathione S-transferases and their potential as acaricide detoxification proteins.

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Background: Poultry red mite, *Dermanyssus gallinae* De Geer, is a major economic and welfare issue for hen health worldwide. The emergence of acaricide resistance has highlighted the need to understand acaricide detoxification mechanisms enabling improved methods of control to be developed. Glutathione S-transferases (GSTs) facilitate detoxification of drugs by catalysing the conjugation of the reduced glutathione (GSH) to electrophilic xenobiotic substrates and, therefore, have a function in multi-drug resistance in many endo- and ectoparasite species. It is envisaged that knowledge of *D. gallinae* GSTs will inform both drug resistance in, and novel interventions for the control of, *D. gallinae*.

Methods: A transcriptomic database representing *D. gallinae* was examined and 11 unique contig sequences were identified with GST BlastX identities. The full-length transcripts represented by 3 contigs, designated *Deg-GST-1*, -2 and -3, were sequenced and further characterised phylogenetically. Recombinant versions of *Deg-GST-1*, -2 and -3 (*rDeg-GST*) were enzymically active and acaricide-binding properties of the *rDeg-GSTs* were established by evaluating the ability of selected acaricides to inhibit the enzymatic activity of *rDeg-GSTs*.

Results: 6 of the identified GSTs belonged to the mu class, followed by 3 kappa, 1 omega and 1 delta class molecules. *Deg-GST-1* and -3 were orthologous with mu class GSTs and *Deg-GST-2* was a delta class GST. Phoxim, permethrin and abamectin significantly inhibited *rDeg-GST-1* activity by 56%, 35% and 17% respectively. Phoxim also inhibited *rDeg-2-GST* (14.8%) and *rDeg-GST-3* (20.6%) activities.

Conclusions: Selectively targeting and disrupting *Deg-GST* function could potentially work in synergy with existing acaricides to increase their efficacy and prolong their use in the field by overcoming resistance.

Thursday 20 August 2015

Concurrent Session 9.2

B: Climate change, epidemiology and modelling

Ref: O228/0696

Can infection with one genotype of *C. parvum* protect against infection with another?

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Currently there is little known about the development of immunity to *Cryptosporidium* in farm livestock; in humans it is possible to become infected with *Cryptosporidium* early in life and again in adulthood. In livestock however, clinical disease caused by the parasite is generally only seen in neonatal livestock and not older animals. It is not known if these animals are resistant to disease because of previous exposure at a young age.

An experimental challenge was carried out to test the development of immunity to homologous and heterologous *Cryptosporidium* infections in lambs using two distinct *C. parvum* isolates. Thirty-six neonatal lambs were split into six groups: two to test age-related susceptibility, two homologous and two heterologous infection and challenge groups. The lambs were kept for 8 weeks with lambs in the age-susceptibility experimental group challenged at 6 weeks of age and lambs in the other groups challenged at <1 week of age and re-challenged at 6 weeks of age.

Total faecal output was collected from each lamb along with weekly blood samples. Clinical data (feed intake, demeanour and faecal consistency) was recorded daily. Oocyst counts were used to determine the shedding profile of oocysts from each group.

Results show that lambs infected for the first time at 6 weeks of age exhibit fewer clinical signs of disease compared to lambs infected at < 1 week of age, although the 6 week old lambs can still shed large numbers of oocysts. We also found evidence that different *C. parvum* isolates can cause more severe and prolonged clinical disease than others. Animals which were re-challenged at 6 weeks of age after initial challenge at <1 week of age also showed a reduction in clinical disease. Sera will be tested by ELISA to measure the antibody titres in each group.

Thursday 20 August 2015

Concurrent Session 9.2

B: Climate change, epidemiology and modelling

Ref: O229/0306

Molecular epidemiological analyses of symbiotic virus of *Cryptosporidium parvum* in Japan

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Cryptosporidium parvum belongs to the phylum Apicomplexa and has been reported to be a zoonotic species that causes severe diarrhea. Therefore, studies on the species/subspecies distribution and transmission routes of *C. parvum* are important for public health and food production.

Some subtyping genes for *Cryptosporidium* spp. have been identified and have been used to investigate its distribution and transmission routes. The 60-kDa glycoprotein (GP60) gene is the major subtyping gene of *C. hominis* and *C. parvum*. The IIa subtype is the most common subtype found in humans and cattle in many countries. In Japan, IIa subtype is the only subtype of *C. parvum* that has been detected to date. Therefore, there are no tools that can trace infection and contamination sources in outbreaks.

We show that *Cryptosporidium parvum* virus 1 (CSpV1), a member of the family *Partitiviridae*, genus *Cryspovirus* that can infect *Cryptosporidium parvum*, is a new candidate for subtyping and could serve as a detection marker of *Cryptosporidium parvum*. CSpV1 was detected in all *C. parvum*-positive samples tested. Phylogenetic analysis of partial sequences of dsRNA1 from CSpV1 samples using the maximum likelihood method revealed that the Japanese samples belonged to a Japanese clade. Sequences detected in samples from Hokkaido (Ashoro and Ishikari), Honsyu, and other islands (Iwate, Tanegashima, and Okinawa) belonged to a single clade. Samples from Iwate, Tanegashima, and Okinawa belonged to a single subclade. Therefore, the *Cryspovirus* dsRNA sequences reflect the regional distribution of their host and have potential as a high-resolution tool for *C. parvum* subtyping.

Recombinant CSpV1 capsid-GST protein was synthesized in *E. coli* and monoclonal antibodies were produced in mice. Anti-CSpV1 capsid protein antibody recognized *C. parvum* sporozoites and localized as dots in the cytosol. The cell biology of *Cryspovirus* infection and localization of other *C. parvum* lifecycle stages remain unknown. Therefore, this antibody has value for the detection of *C. parvum* and study of its cell biology.

Thursday 20 August 2015

Concurrent Session 9.2

B: Climate change, epidemiology and modelling

Ref: O230/0523

Experimental transmission of *Besnoitia besnoiti* by stable flies (*Stomoxys calcitrans*)

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Bovine besnoitiosis is an emerging protozoan disease in cattle. The *B. besnoiti* life cycle is still poorly known. Hematophagous flies, including the stable fly *Stomoxys calcitrans*, could be mechanical vectors. During a blood meal on infected cattle, the puncture of bradyzoites-filled cutaneous cysts or the ingestion of tachyzoites-enriched blood could lead to the contamination of the stable flies' mouthparts by the parasites. However, the release of live parasites within another host after an interrupted blood meal needs to be clearly demonstrated. Two hundred laboratory-reared stable flies were allowed to feed on forty-eight 30 µL drops of heavily contaminated cattle blood (2.5 10⁷ tachyzoites per mL) during five minutes only. This source of contaminated blood was then removed. For most of these stable flies, this initial blood meal was not complete. Thereafter, the flies were allowed to feed on a glass feeder containing non infected blood during one hour. The flies were killed at – 20°C immediately after. Four pools of fifty individual mouthparts and intestines were done before DNA extraction. Presence of parasite DNA was evaluated by a highly sensitive real-time PCR in pooled mouthparts and intestines and in the previously non-infected blood of the glass feeder. This experiment was repeated three times. All pooled intestine contents (N = 12) showed high real-time PCR positive results with Ct values ranging from 20 to 27 whereas eight out of the 12 tested mouthparts pools revealed positive real-time PCR results with Ct values ranging from 34 to 38. The blood of the glass feeder exhibited positive results in 2 out of 3 repetitions with Ct values of 35 and 38. One mL of the remaining glass feeder blood was inoculated into a Vero cells culture for each of the three repetitions. In two repetitions, the presence of tachyzoites in culture medium was visually confirmed fourteen and seventeen days after the culture inoculation. At that time, real-time PCR analyses of the Vero cells exhibited low Ct values (23) indicating that parasite multiplication occurred. This experiment suggests that stable flies are able to transmit live parasites from an infected animal to a safety one. This protocol will be used to assess the factors modulating the efficiency of *Besnoitia besnoiti* mechanical transmission i.e. the number of parasites, the number of flies and the duration of the blood meal interruption.

Thursday 20 August 2015

Concurrent Session 9.2

B: Climate change, epidemiology and modelling

Ref: O231/0555

Masonite oviposition substrate in tiger mosquitoes surveillance: Do the sizes really matter?

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Several systems have been proposed to evaluate *Aedes albopictus* population dynamics, however, ovitraps are still regarded as the most feasible tool. Although the use of standardized features have been suggested for ovitraps no studies have investigated how oviposition substrate sizes may influence the likelihood of capture. The aim of this study was to compare the egg catching in oviposition substrates of ovitraps in terms of paddle's width. Egg count and density on wide surfaces and margins of different sized oviposition substrates have been evaluated in two studies (A and B). In the study A, a total of 29,995 *Ae. albopictus* eggs were counted in 250 "classic" oviposition substrates (14 cm x 2 cm x 0.5 cm). Eggs were found on both wide surfaces (53.1%) and margins (46.9%). Egg density was significantly larger in margins compared to wide surfaces. In the study B, overall 983 *Ae. albopictus* eggs were collected. According to paddle sizes, 51.8% of eggs were on "large" (14 cm x 4 cm x 0.5 cm) and 48.2% on "small" paddles (14 cm x 1 cm x 0.5 cm). Mean egg density of wide surfaces was significantly larger in "small" paddles (0.25 eggs/cm²) compared to "large" paddles (0.06 eggs/cm²). Results indicate that wider oviposition substrates do not mean larger number of *Ae. albopictus* eggs. The results of two field studies revealed that thinner paddles collect the same number of eggs compared to the wider ones, findings suggest that the so called "small" paddles may be routinely employed, thus allowing to save materials and money especially where limited available funds could represent an obstacle to drive public health surveillance activities.

Thursday 20 August 2015

Concurrent Session 9.2

B: Climate change, epidemiology and modelling

Ref: O232/0715

Diversity sand flies in a military training camp in north-eastern Brazil

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Studies on the ecology of phlebotomine sand fly vectors of *Leishmania* spp. are of paramount importance for understanding the eco-epidemiology of the leishmaniasis. We studied the sand fly fauna in a military training camp located in a remnant of Atlantic rainforest in north-eastern Brazil, where outbreaks of cutaneous leishmaniasis have been sporadically described. From July 2012 to July 2014, light traps were placed in 10 collection sites near the burrows of wild animals, trunks and roots of large trees in shady and humid environments. Monthly, light traps worked from 18:00 until 6:00 am, for 4 consecutive nights. The insects captured were kept in 70% ethanol until morphological identification. In total, 24,606 sand flies belonging to 25 species were identified: *Lutzomyia choti* (n=17,951), *Lu. longispina* (n=3,416), *Lu. complexa* (n=1,311), *Lu. sordellii* (n=879), *Lu. amazonensis* (n=243), *Lu. walkeri* (n=186), *Lu. wellcomei* (n=130), *Lu. quinquefer* (n=120), *Lu. evandroi* (n=96), *Lu. ayrozai* (n=48), *Lu. barrettoii barrettoii* (n=49), *Lu. capixaba* (n=40), *Lu. naftalekatzi* (n=34), *Lu. clautrei* (n=31), *Lu. schreiberi* (n=19), *Lu. umbratilis* (n=16), *Lu. whitmani* (n=15), *Lu. brasiliensis* (n=6), *Lu. viannamartinsi* (n=5), *Lu. shannoni* (n=4), *Lu. yuilli pajoti* (n=3), *Lu. migonei* (n=1), *Lu. oswaldoi* (n=1), *Lu. aragaoi* (n=1) and *Lu. furcata* (n=1). The number of males (n=12,683) was higher than that of females (n=11,923). Sand flies were present during all months, being more numerous in March (n=1,691) and April 2013 (n=3,324). *Lu. choti* (72.9%) was the most represented species, followed by *Lu. longispina* (13.8%), *Lu. complexa* (5.3%), representing together >90% of the sand flies. This study indicates a high level of sand fly species diversity, revealing the occurrence of eight potential vectors of *Leishmania* spp. in the examined area, i.e., *Lu. complexa*, *Lu. amazonensis*, *Lu. wellcomei*, *Lu. ayrozai*, *Lu. umbratilis*, *Lu. whitmani*, *Lu. shannoni* and *Lu. migonei*. Indeed, an outbreak of cutaneous leishmaniasis (24 cases) was recorded among soldiers during October-December 2013. In addition to provide new information on the ecology of several sand fly species occurring in the Atlantic rainforest biome, data generated in this study will be useful to define the months of the year when military trainees should avoid undergoing nocturnal activities in the forest so as to reduce the risk of being exposed to high numbers of sand fly vectors.

Thursday 20 August 2015

Concurrent Session 9.2

B: Climate change, epidemiology and modelling

Ref: O233/0146

Feeding phenology of ticks: insights through fat measurement

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Ticks store their energy as fat and utilise this reserve to support their survival between blood meals, questing activity and reproduction. The measurement of fat reserves gives insight into the feeding history of an individual tick, since fat reserves will decline predictably with time since the last meal, and can also elucidate the variation in the seasonal pattern of questing activity within a tick population. However, approaches to measuring fat levels are poorly described in the literature. Here, a study was conducted to compare methods for the measurement of fat levels in field derived samples of the tick, *Ixodes ricinus*. Nymphs of *I. ricinus* were collected fortnightly from fields in south West England and subjected to fat analysis by gravimetric and spectrophotometric methods. Fat levels are analysed in relation to body size and time of year and the value of this technique in uncovering subtle patterns in tick feeding behaviour is demonstrated.

Thursday 20 August 2015

Concurrent Session 9.3

F: Genes and genomics

Ref: O234/0497

RNA-Seq analysis of differentially expressed genes in cattle- and buffalo-derived *Theileria parva* isolates

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Tick transmission of an apicomplexan protozoan intracellular parasite, *Theileria parva*, from infected cattle to susceptible cattle causes a lymphoproliferative disease called East Coast fever, or Corridor disease where transmission originates from contact with infected buffalo. Molecular differences in *T. parva* parasites responsible for the different disease syndromes have not yet been fully established. Consequently, two *Theileria parva* isolates representing cattle- (*T. parva* Muguga) and buffalo-derived (*T. parva* 7014) parasites were investigated for variations in transcriptome profiles using the Illumina next generation sequencing platform.

Theileria parva Muguga genome sequence was used as a reference for mapping of RNA sequences. Overall, 3954 genes (98.4%) of the 4035 predicted coding genes were mapped from the two transcriptomes; 3885 genes were detected in *T. parva* 7014 and 3902 from Muguga. Gene expression variation analysis revealed 1048 differentially expressed genes (DEGs); 742 (70.8%) genes were significantly down-regulated while 309 (29.5%) were up-regulated in *T. parva* 7014 when Muguga gene expression profiles were used as a reference. Although the majority (n=335) of DEGs code for hypothetical proteins (HPs), gene products of interest included members of sub-telomeric fragment-related (SVSP) family, Tar/Tpr protein family, and TashAT/TpHn protein families, membrane-associated proteins, signal peptide containing proteins, transcription/translation/elongation factors and antigenic proteins. Enriched in gene ontology molecular function category were mainly DEGs associated with molecular transducer activity, catalytic activity, and binding. Subsequent to KEGG analysis, gene products of 277 DEGs were mapped to 168 pathways, revealing activities in host cell adhesion, host cell penetration and interaction with signal transduction pathways regulating cell proliferation, differentiation, growth, immune response, survival and death.

The gene expression profiling data generated in this study provide valuable genetic information for revealing transcriptomic variations between cattle- and buffalo-derived *T. parva* isolates and could help explain different disease syndromes resulting from infection with these parasites.

Thursday 20 August 2015

Concurrent Session 9.3

F: Genes and genomics

Ref: O235/0259

Niche-specific gene expression in the parasitic nematode *Teladorsagia circumcincta*

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The primary cause of parasitic gastroenteritis in small ruminants in temperate regions is the nematode, *Teladorsagia circumcincta*. This trichostrongylid has a direct lifecycle involving periods of environmental exposure as eggs and larvae (L1-L3) and prolonged periods of intimate contact with host tissue in the biochemically and immunologically hostile environment of the abomasal mucosa during late L3, L4, L5 and adult stages of development. As such, the repertoire of gene expression in *T. circumcincta* is not only highly stage-specific, it is also niche-specific. We have used a variety of transcriptomic and post-genomic tools and techniques to elucidate the expression profiles and functions of some of the components of the niche-specific gene expression. For the most highly expressed L3-specific transcript, *Tci-cat-4* [which encodes GTP-cyclohydrolase I (GTP-CH)], we used gene rescue, by functional complementation in *Caenorhabditis elegans*, to demonstrate the role of this molecule in cuticle melanisation and, hence, environmental resilience of the infective larvae. Many of the L4-specific transcripts in this species are related to the transition to parasitism (e.g. proteolytic enzymes, globin-like proteins) and rapid growth (collagens, etc.) but, even within this single larval stage, we discovered niche-specific gene expression. We found that L4 harvested from infected sheep were either in direct contact with the mucosal surface of the abomasum ("mucosal larvae") or were present in the luminal contents of the abomasum ("luminal larvae"). Using RNA-Seq we were able to demonstrate the differential expression of a suite of genes between these two sub-populations and identified that several of the up-regulated transcripts in the mucosal larvae as encoding potential immunomodulatory molecules (ASPs and ShK-domain proteins). In conclusion, the stage-specific transcriptome of parasitic nematodes can be further subdivided into niche-specific gene expression, giving us further insights into parasitic lifestyle where appropriate tools are available.

Thursday 20 August 2015

Concurrent Session 9.3

F: Genes and genomics

Ref: O236/0408

The effect of *Psoroptes ovis* infestation on ovine epidermal barrier function

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Sheep scab is a highly contagious ectoparasitic disease of sheep caused by the mite *Psoroptes ovis*. It is an important welfare issue, which causes intense pruritus and severe exudative dermatitis. The mites abrade the stratum corneum, depositing allergens as they progress and this combination of skin abrasion, allergen deposition and self-grooming behaviour triggers a cutaneous inflammatory response, producing an exudate, which supplies the mite with a food source. Terminally differentiated keratinocytes within the stratum corneum represent the first contact between the parasite and the host immune response. The purpose of the present study was to investigate the effect of *P. ovis* infestation on components of the ovine epidermal barrier during the first 24 hours post-infestation (hpi). The expression of epidermal differentiation complex (EDC) genes and epidermal barrier proteins, the nature and severity of epidermal pathology and transepidermal water loss (TEWL) were evaluated following infestation. By 1 hpi a significant dermal polymorphonuclear infiltrate and a significant increase in TEWL with maximal mean TEWL (598.67 g/m²h) were observed. Epidermal pathology involving intra-epidermal pustulation, loss of epidermal architecture and damage to the basement membrane was observed by 3 hpi. Filaggrin and loricrin protein levels in the stratum corneum declined significantly by 24 hpi and qPCR validation confirmed the decrease in expression of the key EDC genes *involucrin*, *filaggrin* and *loricrin*, with 5.8-fold, 4.5-fold and 80-fold decreases, respectively by 24 hpi. This study has demonstrated that early *P. ovis* infestation disrupts epidermal barrier function causing significant alterations in expression of critical barrier components, epidermal pathology, and TEWL. As in atopic dermatitis, barrier disruption facilitates increased transepidermal passage of allergens, provoking a Th2 dominated immune response. Therefore disruption of the epidermal barrier may play a critical role in the development of hypersensitivity reactions that characterise *P. ovis* infestation.

Thursday 20 August 2015

Concurrent Session 9.3

F: Genes and genomics

Ref: O237/0091

Parasite-microbiota interactions – a role in helminth-induced suppression of inflammation?

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Gastrointestinal parasitic helminths share the same environment with a myriad of commensal microorganisms, collectively known as the 'gut microbiota'. While substantial information is available on the interactions occurring at the host-parasite interface, little is known of the impact of infections by gastrointestinal parasites on the composition of the gut microbiota and relative abundance of individual microbial species. Elucidating the complex network of interactions between helminths and the commensal flora is pivotal to a better understanding of the biology and pathogenicity of parasites, as well as of their ability to manipulate the host's immune system and establish chronic infections. In addition, given the critical roles of the gut microbiota in the development of the immune system, as well as in many chronic inflammatory gut disorders, exploring the mechanisms by which helminths regulate the commensal flora may have important implications for the development of helminth-based therapies for the treatment of these diseases. In this study, we assessed the qualitative and quantitative changes in the bacterial communities of human volunteers with a chronic inflammatory gut disorder (i.e. coeliac disease) prior to and following infection with the human hookworm, *Necator americanus*, and following challenge with escalating doses of dietary gluten. While experimental hookworm infection of these subjects resulted in maintenance of the composition of the intestinal flora, even after a moderate gluten challenge, we observed a significant increase in microbial species richness over the course of the study, which could represent a potential mechanism by which parasites can regulate inflammation and maintain intestinal immune homeostasis.

Thursday 20 August 2015

Concurrent Session 9.3

F: Genes and genomics

Ref: O238/0472

Identification of putative vaccine candidates in *Cystoisospora suis* using an *in silico* genomic-based screening

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Cystoisospora suis (syn. *Isospora suis*) is a protozoan parasite of the phylum Apicomplexa that causes coccidiosis in piglets and damages worldwide pig production. Currently, toltrazuril is the only available drug, however it must be administered metaphylactically and resistance is of increasing concern. Thus, a vaccine would be highly desirable. In this project we sequenced for the first time the genome of *C. suis* by Illumina HiSeq technology with the intent of finding potential vaccine candidates using an *in silico* based screening approach. The genome assembly has an estimated length of ~84Mb and contains about 8000 protein-coding genes. To identify eligible vaccine candidates, we screened each protein using a battery of programs (SignalP, TargetP, WoLF PSORT, Phobius, TMHMM) to compute immunogenic features, such as putative transmembrane domains and signal peptides for membrane localisation and secretion. With the aid of the software Vacceed we combined these features and identified a preliminary list of 615 candidates, including proteins known to be involved in the invasion process, such as apical membrane antigen 1 (AMA-1), various microneme (mic2, mic4, mic10, mic13, mic14), rhoptries (rop6, rop14, rop21, rop32, rop39) and dense granule (gra12) proteins, together with 131 uncharacterized proteins with no clear orthologs. We are going to refine this list by incorporating predictions of epitopes recognized by MHC proteins of pig using the IEDB MHC1 prediction software. We plan to validate these candidates through in vitro inhibition assays using recombinant proteins for the production of antisera.

Thursday 20 August 2015

Concurrent Session 9.3

F: Genes and genomics

Ref: O239/0671

Genomic resources to study anthelmintic resistance in *Haemonchus contortus* and *Teladorsagia circumcincta*

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Current methods for identifying anthelmintic resistant gastro-intestinal parasites of livestock are insensitive and time consuming. We are developing molecular markers of anthelmintic resistance (AR) to permit early detection in the field and allow control strategies to be assessed and optimised. Additionally, markers of AR will be used to identify regions of the genome that confer resistance to help to define the underlying mechanisms.

Haemonchus contortus is a key parasite for the study of drug resistance and is itself an economically significant parasite of small ruminants. Several well-characterised anthelmintic resistant and anthelmintic susceptible laboratory isolates are available and it is possible to undertake genetic crosses between different isolates for classical genetic studies. We recently published a high-quality draft genome assembly of a susceptible isolate and improvements to this resource will be described, as will the generation of a genetic linkage map to study ivermectin resistance in this species.

While *H. contortus* is the most important gastro-intestinal parasite of small ruminants worldwide, *Teladorsagia circumcincta* is the most important gastro-intestinal parasite in the UK flock. We are sequencing the genome of a UK isolate of *T. circumcincta*, which was isolated from the field before any modern anthelmintic classes were in use. Preliminary findings from the draft *T. circumcincta* genome will be described, including comparative analysis with *H. contortus*.

These resources will underlie genome-wide association studies in phenotyped field populations of anthelmintic resistant *H. contortus* and *T. circumcincta* from UK farms, using a combination of restriction associated DNA sequencing (RAD-seq) and whole genome resequencing.

Thursday 20 August 2015

Concurrent Session 9.4

Companion animal

Ref: O240/0444

Current status of canine onchocercosis by *Onchocerca lupi* and first case report in Europe of the infection in a cat

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Onchocerca lupi (Spirurida, Onchocercidae) is a zoonotic nematode infecting the ocular tissues of dogs and humans. Clinical signs of canine onchocercosis include conjunctivitis, exophthalmos, periorbital swelling, photophobia and even protrusion of the nictitating membrane, corneal oedema, ulcerative keratitis, anterior and posterior uveitis. The real distribution of this infection in dogs is not well understood mostly due to the difficulties inherent to its diagnosis, which relies on the retrieval of microfilariae in the sediment of skin snips. In addition, data on the life history of this onchocercid is minimal. However, over the last few years, reports of canine onchocercosis are increasing worldwide, in the United States and in Europe (e.g., Greece and Portugal). In the only large epidemiological study performed in Portugal and Greece, where clinical cases were reported, up to 8.4% of the examined animals scored positive for *O. lupi* microfilariae.

This nematode was also diagnosed in two cats from USA. As no data regarding *O. lupi* infection in cats from Europe is available, the aim of this study was to evaluate its occurrence in 155 stray cats from southern Portugal, where this infection has been previously reported in dogs. A skin sample was obtained from each animal from ear, as a part of the neutering program, to easily identify the cats. Of 155 cats, one (0.65%) was positive for *O. lupi* microfilariae. As previously reported in dogs, the infected cat was lacking apparent clinical signs, suggesting the potential role of asymptomatic animals as carriers and reservoirs of this parasite. The detection of *O. lupi* in dogs and cats from Algarve confirms that this parasite is endemic in southern Portugal and highlights the importance to alert the veterinary community, pet owners, physicians and ophthalmologists on the risk of infection by this filarioid and, consequently, to the need of implementing control measures to protect animals and people.

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Thursday 20 August 2015

Concurrent Session 9.4

Companion animal

Ref: O241/0614

Canine and Feline Strongyloidosis in France: a retrospective review of 23 cases with consequences for methods of diagnosis, treatment, and prevention

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Canine and feline strongyloidosis is caused by the nematode *Strongyloides stercoralis* that infect also humans. This disease is endemic in tropical countries mainly on humans but is probably emerging in Europe in dog and cat as more and more cases are observed.

In Alfort veterinary school, *Strongyloides stercoralis* was not detected for more than 30 years before 2006. Since 2006, we observed 23 clinical cases corresponding to 2 cats and 21 dogs. In the majority of the animals, the diagnosis was done during the first year of life. Three diagnostic tests were used: Baermann, Formalin–ether sedimentation, and direct faecal examination will be compared for the diagnosis and the follow-up of clinical cases.

First intention treatment with ivermectin for dogs with no risk of *MDR1* mutation give good results but trial with moxidectin spot-on (advocate®) in two Australian Shepherd dogs give no parasitological cure. At present, in *MDR1* muted dogs, as fenbendazole give unpredictable results, there is no drug to recommend.

As the majority of these animals were probably infected by milk transmission, prevention in breeding units seems to be important and will be discussed.

Thursday 20 August 2015

Concurrent Session 9.4

Companion animal

Ref: O242/0009

Molecular Detection of canine vector borne diseases in dogs from a village in northern Cambodia

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Canine vector borne pathogens such as *Babesia* spp., *Ehrlichia* spp., *Anaplasma* spp., *Hepatozoon* spp., haemotropic mycoplasmas and *Rickettsia* spp. are significant pathogens of dogs and cats worldwide. Moreover, some of them are zoonotic and therefore of public health significance. The objective of this study was to determine the prevalence of canine vector borne pathogens present in dogs from Cambodia using molecular techniques. Blood samples were collected from 101 dogs living in 36 households from Dong Village, Rovieng district, Preah Vihear province in northern Cambodia. The most common canine vector borne pathogen found infecting dogs in this study was *Babesia vogeli* (41.6%) followed by *Ehrlichia canis* (21.8%), *Mycoplasma haemocanis* (12.9%), *Rickettsia felis* (10.9%) and *Hepatozoon canis* (10.9%) respectively. This is the first molecular detection of canine vector borne pathogens in dogs from Cambodia. Of note, this study supports the role of dogs as natural mammalian reservoirs for *R. felis*, the agent of flea-borne spotted fever in humans.

Thursday 20 August 2015

Concurrent Session 9.4

Companion animal

Ref: O243/0341

Prevalence of vector-borne pathogens in dogs from Haiti

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Vector-borne pathogens are a concern for people and dogs on some Caribbean islands, including Haiti, where survey data for canine vector-borne infections are lacking. To determine the prevalence of a variety of vector-borne pathogens in dogs from Haiti, we conducted a molecular and serologic survey of whole blood collected from 210 owned dogs in 2013, 28 (13.3%) of which were infested with *Rhipicephalus sanguineus* ticks at the time of blood collection. The commercially available SNAP® 4Dx® Plus was utilized on all 210 dogs; 37 (17.6%) had antibodies to *Anaplasma* spp., 69 (32.9%) had antibodies to *Ehrlichia* spp., and 0 (0%) had antibodies to *Borrelia burgdorferi*. Antigen to *Dirofilaria immitis* was detected in 55 dogs (26.2%). Blood samples from 207 dogs were tested by PCR; *Anaplasma platys*, *Babesia canis vogeli*, *Dirofilaria immitis*, *Ehrlichia canis*, *Hepatozoon canis*, and *Wolbachia* spp. DNA were detected in 13 (6.3%), 16 (7.7%), 46 (22.2%), 15 (7.2%), 40 (19.3%), and 45 (21.7%) dogs, respectively. None of the 207 canine samples tested PCR-positive for *A. phagocytophilum*, *B. canis canis*, *B. canis rossi*, *B. gibsoni*, *E. chaffeensis*, *E. ewingii*, or *H. americanum*. Active co-infection with 2 or more vector-borne pathogens was detected in 22 (10.6%) dogs; 1 dog was triple-infected. Evidence of past or current infection with at least one vector-borne pathogen was detected in 142 (67.6%) dogs in this study. The common nature of these pathogens, some of which are zoonotic, suggests that canine and human health in Haiti would benefit from vector control programs to prevent infection.

Thursday 20 August 2015

Concurrent Session 9.4

Companion animal

Ref: O244/0688

Molecular detection of zoonotic rickettsiae and Anaplasma in domestic dogs and their ectoparasites in Bushbuckridge, South Africa

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Members of the order Rickettsiales are small, obligate intracellular bacteria that are vector-borne and can cause mild to fatal diseases in humans worldwide. There is little information on the zoonotic rickettsial pathogens that may be harboured by dogs from rural localities in South Africa. Thus the primary aim of this study was to identify and characterise rickettsial pathogens by screening blood samples and ectoparasites collected from domestic dogs

A total of 141 blood samples, 103 ticks and 43 fleas were collected from a convenience sample of owned, free-roaming dogs in the Mnisi community. DNA was extracted and screened for the presence of haemoparasite infections using the reverse line blot (RLB) hybridization assay and for zoonotic rickettsiae using Rickettsia genus and species-specific qPCR assays. Results from RLB showed 49% of blood samples and 30% of tick pools were positive for the genus-specific probes for Ehrlichia/Anaplasma, 16% of the blood samples for E. canis, 9% for Babesia rossi, while 4% were positive for B. vogeli. Haemoparasites' DNA could not be detected in 36% of blood samples and 30% of tick pools. Haemoparasite species detected from tick pools includes Neoehrlichia spp., E. ruminantium, and B. microti. Rickettsiae were not detected in all blood samples analyzed using a Rickettsia genus-specific qPCR assay. Rickettsial infections were detected in 70% of tick pools consisting of H. elliptica, A. hebraeum, R. sanguineus, R. simus, and Ixodes spp. and in 2 of 2 flea pools. Using the Rickettsia species-specific assays, 30% of tick pools tested positive for the R. africae genotype-specific qPCR assay, while both flea pools were positive with the R. felis qPCR assay. Blast analysis on sequences of the 16S rRNA gene of five selected Ehrlichia/Anaplasma positive samples on the RLB hybridization assay revealed an Anaplasma sp. South Africa dog strain closely related to A. phagocytophilum in four of the five samples and an A. phagocytophilum strain in one sample. Furthermore, Candidatus Rickettsia asemboensis a new Rickettsia felis-like organism and Orientia tsutsugamushi-like sequences were also identified in two additional blood samples from next generation sequencing. The detection of zoonotic rickettsiales from blood samples of domestic dogs and their ectoparasites in a rural community in South Africa highlights the potential risk of human infection that may occur with these pathogens.

Thursday 20 August 2015

Concurrent Session 9.4

Companion animal

Ref: O245/0031

Equine parascaris: A different infection pattern in working donkeys

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Background: Parascaris, caused by *Parascaris equorum*, has a worldwide distribution and is of concern due to clinical diseases and the economic losses it causes. Although *P. equorum* has been reported in donkeys from various regions of the world, its age related epidemiological infection has not been investigated.

Methods: Cross-sectional coprological and retrospective post-mortem investigations were conducted to study the epidemiology of parascaris in working donkeys of Ethiopia. Faecal samples collected from 803 donkeys, and data obtained by routine post-mortem examinations of 112 donkeys which were either euthanased on welfare grounds or died of various clinical ailments were analysed. Data were analysed using a generalised linear model and multivariate binary logistic regression in R statistical package. The significance level for all statistical tests was set at $P < 0.05$.

Results: Infection prevalences of 51.1% and 54.5% were obtained in coprologically and post-mortem examined donkeys, respectively. Similar infection prevalence, mean epg and worm burden was obtained between the different age groups of donkeys with no statistically significant differences ($P > 0.05$). 79% of the 61 infected donkeys were above 4.5 years old, and 30 of these had more than 10 parascaris with the mean intensity of 14 worms.

Conclusion: The high faecal parascaris egg counts and worm burdens irrespective of the age of the donkeys suggests, unlike in horses, age-dependent immunity may not develop in working donkeys or they might have been immuno-compromised. This demonstrates that adult donkeys are a potential source of contamination putting young animals at risk, and hence necessitating their inclusion in any control strategy.

Thursday 20 August 2015

Concurrent Session 9.5

One health

Ref: O246/0532

Controlling *Taenia solium* and soil transmitted helminths in a northern Lao PDR village: Impact of a triple dose albendazole regime

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Taenia solium taeniasis-cysticercosis and soil-transmitted helminths (STHs) are parasitic Neglected Tropical Diseases endemic throughout Southeast Asia. Within Lao PDR, a remote northern hill tribe village had been identified as a hyper endemic focus for *Taenia solium*. To reduce this observed prevalence, a One Health intervention covering both pigs and humans was implemented, which included two Mass Drug Administrations (MDA1 and MDA2) for village residents using a triple dose albendazole 400mg treatment regime. In addition to the effect on *T. solium* levels, the dual impact of this anthelmintic regime on STHs within the community was also monitored. Faecal samples were collected pre and post MDA1 and MDA2 and analysed for the presence of *Taenia* species and the STHs *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm species. Quantitative data was obtained using the McMaster technique and molecular characterisation was conducted on *Taenia* and hookworm species to detect zoonotic species. The level of taeniasis within the sampled population decreased by 79.4% after MDA1, remained steady during the five month inter-treatment interval and decreased again by 100% after MDA2. The prevalence of STHs decreased by 65.5% and 62.8% after MDA1 and MDA2 respectively; however an increase to 62.2% of pre MDA1 levels was detected during the inter-treatment interval. Individually, hookworm prevalence decreased by 83.4% (MDA1) and 84.5% (MDA2), *A. lumbricoides* by 95.6% and 93.5% and *T. trichiura* by 69.2% and 61%. Molecular characterisation identified a *T. solium* tapeworm carrier from 21.6% (13/60) of households in the village. *T. saginata* was identified in 5% (3/60) of households. The zoonotic hookworm *A. ceylanicum* was detected in the resident dog population.

These results suggest that the triple dose albendazole 400mg treatment regime achieved a significant reduction in the level of taeniasis whilst simultaneously reducing the STH burden within the village. The increased STH prevalence detected between MDAs reflects the need for behavioural changes and a sustained chemotherapy programme, which may also need to include the resident dog population.

Thursday 20 August 2015

Concurrent Session 9.5

One health

Ref: O247/0028

The epidemiology and public health importance of *echinococcosis* in pastoralist production system in Karamoja region, Uganda

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Echinococcosis is a zoonotic disease caused by *Echinococcus spp* and is one of the most important helminthic diseases worldwide. In Karamoja region the prevalence of *Echinococcus granulosus* in the dog population was recorded to be 66.3% (F.O. Inangolet et al 2007). Cystic Echinococcosis (CE) is highly endemic among the nomadic pastoralists of Karamoja region in Uganda. A recent hospital records among the Karimojong community has shown an average of 20 surgical cases of CE in each of the hospitals in Karamoja. This condition is rare amongst the agricultural based communities of Uganda. These findings indicate that CE is a Public Health and economic problem in the pastoralist production system of Karamoja in Uganda.

In an attempt to establish the epidemiology of CE, a study was conducted between March 2011 and December 2012 in a 19-month period in six districts of Karamoja region of Uganda. During the study period all the slaughter slabs and slaughter houses were visited. A total of 5105 goats, 4675 sheep, 1006 cattle, 612 donkeys and 112 Camels which had been slaughtered had their internal organs (liver, lungs, spleens and hearts) examined for the presence cysts responsible for CE. Logistic regression models were used to explore the association between the prevalence of disease in the livestock and the different risk factors and the number of CE human surgical cases in the different hospitals in the region

It was found that 0.9% of camels, 0.4% of donkeys, 28.6% of sheep, 22.5% of goats and 18.7% of cattle were infected with the disease. The prevalence of CE was higher in the wetter areas compared to the drier areas. The number of cysts per animal also increased with age, with the oldest animals having the largest number of cysts.

Geographical locations, environmental conditions, age, sex, grazing patterns, livestock stocking intensity, civil strife and dog populations were observed to have effects on prevalence. We observed also here that the pastoralist production system had a significant impact on sheep and goat prevalence and those pastoralists were at a higher risk of being infected. This paper discusses strategies that could be used to control CE given the enormous challenges associated with nomadic pastoralism and insecurity in Karamoja region in Uganda

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Thursday 20 August 2015

Concurrent Session 9.5

One health

Ref: O248/0623

The geographical distribution of Ixodidae and their potential vector capacity for the CCHFV in sheep and goats in Greece

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The aim of this study was to determine the abundance of hard ticks (Ixodidae) in small ruminants and their potential to circulate CCHF virus in Greece. A field study was carried out during 3 successive tick seasons in 26 regions of mainland and 5 islands. A total of 2108 ticks (1199 females, 908 males & 1 nymph) were collected from small ruminants. Three species of *Rhipicephalus*, 2 of *Ixodes*, 5 of *Hyalomma*, 3 of *Haemaphysalis* and 1 of *Dermacentor* were detected. The species with the widest distribution were *Rhipicephalus sanguineus* (61.2%), *R. bursa* (25.9%), *Dermacentor marginatus* (4.1%) and *R. turanicus* (3.5%) while the least frequently collected species were *Ixodes ricinus*, *I. gibosus*, *Haemaphysalis parva*, *H. sulcata*, *H. punctata*, *Hyalomma marginatum*, *H. excavatum*, *H. dromedarii*, *H. rufipes*, and *H. impeltatum* representing together less than 5.3% of the collections. *R. sanguineus* was the predominant species, while *D. marginatus* and *I. gibosus* were restricted in North Eastern Aegean sea islands. All ticks were also analyzed single or in pools using RT-nested and q PCR for CCHFV and 20 of them, belonging to the species *R. sanguineus* and *R. bursa*, were found positive. Specifically, one of them with the lineage Europe 2 and the rest with the Europe 1 strain. Moreover, a geographical information system (GIS) was constructed for the study area and distribution maps of host and tick species were created. The availability of geo-referenced data and maps on tick distribution in the Greek area are fundamental for dissemination activities for veterinarians and farmers and to develop future ecological models.

Thursday 20 August 2015

Concurrent Session 9.5

One health

Ref: O249/0646

Spotted fever group rickettsiae in hard ticks (Acari: Ixodidae), Romania

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Tick-borne rickettsioses are caused by obligate intracellular Gram-negative bacteria belonging to the spotted fever group (SFG) of the genus *Rickettsia*, order Rickettsiales. SFG rickettsiae are associated with ticks which act as vectors and/or reservoirs. Currently, they are recognized as important emerging agents of human tick-borne diseases worldwide. The knowledge of bacteria transmitted by ticks in a given area is useful for assessing the risk of infection in humans and animals. The very diverse Romanian tick fauna and abundance of tick populations represent potential risks for animal and human public health. However, agents within the rickettsiales group have been poorly investigated in Romania and the epidemiology of rickettsial diseases it is not well known. Therefore, to explore increased risks for human *Rickettsia* spp. infection in Romania, we investigated for the presence of spotted fever group rickettsiae in ticks. A total of 349 ticks encompassing 3 species (152 *Ixodes ricinus*, 62 *Dermacentor marginatus*, and 135 *Hyalomma marginatum marginatum*) were collected during 2010-2013 from different areas of Romania. Of them, 323 were collected from naturally infested cattle, sheep, goats, or horses and the remainder was questing ticks. The presence of *Rickettsia* in ticks was evaluated using conventional PCR amplification followed by sequencing. The sequence analyses revealed four pathogenic rickettsiae of the spotted fever group of zoonotic concern, as follows: *Rickettsia monacensis* (15.1%) and *Rickettsia helvetica* (5.3%) in *I. ricinus* and *Rickettsia slovaca* (11.3%) and *Rickettsia raoultii* (11.3%) in *D. marginatus* ticks. Moreover, the tick species identified to harbor SFG rickettsiae have been reported to feed on people in Romania. Therefore, the findings should increase the awareness, particular of clinicians, for the risks of transmission of these pathogens to humans. In conclusion, the findings are of major concern to public health as all rickettsiae identified are causing emerging tick-borne diseases and expand the known distribution of SFG rickettsiae in Europe.

Thursday 20 August 2015

Concurrent Session 9.5

One health

Ref: O250/0096

Molecular identification of blood parasites in camels (*Camelus dromedarius*) of Iran

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The aim of this study was to investigate the haemoparasite fauna in Iranian one-humped camels (*Camelus dromedarius*) by molecular techniques.

In this pilot survey a total of 200 apparently healthy camels of both sexes and different ages were sampled randomly. Animals were kept by local farmers in central and south-eastern of Iran. Blood samples were taken from the jugular vein and drops of blood were spotted on Whatman FTA Elute® cards. Genomic DNA was purified according to the filter papers manufacturers' instructions. Polymerase chain reaction was applied for the detection of filaroid worms, *Trypanosoma* spp. and piroplasms. Positive samples were further analyzed by sequencing.

Results showed that 16 animals were infected with filaroid helminths, one with *Theileria annulata* and one with *Trypanosoma evansi*. To the best of our knowledge we document the first sequence-confirmed findings of filaroid helminths in dromedaries, and also tropical bovine theileriosis in one-humped camels. However, further examinations are needed to investigate the parasite fauna of camels in Iran.

Thursday 20 August 2015

Concurrent Session 9.5

One health

Ref: O251/0188

***Tunga penetrans* infections are highly prevalent among animal hosts in human tungiasis-endemic villages of Uganda**

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Tunga penetrans infections cause severe debility and perpetuate poverty in endemic poor communities. Animal infections amplify the prevalence and severity of human disease. Tungiasis affects a variety of mammals which differ among endemic areas and for Uganda *T. penetrans* animal hosts' characteristics are unknown. A cross sectional study was conducted during a dry season in animal rearing households in 10 endemic villages in Bugiri District, Busoga sub-region, Uganda. Pigs, dogs, goats and a cat were detected as animal hosts for *T. penetrans*. Village prevalences of households with tungiasis ranged from 0% to 71.4% (median 22.2%) for animals and 5% to 71.4% (median 27.8%) for humans. Prevalences of human cases in ten villages ranged from 1.3% to 37.3% (median 7%). Pig infections occurred in nine villages with the highest prevalence (median 16.2%, range 0-64.1%) and parasite burden which was higher than that of all other species combined ($p < 0.001$). Dog infections occurred in five of the 10 villages with a median prevalence of 2% (range 0-26.9%). Only two goat kids from two villages and a single cat were affected. There were strong correlations of prevalences of animal and human tungiasis at village ($\rho = 0.89$, $p = 0.0005$) and households ($\rho = 0.4$, $p < 0.001$) levels. Animal tungiasis increased the odds of occurrence of human cases in households six fold (OR=6.1, CI 3.3-11.4, $p < 0.001$). Pigs were identified as the most important animal hosts of *T. penetrans* in Busoga, Uganda. Therefore ectoparasites control in animals is a pertinent aspect of an effective tungiasis control plan under a One Health framework.

Posters with 2 minute Oral Presentation

Oral poster session 1: We can work it out

Tuesday 18 August

10:00-10:30

Ref: 0268

Poster Number: P2C319

Effect of sainfoin (*Onobrychis viciifolia*) pellets on a multi-resistant strain of *Haemonchus contortus* in lambs

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The worldwide spread of resistance to anthelmintics (AH) in gastro-intestinal nematodes (GINs) imposes to explore alternative solutions. Amongst those, the possible use of tannin-containing nutraceuticals is widely explored. Our objectives were i) to test the AH efficacy of sainfoin pellets against a multiresistant strain of *Haemonchus contortus* in experimentally infected lambs; ii) to examine the hypothesis of any possible synergy between oral ivermectin and CT-rich forage. Twenty-four lambs were inoculated (Day 0) with *H. contortus* infective larvae from the multiresistant White River strain. On D21PI, the lambs were stratified based on Fecal Egg Counts (FEC) and Packed Cell Volume (PCV) and then assigned to two dietary treatments (sainfoin vs lucerne control pellets). Blood samples were taken weekly to measure PCVs. Individual fecal samples were collected twice weekly to measure FEC. On D39, half of the animals per group received 0,25ml/kg of an oral ivermectin treatment. On D46PI, worm counts were performed. The consumption of sainfoin pellets was associated with improved PCV values ($P<0.05$) and reduced FECs ($P<0.05$). For the experimental feeding period, the FECs were overall reduced by 50% for the sainfoin group. The diet did not induce any significant effect on the worm number. The results based on the pharmacological measurements of blood IVM in the 2 groups suggest an overall decrease of IVM efficiency when lambs were fed sainfoin. Our data confirm that tannin-containing nutraceuticals disturb the biology of multiresistant nematodes, thus representing an option for their sustainable control. The interactions between nutraceuticals and synthetic drugs need further investigation.

Oral poster session 1: We can work it out

Tuesday 18 August
10:00-10:30

Ref: 0300

Poster Number: P2C320

Elucidation of *Leucaena leucocephala* anthelmintic-like phytochemicals, and the ultrastructural damage generated over *Cooperia* spp., eggs

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Leucaena leucocephala (LL) is a tropical forage legume suggested as an alternative to control gastrointestinal parasitism. Tannins have been proposed as main effectors of its anthelmintic (AH) activity, though, LL could also have other AH-like phytochemicals. This study aimed to: 1) elucidate bioactive compounds present in LL, with AH activity against *Cooperia* spp., and 2) assess ultrastructural damage engendered over *Cooperia* spp., eggs. Anthelmintic activity of LL was traced from an aqueous extract using the egg hatching inhibition assay (EHIA; 9.6 mg/ml). Phytochemicals were isolated through silica gel columns, and identified throughout a high performance liquid chromatography using standards for comparison. Final fraction was evaluated with EHIA at: 1.1; 0.50, 0.250, 0.125, 0.06 mg/ml. Scanning and transmission electron microscopy allowed the observation of ultrastructural changes of eggshells. Kruskal-Wallis test was used to compare activity among fractions. Hatching inhibition was calculated with the formula: $100 \times (1 - H_T/H_C)$. Lethal concentrations were calculated using a Probit analysis. Bio-guided isolation procedures lead to the recognition of an active fraction mainly composed of Quercetin (82.21%) and Caffeic acid (13.42) which inhibited $90.49 \pm 2.8\%$ of *Cooperia* spp., egg hatching ($P < 0.05$). Further purification provided two less active mixtures, decreasing the ovicidal activity in over 50%. Thickening and fractures were observed in *Cooperia* spp., eggshells. Quercetin and Caffeic acid were identified as main constituents of the active fraction; however, changes in bioactivity after purification suggest synergistic interactions between them.

Oral poster session 1: We can work it out

Tuesday 18 August

10:00-10:30

Ref: 0398

Poster Number: P2C322

Efficacy of *Artemisia annua* hydroalcoholic extract against poultry beds contaminated with *Eimeria* sp.

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Artemisinin, a sesquiterpene lactone present in *Artemisia* sp. plants, has received much attention in the treatment of some Protozoa organisms and it is considered a candidate to reduce others protozoa infections such as *Eimeria* sp. in chickens. The aim of this study was to determine the efficacy of hydroalcoholic extract of *Artemisia annua* against oocysts of *Eimeria* sp. in contaminated poultry beds. The extract was produced after 7 days of storage at 4°C that was then lyophilized to perform (1) the phytochemical screening; (2) the artemisinin measurement by HPLC; (3) the total phenolic; (4) DPPH antioxidant testing; and (5) the *Artemia salina* Leach toxicity test. To test the anti-coccidian activity, the birds area composed of wood shaving with 2.18m² were naturally contaminated with approximately 5000 oocysts. Four treatments were applied in triplicates of herbal preparations: G1: 12mg/ml; G2: 9mg/ml; G3: 6mg/ml; and C: dist. water. The herbal product was applied (800 ml) over the area and triplicate samples of 10 cm² were collected in each box at 6 time points (0, 3, 6, 24, 48, and 72 h after application). The bed content was washed passing the material through sieves (sizes 250 to 20µm). The content was centrifuged at 2000 rpm for 5 min and the supernatant was discarded. The volume was completed with saturated saline solution and after 15 min was examined. The phytochemical analysis, revealed many compounds with antiparasitic properties, such as flavonoids and tannins. The measured amount of total phenols was 203 mEq/g (±0.569). Artemisinin had a concentration of 59.409 mg/dl (±1.47). The *A. annua* extract had a low toxicity (LC₅₀ 3,827 µg/ml) against *Artemia salina*. The extract at 12.0; 9.0; and 6.0 mg.ml⁻¹ had an efficacy of 42.52; 26.8; 17.1% respectively, demonstrating a clear dose-dependent effect. The results of the biochemical together with the *in vitro* test showed that the extract is a good candidate for controlling *Eimeria* sp. in the environment. Further studies should be conducted to maximize the effectiveness of this and other extracts of *A. annua*, against *Eimeria* sp. oocysts. The toxicity test certifies that the product is safe for the environmental.

Oral poster session 1: We can work it out

Tuesday 18 August
10:00-10:30

Ref: 0599

Poster Number: P2C324

In vivo and in vitro effects of Propolis extracts on acute and latent *T. gondii* viability

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Propolis is a honey bee product has a multifunctional substances used by bees in the construction and maintenance of their hives, being used as human dietary supplement, and gained popularity in alternative medicine due to its anti-microbial activity without draw back or side effects. The treatment of recurrent **acute** and **chronic** toxoplasmosis is a significant problem of clinical medicine due to dormant in-cyst bradyzoites stage. The present study **aimed** to qualify the antiprotozoal activities of Propolis extracts in vivo and in vitro trials against acute and latent stages of *Toxoplasma. gondii*. A total of two hundred and eighty Swiss mice were used as a long as the term of the study. For *in vitro* trials, $10^{(3)}$ of the peritoneal harvesting RH tachyzoites were exposed at 4°C to the original and serial dilution of Propolis extract as; 100%, 50%, 25%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% and 1%. While *in vivo* trials were conducts after and according to feed back of efficient significant dilutions reference to in vitro trials. Wherever, the infected acute and chronic mice groups were inoculated by $3 \times 10^{(3)}$ of either RH or ME49 strains respectively, followed by oral or intra-peritoneal treatment by serial dilution of Propolis extract at; 100%, 50%, 25%, and 1%. Mice was inoculated by 1.5mg Propolis extract for consequence 3 days course beginning at (60HPI) or 7days course beginning at (45HPI) corresponding to the acute or chronic groups. **The results** validate in vitro diluted propolis extract at 5, 6, 7 and 8% were effective to enhance preserving values, Where 7% symbolize maximum optimal reactive value, successfully increase the percent of persisting viable tachyzoites longer time up to (174.5 HPE) and (240 HPE), which exceeding over the normal control saline (100 HPE) and (140 HPE) at the same chilling temperature at 4°C, and corresponding to LD50 & LD100 of in vitro viability. The higher concentrations of propolis extract the higher destructive and degenerative power agenist in vitro viable tachyzoites, the faster time was recorded with 100, 50, 25 and 10%, through maximum values with 100% at less than 1.8 HPE, estimated reference to the normal control saline (100 HPE) at chilling temperature (4°C), corresponding to LD50 and at 4 HPE corresponding to LD100. Concerning in vivo trials; the average percent of dead and survive acute infected mice groups showed significance higher survived and lower dead with intra-peritoneal treated groups than the oral treated ons, with The most optimal reactive value of propolis dilution was 50% in both treated groups, with maximum survived mice percent up to 60 and 15 corresponding to intra-peritoneal and oral treatment. the lowest APL was corresponding to intra-peritoneal treatment 14.2 and 23.5 APL/10mg/ in both chronic and acute groups respectively and corresponding to brain and visceral tissue. APL was recording lowering values in oral-chronic groups than in oral-acute ones. Also, APL was recording lowering values in intra-peritoneal-chronic groups than in intra-peritoneal acute ones. The 50% dilution of propolis extract recording optimal reactive value higher than 100% dilution, creating unlogic curve. We are **concluded** that the significance lowering of APL with all varied propolis dilution corresponding to in vitro and in vivo trials of all acute and chronic treated groups even with varieties of dose, route, long or short treatment course, indicting significance use of higher concentration of propolis in treatment acute or latent human toxoplasmosis, besides the possible use of propolis lower concentrations for in vitro cryo-preserving tachyzoites.

Oral poster session 1: We can work it out

Tuesday 18 August
10:00-10:30

Ref: 0624

Poster Number: P2C325

Efficacy of *parthenium hysterophorous* as a therapeutic source against *Haemonchus contortus*

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Rapid development of anthelmintic resistance against synthetic drugs and toxicity of these drugs has triggered the identification of new anthelmintic drugs. In this regard, traditionally used ethnobotanicals with anthelmintic properties being considered among the novel approaches particularly in developing countries. The goal of this study was to evaluate the effectiveness of *Parthenium hysterophorous* for controlling gastrointestinal parasites in young female goats. Anthelmintic potential of *P. hysterophorous* against *Haemonchus contortus* *in vitro* was investigated in different solvents (methanol, ethanol, hexane) at different concentrations (100, 50, 25, 12.5, 6, 3, and 1.5mg/ml) using adult worm motility inhibition assay. Results demonstrated that crude methanolic extract (CME) exhibited highest wormicidal activity (53%) against *H. contortus* as compared to ethanol (46%) and hexane extracts (30%), respectively. Moreover, at the lower concentration of CME (25mg/ml concentration) all the worms died at 6 hr of post treatment with mean mortality 3.67 ± 0.33 as compared to positive control Albendazole (0.33 ± 0.16). Thus the CME of *P.hysterophorous* exhibited significant ($P < 0.05$) anthelmintic activity in dose-dependent ($P < 0.05$) manner at the lower concentration. Additionally through staining an attempt has been made to observe cuticular changes in different solvents treated *H. contortus* by comparing with positive and negative control. Moreover, *in vivo* the impact of *P.hysterophorous* in dairy goats (beetle) which naturally parasitized with abomasal parasites (*H.contortus* epg ≥ 200) was evaluated by measuring fecal egg count reduction test. For this purpose Group one (G-I), two and three received two oral doses viz 3, 6, and 9g/kg of crude plant powder (at 0 and 45 day) for three months respectively. The group four considered as positive control, which was given single dose of albendazole on the day zero, while group five was negative control and received no treatment. Throughout the trial, goats G-III which were exposed to dose (9g/kg) of crude powder of plant showed a higher efficacy ($P < 0.05$) against *Haemonchus contortus* (73%) followed by *Oesophagostomum columbianum* (72%) *Trichostrongylus colubriformis* (68%), *Trichostrongylus axi* (66%) as compared to positive, control and other treated groups. Overall G-III animals showed a significant ($P < 0.05$) improved hematocrit level with less anemic score of FAMACHA. Study depicts that *P.hysterophorous* plant could act as a good alternative source to cope abomasal parasitic infections in small ruminants.

Oral poster session 1: We can work it out

Tuesday 18 August
10:00-10:30

Ref: 0640

Poster Number: P2C327

Sesquiterpene lactone-containing extracts from two chicory cultivars show different anthelmintic activity *in vitro* against *Ostertagia ostertagi*

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Mechanisms behind reported *in vivo* anthelmintic effects of chicory (*Cichorium intybus*) in ruminants are poorly understood but it is likely that plant compounds, like sesquiterpene lactones (SL), play a role. **Objectives:** The aim was to test the inhibitory activity of SL-containing extracts from two chicory cultivars on free-living and parasitic stages of *Ostertagia ostertagi*. **Methods:** Leaves from chicory cv. Spadona and cv. Puna II were freeze-dried and SL extracted with methanol/water. Resulting extracts were incubated with cellulase enzyme and SL were purified from other plant compounds by normal solid-phase extraction. Purified extracts were dissolved in DMSO. *O. ostertagi* eggs from a mono-infected calf were hatched and first-stage larvae (L1) were used in a larval feeding inhibition assay (LFIA), while L3 cultured from faeces were used in a larval exsheathment inhibition assay (LEIA). *O. ostertagi* adult worms recovered post-mortem were used for motility inhibition assays (AMIA) and worm motility was evaluated after 6, 24 and 48 h of incubation (37°C). In all *in vitro* assays, decreasing concentrations of chicory extracts in PBS (1% DMSO) were tested in triplicates with 1% DMSO in PBS as negative controls. Chemical profiles of the extracts were analysed by liquid chromatography (LC). **Results:** In the LFIA, Spadona-extract inhibited larval feeding at significantly lower concentrations than Puna II-extract ($EC_{50}=31.5$ [CI=25.9-38.3] μ g Spadona-extract/mL vs. $EC_{50}=121.1$ [CI=95.2-153.8] μ g Puna II-extract/mL; $p<0.0001$). In the LEIA, extracts from neither of the two cultivars interfered with the exsheathment of L3 at any of the tested concentrations. In the AMIA, Spadona-extract showed a significantly higher potency and exerted faster worm paralysis than Puna II-extract at all time points when tested at equal concentrations ($p<0.0001$). Preliminary LC analyses revealed different SL profiles of the extracts and further chemical characterization is undergoing. **Discussion:** This is the first study confirming direct effects of SL against *O. ostertagi*. However, the observed anthelmintic effects of SL-containing extracts from chicory seem to be stage-specific as L1 and adult *O. ostertagi* but not L3 were affected. Different anthelmintic potency of SL from different chicory cultivars may help the identification of the most active(s) molecule(s) and the selection of cultivars with higher antiparasitic potential.

Oral poster session 1: We can work it out

Tuesday 18 August
10:00-10:30

Ref: 0642

Poster Number: P2C328

Feeding chicory (*Cichorium intybus*) selectively reduces *Ostertagia ostertagi* infection levels in cattle

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Objectives: Studies were conducted to test the potential use of chicory against gastrointestinal nematode infections in cattle. **Methods:** In study 1, fifteen 2-4 months-old dairy calves were allocated into a chicory (CHI, n=9) or control (CTL, n=6) group. CHI and CTL were stabled and fed with chicory silage or hay, resp., *ad lib* for 56 days. Protein/energy intakes were equalized between groups throughout the study. After 14 days on the diet all calves were infected with 10,000 *Ostertagia ostertagi* and 66,000 *Cooperia oncophora* third-stage (L3) larvae. In study 2, twenty 4-6 months-old dairy calves grazed a second-year, pure chicory sward (CHI, n=10) or a ryegrass/white clover pasture (CTL, n=10) for 43 days. After 7 days on the diet all calves were infected with 20,000 *O. ostertagi* L3. In both studies, individual live weights were recorded and faecal egg counts were calculated as number of eggs per g of dried feces (FECDM). At day 56 (study 1) calves were killed for worm recovery. Live weights and log-transformed FECDM were analysed by ANOVA using repeated measurements. Log-transformed worm counts were analysed by t-test. **Results:** In study 1 daily live weight gains were 500 and 329 g/day in CHI and CTL animals, resp. (p=0.02). Mean FECDM were not significantly different between groups (p=0.19). *O. ostertagi* geo mean worm counts (SD) were 1599 (886) and 3752 (633) in CHI and CTL groups, resp. (p<0.001). *C. oncophora* geo mean worm counts were not statistically different between groups (p=0.10). In study 2 daily live weight gains were 366 and 748 g/day in CHI and CTL calves, resp. (p<0.001). Mean FECDM of CHI and CTL at day 20 post-infection were 1589 and 1474, resp. (p>0.05). From this point, egg excretion in CHI calves was significantly reduced and by day 36 post-infection FECDM was decreased by 48-65% compared to CTL (P<0.05). **Discussion:** Feeding on a chicory diet demonstrated a marked anthelmintic effect against *O. ostertagi* in both trials, whereas *C. oncophora* in study 1 was unaffected. Adult worm counts and egg excretion of *O. ostertagi* were significantly reduced in chicory-fed calves, suggesting that the survival of adult worms is reduced and/or worm expulsion is enhanced. The lower weight gains of CHI in study 2 probably reflect lower energy consumption and suggest that duration of grazing of pure chicory should be limited to selectively target established *O. ostertagi* adult populations.

Oral poster session 1: We can work it out

Tuesday 18 August
10:00-10:30

Ref: 0703

Poster Number: P2C329

Anthelmintic activity and metabolomic profiling of extracts of *Daniellia oliveri* and *Psorospermum febrifugum*

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Helminth parasites are a serious threat to animals worldwide, causing large economic losses in livestock. In the absence of effective vaccines, control of helminth infections continues to rely on the use of anthelmintic drugs. Regrettably, the increased use of currently available anthelmintics has led to the development of resistance in numerous parasite species of veterinary importance. As a result, control of helminth infections has become increasingly difficult with the available anthelmintics. Moreover, effective control of many filarial nematodes including *Brugia malayi*, *Onchocerca ochengi* and *Dirofilaria immitis* is hindered by the lack of an adulticide, leaving the adult worms to continue to reproduce. There is therefore the urgent need for the discovery and development of new anthelmintic drugs. We have employed quantitative biochemical (MTT/formazan) and motility assays (including the WormLab software) to screen extracts from the plants; *Daniellia oliveri* and *Psorospermum febrifugum*, for nematocidal activities against several nematode species. Extracts of these plants showed broad-spectrum activity against the soil-transmitted helminth, *Oesophagostomum dentatum*, and the adult filarid parasites; *Brugia malayi* and *Onchocerca ochengi*. Interestingly, the majority of the active extracts showed little or no toxicity on N27 rat dopaminergic cells. Metabolomic profiling using GC-MS revealed identifiable peaks for compounds which can be isolated and further tested for enhanced activity. Our results indicate that *Daniellia oliveri* and *Psorospermum febrifugum* have potential as sources of lead compounds for the development of novel anthelmintics. Currently, we are assaying the activity of promising extracts on the motility of the non-parasitic nematode, *Caenorhabditis elegans*, using the WormLab system. The results obtained will be combined with mutagenesis studies in *C. elegans* with the hope that this platform can help reveal the mode of action of our anthelmintic extracts/compounds.

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Oral poster session 2: Here, There and Everywhere (1)

Tuesday 18 August
12:00-13:00

Ref: 0055

Poster Number: P1A184

Effectiveness of selected anthelmintics on nematode parasites of sheep in KwaZulu-Natal, South Africa

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This study determined the effectiveness of selected anthelmintics (Ivermectin 1% (IVM), Closantel 7.5% (CST) and a combination Abamectin 0.08% and Praziquantel 1.5% (CAP) currently being used in SA. Gender, initial egg per gram (EPG) and initial live weight aided in blocking animals into groups, each group was randomly treated with one of four drug treatments comprising: the untreated control (D0), IVM, CST, and CAP. Animals grazed throughout on infested pasture. Rectal faeces were collected on days 0, 7, 14 and 21 for determining EPG. Faeces were mixed per group and incubated to identify and determine the abundance of larval forms of *Haemonchus*, *Trichostrongylus*, *Strongyloides*, *Nematodirus*, and *Cooperia* species. Differences between treatments changed over time. On day7 IVM, CST, and CAP depressed EPG to 0.66, 0.37 and 0.80 of their respective starting values whilst EPG increased 1.39 times for D0. Thereafter, EPG increased consistently for all drugs; CST recorded the lowest values. *Haemonchus*, *Trichostrongylus*, *Strongyloides*, *Nematodirus* and *Coperia* species contributed respectively 60%, 30%, 6%, 3% and 1% of the larval forms on day0; and 78%, 8%, 11%, 1%, 2% on day21. Larval forms increased for *Haemonchus* species but decreased for *Trichostrongylus* species over time. Closantel was the most effective dewormer. *Haemonchus* Spp. were least affected whilst *Trichostrongylus* Spp. were the most affect by all drugs.

Oral poster session 2: Here, There and Everywhere (1)

Tuesday 18 August
12:00-13:00

Ref: 0075

Poster Number: P2E392

Prevalence and therapeutic trials against anaplasmosis in sheep and goats in Lahore, Pakistan

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Anaplasmosis is deadly infectious disease of small ruminants throughout the world which is characterized by rise in body temperature, loss of appetite, weight loss, anemia, pale mucous membrane, jaundice and recumbency at final stage of the disease. Looking the importance and utilization of the small ruminants in our country and the substantial losses because of the Anaplasmosis the prevalence and chemotherapy of anaplasmosis was studied in clinically effected small ruminants of Lahore. A total of 300 blood samples from small ruminants including 150 sheep and 150 goats were examined microscopically. Hematological analyses of the entire blood sample were carried out in quality enhancement cell of the University of veterinary and animal science Lahore. For therapeutic trials a total of 24 small ruminants (12 sheep; 12 goats) positive for anaplasmosis were divided into three groups A, B and C. The animals of group A was treated with oxytetracycline, (Oxy-fen, Selmore, Pakistan) @ 20 mg/kg BW I/M, group B was treated with imidocarb [dipropionate \(Imizol, ICI, Pakistan\) @ 3 mg/kg BW I/M where](#) as the animals of group C was treated with diminazene aceturate (Fatrybanil, Fatro, Pakistan) @ 3.5-7mg/ kg BW I/M. Data on prevalence of anaplasmosis in clinical affective cases was estimated by Pearson's chi-square test for significance where as data on hematology was analyzed by Student's *t*-test while data on therapeutic trail was analyzed by one way ANOVA using SPSS.

Out of 300 animals 129 (43%) were found positive for anaplasmosis in small ruminants. The prevalence was more in sheep than goat which was 55.33% in sheep and 30.67% in goats. On blood analysis Hemoglobin and white blood cells (WBC) values were increased significantly while packed cell volume (PCV) and red blood cells (RBC) values were decreased non-significantly. Overall efficacy of oxytetracycline, imidocarb dipropionat and diaminazin aceturate was 100, 87.5 and 62.5% respectively in small ruminants. The most effective drug is oxytetracycline which showed 100% efficacy against anaplasmosis.

Key words: Prevalence, Anaplasmosis, Oxytetracycline, Sheep, Goats.

Oral poster session 2: Here, There and Everywhere (1)

Tuesday 18 August
12:00-13:00

Ref: 0147

Poster Number: P1A185

Practices in controlling helminth infections of small ruminants by veterinarians in clinical practice in Greece

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Questionnaires from 46 veterinarians active in farm animal practice in Greece (19% of veterinarians active in that branch of vet medicine) were processed. Return rate of completed questionnaires was 35%; mean time to returning them was 13.6 days.

Mean numbers of administrations of anthelmintic drugs annually were 2.6 to sheep and 2.5 to goats. Frequent administration of anthelmintic drugs was reported in autumn (>95% of responders) or spring (>79%) ($P < 0.001$ between seasons), as well as during last part of pregnancy (>93%) or around mating period (including first part of pregnancy) (>62%) ($P < 0.03$ between reproductive periods). No significant differences were reported between sheep or goats ($P > 0.55$).

Benzimidazoles or pro-benzimidazoles were the anthelmintic drug family more commonly administered. Macrocyclic lactones, levamisole and various other drugs were also reported to be administered. Of responders, 91% indicated administration of benzimidazoles or pro-benzimidazoles and 56.5% indicated administration of macrocyclic lactones ($P < 0.001$ between drugs, $P > 0.11$ for differences between sheep or goats). Administration of formulations with two anthelmintics was reported by 44% of responders. Responders reported most frequent use of 'bolus' or 'oral drench' (>62% of responders), with smaller use of 'injectable solution' (42%) ($P > 0.065$ between forms, $P > 0.29$ for differences between sheep or goats). There was an association between use of 'injectable solution' and administration of macrocyclic lactones ($P < 0.001$).

The results provide for the first time information regarding use of veterinary drugs in small ruminant veterinary practice in Greece and they are useful for understanding administration of anthelmintics to sheep or goats in clinical veterinary practice in Greece.

Oral poster session 2: Here, There and Everywhere (1)

Tuesday 18 August
12:00-13:00

Ref: 0178

Poster Number: P2I352

IgA and IgG response of beef cattle against gastrointestinal nematodes in warm humid tropic of Mexico

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The main goal of the present study was determining the IgA and IgG levels against gastrointestinal nematodes in infected grazing beef cattle as a possible method of diagnosis. Nine infected young cattle of each one of four breeds (Guzerat, Brangus, Charolais and Brown Swiss) were sampled to determine the number of fecal eggs per gram (EPG), peripheral Ig levels (A and G), as well as the hematological parameters, i.e. percentage of packed cell volume (PCV), serum protein and leukocytes, for five months. The experiment was divided into two stages: the first before anthelmintic treatment, and the second after treatment. The ELISA assay was performed using crude worm antigen of *Mecistocirrus digitatus* and *Cooperia punctata*, respectively. One statistical fixed model was used to analyze data. The number of EPG does not show statistical differences among the breeds: Brangus (122±115), Charolais (391±507), Guzerat (294±326) and Brown Swiss (413±395). Differences in hematologic indicators were observed among breeds. The Guzerat calves showed the highest PCV (42.6 ± 4.7%). The lowest number of leukocytes was observed in Charolais breed (9.2 ± 2.4). Difference between Guzerat and Charolais were only detected in eosinophils, neutrophils and lymphocytes. Major level of IgA (OD) was observed against *M. digitatus* (0.41) compare with the response against *C. punctata* (0.22 OD) in serum samples. This same behavior was observed in saliva (0.75 vs 0.52, respectively). In serum and saliva the lowest OD value recorded corresponded to Guzerat calves (0.11±0.02 and 0.39 ± 0.06, respectively), while Charolais and Brown Swiss had the highest OD values in serum and the highest value in Brangus (1.00±0.10) in saliva, had no relationship of IgA between serum and saliva.

Oral poster session 2: Here, There and Everywhere (1)

Tuesday 18 August
12:00-13:00

Ref: 0192

Poster Number: P1A187

Anthelmintic resistance in gastrointestinal nematodes of goats in Slovakia detected by in-vitro and in vivo methods

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Resistance to anthelmintic compounds in gastrointestinal parasites of goats is presently cosmopolitan, and single- and multiple-drug resistant species are common throughout the world. The extent and significance of anthelmintic resistance in goat farms in Slovakia have been previously studied in survey conducted two decades ago and identified multiply resistance strains on imported goats. The main aim of this survey was thus to detect the occurrence of benzimidazole (BZ) resistance on 11 goat farms in Slovakia by using in vivo and vitro methods. A total of 11 goat farms were visited during the period from September to November 2014. Only 9 farms were examined for anthelmintic resistance using in vivo FECR test and in vitro egg hatch test and larval development test; the remaining 2 farms were not included because of low strongyle eggs per gram. The goats were treated with albendazole at 5 mg/kg body weight. If sufficient number of animals were available, another group were treated with double dose of albendazole. There was agreement in the declaration of BZ resistance between the FECR test and both in vitro tests. BZ-resistant gastrointestinal nematodes were found on all farms. Three farms had low levels of resistance (5%-10%), and 6 farms had high levels (10%-50%) of resistant nematodes. The status of BZ resistance on Slovak goat farms changed, assuming a high persistent prevalence of BZ resistance parasites during the intervening, untested period.

The study was supported by Grant Agency VEGA, Grant No. 2/0151/13 of the Scientific Agency of the Slovak Academy of Sciences and Slovak Research and Development Agency Project No. 0539-10.

Oral poster session 2: Here, There and Everywhere (1)

Tuesday 18 August
12:00-13:00

Ref: 0312

Poster Number: P1A191

Nematode anthelmintic resistance on a government sheep breeding farm

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Worm Egg Count Reduction Test was conducted to evaluate the current status of nematode anthelmintic resistance towards few commonly used anthelmintics in Malaysia namely albendazole, fenbendazole, levamisole and ivermectin at a government breeding farm located in Terengganu, Peninsular Malaysia. Twenty five (25) female Barbados Blackbelly sheep was divided to four treatment groups and one control group, where the sheep were treated respectively by groups following the manufacturers recommended dosage based on the live bodyweight. Worm egg count and larval culture were conducted on two occasions, at pre-treatment and on day 13 after treatment. Worm egg count reduction percentages showed that albendazole, fenbendazole and ivermectin did not reduce the worm egg counts (-16%, -2% and -18%), while levamisole only reduced the egg count by 90%. *Haemonchus contortus* and *Trichostrongylus* sp. were the nematodes found in pre-treatment and post-treatment larval culture for albendazole, fenbendazole and levamisole group. Only *Haemonchus contortus* was found in the post-treatment larval culture for ivermectin group. The increasing nematode worm egg count in albendazole, fenbendazole and ivermectin groups after treatments indicates that these three anthelmintics are not suitable to be used in this farm due to nematode resistance. The management of the farm now practised zero grazing for most of the sheep and will apply rotational grazing for sheep which are allowed to graze. As reports on multiple anthelmintic resistance of nematodes in small ruminants are increasing in Malaysia, further studies should be conducted in order to diminish this problem.

Oral poster session 2: Here, There and Everywhere (1)

Tuesday 18 August
12:00-13:00

Ref: 0473

Poster Number: P1A199

Simple, rapid and low cost detection methods of helminthes can promote refugia and decrease Nguni goat anthelmintic resistance in communities around Empangeni in South Africa

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In humans and animals, helminthes have been observed to manipulate the immune system by weakening the host cellular and humoral responses. Such abilities promotes anthelmintic resistance (ANR) and has been a major concern to many international organizations, UNICEF, WHO, AU and developing countries. Management practices such as treatment frequency, under-dosing, group treatment, single drug treatment programs and animal movement have all been documented to play a part in ANR. Treatment of only sick animals and leaving behind a parasite population that has not been exposed to a particular drug is known as refugia. Refugia that has escape selection provides a pool of genes that dilutes the frequency of resistant genes in animals hence a decrease in ANR. Management techniques that can assist in treating only parasite infested animals can decrease ANR arousing from frequent and group treatment. The application of FAMACHA[®] with no formal training by resource-poor farmers in the community to identify anaemic or helminthes infested animals as a method of increasing refugia and decreasing ANR was investigated. Two sites (KwaMthethwa (KM) village and Owen Sitole College of Agriculture (OSCA) farm) with 40 animals each of mixed sex were used in this study. The animals grazed on natural pasture during the day and housed in Kraal at night. FAMACHA[®] chart scored animals on eye colour; 1 (Red, non-anaemic and acceptable), 2 (red-pink, non-anaemic and border line), 3 (pink, mildly anaemic and dangerous), 4 (pink-white, anaemic and fatal) or 5 (porcelain white, severely)). Eye scoring was over four seasons (autumn, winter, spring and summer) alongside faecal egg count (FEC) as a positive control to FAMACHA[®] diagnosis method. Anaemic animals varied ($P < 0.05$) between the sites, 76.72, 40.34 and 49.37% for KM, OSCA and KM+OSCA, respectively. Comparison of FAMACHA[®] with FEC showed that only 61.53, 33.5 and 40.12% of the animals were anaemic at KM, OSCA and KM+OSCA, respectively, due to false positive results. Approximately 80% of all anaemic animals were identified by no skilled resource-poor farmers using FAMACHA[®]. This reinforces the economic (cheap) importance and reliability of FAMACHA[®] chart in parasite resistance management since an increase in the size of refugia implies that the rate of evolution towards resistance also decreases.

Oral poster session 2: Here, There and Everywhere (1)

Tuesday 18 August
12:00-13:00

Ref: 0622

Poster Number: P1A206

Ivermectine resistance in nematodes of sheeps in Colombia

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The objectives of the present study were to report the percentage of ovine farms with gastrointestinal nematodes (GINs) resistant to ivermectine in some regions of Colombia and identify the genera of GINs involved in resistance. The departments evaluated with at least one farm were Cundinamarca, Boyacá, Cesar, Guajira, Cordoba and Sucre. The faecal egg count reduction test (McMaster technique) was used to detect the presence of resistant GINs with which it was found percentage of ovine farms with GINs resistant to ivermectine was 100% (10/10). The percentage of faecal egg count reduction on resistant farms was between-61 and 80%. Only one farm had GIN populations (*Trichostrongylus spp*) in Sucre susceptible to ivermectine. *Haemonchus spp.* was the genus most commonly found to be resistant, followed by *Trichostrongylus spp.*, *Teladorsagia circumcincta* and *Cooperia spp.* None of the farmers weighed their animals in order to dose them correctly with anthelmintics but is necessary to identify factors that influenced the presence of GIN resistance. This is the first report of ivermectine resistant GINs of sheeps in Colombia. Improving the use of anthelmintics and use of alternative control will help control the spread of resistance.

Oral poster session 2: Here, There and Everywhere (1)

Tuesday 18 August
12:00-13:00

Ref: 0655

Poster Number: P1A208

First evidence for triple anthelmintic resistance on an Irish sheep farm

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While resistance to benzimidazole (BZ) and levamisole (LEV) in Irish ovine nematode populations is clear, evidence for resistance to ivermectin (IVM) has been unconfirmed. Lambs from 4 geographically disperse flocks with suspect IVM resistance were recruited. Faecal Egg Count Reduction Tests (FECRT) in accordance with WAAVP guidelines indicated IVM resistance on 2 farms; reduction in egg count of 94% (C.I. 86.82-97.82), 77% (C.I. 59.9-87.1) was observed in the flocks from counties Cavan and Wicklow respectively. A minimum of 3 sheep per farm were subsequently purchased, housed on a concentrate diet with free access to water and administered IVM twice, 14 days apart in accordance with manufacturer recommendations. Nematode eggs were recovered post treatment and cultured to recover third stage larvae. The gastrointestinal tract was recovered at post-mortem to identify worms. *Teladorsagia circumcincta* was the only species recovered at post mortem from sheep from both farms. This represents the first confirmed cases of IVM resistant *T. circumcincta* in the Rep. of Ireland. The Cavan *T. circumcincta* isolate was further characterised in terms of its levels of resistance to BZ and LEV and Macrocytic Lactone (ML). A total of 60 helminth naïve lambs were each experimentally challenged with a single dose of approximately 7,500 third stage *T. circumcincta* larvae. 28 days post infection lambs were divided into four groups, each lamb being assigned to a BZ, LEV, IVM or Control group. Anthelmintics were administered as per manufacturers instructions and faecal samples were taken 7, 14 and 16 days post treatment. FECR of 53%, 93% and 50% were observed for BZ, LEV and IVM respectively. Following the FECRT, a Controlled Efficacy Test (CET) was carried out to further characterise the level of ML resistance. The 15 untreated control animals were randomly assigned to either Moxidectin (MOX), IVM or control treatment groups (n=5 per treatment). At 5 & 6 days post treatment, worms were recovered at post mortem and the worm burden was established. *T. circumcincta* was the only species recovered and the mean reduction in worm burden was 96.6% and 78% for MOX and IVM treated groups respectively. Further characterisation of the Wicklow isolate is underway. This study provides the first confirmed evidence of a triple resistance isolate in the Republic of Ireland.

Oral poster session 2: Here, There and Everywhere (1)

Tuesday 18 August
12:00-13:00

Ref: 0668

Poster Number: P1A209

Drench test failure on Irish sheep farms

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As part of a nationwide sheep technology adoption program (STAP), over 3,500 sheep producers availed of the opportunity to test the efficacy of their anthelmintic treatment by means of a drench test. Tests were carried out between June and October on spring born lambs in 2013 and 2014. The interval between faecal sampling the same cohort of lambs pre and post anthelmintic treatment was 7 days for Levamisole (LEV) and 14 days for Benzimidazole (BZ) and Macrocytic Lactones (ML). The faecal samples and treatment information were then sent to Department of Agriculture Food and the Marine (DAFM) approved laboratories to determine the Faecal Egg Counts (FEC). FEC were categorized as *Nematodirus* spp. (FEC_{NEM}) and other trichostrongyles, excluding *Strongyloides papillosus*, (FEC_{OT}). To calculate a reduction, a pre-treatment FEC of ≥ 200 eggs per gram (epg) was required in addition to the post-treatment faecal sample taken between 10-14, 4-7 or 14-18 days for BZ, LEV and ML, respectively. An anthelmintic treatment resulting in a reduction in FEC $>95\%$ was deemed effective. In 2014, 50% of participants carried out the task as required compared to 30% in 2013. In both years BZ was the most popular drug class tested followed by ML and LEV. Approximately half of the compliant drench tests for both years were effective against Trichostrongyles. There was a significant difference in the efficacy of the three drugs (χ^2 test; $P < 0.001$) against these species. In both years drench test failure was more prevalent in the BZ group. In 2013 a reduction of $>95\%$ was observed for 30%, 52% and 76% of cases for BZ, LEV and ML respectively. In 2014 a similar pattern was observed with 29%, 56% and 71% of BZ, LEV and ML treatments being effective. In 2014, similar criteria were used to calculate the drug efficacy against *Nematodirus* spp, with the majority of these 109 tests being effective. Of these, 95 met the criteria to also calculate a Trichostrongyle egg reduction. While being completely effective against *Nematodirus*, evidence for inefficacy against Trichostrongyles following BZ, LEV and ML was observed in 75%, 32% and 17% of tests, respectively. This evidence suggests that the treatment failure is as a result of anthelmintic resistance as opposed to an inappropriate dosing technique. While it is not possible to definitively conclude that treatment failure in the majority of cases is due to anthelmintic resistance, data from a cohort of flocks suggest that it is responsible.

Oral poster session 2: Here, There and Everywhere (1)

Tuesday 18 August
12:00-13:00

Ref: 0690

Poster Number: P1B075

Gastrointestinal nematode populations in Canaria Hair Breed Sheep farms from Gran Canaria

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Gastrointestinal nematodes (GI) are one of the main causes of production losses in sheep farms worldwide, including the Canary Islands. In essence, GI control relies upon different anthelmintic compounds that have contributed to parasite drug-resistance all over the world. Hence, the development of alternative methods of nematode control in small ruminants is essential to reduce anthelmintic-resistance in these nematode populations. Targeted selective treatment (TST) of infected animals is an alternative approach that maintains worms in refugia, therein delaying selection for anthelmintic-resistance. Worm epidemiology is one of the criteria to establish a specific TST in farms.

Although Gran Canaria is a small island, significant climatic differences between north and south areas due to the topography of the island, could significantly modulate parasite loads and distribution of species between different sheep farms. Furthermore, two sheep breeds are exploited, Canarian sheep (CS) and Canarian Hair sheep (CHB), in which differences in resistance to infection with *Haemonchus contortus* has been observed. However, these factors are not considered in nematode control plans that are applied on sheep farms on the island.

This study was designed to estimate the worm epidemiology in a CHB sheep farm. In total, 390 stool samples were processed, two samples per season for thirty months. In this specific climatic region, the results show differences between parasite loads and seasons. In this study we discuss the implications of these results may have in controlling infections by these parasites in sheep farms on Gran Canaria Island.

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Oral poster session 2: Here, There and Everywhere (1)

Tuesday 18 August
12:00-13:00

Ref: 0693

Poster Number: P1A212

Anthelmintic resistance in nematodes of sheep in Uruguay: a retrospective study

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Anthelmintic resistance (AR) in sheep gastrointestinal nematodes (GIN) is wide-spread in sheep farms in Uruguay. Results from a survey published in 1996 revealed that benzimidazole (BZ), levamisole (LEV) and ivermectin (IVM) resistance was present on 80%, 71% and 1.2% respectively, of the studied farms.

The aim of this work was to present an update on AR in sheep GIN in Uruguay.

A retrospective study was performed on 36 six farms that evaluated AR during 2011 and 2015. Faecal egg count reduction tests (FECRT) were conducted following WAAVP guidelines.

At each farm, animals were allocated to nine groups of 10 sheep and each was randomly assigned to: Untreated Control; BZ; LEV; moxidectin (MOX); closantel (CLT); naphtalophos (NPT); combination BZ+LEV+IVM (BZ-LEV-IVM); triclofon (TRN); monepantel (MPL).

Oral formulations were used and efficacy was evaluated using Dash formula.

Reduced efficacy to BZ was found in 100% of the studied farms, in 91.2% to LEV; in 94.4% to MOX, in 93.1% to CLT, in 13.8% to NPT; in 62.9% to triple combination; in 88.5% to TRN and in 6.0% to MPL.

Haemonchus sp and *Trichostrongylus* spp were identified as the main genera involved in the resistance.

Although these results do not allow comparisons, they suggest that development of AR continues developing among sheep farms, in Uruguay.

Oral poster session 3: With a little help from my friends

Tuesday 18 August
12:00-13:00

Ref: 0013

Poster Number: P1A183

Synergic effect between permethrin and dinotefuran on ganglionic synaptic transmission in an insect model (*Periplana americana*) assessed by mannitol-gap recording

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Introduction: Synergistic effects were reported between neonicotinoids and pyrethroids. These two classes of insecticides are combined (dinotefuran:permethrin, 1:7.3) in an ectoparasiticide (Vectra®3D). This study was designed to investigate the activity of dinotefuran, permethrin alone and their combination on the synaptic transmission in isolated insect nerve preparation.

Methods: The abdominal nerve cord, one circus and the corresponding cercal nerve XI were isolated from adult cockroaches (*Periplana americana*) and flooded in saline solution, in the recording chamber. The preparation was continuously superfused with saline and mannitol solution (87 g/L). Permethrin (36.5 µM), dinotefuran (5 µM) or their combination were perfused in the ganglia (synaptic level) at a constant solution exchange (500 µL/min). The excitatory postsynaptic potentials (EPSPs) were obtained by electrical stimulation of the ipsilateral nerve XI. Ganglionic depolarizations were recorded with a high-impedance amplifier.

Results: The spontaneous activity (15.9 ± 1.0 counts/s) was increased by dinotefuran within 1 min while permethrin had no significant effect ($3 - 5$ counts/s). Their combination induced a higher spontaneous activity (44.5 ± 1.0 counts/s) than the sum of the activities induced by either the compounds alone. While EPSPs were not blocked by dinotefuran or permethrin alone, EPSPs were all blocked by the combination when the ganglionic depolarization reached a peak. The ganglionic depolarization was increased by dinotefuran (11.6 mV) but not by permethrin (0 mV). The mean ganglionic depolarization induced by the mixture was 22.4 mV.

Conclusion: This study demonstrates that the combination of dinotefuran and permethrin at the ratio 1:7.3 is synergistic and more potent on insect synaptic transmission than each individual active.

Oral poster session 3: With a little help from my friends

Tuesday 18 August
12:00-13:00

Ref: 0040

Poster Number: P2E389

Molecular Xenomonitoring of Trypanosome in tsetse for prioritizing vector control in northern Tanzania

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Tsetse flies (*Glossina*) transmit the blood-borne Trypanosomes that cause Human African Trypanosomiasis (HAT) and Animal African Trypanosomiasis (AAT). Diagnostic tools appropriate for undertaking interventions to control trypanosome infections are key to their success; however, many have unsatisfactory performance and are not well suited for their use in the parasite control programs being implemented. Monitoring trypanosome infections in wild tsetse flies in a given area is important in prediction of epidemic outbreaks of diseases and spread and could help focus control programs in areas requiring immediate attention to halt disease transmission especially trypanosomiasis of zoonotic importance. The objective was to roll out the use of the recently developed RIME LAMP for screening large numbers of tsetse flies for trypanosomes found in Serengeti areas in order to predict the epidemic outbreak and spread of HAT for immediate attention and institution of control programs. Flies were collected from Simiyu, Kirawira, Birila, Serena and Moru during the dry and wet seasons, sundried and DNA extracted using Qiagen Kit; and analysed by ITS-PCR and Rime LAMP. A total of 125 flies were analysed for each season. During the wet season, the common trypanosomes were *T. brucei* (11/125); whereas during the dry seasons, the common trypanosomes were *T. godfreyi* (14/125) and *T. vivax* (11/125). ITS-PCR picked all trypanosomes present in the fly; however RIME LAMP detected the trypanozoon *T. brucei* only. The study shows that carrying out LAMP tests in the field provides the simplest and quickest means to estimate trypanozoon trypanosome infection rates in tsetse for decision support to implement intervention programs.

Oral poster session 3: With a little help from my friends

Tuesday 18 August

12:00-13:00

Ref: 0048

Poster Number: P2C313

Effects of shampooing, water, and sunlight on the efficacy of topical and systemic treatments against adult and immature fleas (*Ctenocephalides felis*) on dogs

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To study environmental stressors on adulticides and ovicides, 5 groups of 7 beagles were assigned as follows: 1) Untreated, 2) Sentinel (S), 3) Frontline Plus (FL), 4) Vectra 3D (V3D), 5) Sentinel Spectrum (SS). Each dog was treated, per label, day 0 and infested with approximately 250 unfed, adult fleas (KS1 strain) days -7, 1, 8, 15, 22 and 29 with flea counts (comb) days 2, 9, 16, 23, and 30. Dogs were exposed to sunlight (4 hr/day, 5 days/week), shampooing (once) and water immersion (twice). Lufenuron (S, SS) was highly ovicidal against eggs collected from treated dogs (99.5% or 100%, respectively, for the length of study) and had no adulticide effect. Adulticidal efficacy of FL and V3D declined from 100% and 99% (Day 2) to 20% and 40% (Day 30) respectively while ovicidal efficacy declined from 92% to 8% and 93% to 6.1% respectively. Hair clipped from dogs treated with FL (s-methoprene) or V3D (pyriproxifen) showed greater ovicidal efficacy at midline than at the rump or tail against Auburn colony flea eggs. Hair clipped from lufenuron dogs had no ovicidal effect. These data indicate environmental factors can affect efficacy of topical products (FL, V3D), while systemic products (S, SS) remain unaffected.

Oral poster session 3: With a little help from my friends

Tuesday 18 August
12:00-13:00

Ref: 0223

Poster Number: P2E396

Invasive alien species of Europe: ecology, biology and vector competence of *Aedes koreicus*, a mosquito recently introduced in north-eastern Italy

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The last invasive container-breeding mosquito introduced in Europe is *Aedes (Finlaya) koreicus* (Edwards), a species native of Korea, China, Japan and of the Asian part of Russia. Since its first report in Belgium in 2008, this mosquito has been identified in north-eastern Italy in 2011, in Switzerland and the south of European Russia in 2013. Here we report our advancement in the knowledge of the behavior and vector competence of this mosquito from field and laboratory experiments performed since 2011. The species was found to be already established in north-eastern Italy over more than 3000 km² with an expanding trend. *Aedes koreicus* is well adapted to urban settlements, colonizing mainly urban areas where it breeds in a variety of artificial man-made containers. The species was found in sympatry with *Ae. albopictus*, but also in other empty niches. Indeed, the highest density of *Ae. koreicus* was in hilly and mountainous habitats, with low winter temperatures, poorly or not yet colonized by *Ae. albopictus*. Laboratory and field studies suggested a preference of *Ae. koreicus* for humans, however the species was able to complete its life cycle when fed with poultry, cattle, sheep and canine blood. Dog blood was also found in a specimen collected from the field.

A colony of *Ae. koreicus* was infected with canine blood containing 3000 microfilariae/ml of *Dirofilaria immitis*. At the end of the study a total of 18 mosquitoes developed L3 giving an estimated infection rate at 12 days post infection of 68.2% and a vector efficiency index of 25.2%. This poses a new threat for animal and human health in endemic areas for dirofilariosis and enhances the risk of spreading the infection in previously non-endemic areas.

Funding: Veneto region and LExEM project (www.lexem.eu).

Oral poster session 3: With a little help from my friends

Tuesday 18 August
12:00-13:00

Ref: 0279

Poster Number: P2D424

Immune response of lambs naturally infested by *Oestrus ovis* (Diptera: Oestridae)

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Oestrus ovis are an important parasite of small ruminants raised in tropical and subtropical climates. Twenty-six newborn Bergamacia lambs were evaluated for 150 days to assess the level of serum immunoglobulin (IgG) against a crude extract antigen from second instar (L2) of *O. ovis* larvae. The trial was conducted in a farm in Botucatu, Sao Paulo State, Brazil. Serum samples were taken from lambs fortnightly from birth to slaughter to determine the IgG levels using the ELISA technique. After slaughter, the *O. ovis* larvae were collected, quantified and identified accordingly with larval stage (L1, L2 or L3). The results showed that all lambs were parasitized with different larval stages of *O. ovis* with an average of 39 larvae per lamb (intensity of the infestation from 10 to 97 larvae). The results of IgG were expressed as the percentage of the optical density value (OD) of the positive standard serum. High levels of IgG anti-*O. ovis* were observed at the first 15 days of age (averages of 70% in newborns and 61% at 15 days of age). These results mean that antibodies are transferred via colostrum from ewes to lambs. Then, the antibodies levels declined until 75 days of age with an OD of 23%, followed by a progressive increase until the end of the trial, when the highest average was recorded (OD of 93%). The Spearman's correlation coefficient between IgG x Larval burden was very low ($r = -0.039$; $p > 0.05$). It is clear that after the beginning of the infestation the lambs acquired a natural immune response, but it seems that the presence of antibodies in the serum were not efficient to protect the lambs against *O. ovis* infestation.

Acknowledgements:

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Oral poster session 3: With a little help from my friends

Tuesday 18 August
12:00-13:00

Ref: 0402

Poster Number: P1B069

Characteristics and impact of flea (*Vermipsyllidae*) infestation on ruminants in Mongolia

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Very few data exist on the infestation of domestic ruminants by *Vermipsylla alakurt* and *Dorcadia ioffi*, two species of fleas belonging to Vermipsyllidae family. These fleas are distinctive by their huge size at the stage of engorged female (6 to 16 mm*), which allows them to be visible to the naked eye. A questionnaire was submitted to 132 herder families in Arkhangai, a mountainous province of Mongolia (1290 to 3529 m of altitude) with a highly continental climate. Seven fleas have also been sampled on sheep. Respectively 99 %, 94 % and 93 % of the families had bovines (*Bos taurus* or *Bos grunniens*, i.e. yak), ovines and caprines. The husbandry system is semi-nomadic: families migrate four times annually, following the same rotation year after year. Animals give birth from March to May. Nowadays, flea infestation is particularly frequent in a limited (but expanding) zone in the northern part of Arkhangai. The occurrence of adult stages of Vermipsyllidae is very seasonal, focused on winter, from November until January, which is the dry period (10 mm of accumulated snowfall) with average temperatures ranging from -15°C to -4°C. Fleas are observed on ovines, caprines, *Bos taurus* and *Bos grunniens* in respectively 98 %, 35 %, 38 % and 27 % of infested herds. Lambs are less often infested than adult sheep (64 % versus 94 %) while for other ruminants, young animals seem more often parasitized, especially for *Bos taurus* (six times more). The localisations of fleas seem to vary according to the host, but the most frequent sites are the neck, followed by the elbows and the tail (respectively 30 to 55 %, 24 to 40 % and 9 to 25 % of the localisations, depending on host species). Although a previous study described the presence in Arkhangai of only *Vermipsylla alakurt* and explained the absence of *Dorcadia ioffi* by the harshness of the climate*, we have identified the presence of *Dorcadia ioffi* on the neck of sheep. According to the localisations of the fleas, it seems that *Dorcadia ioffi* is even more frequent than *Vermipsylla alakurt* in this province. For 80 % of affected herders, fleas greatly weaken animals. This parasitosis, currently expanding via animal movements, represents one the most worrying livestock diseases in this region.

* Zedev, *Monatshefte für Veterinärmedizin*, 1976

Oral poster session 3: With a little help from my friends

Tuesday 18 August
12:00-13:00

Ref: 0423

Poster Number: P1A196

Quantitative Expression levels of Acetylcholinesterase and Esterase genes in Resistant *Rhipicephalus microplus* population treated with Organophosphates

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Currently, the resistance to pesticides in cattle ticks *Rhipicephalus microplus* population is considered one of the major problems on the livestock and their control is based on the intense use of pesticides. The intensive and indiscriminate use of these compounds led to the selection of Brazilian population of resistant ticks. In this work, we developed a quantitative methodology to evaluate the expression levels of *Acetylcholinesterase* (AChE) and *Esterase* (EST) genes on tick population resistant to organophosphates pesticides. The gene expression was quantified by real time PCR by mRNA detection extracted from ticks treated to different concentrations of Diazinon. The samples were identified as resistant and susceptible after immersion for 30 min in Diazinon solutions to 1%, 2.5% and 5%. A group of twenty molecular targets were designed to recognize alleles of AChE and EST genes from *R. microplus*, where only three targets were chosen from the high expression verification in gene quantification. The quantitative measurements were normalized with the expression level of endogenous control. Regarding the EST gene, the values of relative quantity shown high relative expression levels, as 4.2602 ± 0.6585 and 1.4998 ± 0.1913 related to immersion in 2.5% and 5% of Diazinon concentration, respectively. Also, the AChE gene shown considerable levels of relative expression as 1.7328 ± 0.5905 and 0.8747 ± 0.0501 in 2.5% and 5%, respectively. For both genes, there was no expression for samples treated with 1% of Diazinon, this proves that the selected samples were considered very resistant to high concentrations of organophosphates. Also, the results demonstrated there was an overexpression of AChE and EST genes at the singanglion of ticks who survived after exposure to Diazinon 2.5%. These results, probably, are related to the intensive control of ticks in the dairy herd where the samples were collected. The selected molecular probes for detection of AChE and EST expression levels proved to be viable and appropriate for molecular diagnostic purposes of resistant *R. microplus* populations to organophosphate pesticides.

Oral poster session 3: With a little help from my friends

Tuesday 18 August
12:00-13:00

Ref: 0478

Poster Number: P2C323

Development of a viable standardized method for testing chemical disinfectants against harmful arthropods — *Dermanyssus gallinae* and *Musca domestica* as Indicators

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In parasitic disease- control, disinfection against exogenous parasite stages in animal health takes a fixed position as a subcategory of hygienic-prophylactic measures and accompanies both Prophylaxis and Mesophylaxis as well Metaphylaxis. Although most of Helminths, pathogenic Protozoa and harmful Arthropods have exogenous stages, the current level of the chemical disinfection against exogenous parasite stages is unsatisfactory. Currently in Germany only *Eimeria tenella* and *Ascaris suum* are specified as indicators of Protozoa and Helminths; the external stages of arthropods were previously ignored. From the currently 104 registered disinfectant products for animal husbandry in Germany by the Disinfectant Commission of the DVG (German Society of Veterinary Medicine), there are only 15 products which are used as antiparasitic agents and are exclusively for Helminths and Protozoa. Three chemical poly component disinfectious products:

Ascarosteril AB[®] (o-hydroxybiphenyl-fatty acids & eutectic peracetic acids), Neopredisan 135-1[®] (cresols), Preventol VET 100[®] (phenols) were tested against several Insect- and Acarid species in extensive experiments to review their efficacy. Eggs, larvae, pupae and adults of *Musca domestica* as well as eggs, larvae, nymphs and adults of *Dermanyssus gallinae* were included, for the spatial disinfection, as capable indicators for both suspension and germ-carrier tests. The efficacy of the three disinfectants were tested with own developed methods. The suitability of the two indicators *D.gallinae* and *M.domestica* could be demonstrated with the suspension and an own developed germ carrier test. More methodology and obtained results will be presented in details.

The targeted search for new highly effective, environmentally friendly additives to chemical disinfectants against exogenous stages of harmful arthropods is considered urgent.

For a definitive assessment of the results of efficacy tests in our previous studies we could find that, in addition to suspension and germ carrier test, it is very important to have tests under practical conditions – so called field test - with different application techniques, before a new registration occurs.

Oral poster session 3: With a little help from my friends

Tuesday 18 August
12:00-13:00

Ref: 0691

Poster Number: P1A211

Assessment of the efficacy of biocides or repellents for the control of *Sarcoptes scabiei* in the environment

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Sarcoptes scabiei infection is a contagious disease affecting both humans and animals. The transmission occurs either by direct contact or exposure to fomites in where mites could survive up to 24-36h at room conditions and may remain infective. The number of products available for the environmental control of *S. scabiei* is very limited. The objective of the present study was to assess the efficacy of biocides or repellents against *S. scabiei* var *suis*. *Sarcoptes scabiei* var *suis* mites were collected from experimentally-infected pigs maintained at the veterinary college of Alfort, France. For each test, 20 live mites of all motile stages were placed in a plastic Petri dish (3 cm in diameter) and sprayed uniformly by each product. The control was sprayed by distilled water. The study was performed in triplicate under room condition and the mites were inspected under stereomicroscope in 5, 10, 15, 20, 25, 30, 40, 50, 60 min, 2, 3, 4, 5 and 24 hrs. The viability of the mites was checked by stimulation with a needle and mites were recorded as dead if no reaction occurred. The data were analyzed by Kaplan Meier survival curves and significant differences between survival curves were calculated by Log-rank tests using JMP 11.0 software.

All the products, except the combination of tetramethrin and sumithrin (A-PAR®), killed all mites within 24h. For pyrethroids, the median survival time was 50 ± 30.4 min, 120 ± 309 min, 10 ± 5.9 min, 40 ± 36.8 min, 15 ± 7.3 min, 180 ± 417 min and 1440 ± 600 min when mites were exposed to permethrin 4% (Insect Ecran®), permethrin 0.6% (Parastop®), esdepallethrine 0.315g and bioresmethrin 0.067g (Pyréflor®), bifenthrin 0.1g (Pyréflor® bifenthrin), cypermethrin 0.1% and imiprothrin 0.1% (Raid®), cyfluthrin 0.04g (AdvantHome®), tetramethrin 0.19g and sumithrin 0.19g (A-PAR®), respectively. For repellents, the median survival time was 20 ± 6.5 min, 15 ± 4.3 min, 30 ± 42.1 min and 15 ± 4.9 min for DEET 25% (Insect Ecran®), DEET 50% (Insect Ecran® 1), icaridin 20% (Insect Ecran® 2) and IR3535 20% (Paranix®), respectively.

The results of the present study could give support for evidence-based use of biocides and repellent for household, hospitals and farms.

Oral poster session 3: With a little help from my friends

Tuesday 18 August
12:00-13:00

Ref: 0718

Poster Number: P2C330

Comparative field study of the efficacy of Fluron® Gold Pour On and a commercial product with 2.5% fluazuron in the treatment of cattle naturally infested with *Rhipicephalus (Boophilus) microplus* in Brazil

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The objective of this study was to evaluate the therapeutical efficacy of **Fluron® Gold Pour On** (a novel association of 5% of cypermethrin, 7% of chlorpyrifos, 2.5% of fluazuron and 5% of piperonyl butoxide) when comparing to a commercial product with 2.5% of fluazuron in the treatment of cattle naturally infested with *Rhipicephalus (Boophilus) microplus* in a field study in Brazil. Thirty bovines were selected after the counting of engorged female ticks measuring $\geq 4.5\text{mm}$ on the whole body of the animal. The count was performed on days -7 and -3 prior to treatment and weekly after the treatment. The animals were kept in separate pastures with mineral salt and *ad libitum* water. The animals were allocated to 3 groups of 10 animals each considering the mean of engorged female ticks counted before treatment. One group did not receive any treatment (negative control group), another group was treated with **Fluron® Gold Pour On** and the last one received the commercial product with 2.5% fluazuron (positive control group). All products were used according to the label via pour on route at a rate of 1mL/10 kg b.w. in a single administration. Mean number of engorged female ticks observed prior to treatment was 84.23, 77.9 and 78.6 respectively for animals of negative control, positive control and **Fluron® Gold Pour On**. Efficacy levels observed after the treatment for the positive control group were 26.75%, 56.85%, 94.90%, 99.79%, 97.98%, 88.16% and 96.71%, for days +3, +7, +14, +21, +28, +35 and +42, respectively. Efficacy levels after treatment with **Fluron® Gold Pour On** were 53.90%, 65.14%, 100.00%, 99.79%, 96.00%, 99.00% and 92.09%, for days +3, +7, +14, D+21, +28, +35 and +42, respectively. The results have shown that when comparing to negative control group, the commercial formulation with 2.5% of fluazuron did not show any statistical difference on the first week post-treatment (D+3 until D+7). However, **Fluron® Gold Pour On** showed statistically superiority at this moment. It was also possible to observe that the association of fluazuron, cypermethrin, chlorpyrifos and piperonyl butoxide conferred statically superior immediate efficacy (D+3 and D+14) than the single use of fluazuron.

Oral poster session 4: Penny Lane

Tuesday 18 August
12:00-13:00

Ref: 0139

Poster Number: P2I351

Fasciola hepatica – could diagnostic indicators replace necropsy?

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At present the diagnosis of true resistance and determination of drug efficacy rely solely on terminal experiments. The range of fecal diagnostic tools available has increased in the last decade, although not all have been commercialised. Fecal indicators accessible to diagnostic laboratories include the traditional techniques of fecal egg count (FEC) and the coproantigen ELISA (Bio-X, Jemelle Belgium). Data statistically correlating these tests with actual fluke burden is limited, although small cohorts of cattle and sheep have been analysed (Brockwell *et al* 2013 and Mezo *et al* 2004). Fluke burdens of necropsied sheep were compared to FEC and coproantigen ELISA at necropsy. Regression analysis demonstrated that coproantigen ELISA correlated strongly with fluke burden, whilst FEC correlated weakly with coproantigen ELISA. The correlation of FEC and fluke burden was also weak, although stronger than that of FEC with coproantigen ELISA.

References:

Brockwell *et al* 2013, 'Comparative kinetics of serological and coproantigen ELISA and faecal egg count in cattle experimentally infected with *fasciola hepatica* and following treatment with triclabendazole', *Vet Parasitol*, vol. 196, no. 3-4, pp. 417-426.

Mezo *et al* 2004, 'An ultrasensitive capture ELISA for detection of *fasciola hepatica* coproantigens in sheep and cattle using a new monoclonal antibody (mm3)', *J Parasitol*, vol. 90, no. 4, pp. 845-852

Oral poster session 4: Penny Lane

Tuesday 18 August
12:00-13:00

Ref: 0143

Poster Number: P1G087

The feasibility of testing whether *Fasciola hepatica* is associated with increased risk of verocytotoxin producing *Escherichia coli* O157 from an existing study protocol

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Background: The parasite *Fasciola hepatica* is a major cause of economic loss to the agricultural community worldwide as a result of morbidity and mortality in livestock, including cattle. Cattle are the principle reservoir of Verocytotoxigenic *Escherichia coli* O157 (VTEC O157), an important cause of disease in humans. To date there has been little empirical research on the interaction between *F. hepatica* and VTEC O157. It is hypothesised that *F. hepatica*, which is known to suppress type 1 immune responses and induce an anti-inflammatory or regulatory immune environment in the host, may promote colonisation of the bovine intestine with VTEC O157.

Objectives: Here we assess whether it is statistically feasible to augment a prospective study to quantify the prevalence of VTEC O157 in cattle in Great Britain with a pilot study to test this hypothesis.

Methods: We simulate data that conforms to known individual-level and farm-level prevalence on average under a mixed-effects logistic regression model framework. We use Monte Carlo sampling to empirically calculate the power to detect an association effect size (odds ratio) of 2. In order to reduce the resources required for such a study, we exploit the fact that the test results for VTEC O157 will be known in advance of testing for *F. hepatica* by restricting analysis to farms with a VTEC O157 sample prevalence of >0% and <100%.

Results: From a total of 270 farms (mean 27 cows per farm) that will be tested for VTEC O157, power of 87% can be achieved, whereby testing of *F. hepatica* would only be necessary for an expected 50 farms, thus considerably reducing costs.

Conclusions: Pre-study sample size calculations are an important part of any study design. The framework developed might be applicable to the study of other co-infections.

Oral poster session 4: Penny Lane

Tuesday 18 August
12:00-13:00

Ref: 0319

Poster Number: P2C321

Helminth infection in dairy cattle herds: development of a questionnaire for risk assessment of gastrointestinal nematodes, *Dictyocaulus viviparus* and *Fasciola hepatica* infections

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Risk assessment (RA) of gastrointestinal nematodes (GIN), *Dictyocaulus viviparus* and *Fasciola hepatica* infections in dairy cattle herds requires to carry out a field audit and/or to perform laboratory examinations that are costly, time- and labour-consuming and need deep and accurate knowledge about parasitic infection. The aim of this study was to develop a RA questionnaire for the 3 major helminth infections of grazing dairy cattle that should be simple, concise, accurate and usable by any person involved in animal health on the field (technician, engineer, veterinarian). For each parasite, on the basis of a review of technical and scientific literature, we selected a large range of factors related to the risk of exposure or to the risk of negative consequences (clinical disease or production impairment). First of all, these factors were transformed into a draft questionnaire with preliminary questions. This draft was thereafter submitted to a panel of 10 experts in cattle parasitology originating from academic, research or field sectors. A Delphi method in 3 rounds was used to replace, modify, refine or validate the preliminary questions. The experts were asked to judge each preliminary question according the following criteria: suitability (is it relevant?), wording (is it understandable?), functionality (can the farmer answer the question?). New questions from the experts could be proposed as well and incorporated into a consolidated questionnaire for the next round. At the end of the 3rd round, 29 questions were retained as consensual: 9 questions about *Dictyocaulus*, 12 about GIN, 7 about *Fasciola* and 1 general question at the beginning of the form regarding the access of animals to pastures. A global RA scale based on a simple addition of points from each question completed the questionnaire and provided a grossly and preliminary estimation of risk for each parasite and each category of animal (heifer or cow) through a coloured chart from green (lowest score) to red (highest score). The questionnaire was tested in 20 dairies in western France and took 20 minutes to be fulfilled. Some minor editings (wording) were made accordingly. The implementation of the questionnaire will be performed in 500 herds in western France and will be compared with bulk tank milk ELISA results for the 3 parasites.

Oral poster session 4: Penny Lane

Tuesday 18 August
12:00-13:00

Ref: 0407

Poster Number: P1A195

Evaluation of *Fasciola hepatica* *in vitro*-assays to search for new flukicides

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The increasing number of documented cases of resistance of the liver fluke *Fasciola hepatica* against treatment with triclabendazole has put pressure on identifying novel compounds with new modes of action. *In vitro* tests can be a tool for identifying potentially active compounds and serve as a first filter before testing in animals.

Materials and Methods: We compared a juvenile *in vitro*-test (JIV) using newly excysted juveniles of *F. hepatica* with an adult *in vitro*-test (AIV). Standard compounds with known efficacy against juvenile or adult stages of *F. hepatica* like triclabendazole, closantel, clorsulon, benzimidazoles and negative controls like monepantel were used. To evaluate the activity of the compounds, a scoring system was used in the JIV-test, while a video-camera-system allowed the quantification of movements of the adult stages.

Results: From 13 tested compounds, 8 were considered active in the JIV-test, while only 6 were found positive in the AIV-test. The two compounds positive only in the juvenile test were found to be inactive against 10-week old flukes in sheep. Three of the 6 compounds found positive in both tests could not be confirmed in sheep, while 3 showed a high activity, including triclabendazole and closantel.

Discussion: The AIV-test may be a good basis for the evaluation of active compounds against liver flukes. The registration of movements with a video-camera-system allows an objective determination of the activity. However, the sourcing and the large size of adult flukes limits their use for a high throughput screening.

Oral poster session 4: Penny Lane

Tuesday 18 August
12:00-13:00

Ref: 0496

Poster Number: P2I356

Prevalence of Liver Fluke Infection in Irish Horses and Assessment of a Serological Test for Diagnosis of Equine Fasciolosis

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Fasciola hepatica is a common parasite of livestock in Ireland, causing significant economic losses and affecting animal welfare. To date there is little information on the prevalence of infection in the Irish horse population and the degree of liver pathology due to infection. Definitive diagnosis relies on faecal egg counts (FECs) or post-mortem identification of the parasites. However, FECs are known to have low diagnostic sensitivity, and post-mortem examination is highly labour-intensive. We conducted an abattoir study to determine the prevalence of *F. hepatica* infection in horses in Ireland, and to validate the diagnostic potential in horses of an indirect ELISA based on the *F. hepatica* recombinant cathepsin L1 antigen. Data was collected from 200 horses over a period of 12 months (May 2013 - April 2014) and correlations from ante- and post-mortem examination, complete blood count, serum biochemistry and FECs with serum ELISA results will be presented. Overall, the prevalence of liver fluke infection was 9.5%. The sensitivity and specificity of the cathepsin L1 ELISA were determined to be 47.4% (95% C.I. 24.5- 71%) sensitive and 95.6% (95% C.I. 91.5%-98%) specific. Although the cathepsin L1 ELISA was highly specific, the sensitivity was surprisingly low. The humoral immune response in horse following liver fluke infection appears to be significantly different from ruminants and this is currently under investigation.

Oral poster session 4: Penny Lane

Tuesday 18 August
12:00-13:00

Ref: 0502

Poster Number: P2I357

Comparison of diagnostic tests for *Fasciola hepatica* infection in sheep and their ability to assess the effect of triclabendazole treatment

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Sheep are at risk of fasciolosis, caused by the trematode, *Fasciola hepatica*. The acute/subacute form of the disease adversely affects animal health and welfare and has a significant economic impact. To date, triclabendazole (TCBZ) is the drug of choice as it kills the early, immature stages of the parasite. However to most effectively target treatment with TCBZ there is a need for sensitive diagnostic tests to detect these early stage infections. Moreover, TCBZ is becoming increasingly ineffective due to emergence of resistance. Three different diagnostic techniques are available for *F. hepatica*; faecal egg counts (FEC), coproantigen BIO K201 ELISA and molecular PCR-based assays. PCR was designed to specifically amplify the *F. hepatica* internal transcribed spacer 2 region (ITS2) using faecal samples (Robles-Perez et al., 2013).

In this study, 12 sheep were infected with 200 metacercariae of a known TCBZ susceptible isolate of *F. hepatica*. Faecal samples were taken weekly from pre-infection until 14 weeks post-infection (wpi) at which point sheep were randomly assigned to either control (n=6) or TCBZ treated (n=6) groups. Faecal samples were collected daily until 10 days post-treatment (dpt) and then sheep were euthanised and liver fluke enumerated. The tests were compared statistically to determine which method: a) detected infection with *F. hepatica* with the greatest sensitivity and b) most accurately assessed the impact of TCBZ treatment.

Results showed that the coproantigen ELISA could detect infection from 5 wpi (2/12 sheep), and all sheep were positive by 9 wpi. FEC was less sensitive for early infection, 4/12 animals were first positive at 9 wpi and all sheep were positive at 11 wpi. The PCR failed to detect infection at any time point. The efficacy of TCBZ against *F. hepatica* was 97% with $p < 0.001$. In treated animals both the coproantigen ELISA value and FEC epg decreased post treatment but remained positive 10 dpt. Finally, there was no correlation between fluke burden and either coproantigen levels or FEC for the six control sheep, $R^2 = 0.265$ and $R^2 = 0.001$, respectively.

Overall, the study confirmed that the coproantigen ELISA is more sensitive in detecting early fluke infection compared to FEC. Sheep infected with a TCBZ sensitive isolate remained positive by FEC and coproantigen ELISA 10 days post TCBZ treatment even though they were negative for a fluke burden.

Oral poster session 4: Penny Lane

Tuesday 18 August
12:00-13:00

Ref: 0504

Poster Number: P1A202

Efficacy of flukicides against *Fasciola gigantica* and paramphistomes in naturally infected cattle in Arumeru and Iringa Districts in Tanzania

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The tropical liver fluke, *Fasciola gigantica*, and possibly paramphistomes can be important causes of lost production in cattle in Africa. To check the efficacy of fasciolicides, controlled trials were run in the Arumeru and Iringa Districts of Tanzania. In Arumeru almost all cattle were zero-grazed, but were fed herbage from irrigated pastures where goats were also kept. The cattle in Iringa District were infected through grazing. Albendazole, closantel, nitroxynil, oxclozanide and triclabendazole were administered to weighed and randomly selected animals and faecal egg counts were made on days 0, 7, 14 and 28 by the Flukefinder® (Richard Dixon, ID, US) method. By day 14 fluke egg counts had fallen to zero in faeces from cattle treated with closantel, nitroxynil oxclozanide and triclabendazole in both Districts. However, albendazole only reduced egg counts by 49% in the Arumeru district and 89% in the Iringa District. Oxclozanide was the only drug to control shedding of paramphistome eggs (99% reduction). The failure of albendazole is probably due to its widespread use for controlling nematodes in sheep, goats and cattle. Combined with data on periods of transmission the information may help in the control of trematodes of cattle and small ruminants in Tanzania. This work was supported by the Leverhulme-Royal Society African Award Scheme.

Oral poster session 4: Penny Lane

Tuesday 18 August
12:00-13:00

Ref: 0529

Poster Number: P1B071

Farm level risk factors for *Fasciola hepatica* infection in Danish dairy cattle

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Introduction: Recent studies suggest that liver fluke (*Fasciola hepatica*) infections in cattle have increased in Denmark in recent years. This case-control study aimed to identify potential farm level risk factors for liver fluke infection in Danish dairy farms using two different diagnostic methods.

Methodology: Based on liver condemnation data of all individual cattle slaughtered in Denmark, 145 and 77 farms were selected as cases and matched controls. The selection criteria were; 1) minimum 50 animals were slaughtered in 2013, 2) minimum three cases of liver condemnation due to liver flukes in 2013 (case) / no history of liver condemnation due to liver flukes for the last three years (control), and 3) control farms were located within 10 km from the case farms. Bulk tank milk (BTM) samples from the farms were analysed using enzyme-linked immunosorbent assay (ELISA), and telephone interviews were used to obtain information on the type of production, the farmers' knowledge about liver fluke infection, grazing pattern, anthelmintic treatments and management routines.

Results and discussion: Preliminary results based on 132 case and 64 control farms indicate that grazing was significantly associated with liver fluke infection ($p=0.006$). However, in 12 case herds, grazing was not applied (all-in systems), suggesting indoor rearing does not completely prevent liver fluke infections, although misdiagnosis or incorrect registration at slaughter is possible. The percentage of farms allowing heifers to graze on wet areas was significantly higher in case than control farms ($p<0.001$). Using grazing for heifers and drinking from natural waterways were associated with liver fluke infections ($p=0.07$ and $p=0.01$). Approximately 30% of case farms tried actively to avoid infection by anthelmintic treatment or preventive management measures. The prevalences of liver flukes estimated by BTM ELISA were 75% and 12.5% for case and control groups, respectively. The negative ELISA results in some of the case herds may be due to their low in-herd prevalence, but it is still under investigation.

Conclusion: Based on the risk analysis, we expect grazing management can be improved on many infected farms as part of the control of liver fluke. The substantial discrepancy between different diagnostic methods should be taken into account for future studies.

Oral poster session 4: Penny Lane

Tuesday 18 August
12:00-13:00

Ref: 0568

Poster Number: P1A204

Development of resistance to triclabendazole of liver fluke in The Netherlands

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Resistance of *Fasciola hepatica* (liver fluke) to triclabendazole (TCBZ) has been reported in many European countries. In the winter of 1998/1999, sheep on a farm in the province of North Holland died from distomatosis despite several previous TCBZ treatments. In a randomised clinical trial, using TCBZ and closantel in sheep, and TCBZ and clorsulon in cattle, fluke egg output was monitored weekly for 3 weeks. The results showed a significant reduction in fluke egg output at 21 days in animals treated with clorsulon/closantel compared to those treated with TCBZ, indicating the presence of TCBZ-resistant *F. hepatica* on this farm. In the following years, TCBZ resistance was found on several farms in the same area, probably indicating spread of resistance. This area has excellent conditions for survival of a large population of *Galba truncatula*.

On the farm with proven TCBZ-resistance, closantel was used as a sole treatment to liver fluke in sheep for four years. Afterwards, TCBZ was used again but resistance was still present and no return to susceptibility was noticed. Consequently, in the years after, spread of TCBZ-resistance in the Netherlands was expected to become a growing problem. In a study in 2007 on farms with suspected TCBZ resistance in sheep, sheep were confirmed as sentinel for TCBZ resistance in cattle. On 27% of these farms no TCBZ resistance was found and on several farms the dose rate seemed incorrect. In 2008, TCBZ resistance was first found on sheep and cattle farms outside the original area in North Holland, and is from then on starting to spread all over the country. The total number of farms where resistance to TCBZ, proven by a faecal egg count reduction test (FECRT), is now 99. After an active approach of veterinary practitioners in 2013 another 177 farms with suspicion of TCBZ resistance to liver fluke were indicated. FERCT on 17 of these farms confirmed TCBZ resistance to liver fluke on 10 farms. In the Netherlands the only registered anthelmintic against fluke in sheep, beside TCBZ, is closantel. Till now resistance to closantel is not found.

Oral poster session 4: Penny Lane

Tuesday 18 August
12:00-13:00

Ref: 0573

Poster Number: P2I359

Secretory/excretory proteins identified from the rumen fluke *Calicophoron daubneyi*

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Paramphistomosis is a significant cause of morbidity and mortality in ruminant livestock in subtropical and tropical areas, caused by several genera of digenean trematodes within the family Paramphistomidae. In recent years there has been a rise in paramphistome infections of ruminant livestock in the temperate climates of western Europe, with a number fatalities reported in association with severe haemorrhagic enteritis, produced as immature paramphistomes invade the submucosa of the small intestine. To date, *Calicophoron daubneyi* is the only paramphistome found infecting livestock in western Europe, with a prevalence of up to 100% of cattle in some Irish herds infected, and average prevalence of ~20% reported in cattle in north-western Spain. Current understanding of the fundamental biology of this parasite is surprisingly fragmented relative to other helminths of veterinary importance. Disease from *C. daubneyi* infection cannot be considered as a current concern for livestock producers and veterinarians, given the other headline issues of parasitic infections such as fasciolosis and anthelmintic resistance. However, *C. daubneyi* has the potential to cause significant pathology within the rumen, clinical disease and production losses. Therefore, a detailed discovery biology study is warranted. Here, we present findings of the host-exposed excretory/secretory proteome of mature *C. daubneyi*, collected from the rumen of naturally infected cattle at a local abattoir. Our results indicate the release of parasite proteins into the rumen environment which may have important implications for future diagnostic, immunological and production focussed studies.

Oral poster session 4: Penny Lane

Tuesday 18 August
12:00-13:00

Ref: 0638

Poster Number: P2C326

Use of FAMACHA© scoring of anaemia to detect chronic *Fasciola hepatica* infection in sheep

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While targeted selective treatment (TST) of individuals within a flock is increasingly advocated to support the sustainability of gastrointestinal nematode infections in sheep, similar approaches for the liver fluke *Fasciola hepatica* have received little attention. In part, this could be due to the lack of simple, validated indicators of the level of infection and disease in individuals. FAMACHA© is a well-proven system for the detection of anaemia in sheep, which is widely used to support TST of haemonchosis in sheep and goats. FAMACHA© scores were estimated in 288 sheep presented for slaughter in winter in the UK, and compared with liver condemnation rate and the adult fluke burden in condemned livers. Those individuals whose livers were condemned had significantly higher FAMACHA© scores, indicating a greater degree of pallor. Condemned livers were dissected for adult flukes, and all were found to be infected (n = 62, fluke burden 2-485). The number of flukes recovered from livers was highly correlated with FAMACHA© score ($r^2 = 0.54$, $p < 0.001$), and with plasma packed cell volume ($r^2 = 0.24$, $p < 0.001$). FAMACHA© score was not correlated with nematode faecal egg count (FEC), and it was estimated that treatment of sheep tested in winter with a combination fluke-nematode anthelmintic would have preserved between 27 and 100 % of nematodes in refugia on the basis of FEC (n = 11 groups). Results suggest that FAMACHA© could provide a tool for the detection of individual sheep in need of treatment for *F. hepatica*, in the chronic phase of infection. This could support TST for liver fluke. Additionally, its application when using combination anthelmintic products could help to preserve drug efficacy against nematodes. The dangers of failing to treat animals in the acute phase of infection that are not yet pale should be considered, especially if changing epidemiology increases the likelihood of such infections in winter.

Oral poster session 5: Moo-ving on

Tuesday 18 August
12:00-13:00

Ref: 0064

Poster Number: P2E390

***Neospora caninum* nucleic acid can be detected in caprine and bovine colostrum**

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Neosporosis causes abortion in cattle with serious economic impact worldwide. To elucidate the presence of this parasite in milk, we examined 582 goat milk and 55 cattle milk, especially colostrum, by conventional PCR and nested PCR, for the presence of *Neospora caninum* DNA. Of the 582 goat milk examined, 544 were colostrum, and of the 55 bovine milk, 52 were colostrum, respectively. Among the 582 goat milk, 3 were positive by the conventional PCR method and 130 positive by the nested PCR. Of 130 positive milk samples, 122 were colostrum. Thus, the percentage of caprine colostrum positive for *N. caninum* DNA was 22.4% (122/544). Among the 55 bovine milk, none was positive by the conventional PCR method but 10 were positive by the nested PCR. Of 10 positive milk samples, 9 were colostrum. Thus, the percentage of bovine colostrum positive for *N. caninum* DNA was 17.3% (9/52). All products amplified by conventional PCR and nested PCR were sequenced to confirm that the amplicons were those of *N. caninum*. This is the first finding of *N. caninum* DNA in goat milk. Loop-mediated isothermal Amplification PCR (LAMP) was also developed for the rapid diagnosis of neosporosis using milk samples. This method may be useful for application in the dairy farm. Since our results showed that *N. caninum* DNA can be detected in both caprine and bovine colostrum, it indicates the possibility of transmammary infection route as well as its implication for milk hygiene.

Oral poster session 5: Moo-ving on

Tuesday 18 August
12:00-13:00

Ref: 0106

Poster Number: P2C316

Impact of coccidia-specific control protocol on medium term growth performance in a commercial Spanish feedlot-farm

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In the study, 154 animals (56.40 ± 21.38 days old) were randomly allocated into three groups (V1, V2 and control group (C, n=79)) at the feedlot entry. Each group was housed separately. Groups V1 and V2 were randomly administrated either once a single dose (day 14, n= 37, V1) or twice a single dose separated by 14 days (days 0 and 14, n=38, V2) of diclazuril (Vecoxan®, 1 ml/2.5 kg BW). Based on clinical symptomatology and fecal score from 0 to 4 (Taylor and Catchpole, 1994), incidence of clinical disease on the different groups was evaluated. No clinical disease was diagnosed and the maximum fecal score was 2 (liquid faeces). Fecal score 2 has only been found in the control group. During the first 55 days in the feedlot phase, calves in group V2 gained 149 g/day more than calves allocated in the control group (1353 Vs 1204 g/d; $p=0.03$; V2 Vs C, respectively). Group V1 was not statistically different from V2 and C, however calves from group V1 grew 61 g/day more than those in group C (1265 Vs 1204 g/d; $p=0.38$; V1 Vs C, respectively). These effects on growth performance in a period longer than 2 parasitic cycles (42 days) could be due to the capacity of diclazuril to allow development of natural immunity against coccidiosis, as previous research have found. The choice of correct timing for the beginning of preventive treatment must be carefully determined based on history and farm management. In this kind of feedlot systems, the sanitary status of the calves is unknown at arrival, and the life-cycle status of the parasite differs in each animal; some calves could be heavily infected and others not. In conclusion, treatment with diclazuril at 1 mg per kg bodyweight improved the performance of feedlot calves during first 55 days, and this effect was significant when calves were treated twice with 14 days interval.

Oral poster session 5: Moo-ving on

Tuesday 18 August
12:00-13:00

Ref: 0258

Poster Number: P2D423

Molecular and serological survey of bovine besnoitiosis in selected provinces of South Africa

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Bovine besnoitiosis is a chronic and debilitating disease characterized by cutaneous and systemic manifestations and is caused by a cyst forming apicomplexan parasite (*Besnoitia besnoiti*). It is an economically important disease as it causes weight losses, decreased milk production, abortions, sterility and reduced hide quality. In South Africa, the disease has been previously reported in Limpopo, North-Western, Mpumalanga and KwaZulu-Natal provinces, with reported serological prevalence of less than 10%. The aim of our study was to determine the distribution of the parasite within South Africa using both serology and molecular detection of the parasite.

A total of 315 blood samples from three (3) provinces [189 from Gauteng (12 herds), 71 from Limpopo (2 herds) & 60 from Eastern Cape (2 herds)] were collected from cattle. Five (5) blood samples were collected from goats in Limpopo. Conventional PCR was done using primers targeting the ITS region of ribosomal DNA of *B. besnoiti*, as described by Cortes et al. (2007) on all the samples. ELISA was done on 195 serum samples to detect presence of antibodies against *B. besnoiti* using the PriorCheck® Besnoitia AB 2.0 kit (Life Technologies).

Using PCR, the prevalence from Gauteng, Limpopo & Eastern Cape provinces were 12% (22/189), 26% (17/71) and 17% (10/60) respectively. Antibody prevalence for Gauteng, Limpopo and Eastern Cape provinces were 64% (70/110), 85% (34/40) and 76% (34/45), respectively. 20% (1/5) of the goats were positive by PCR to *B. besnoiti*. The herd prevalence were 83% (10/12), 100% (2/2) and 100% (2/2) for Gauteng, Limpopo and Eastern Cape provinces, respectively.

Our study highlights the high prevalence of the parasite within South African herds as well as new reports of the presence of the parasite circulating in Gauteng and Eastern Cape provinces. As far as we know, this study also shows first report of *B. besnoiti* in goats. There is thus a need to carry out more extensive studies to determine the distribution of the parasite across all the provinces of South Africa as well as to sample more goats in these farms/communities to see if they harbour the *B. besnoiti* parasite as well, thus possibly indicating their importance in the epidemiology of the disease.

Oral poster session 5: Moo-ving on

Tuesday 18 August
12:00-13:00

Ref: 0329

Poster Number: P2I354

Development of improved diagnostics for the detection of *Neospora caninum* chronically infected cattle

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Neospora caninum represents a major cause of reproductive failure due to abortion and neonatal mortality in cattle worldwide. At present, control of bovine neosporosis relies mainly on biosecurity and diagnostics as effective vaccines and licensed pharmacological treatments are unavailable. Although widely used for the *in vivo* diagnosis, serological tests show limitations deriving from the drop of antibody levels against the actively growing tachyzoite stage of the parasite in chronically infected cows. In these animals, *N. caninum* resides in form of the slowly replicating bradyzoite stage developing within tissue cysts and expressing a different antigenic repertoire. In order to identify *N. caninum* antigens expressed by the bradyzoite stage, a set of genes putatively encoding antigenic proteins was selected. As *N. caninum* and *Toxoplasma gondii* genomes display a high degree of synteny with many protein-coding genes showing a one-to-one correspondence, *T. gondii* was used as a model. Selection of *N. caninum* candidate genes was performed either by orthology or predicted protein homology with already characterised *T. gondii* antigens that are known to be expressed by the bradyzoite stage. Recombinant proteins were produced using bacterial expression systems and their immunoreactivity was evaluated through Western-Blot using a panel of reference sera. Besides reacting with sera from both naturally and experimentally infected animals, some of the candidate antigens reacted with sera from animals that tested negative with commercial serological tests but in which tissue cysts were detected. The aim is to employ these recombinant antigens for the development of a serum antibody ELISA able to identify cows chronically infected with *N. caninum*.

Oral poster session 5: Moo-ving on

Tuesday 18 August
12:00-13:00

Ref: 0352

Poster Number: P1A193

Confirmation of Macrocyclic Lactone Resistance in *Ostertagia ostertagi* from Cattle in New Zealand

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Although resistance to macrocyclic lactone anthelmintics in *Ostertagia ostertagi* had been suspected in New Zealand, it had never been confirmed.

In this study, four suspected cases were investigated after routine anthelmintic testing on commercial farms. Initially a comprehensive faecal egg count reduction test (FECRT) was undertaken using oral formulations of ivermectin (0.2 mg/kg), albendazole (5.0 mg/kg) and levamisole (7.5 mg/kg) on all four farms. The proportions of *O. ostertagi* in the untreated control and post-treatment larval cultures were used to determine efficacy against this species.

Subsequently, the *O. ostertagi* isolates from two of the farms were recovered and used to infect 18 six month old calves for each isolate. The efficacy of oral ivermectin and moxidectin, both at 0.2 mg/kg, against these two isolates was determined by slaughter and worm count trial.

The efficacy of ivermectin against *O. ostertagi*, based on differentiated FECRT for each of the farms was 45%, 39%, 71% and 0%. Efficacy results from worm counts in the slaughter trial for the first two isolates were 54% and 12% respectively for ivermectin and 100% for both with moxidectin. In addition albendazole, at a dose rate of 5mg/kg, failed to achieve 95% efficacy for two of the isolates (82 and 85%). These results confirm the presence of macrocyclic lactone resistant *O. osteragi* in cattle in New Zealand and the likely presence of dual resistance, to macrocyclic lactones and albendazole, in some isolates.

Oral poster session 5: Moo-ving on

Tuesday 18 August
12:00-13:00

Ref: 0371

Poster Number: P1G089

Liver fluke, rumen fluke and *Ostertagia spp.* occurrence in slaughter cattle: associated factors and impact

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Liver, rumen and abomasum of slaughter cattle were examined for fasciolosis, paramphistomosis and ostertagiasis according to the methods described in Forbes et al. (see concurrent abstract). A total of 935 abomasa, 938 rumen and 953 livers, originating from 156 herds in England and Wales, were examined. The factors and carcass parameters (cold carcass weight, conformation score and fat coverage) impact associated with the occurrence and parasitic-specific gross lesions were investigated by multinomial and linear mixed models respectively, with herd as a random effect. Preliminary results are presented here. After controlling for other factors, there were significant differences in the distribution of abomasal and liver damage among cattle by type of breed, with dairy cross breeds more likely to have lesions due to *Ostertagia spp.* and beef breeds (pure and cross) more likely to have liver damage compared to pure dairy breeds. Animals older than 30 months were more likely to present rumen fluke and *Ostertagia spp.* lesions than animals younger than 24 months and heavier carcasses (>300kg) less likely to present *Ostertagia spp.* lesions than carcasses weighing less than 300 kg. Steers, heifers and young bulls were more likely to present either abomasal lesions or rumen fluke, but less likely to present liver damage compared to cows. A seasonal pattern was present for all types of parasites lesion/presence, with a general tendency of more occurrence and severe lesions for animals slaughtered in October-January compared to March-June. Carcasses with evidence of any one of the parasite presence/lesion were more likely to have presence of another concurrent parasite presence/lesion. A rumen surface damage of 10-50% was associated with a decrease in cold carcass weight. Moderate to severe abomasal lesions and presence of rumen fluke were associated with a decrease in the continuous numerical scale of conformation grades. There were no significant associations between parasite presence/lesions and carcass fat coverage.

Oral poster session 5: Moo-ving on

Tuesday 18 August
12:00-13:00

Ref: 0372

Poster Number: P1G090

The occurrence of liver fluke, rumen fluke and abomasal pathology associated with *Ostertagia spp.* in slaughter cattle

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Specific viscera from slaughter cattle were examined post-mortem quarterly on four days within a 12-month period in an abattoir in south-west England. On each occasion the liver, rumen and abomasum were examined from every animal and the organs scored for the severity of: liver pathology (scores 0-2) and presence of liver fluke (+/-); proportion of the ruminal epithelium that was colonised (scores 0-3) and presence of adult rumen fluke (scores 0-4); and abomasal lesions characteristic of *Ostertagia spp.* (scores 0-3). The scoring was conducted by the same operators without knowledge of the identity of the animal. Standard abattoir records were collected in order that the results of the examinations could be further analysed by age, breed and other demographic information. Over the four data collection days, viscera from 974 animals were examined, comprising 53% steers, 20% cows, 16% heifers, 11% young bulls and <1% mature bulls. The overall proportion of livers with pathology typical of *Fasciola hepatica* infection was 29% and of these, 85% also had liver fluke. Rumen flukes were observed in 25% of the cattle and in 49% of these, more than 10% of the rumen surface was colonised. The proportion of abomasa that showed lesions of ostertagiasis was 89% and of these, 40% had scores of 3 (>1000 lesions). The number of cattle that were co-infected with two or more helminth parasites was 353 (39%). Of the 231 animals positive for rumen fluke, 103 (45%) were also co-infected with liver fluke. The highest prevalence of rumen fluke occurred in the October sample (29%), the lowest in March (17%). The prevalence of liver fluke was highest in both October and January (32%) and lowest in March (22%). These data will be used to investigate various host-parasite interactions and their impact on carcass characteristics.

Oral poster session 5: Moo-ving on

Tuesday 18 August
12:00-13:00

Ref: 0388

Poster Number: P1B067

Results of bovine trichomonosis diagnosis in bulls from beef cattle herds in Spain

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Bovine Trichomonosis (BT) is a sexually transmitted diseases (STD) which is prevalent in areas where natural breeding in beef cattle is widely practiced. In the last years, Spanish beef cattle population managed in extensive outdoor grazing systems has increased significantly. These changes in farming practice have led to the re-emergence of BT due to the presence of risk factors for STD in these systems. The systematic approach for BT control relies upon identification of infected bulls followed by their removal from the herd. The purpose of this study was to analyze the diagnostic results for BT generated in the SALUVET laboratory from 2009 to 2014. From 2009 to 2010, bull testing was required by the veterinary for the differential diagnosis of early reproductive failure in the herd. In this period, BT were identified in 30.8% (36/117) of bulls tested. From 2011 to 2014, an increase in the BT diagnosis demand was observed and bull testing was starting to be considered as a part of the herd health scheme (only in 2014 a total of 900 bulls were testing). Consequently, a significant decrease in the number of infected bulls was detected compared to 2009-2010 years (16.4%, 289/1763, $P<0.001$). Nevertheless after 4 years of testing and culling policy, bull prevalence did not vary between 2011-2012 (15.07%) and 2013-2014 (16.9%) periods ($P>0.05$). In addition, a significant higher BT prevalence was found in bulls from beef herds managed in mountain areas where several owners use common pastures (19%) than in those from cattle reared in fenced grasslands from Western Spain (14%) ($P<0.001$). This study highlight the importance of BT in beef cattle kept in extensive husbandry systems and the need for implementation of routine BT diagnosis in breeding bulls in Spain. Vaccination could be as a feasible and efficient measure for BT control, specially an areas where several owners use common pastures.

Oral poster session 5: Moo-ving on

Tuesday 18 August
12:00-13:00

Ref: 0490

Poster Number: P1A201

Anthelmintic efficacy in cattle: an investigation of new methodologies for robust interpretation of a FECRT

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Anthelmintic Efficacy in cattle is assessed in the field using the Faecal Egg Count Reduction Test (FECRT) (1). FECRT outcome is heavily influenced by group size, pre-treatment faecal egg count (FEC) aggregation & FEC technique sensitivity (2). WAAVP guidelines (1) do not take full account of these or other factors (such as incorrect dosing or sample handling) which could result in a “negative” FECRT, and offer a prescriptive interpretation of FECRT with pre-defined pass/fail criteria (failure is often interpreted as “Resistance”). FEC data from 88 FECRTs conducted in first-season animals in geographically distinct English regions were analysed by calculating percentage reductions in egg counts using pre- and post-treatment group means (3), and 95% confidence intervals (CI) constructed. Macrocytic Lactone (ML; ivermectin (IVM) and doramectin (DOR) pour-on & injectable) and fenbendazole (FBZ; drench) were used. Results were interpreted using WAAVP criteria, and using two “expert systems” taking into account group size, pre-treatment FEC and aggregation (Expert System 1 (ES1)), and in addition the season in which the FECRT was conducted and larval speciation data generated from culture and larval identification of pre- and post-treatment samples was considered (Expert System 2 (ES2)). Using WAAVP criteria, 25/88 FECRTs demonstrated efficacy (9/19 DOR injection, 4/8 DOR pour-on, 10/18 FBZ, 1/27 IVM injection, 1/16 IVM pour-on), the remainder being classified as “Lack of Efficacy”; Using ES1, 7/88 FECRTs demonstrated efficacy (3/19 DOR injection, 4/18 FBZ), 8/88 demonstrated “Lack Of Efficacy” (LOE) (2/8 DOR pour-on, 5/27 IVM injection, 1/16 IVM pour-on) with the remaining 73 FECRTs “inconclusive”. Using ES2, 18/88 FECRTs demonstrated efficacy (11/19 DOR injection, 1/8 DOR pour-on, 6/18 FBZ), 40/88 demonstrated “(Suspect) Lack Of Efficacy” ((S)LOE) (1/19 DOR injection, 2/8 DOR pour-on, 6/18 FBZ, 19/27 IVM injection, 12/16 IVM pour-on) with the remaining 30 FECRTs “inconclusive”. An “intelligent” analysis of FECRT data including level and distribution of FEC and parasite species information classified many WAAVP “LOE” FECRTs as “inconclusive”. Caution is necessary in interpreting farms showing “LOE” under current WAAVP guidelines, and clear industry guidance on statistical methodologies for efficacy calculation is required to avoid misclassifying “inconclusive” farms as “LOE” or Resistance.

Oral poster session 5: Moo-ving on

Tuesday 18 August
12:00-13:00

Ref: 0620

Poster Number: P1A205

Implementing rational drug use in smallholder cattle populations of northern Togo

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TRYRAC is a 5 year project (2012-2017) funded by the European Commission. The overall objective is improving trypanosomosis management of livestock smallholders in western, eastern and southern Africa- represented by Togo, Ethiopia and Mozambique. State-of-the-art control strategies were designed for northern Togo after having identified main constraints such as high vectorial pressure, illegal drug markets, high calf mortality and limited access to veterinary care. Farmers' and local veterinarians' knowledge is enhanced and rational drug use as well as affordable vector control in selected study herds are being implemented. Concurrent extension messages for dissemination of these best-bet strategies are developed. A core element of this strategy is usage of readily available resources such as operating research facilities, field personal and veterinary drugs in order to ensure sustainability. In the process, the combined application of trypanocides (diminazene aceturate and isometamidium chloride), anthelmintics (Albendazole) and an insecticidal formulation (deltamethrin) on endo- and ectoparasites was tested in northern Togo. While an overall impact assessment will take place after 24 months of implementation, preliminary results indicate a mean decrease in faecal egg counts of 98.2% (CI 95%-100%) and a significantly lower tick infestation compared to control animals. The efficacy of targeted trypanocidal treatments has yet to be evaluated. Ongoing studies will show if the implemented herd management program has the potential of improving the trypanosomosis chemo-resistance situation and animal health in Togo.

Oral poster session 6: Help!

Tuesday 18 August
15:00-15:30

Ref: 0122

Poster Number: P2E393

Prevalence, species identification and genotyping *Cryptosporidium* from livestock and deer in a catchment in the Cairngorms with a history of a contaminated public water supply

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Cryptosporidium, in particular *C. parvum*, is an important zoonotic parasite which represents a threat to animal health, water quality and public health. The main reservoirs of *C. parvum* are known to be farm livestock, particularly neonatal calves, but the contribution from other livestock and wildlife to the prevalence of *C. parvum* oocysts in water catchments is unclear. This study aimed to establish *Cryptosporidium* prevalence, species and genotypes present in livestock, deer and water samples from a water catchment with a history of *Cryptosporidium* contamination in the public water supply. A novel method of processing adult ruminant faecal samples was used to concentrate oocysts, followed by nested species specific multiplex (nssm) PCR, targeting the 18S rRNA gene to detect and speciate *Cryptosporidium*. Results indicated a very high prevalence of *Cryptosporidium* with a predominance of *C. parvum* in livestock, deer and water samples. A multilocus fragment typing (MLFT) tool was used, in addition to GP60 sequencing, to genotype *C. parvum* positive samples. Four GP60 subtypes were detected within *C. parvum*, with the majority IIaA15G2R1, which was detected in all host species and on all farms. MLFT further differentiated these into 6 highly related multilocus genotypes. It is anticipated that project outputs, including knowledge exchange activities with farmers and land owners, alongside fencing off the water courses above the public water supply and provision of water troughs for livestock, will reduce the cycles of *Cryptosporidium* contamination previously recorded in the public water supply.

Oral poster session 6: Help!

Tuesday 18 August
15:00-15:30

Ref: 0130

Poster Number: P2E394

Antibody prevalence of rickettsial infections in different human populations and molecular detection of rickettsiae in animal ectoparasites in Malaysia

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Rickettsioses are emerging infectious diseases transmitted through bites of arthropods such as ticks and fleas. As limited information is available on rickettsial infection in Malaysia, this study was designed to assess the antibody prevalence of spotted fever group rickettsia (*Rickettsia conorii*), and flea-borne rickettsia (*Rickettsia felis*) among 87 farm workers and 102 rural residents. Ticks and fleas collected from pet and domestic animals were screened for the presence of rickettsiae using PCR assays targeting *gltA*, *ompA* and *ompB* genes. IgG antibodies against *R. conorii* and *R. felis* were detected from 50% and 22.5% of rural residents, respectively. The seroprevalence of *R. conorii* and *R. felis* among farm workers were 13.8% and 16.1%, respectively. The seroprevalence of rickettsial infections in both populations were significantly ($p < 0.001$) higher than those of urban blood donors (3.3%, 0%). Of 153 fleas collected from cats and dogs in rural area, 92 (68.4%) fleas were positive for rickettsiae. Rickettsial DNA was amplified from 46.4% (26/56) ticks collected from pet animals in rural area, 9.3% (25/270) of cattle ticks, 39.4% (13/33) of dog ticks and 9.3% (17/182) of cat fleas in the urban area, respectively. The high carriage rates of rickettsiae in ticks in the rural areas could be associated with the high seroprevalence of spotted fever group rickettsial infections in the population understudied. The seroreactivity of *R. felis* in the local population was reported for the first time. Appropriate prevention and control measure are necessary to reduce the exposure of humans to rickettsial infections in Malaysia.

Oral poster session 6: Help!

Tuesday 18 August
15:00-15:30

Ref: 0132

Poster Number: P2E395

Genetic diversity of *Toxoplasma gondii* in St. Kitts, West Indies

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Toxoplasma gondii is a ubiquitous protozoan parasite capable of infecting all warm-blooded animals. Humans become infected with *T. gondii* post-natally by ingesting tissue cysts from meat, or by ingesting oocysts from contaminated food or water, or directly from the environment. Disease outcome can vary depending on a number of factors, including genetic diversity of the infecting strain. We demonstrated a high prevalence of *T. gondii* in livestock on St. Kitts suggesting widespread environmental contamination with oocysts. The aim of this study was to determine the genetic diversity of *T. gondii* in free-roaming chickens on St. Kitts. Eighty one chickens were collected from 9 locations around the island and their hearts and brains were digested and inoculated into 243 CD1 mice in a bioassay. Aliquots of digested chicken material were examined for *T. gondii* DNA using quantitative PCR and 25% (20/81) were positive. Sera from chickens were examined for *T. gondii* antibodies using an in-house ELISA, and reactive antibodies were detected in 93% (75/81) of samples. Left lung was collected from all mice following bioassay for *in vitro* culture in Vero cells. Live *T. gondii* was isolated from 20% (16/81) of chickens, from 7 out of 9 locations on the island, highlighting the potential public health risk should chicken meat be consumed (or handled) raw or undercooked. *T. gondii* isolates were cryopreserved for genotyping and virulence studies. Right lung and brain were also collected from all mice following bioassay for genotyping using 11 genetic markers (5'SAG2, 3'SAG2, SAG3, GRA6, BTUB, SAG1, C22-8, C29-2, L358, PK1 and Apico). These results will be discussed.

Oral poster session 6: Help!

Tuesday 18 August
15:00-15:30

Ref: 0266

Poster Number: P1B066

The Identification of New *Blastocystis* Subtypes in Non-Human Primates

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Blastocystis is an enteric protist which is known to infect humans and a variety of animal hosts, including numerous non-human primate (NHP) species. The genetic diversity of *Blastocystis* isolates from captive and wild NHPs in four countries is evaluated. This study is one of the first to report the detection of *Blastocystis* in the Guatemalan black howler monkey (*Alouatta pigra*), and to characterise *Blastocystis* isolates from NHPs in Central America. Isolates belonging to subtypes (STs) 1, 2 and 8 were identified. ST 1 primarily consisted of isolates from captive NHPs, and was not detected in wild NHPs. Both wild and captive NHPs harboured ST 2. ST 8 was detected in wild NHPs. Three new STs assigned 18, 19 and 20 were isolated from the Javan lutung (*Trachypithecus auratus*), colobus monkey (*Colobus vellerosus*) and Guatemalan black howler monkey, respectively.

Oral poster session 6: Help!

Tuesday 18 August
15:00-15:30

Ref: 0337

Poster Number: P1G088

Gastro-intestinal Parasites of Captive Olive Baboons, *Papio cynocephalus anubis*

Lydia-Helen Kutima, Michael Oduor

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A wide array of parasites may infect non-human primates (NHPs) that are often valuable in studies of either human disease processes or vaccine and drug development. Assessment of gastrointestinal parasites of colony experimental baboon of different age and sex was done using non-invasive technique to determine the identity; prevalence and intensity of these parasites since parasitic infections may affect animal health and ultimately the studies for which the animals are used. A total of 80 faecal samples were collected from 20 baboons: [10 Adults (5 males and 5 females) and 10 Juveniles (5 males and 5 females)]. Four faecal samples were collected per week from each animal. Examination of these faecal samples through Direct smears method to detect trophozoites of amoebae and flagellates, Formal ether sedimentation technique for the identification of parasite eggs, cysts, and larval stages. Harada-Mori culture technique for identification of larvae from L1-L3 stage and MacMaster egg counting technique for intensity of parasite eggs per gram of faecal material. *Balantidium coli*, *Entamoeba coli*, *E. histolytica*, *Trichuris spp*, *Oesophagostomum spp* and *Strongyloides spp* were isolated from the baboons.

Oral poster session 6: Help!

Tuesday 18 August
15:00-15:30

Ref: 0382

Poster Number: P2E397

Transmission of *Echinococcus* species in pastoral communities in southern Kyrgyzstan

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Echinococcus granulosus and human cystic echinococcosis (CE) are highly endemic in Central Asia and western China. Human alveolar echinococcosis (AE), caused by *Echinococcus multilocularis*, is an emergent public health problem in Kyrgyzstan and in the region. Community, veterinary and ecological investigations were undertaken in 2012 in the Alay Valley, south Kyrgyzstan. Mass ultrasound screening of the local population detected hepatic lesions confirmed to be AE (7% prevalence); no human CE cases were identified. Voluntary arecoline purgation of 20 owned dogs revealed 8 (40%) infected with *Echinococcus* spp.; PCR analysis of worms revealed presence of *E.granulosus* (G1), *E. canadensis* (G6) and *E.multilocularis*. An *Echinococcus* spp coproantigen ELISA-based survey of owned dogs (n=333) in four villages gave a copro-prevalence of 26.4% (range 21.4% to 25.7 %). PCR testing confirmed the presence of all three species in owned dogs. A logistic regression analysis found that sheepdogs had lower odds of coproantigen positivity, as did households with donkeys; some knowledge of echinococcosis; and no involvement in home slaughtering. There was no evidence of an association between free roaming or previous praziquantel dosing and coproantigen positivity, as has been found in previous studies. There was also no evidence of a difference in coproantigen prevalence between villages. Environmental sampling of canid faeces indicated high contamination levels in villages with some faecal samples positive for DNA from *E.canadensis* or *E.multilocularis*. A small mammal survey indicated high densities of Zaisan mole voles (*Ellobius tancrei*) in and around villages; *E.multilocularis* lesions were confirmed in *E.tancrei*. Ongoing surveillance from 2012 to 2014 in the face of a control programme aimed at dosing owned dogs four times annually with praziquantel appeared to reduce the coproantigen and coproPCR prevalence amongst owned dogs over a period of 28 months. Human AE is a highly pathogenic emergent zoonosis in the Alay Valley which requires sustained and coordinated intervention to reduce a considerable potential public health problem.

Oral poster session 6: Help!

Tuesday 18 August
15:00-15:30

Ref: 0531

Poster Number: P2E398

Zoonotic parasites in faecal samples from different dog, cat and human populations in Greece

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A cross-sectional study was conducted to investigate to what extent dogs, cats and humans in Greece are infected with intestinal parasites, including zoonotic species. Between October 2011 and January 2015, 884 faecal samples were collected from different dog populations (shelter dogs, household dogs and shepherd dogs), 253 faecal samples were collected from shelter cats and household cats and 874 stool samples were collected from humans (children and adults). Samples were examined for the presence of worm eggs and protozoal oocysts using both a sedimentation and a flotation technique. For the detection of *Giardia* spp and *Cryptosporidium* spp a direct immunofluorescence assay was used. In different canine populations high infection rates of zoonotic parasites were recorded such as *Giardia* (4-54%), *Cryptosporidium* (1-15%), hookworms (6-33%), ascarids (4-18%) and *Echinococcus/Taenia* (0-7%). In cats, the zoonotic parasites found were *Giardia* (16-38%), *Cryptosporidium* (6-12%), *Toxocara* (8-11%) and hookworms (5-9%). In humans, *Giardia* (1.1%) and *Cryptosporidium* (0.3%) were detected. Samples that were positive for *Giardia* or *Cryptosporidium* are being genotyped to assess their zoonotic potential. PCR protocols targeting the 18S rDNA gene, the β -giardin, the tpi and gdh gene, are used for the molecular identification of a subset of *Giardia* isolates. Interim results revealed the presence of dog-specific *G.duodenalis* assemblage C and D, as well as potentially zoonotic assemblages A and B, in different dog populations. For the molecular characterization of *Cryptosporidium* isolates PCR protocols targeting the 18S rDNA gene and HSP70 gene are used.

Oral poster session 6: Help!

Tuesday 18 August
15:00-15:30

Ref: 0533

Poster Number: P1B072

Occurrence of parasitic infestations in Myanmar Timber Elephants

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Introduction: World's largest semi-captive Asian elephant population (n≈4000) is employed in timber industry of Myanmar. The highly seasonal climatic and variable ecological conditions of Myanmar are favourable for the growth, multiplication and transmissions of parasites which cause significant health problems in timber elephants where they can act as disease carriers to wild counterparts sharing the same foraging grounds.

Materials and Methods: Between September, 2012 and December, 2014, a total of 548 (males=234 males; females=324 females; age range =5 yr to 50 yr) fecal samples of timber elephants from Nay Pyi Taw (South and North) and Taungoo timber extraction agencies were sent to the Department of Pharmacology and Parasitology, University of Veterinary Science, Nay Pyi Taw for the presence of parasitic infestation. Sedimentation method was used to determine the parasite egg count per gram of stool. A Pearson's chi-square test was used to test a correlation between occurrence of parasites (eggs per gram) and age, sex and months of collection using SPSS for windows (version 12.0).

Results: Amphistome and Strongyles were the most common parasites with the prevalence of 34.85% (191/548) and 27.91% (153/548), respectively. The highest prevalence was found in August and September. Age and sex were found no relationship with parasitic infestations.

Oral poster session 6: Help!

Tuesday 18 August
15:00-15:30

Ref: 0674

Poster Number: P2E399

Gastrointestinal and liver helminthic infections in house mice (*Mus musculus domesticus*) from the Madeira Archipelago: implications on Animal and Public Health

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Rodents are major reservoirs for parasites with important implications for human and animal health. The house mouse, due to its commensal habits and proximity with human dwellings is one of the most problematic species concerning the spread of zoonotic parasites. In Madeira and Porto Santo islands, this risk is potentiated by high population densities and a close relationship with humans and agricultural products. In this study, we assessed the parasitofauna of these insular populations for the first time. Livers of 120 mice, 100 mice from Madeira and 20 from Porto Santo, were screened for lesions and parasites, being 67 (55.8%) positive. In Madeira, mice were positive for 56% of the samples and in Porto Santo for 55%. *Calodium hepaticum* was found in 28 mice (23.3%), *Cysticercus fasciolaris* in 25 (20.8%) and *Cysticercus* sp. in 19 (15.8%). These last two species were found on both islands while *C. hepaticum*, a zoonotic species, was only detected in Madeira and spread throughout the island. When compared with results from previous studies on other archipelagos, namely Azores and Canary islands, *C. hepaticum* infection showed an intermediate prevalence in Madeira (28%). *C. fasciolaris* was more prevalent in Madeira (23%) than in the Azores and Canary Islands. The digestive systems of the 120 mice were also dissected and screened, 109 (90.8%) of which were positive for parasites. 90% were positive for at least one parasite species for both Madeira (n=100) and Porto Santo (n=20). Much like for the hepatic parasites, differences were observed between parasite species composition of the two studied islands. *Mastophorus muris* (10%), Trichostrongylidae (4%), *Syphacia obvelata* (75%), *Trichuris muris* (50%) and *Heterakis spumosa* (5%) were found in Madeira, whilst in Porto Santo only *Syphacia obvelata* (90%) was detected. This variation across islands may be due to differences in mouse population densities, genetic pool, colonization origin and/or different levels of interaction with definitive hosts. *C. hepaticum* and *C. fasciolaris* are zoonotic agents whose life cycles involve domestic carnivores in the proximity with humans. *S. obvelata* also has a zoonotic potential due to its life cycle and was detected throughout both islands. The human infections by these parasites are usually underestimated, possibly becoming a serious concern in terms of Public Health.

Oral poster session 7: Here, There and Everywhere (2)

Thursday 20 August
12:00-13:00

Ref: 0052

Poster Number: P2C314

ParaBoss: Australia's sheep parasite control resource

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ParaBoss is the national organisation that leads the development and extension of best practice information, training and tools to improve parasite management of sheep in Australia. Information sharing and better communication through ParaBoss has been a timely response to loss of State funds for animal health services with benefits for cross-regional parasite control. Steering and Technical Committees provide governance for ParaBoss with the latter contributing specialist expertise in the control of worms, flies and lice across all sheep producing regions of Australia, and close connections with industry.

The ParaBoss suite includes WormBoss, FlyBoss and LiceBoss websites, a monthly subscriber based e-newsletter, an on-line Technical Forum and a biennial national workshop. Web user numbers doubled during 2014 to average 7,000 per month with 30% of sessions originating from outside of Australia. On-line learning providing both a structured reading and a question and answer process has been incorporated into the Boss websites. The monthly e-newsletter containing State outlooks and feature articles is sent to 3,500 subscribers. The ParaBoss Technical Forum is restricted to animal health professionals, with current membership just over 100, where important and contentious issues for the control of worms, flies and lice are debated as part of the process of forming industry messages. The national workshop is planned to extend this process and thereby identify important R, D and E issues requiring attention by funders.

ParaBoss is managed by the University of New England, with the initial phase under licence from the Sheep CRC and financial support provided by Australian Wool Innovation Ltd and Meat and Livestock Australia. The ParaBoss Business Plan is the basis for developing into a cost-recovery organisation and identifies the key strengths being a respected, independent and trusted brand, having high traffic web sites providing exposure to national and international markets and providing an opportunity for engagement with industry and the education sector.

Oral poster session 7: Here, There and Everywhere (2)

Thursday 20 August
12:00-13:00

Ref: 0110

Poster Number: P2C317

Effect of protein supplementation when using creep feeding on the susceptibility of suckling lambs to gastrointestinal nematodes

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The effect of protein supplementation on the susceptibility of suckling pasture-raised lambs to gastrointestinal nematodes was evaluated at the present study. Lambs were separated at birth in two treatment groups: control (n=15) and creep feeding (n=16) that received 6 g crude protein/day. Further classification of the participating animals was made according to birth order, birth type, and sex of the lambs in two and 3 area repetition. Significant interaction between the age of the lambs and the obtained treatment was observed. Suckling lambs under supplementation were characterised by lower average of EPG ($P<0.05$) (404 - 100 to 1,250) in comparison to the control group (3,798 - 0 to 44,150). The majority of the treated animals (80%) indicated an EPG lower than 1,050 while 74% of the control group showed an EPG rate above 1,000. A subset of 49% of the latter group presented critical EPG values above 5,050. Administration of 10 and 27 different anthelmintic schemes was performed to lambs treated and not, respectively. However, a significant effect ($P<0.05$) was observed 60 days post treatment. when only 3 anthelmintic treatments were performed in supplemented lambs and 15 treatments were performed in the animals from the control group. Creep feeding appeared to be a practical and efficient alternative to the nutritional supplementation of the lambs, leading to the reduction of gastrointestinal parasitism.

Oral poster session 7: Here, There and Everywhere (2)

Thursday 20 August
12:00-13:00

Ref: 0263

Poster Number: P1A189

Use of pyrosequencing to genotype benzimidazole resistant and susceptible isolates of *Haemonchus contortus* during patent infection in lambs

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Resistance to anthelmintic compounds in gastrointestinal parasites of sheep is presently cosmopolitan, and single- and multiple-drug resistant species are common throughout the world. The growing importance of anthelmintic resistance has led to an increased need for reliable and standardized methods of detection. A problem of *in vitro* tests was the variable relationship between the age of the parasitic infection and the ED₅₀/LD₅₀ (concentration of drug required to kill 50 % of eggs or larvae) values in infections with *Haemonchus contortus* and *Ostertagia circumcincta*. Changes in ED₅₀/LD₅₀ values in egg hatch tests and larval development test have been described by several authors. The present study was designed to evaluate if a similar variation in numbers of resistant alleles occurs during patent period when the susceptible and resistant isolates of *Haemonchus contortus* are subjected to pyrosequencing. Lambs were infected with 5000–6000 L3 larvae of each isolate. Faecal samples were collected on 28 separate days (20-90 days) after experimental infection and coprocultures were subsequently prepared to produce L3 larvae for genotyping. Pyrosequencing (DNA from 1000–5 000 L3s) was used to measure resistant beta-tubulin isotype 1 allele frequencies at codon 200 of both isolates. The average resistant (TAC) allele frequency in the susceptible isolate was 6.5 % and varied between 0 and 10.8% to give a coefficient of variation of 37.7 %. The TACa-allele frequency for the resistant isolate was 35.7 % (range 25.6-49.25%; coefficient of variation 12.6 %). Our results confirm that there is considerable variation concerning the percentage of the beta-tubulin resistant allele frequency in susceptible and resistant *Haemonchus contortus* isolates during the course of infection.

The study was supported by Grant Agency VEGA, Grant No. 2/0151/13 of the Scientific Agency of the Slovak Academy of Sciences and Slovak Research and Development Agency Project No. 0539-10.

Oral poster session 7: Here, There and Everywhere (2)

Thursday 20 August
12:00-13:00

Ref: 0296

Poster Number: P1A190

Evaluating the efficacy of closantel against ivermectin- and fenbendazole-resistant *Haemonchus* sp. in sheep in Ontario, Canada

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In Canada, widespread resistance to both ivermectin and fenbendazole, the only readily available ovine anthelmintics, has been documented primarily in *Haemonchus* sp. In other parts of the world, closantel has been used to control such infections; however, the drug is not readily available and has not been approved for use in Canada. A study was therefore conducted on six client-owned farms in Ontario, Canada in 2013 and 2014 to determine the efficacy of closantel (Flukiver 5% Oral Suspension, Elanco Animal Health) against ivermectin- and fenbendazole-resistant *Haemonchus* sp. infections in both recently lambed ewes and grazing lambs. All treated animals were weighed and drenched with 10 mg/kg closantel. Fecal samples were collected from a minimum of 15 randomly selected animals on each farm at the time of treatment and 14 days later, with trichostrongyle-type fecal egg counts (FEC) performed using a modified McMaster technique (detection limit: 8 epg). *Haemonchus*-specific FECs were determined by multiplying total egg counts by the proportion of *Haemonchus* sp. identified from coproculture for each farm. Fecal egg count reductions were then calculated for each farm. Pre-treatment FEC ranged from 200-4,539 epg, and *Haemonchus*-specific FECs ranged from 26 – 3,359 epg. Closantel efficacy against *Haemonchus* sp. ranged from 97% (95%CI: 93%-100%) to 100% in recently lambed ewes on all farms in both years (total n=284), and from 99% (95% CI: 98% - 100%) to 100% in grazing lambs in both years on all but one of the farms tested (total n=181). On the latter farm, closantel efficacy was 84% (95%CI: 81%-88%) in the first year, but 100% in the second year. Treatment with closantel was shown to be a viable alternative for farms with documented anthelmintic resistance and problems with haemonchosis.

Oral poster session 7: Here, There and Everywhere (2)

Thursday 20 August
12:00-13:00

Ref: 0338

Poster Number: P2J246

Low repeatability of faecal nematode egg counts in British Boer goats

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Most breeds of goat are very susceptible to gastrointestinal nematode infection compared to sheep. The reasons are unclear. As part of a comparative study, we estimated the repeatability of faecal nematode egg counts (FEC) in naturally infected British Boer goats. This study was conducted on a farm in Lancaster, England from August to October 2014. A total of 161 goats of both sexes were sampled at 3 to 5 months of age. The goats grazed on the pasture throughout the summer. All goats were drenched with 2.5% benzimidazole in August and 0.1% moxidectin in September and October according to the manufacturer's recommended dosages. Faecal samples were collected per rectum from each goat in the morning within one week before drenching. The faecal samples were counted with a modified McMaster technique which was sensitive to 25 eggs per gram of faeces. Pooled faecal samples collected in each month were cultured to harvest infective stage larvae. *Teladorsagia circumcincta* was the most predominant species harvested from the faecal culture between 88% to 93% followed by *Trichostrongylus* sp. The FEC were highly over dispersed and similar to a negative binomial distribution. In this distribution the variance is equal to $\mu + k(\mu)^2$. The index of overdispersion (k) ranged from 1 to 3 throughout the study. Internal repeatabilities of FEC taken from the same sample were high at 0.74, 0.89 and 0.96 with a mean repeatability of 0.86 ($P < 0.0001$) indicating that the technique used was reliable. However, a very low repeatability was detected between FEC of each month. FEC between August and September was $r = -0.008$, FEC between August and October was $r = -0.079$ and FEC between September and October was $r = 0.077$. The relatively low correlation between the FEC suggests that resistance developed much more slowly in goats than sheep where comparable repeatabilities were much higher. The low repeatabilities also indicate that selective breeding of young Boer goats to resist infection may be difficult. In lambs, immunity depends upon IgA, IgE, eosinophils and mast cells. We now plan to follow the development of these responses in kids.

Oral poster session 7: Here, There and Everywhere (2)

Thursday 20 August
12:00-13:00

Ref: 0432

Poster Number: P1B070

Barcoding helminths in Austria - the ABOL initiative

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Barcoding of species inventories has received increasing attention in recent years, and the Austrian Federal Ministry of Science recently initiated a start-up project on barcoding various groups of organisms from Austria, including parasitic worms (helminths) - the Austrian Barcoding Of Life (ABOL) project. DNA barcoding is used to identify species of different organismic groups with standardised techniques of classical morphological identification in combination with DNA typing. To fulfil the criteria of international barcoding databases, each specimen has to be identified and described in detail, and both the data and the material have to be deposited to be accessible for the research community. While ongoing barcoding projects include parasitic metazoa as part of the lower invertebrates, we specifically initiated a project targeting nematodes, trematodes, cestodes and acanthocephalans endemic in Austria. We developed standardised protocols for specimen collection (or the use of available scientific collections) and identification as well as DNA barcoding of the cytochrome c oxidase subunit I for the available groups of helminths which will permit (a) identification of parasite material which cannot fully be identified by morphology (e.g. damaged specimen, eggs, larvae...) (b) identification of sub-species level heterogeneity (including cryptic species) in a restricted geographic area and (c) comparison of data with those already available. Barcoding of parasitic metazoa is an additional tool that complements classical morphological identification, and we are aiming to contribute to the growing data pool of the barcoding community as well as to the taxonomy and systematics in parasitology.

Oral poster session 7: Here, There and Everywhere (2)

Thursday 20 August
12:00-13:00

Ref: 0501

Poster Number: P1G091

DISCONTTOOLS: a novel initiative to prioritise research on vaccines, pharmaceuticals and diagnostics for the control of infectious diseases of animals

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It is estimated that the public and private sector in the E.U. spend around €800 million per year on animal health and welfare related research. An objective process to prioritise research by focusing on the critical gaps in the knowledge and available control tools for the important diseases can help to speed up the development and delivery of new or improved diagnostics, vaccines and pharmaceuticals. This in turn can reduce the burden of animal diseases. Expert groups were established for 52 infectious diseases of animals, with a total of 342 animal health scientists contributing to the process. For each disease, the expert groups produced a disease and product analysis document that formed the basis for gap analysis and prioritisation. The diseases were classified into three groups: epizootic diseases, food-producing animal complexes or zoonotic diseases. The highly ranked diseases in the prioritisation model comprised mostly zoonotic and epizootic diseases with important gaps identified in vaccine development and pharmaceuticals, respectively. The most important outcome is the identification of key research needs by disease. The rankings and research needs by disease are provided on a public website (www.discontools.eu) and comments may be provided on the disease specific information. The aim is to update the database in a regular cycle with new information and new diseases, so that it can become a reference point for funders of research including the European Commission, member states, foundations, trusts along with private industry to prioritise research. This will deliver benefits in terms of animal health and welfare but also public health, societal benefits and a safe and secure food supply.

Oral poster session 7: Here, There and Everywhere (2)

Thursday 20 August
12:00-13:00

Ref: 0503

Poster Number: P2I358

The recovery of added nematode eggs from horse and sheep faeces by three methods

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Equine nematodes are clinically important and anthelmintic resistance is becoming increasingly common. To obtain comparable data from different countries a standardised accurate faecal egg counting method is required. Three methods, Mini-FLOTAC (analytical sensitivity 5 epg), modified McMaster (analytical sensitivity 15 and 50 epg) and Cornell Wisconsin (analytical sensitivity 1 epg) were compared by adding known numbers of either ovine or equine nematode eggs to equine faecal samples that were egg free. Eggs were added to give final egg counts of 10, 50, 200 and 500 epg and the samples thoroughly homogenized. In addition a new method, Fill-FLOTAC, for weighing faecal samples using a measuring cone was tested. The McMaster technique suffered from debris and fine particles that reduced the illumination making counting more difficult. This was overcome with Mini-FLOTAC by translation that moves the top layer containing the eggs away from the debris below. Mini-FLOTAC gave the highest rate of recovery (90.6 to 100%), compared with McMaster (76.6 to 104.7%, thus showing overestimation) and Cornell Wisconsin (41 to 58.5%). Thus Mini-FLOTAC is the optimal method of counting equine nematode eggs. Combining Mini-FLOTAC and Fill-FLOTAC, provides the simplest and most accurate method for faecal egg counting and, as it avoids the need for weighing samples, is ideal for use on yards.

Oral poster session 7: Here, There and Everywhere (2)

Thursday 20 August
12:00-13:00

Ref: 0631

Poster Number: P1A207

Slow change in nematode control practices among sheep farmers in south-west England

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A total of 315 interviews were conducted with sheep farmers in person, at a biennial trade show in the south-west of England, over a period of eight years from 2005 to 2013. Awareness of anthelmintic resistance was high, with 83% of interviewees admitting to being concerned about the issue. Despite this, only 20% (rising from 14 to 25% over the period of study) had conducted a formal test for resistance on their farm, with 40% citing evidence of resistance from such a test. On average, farmers reported treating their lambs against nematode infection 3.3 times per year (standard deviation, sd 1.7), and their ewes 1.7 times (sd 0.8). Overall, 60% of farmers reported dosing and moving lambs to clean pasture, and 61% applied quarantine treatments on new stock brought onto the farm. Neither average routine treatment frequency nor the proportion of farmers practising dose-and-move or quarantine changed over the study period. However, in 2011-13, 14% reported treating their lambs only when needed, or employing part-group (targeted selective) treatments (7% for ewes), compared with none in 2005-07. When asked what steps they took to slow the development of resistance, farmers listed changing or rotating anthelmintic class between years (38%), reducing the number of treatments given (18%), grazing management (11%), using faecal worm egg counts (FEC) (8%), dosing to animal weight (4%), applying quarantine (2%), breeding to improve host resistance (1%), and monitoring drug efficacy (1%), while 17% of farmers stated making no specific changes. The proportion of farmers taking specific steps was not different between years, except for TST (see above) and using FEC, which rose from 1% (2005-07) to 14% (2011-13). Of farmers reporting that they used quarantine dosing, 69% stated an inappropriate drug choice (usually the single active used routinely in that year), and 37% reported turning out onto clean pasture, while there was also apparent confusion over the appropriate duration for which animals should be yarded. The proportion choosing inappropriate drugs for quarantine declined over the study period from 89% to 51%. Results suggest limited popular uptake of recommendations for sustainable anthelmintic use, but some impact among a subset of farmers, which could drive wider change in future.

Oral poster session 7: Here, There and Everywhere (2)

Thursday 20 August
12:00-13:00

Ref: 0679

Poster Number: P1B074

Spatial analysis of *Toxoplasma gondii* and *Neospora caninum* infections in small ruminants bred in Northern Italy

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Geospatial analysis allows increasing the comprehension of the association between disease processes and explanatory spatial variables. Epidemiological data on *T. gondii* (Gazzonis et al., Ann Agric Environ Med 2015 22(1):68-74) and *N. caninum* (Gazzonis et al., SIPAOC Proceedings 2014 138p.) infections in sheep and goats bred in Northern Italy were submitted to geospatial analysis with the aim to investigate on geographical factors influencing the distribution of the infections. Farms locations were plotted using Google Earth. Coordinates were calculated using Kml2x, associated to infection and imported to SaTScanTM 9.3; Kulldorff's scan statistic was performed. A Bernoulli model was performed using multiple dataset considering data on individual age, rearing system and farm size (Kulldorff et al.; Stat Med 2007 26:1824-1833). Statistical analysis on climatic data (temperature and rainfall) obtained from WorldClim 1.41 were performed. A GIS database, including information on farm location, serological results and climatic data, was generated using Quantum GIS 2.4.0. Unadjusted spatial analysis highlighted a heterogenic distribution of cases of infection, creating clusters of infection for sheep and goats for both pathogens. Adjusted analysis was then considered. For *N. caninum* all clusters resulted dependent on spatial distribution of managerial variables and any climatic variables did not enter in the final model. Regarding *T. gondii*, a cluster of high risk for goats and a cluster of low risk for sheep not dependent on considered variables were calculated; for both species, mild temperature and rich rainfall resulted to be predictors of the infection. Concluding, geographical and environmental factors enhancing the risk of the infection by *T. gondii* in small ruminants were identified. On the contrary, *N. caninum* infection appeared not dependent on geographical features but related to managerial variables. Geospatial analyses revealed useful tools for the identification of areas at high/low risk of infection; nevertheless, data should be interpreted considering individual and managerial factors, since a not homogeneous distribution of cases (due to territorial, zootechnical and economical characteristics) could lead to calculation of areas of infections not depending on geographical or environmental features but to structural characteristic of sampled herds.

Oral poster session 8: Ticket to Ride

Thursday 20 August
12:00-13:00

Ref: 0095

Poster Number: P1B065

The seroprevalence and risk factor associated with *Toxoplasma gondii* infection in horses from Thailand

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Toxoplasma gondii, an obligate intracellular protozoan parasite, is the causative agent of toxoplasmosis, recognized and infected most species of warm-blooded animal including humans, birds and almost all species of mammals over the world. The aim of this study was to determine seroprevalence and risk factors associated with *T. gondii* infections in horses, Thailand. A total of 1099 horse serum samples from 4 regions of Thailand (northern, northeastern, central and southern) were collected and examined for the presence of *T. gondii* infection by using the commercial latex agglutination test (LAT) kits (TOXOCHECK-MT; Eiken Chemical, Tanabe, Japan). Forty-seven out of 1099 (4.3%) were found to be positive to *T. gondii*, among 4 regions in this survey. Additionally, a northern region has the highest seroprevalence (6.3%) than other 3 regions. However, there was no significant age or sex associated with *T. gondii* infection in this study.

Furthermore, all seropositive samples by LAT will be tested by immune fluorescence antibody test (IFAT) to confirmed *T. gondii* infection. Moreover, further research to assess the risk for infection in human associated with the ingestion of raw or undercooked horse meat infected with *T. gondii* will be needed.

Key words: seroprevalence, *Toxoplasma gondii*, horses, agglutination, Thailand

Oral poster session 8: Ticket to Ride

Thursday 20 August
12:00-13:00

Ref: 0113

Poster Number: P2C318

Strategic and targeted selective treatment against gastrointestinal parasites of horses in Brazil

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Targeted selective treatment (TST) is a method aiming the treatment of animals with higher parasite burden or lower body weight, contrary to the traditional strategic control (TSC), based on the epidemiology of the parasites in the livestock. In the present study, we compared both TST and TSC schemes in grazing adult horses in Brazil. A randomized study performed in 15 animals, distributed in two groups according to the received treatment, was carried out in the period from April 2011 to March 2012. The TST group (n=9) was treated with ivermectin oral paste (200mg/Kg) when the number of eggs per gram (EPG) in faeces was higher than 500. The second group (n=6) received the TSC in two doses, performed in the fall and in the spring. The animals were infected almost exclusively by small strongyles (*Cyathostomes*). Ivermectin showed efficacy of 100% on the 28th day post treatment. The observed mean EPG values of the animals submitted to the TSC were constantly above 500. Performance of TST resulted in a lower overall EPG average (p=0.0423) in comparison to the group receiving the TSC. Additionally, records of the number of treatments per animal per year were made. In average, 2.22 and 3 treatment per animal were performed for the TST and TSC group, respectively. The TST resulted in effective parasite control, in spite of the overall cost, which is approximately five times higher than the TSC, including the costs of the performed examinations. The TSC was proved inefficient to maintain EPG counts at low level.

Oral poster session 8: Ticket to Ride

Thursday 20 August
12:00-13:00

Ref: 0212

Poster Number: P1A188

A field study on the efficacy of moxidectin and its egg reappearance period in donkeys naturally infected by Cyathostominae in Italy

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Moxidectin (MOX) has a wide range of ecto-endoparasitic activity in many species including horses. There is a paucity of data on the efficacy of anthelmintics used in donkeys. The aims of the present study were to evaluate the field efficacy and Egg Reappearance Period (ERP) of MOX up to 84 days at horse dose against natural infection of Cyathostominae in donkeys. The trial was conducted in northern Italy, twenty-nine donkeys, were selected on the basis of faecal egg counts (FEC) > 300 EPG. Animals were treated on Day 0 with an oral gel containing MOX, at the manufacturer's recommended horse dose (0.4mg/kg BW). FECs were performed on each donkey at least every fortnight during 84 days after treatment using a modified McMaster technique with a sensitivity 10 EPG. The efficacy and ERP values were determined following the AAEP Parasite Control Guidelines. The percentage reductions in FEC were 99.7% at the day 14, 99.5% at the day 28, 97.7% at the day 56 and 73.3% on day 84 post treatment. This trial demonstrates that MOX at horse dose was effective and safe for the treatment of Cyathostominae in donkeys, however with a shortened ERP this could be the first indication of developing anthelmintic resistance. Considering that the donkeys have a greater capacity to metabolize certain drugs compared with horses, a higher dosage or shorter intervals could be required for maintaining effective drug concentrations. The shorter ERP observed in the studied donkeys could be associated with a sub-therapeutic level of MOX.

Oral poster session 8: Ticket to Ride

Thursday 20 August
12:00-13:00

Ref: 0340

Poster Number: P1A192

A national questionnaire survey of helminth control practices on horses in Italy – preliminary results

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A national survey to obtain information of helminth control practices on horses and farm management was carried out in 2014 in Italy. Data were collected using 387 face-to-face questionnaire with questions regarding type and size of farms, use of horses, grazing management, parasite control protocol, interval worming, time of treatments, number of treatments per year, anthelmintic class used, specific treatments (tapeworms and encysted cyathostomins). In total, data of 7,616 horses were obtained. In Italy, most control protocols were strategic. Sixty eight percent of respondents administered anthelmintics at particular times of the year. With respect to worming intervals, most of them (45%) treated animals at 6 months intervals and 4% wormed horses bi-monthly. Six percent of respondents declared that they left animals untreated. With regard to the time of treatment administration, most reported that dosing was carried out independently from the season, although someone emphasized a greater attention to dosing during autumn and spring months. The mean number of treatments per year was 2.6 (range 1-12). Over half (64%) of respondents indicated that ivermectin and moxidectin or their associations (e.g. plus praziquantel) were the most commonly used anthelmintics in the previous 12 months. Treatments against cyathostomin encysted larvae and tapeworms were administered by 11% and 21% of respondents, respectively. Most owners obtained information on parasite control from veterinarians (93%), in addition to specialized journals and internet forums in decreasing order of importance. This is the first national survey investigating parasite control practices and anthelmintic use in horses in Italy.

Oral poster session 8: Ticket to Ride

Thursday 20 August
12:00-13:00

Ref: 0374

Poster Number: P1A194

Trends in ivermectin and moxidectin efficacy in client owned horses over a four year period: a retrospective study

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Rationale: The macrocyclic lactones (ML) ivermectin and moxidectin are both licensed for treatment of equine nematodes. Both drugs demonstrated high efficacy and long egg reappearance periods (ERP) post dosing when first marketed. Reductions in efficacy to this class of anthelmintics have been reported in Kentucky, South America and parts of Europe. Early egg reappearance has been highlighted as the first step towards anthelmintic resistance.

Objectives: This retrospective study was performed to determine trends in anthelmintic efficacy of ivermectin and moxidectin in a selected population of UK pleasure horses deemed at high risk of parasitic infection.

Method: Parasite control records from a commercial provider of horses that had been ML efficacy tested, between 1st January 2008 and 29th August 2011 were reviewed. Efficacy tests were performed due to unusual faecal egg count (FEC) patterns or continually positive FECs post treatment. Results from a modification of the FEC reduction test, within the licensed ERP for the product, were compiled into a database for each year. Initial FECs <150epg were rejected, following WAAVP guidelines. From 200 horses tested 153 met the inclusion criteria, moxidectin (n=96) and ivermectin (n=57). Mann-Kendall trend test was performed from 2008-2011 for both ivermectin and moxidectin groups.

Results: Moxidectin mean FEC reduction 2008-2011 was 93.3%, 89.9%, 86.5% and 79.5% respectively. Ivermectin mean FEC reduction 2008-2011 was 74.4%, 41.4%, 87% and 68.2% respectively. Moxidectin demonstrated a significant negative trend ($r=-.172$) ($P=.022$) over the 4 year period whereas ivermectin did not demonstrate a significant trend.

Conclusions: The mean FEC reduction for each year studied, for both ivermectin and moxidectin, was below the expected $\geq 95\%$ reduction. Moxidectin demonstrated a continual negative trend in efficacy over the period studied. Ivermectin did not demonstrate a significant downward trend but the mean reduction was low and ranging for this anthelmintic over the period studied. Here we report reduced efficacy of ivermectin and reducing efficacy of moxidectin, through early egg reappearance of horse strongyles in selected high risk UK pleasure horses.

Acknowledgements: EPLA Ltd provided the data for this study.

Oral poster session 8: Ticket to Ride

Thursday 20 August
12:00-13:00

Ref: 0406

Poster Number: P2I355

The development of a diagnostic ELISA for informing on cyathostomin encysted larval burdens in horses

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Cyathostomins are common pathogenic nematodes of horses. They have similar life cycles that involve encystment of larval stages in the large intestinal wall. The encysted larvae (EL) play a critical role in the pathogenicity of the infection, because they can represent up to 90% of the total worm burden, with several million being present in individual animals. In some cases, a large number of EL emerge from the cysts to recommence development (larval cyathostominosis) and this can cause life-threatening colitis in approximately 50% of the cases. Currently, EL cannot be detected in live horses. Previous studies identified two native antigen complexes that could be used as markers for EL burden. Cyathostomin gut associated larval antigen-1 (Cy-GALA-1), a component of these complexes, was identified by immunoscreening a complimentary (c)DNA library using sera from experimentally-infected ponies. Specific IgG(T) responses against rCy-GALA-1 were shown to correlate with the EL burden in infected individuals. It was found that Cy-GALA-1 was derived from *Cyathostomum pateratum*. However, the Cyathostominae group comprises approximately 50 species, which should be taken under consideration in the development of a diagnostic test. Segments of the *Cy-gala* gene from an additional four species (*Cylicocyclus ashworthi*, *Cylicostephanus goldi*, *Cylicostephanus longibursatus* and *Cyathostomum catinatum*) were obtained, sub-cloned and expressed as recombinant proteins. The predictive ability of a cocktail comprising all five recombinant GALA proteins in informing on EL burden was assessed. Receiver operator characteristic curve analysis showed that the CT5 ELISA can effectively discriminate between test-positive and test-negative results, indicating potential predictive value for EL burdens.

Oral poster session 8: Ticket to Ride

Thursday 20 August
12:00-13:00

Ref: 0448

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Investigations into the performance of the faecal egg count reduction test in equids

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The faecal egg count reduction test (FECRT) is routinely used to monitor anthelmintic efficacy against equine helminths. The performance of the FECRT was investigated through a simulation approach. The effects of the following factors on efficacy classification (based on an adapted WAAVP method, Coles et al., 1992) were investigated: faecal egg count (FEC) method used to analyse pre-treatment and post-treatment samples, egg detection limit (dl) of the FEC method, underlying mean pre-treatment FEC, underlying distribution of the Day 0 FEC (negative binomial parameter, k), number of horses included in the test, and true efficacy of the anthelmintic. The overall aim was to produce recommendations for the conduct and interpretation of equine FECRT data that are practical, take account of the underlying influences of methods used, and validated by field data. For each set of parameters investigated, 100 sets of simulated FECRT data were generated, and from these, efficacy misclassification rates were estimated. The results demonstrated that the most critical factors affecting the outcome of the FECRT were the mean pre-treatment FEC and the egg dl. The misclassification rate could be reduced by using a FEC method with a low egg dl (<10 eggs per gram). However, when drug efficacy and the threshold for classifying an anthelmintic as resistant/efficacious were close together then the false positive misclassification rates were high, regardless of increasing the pre-treatment mean FEC, the number of horses included or lowering the egg dl, indicating that when a single threshold is applied then there is a greater chance of misclassifying the outcome of a FECRT. As such, 95% confidence limits should be included to aid interpretation of FECRT results. The results from this work will help to support evidence-based recommendations for conducting equine FECRT to reduce the likelihood of misclassification and optimise the usefulness of the FECRT in monitoring anthelmintic efficacy.

Oral poster session 8: Ticket to Ride

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Differential expression of β -tubulin isotype 1 and 2 in the equine roundworm *Parascaris* sp

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Introduction: Anthelmintic resistance to macrocyclic lactones (ML) has been described in *Parascaris* sp. world-wide, whereas reduced efficacy of the pyrantel pamoate has only been reported in the U.S. Thus, the anthelmintic group benzimidazoles (BZ) are the only drug class still effective for treatment. The mode of action of BZ is binding to β -tubulin and thus preventing the polymerisation of microtubuli. In this study the β -tubulin gene expression of isotype 1 and 2 in *Parascaris* sp. was investigated at different developmental stages of worm development. In addition, the effect of thiabendazole (TBZ) on β -tubulin gene expression was studied after exposure for five days.

Material and Methods: *Parascaris* sp. eggs were purified from faecal samples. Gene expression was investigated with qPCR. To evaluate if TBZ alters the gene expression of β -tubulin isotype 1 and 2 a modified in ovo larval developmental test was developed.

Results: There was a significant difference in expression level of isotype 1 and isotype 2 in the different development phases of *Parascaris* sp. Isotype 1 was expressed in adult worms and throughout the developmental stages, although a 5 fold increase was observed the first 48h. In contrast, isotype 2 was mainly expressed in the first 24h and thereafter it rapidly decreasing about 10 fold to very low levels. Increasing concentration of TBZ did affect the gene expression of isotype 1 but not 2 when measured in L3 after incubation for five days.

Conclusion: There was a strong pattern of differential expression of β -tubulin isotype 1 and 2 in different life cycle stages. This assumes that each isotype has specific functions, which is in agreement with the "multi-tubulin" hypothesis, stating that different tubulin isotypes are required for specialized microtubule functions. Moreover, increasing concentration of TBZ did alter the β -tubulin isotype 1 gene expression in L3 exposed for five days. This was not seen for isotype 2.

Oral poster session 8: Ticket to Ride

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Poster Number: P1B073

Epidemiology and control of intestinal helminths in two Lusitano horse stud farms in Center and South of Portugal

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Horses are usually infected with several different helminth species, most of them very pathogenic. Therefore, the implementation of anthelmintic control programs should be considered to keep the parasite burden at minor levels, compatible with a good animal health status. In Portugal, available data are scant and outdated. In order to assess the epidemiology of intestinal helminths and each control approach, faecal samples were collected monthly, from December 2012 to January 2014, from 60 horses (20 mares, their foals and respective yearlings), raised in extensive system, equitably distributed per two study areas, one in Ribatejo - Azambuja (MA) and the other in Baixo Alentejo - Serpa (MS). Quantitative and qualitative coprological techniques, including faecal cultures, were performed, as well as the scotch tape technique to search for eggs and adults of *Oxyuris equi*. Anthelmintic efficacy on strongyles and roundworms were evaluated using the Faecal Egg Count Reduction Test, and on pinworms was evaluated using Positive Animals Reduction Test. For statistical inference analysis, ANOVA was performed using the program SAS[®] 9.3. The following intestinal helminths were found: *O. equi* (MS-70%; MA-67%), equine strongyles [ES] (MS-100%; MA-93%), *Parascaris* spp. (MS-59%; MA-52%), *Strongyloides westeri* (MS-10%; MA-4%) and *Anoplocephala* spp. (MS and MA-3%). Mares's ES egg shedding was subtle, whereas a seasonal pattern was noticed on foals, with rises in spring, summer and autumn. The genus *Cyathostomum sensu latum* was the most prevalent (85%) and abundant (99.7%). The immune system seems to play a crucial role in roundworm egg shedding, since very high EPG values were observed in suckling foals and null values in mares. Pinworms were identified in adult mares and also in foals with two and three months old, suggesting a shortening of the prepatent period at approximately 50%. Doramectin was not very efficacious in controlling these helminths, particularly *O. equi*, while Febendazole and Ivermectin/Praziquantel proved to be. Approximately 94% of the mares were dewormed without a valid justification, once their EPG values were under 500. Finally, based on the data analysis, it was possible to propose an integrated and sustainable control scheme of the identified parasites.

Oral poster session 8: Ticket to Ride

Thursday 20 August
12:00-13:00

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Poster Number: P1A210

“Survey stop”: a sustainable approach for improvement of horse parasite control in Portugal

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Equine parasite control in Portugal is still focused in strategic /empirical deworming like 50 years ago, mainly due to the passive role of Veterinarians in its design, follow up and also to the consequent devaluation by owners of this important feature of horse health programs. Aiming the development of “Survey STOP” (Simple Test On Parasites Eggs), a partnership began in 2012 between CIISA-FMV-ULisboa and Merial Animal Health, Portugal, focusing on faecal parasitological analysis and the implementation of evidence based parasite control programs in equids.

Faecal samples were collected on day 0 and 21 of deworming with Ivermectin (IVM, Eqvalan®) or Ivermectin+Praziquantel (IVM+PRZ, Eqvalan Duo®), sent by prepaid envelope to FMV-ULisboa and analysed with flotation/sedimentation techniques. EPG level and IVM efficacy on strongyles were evaluated with McMaster and Faecal Egg Count Reduction Test (FECRT) on day 21. 500 EPG was the threshold to consider low or high egg shedding. The genus/species of strongyles were assessed with faecal cultures. Parasitological mapping was done per district.

Till present moment, 465 faecal samples were received (96.1% horses, 3.9% donkeys), most from Lisbon area and southern districts like Évora, Beja and Setúbal. 48.2% of equids were positive in McMaster test, with lower average EPG in donkeys when compared with horses (593.5 vs. 1427.4), suggesting donkeys are more resilient than horses, taken in consideration the 500 EPG threshold.

Faecal cultures confirmed *Cyathostomum* spp. as the most prevalent nematodes in equids with abundance higher than 90%, with higher nematode diversity in horses, but higher overall prevalence for all genera and species in donkeys, namely pathogenic species like *Strongylus vulgaris*, mostly found in the Center and South districts of Portugal, while the distribution of cyathostomins remains ubiquitous. By these results it can be concluded that portuguese equids are still dewormed without a prior parasitological assessment, since more than 50% negative animals were dewormed throughout the whole survey. However every IVM and IVM+PRZ deworming showed efficacy of 100%.

Oral poster session 9: "Bits and pieces" (cells, molecules and genes)

Thursday 20 August
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Ref: 0033

Poster Number: P2D421

Neutrophil and eosinophil extracellular trap formation induced by *Haemonchus contortus* significantly contributes to larval entrapment

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Haemonchus contortus is a haematophagous gastrointestinal nematode parasite with a worldwide distribution. This nematode causes considerable economic losses in ruminant livestock due to severe haemorrhagic gastritis in affected animals. Neutrophil and eosinophil extracellular trap (ET) formation has recently been demonstrated as an important effector mechanism of leukocytes acting against several invading pathogens. To date, little evidence on ET formation directed against metazoan parasites is available. We here investigated the ET formation of bovine PMN and ovine eosinophils after exposure to viable *H. contortus* third stage larvae (L₃). Significant larval entrapment by ET structures was observed after 60 min of incubation via scanning electron microscopy (SEM) and fluorescence microscopy. Interestingly, the aggregated type of ET formation (aggET) was here predominantly observed. AggETs firmly ensnared *H. contortus* larvae in a time dependent manner. A significantly stronger reaction was observed when ensheathed larvae were tested versus exsheathed ones. However, ETs had no lethal effect on *H. contortus* larvae but led to immobilization of these parasitic stages. Furthermore, also dead parasites significantly induced ETs. Co-localization of extracellular DNA with histones, neutrophil elastase (NE) and myeloperoxidase (MPO) in parasite entrapping structures confirmed the classical characteristics of ETs. In addition, *H. contortus*-triggered ET formation was significantly diminished by treatments with inhibitors of NADPH oxidase and MPO, demonstrating this parasite-triggered effector mechanism to be NADPH oxidase- and MPO-dependent.

Oral poster session 9: “Bits and pieces” (cells, molecules and genes)

Thursday 20 August
12:00-13:00

Ref: 0184

Poster Number: P1A186

Monepantel and combination anthelmintics – are cytochrome P450's clinically relevant?

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Recent reports speculate that induction of metabolic P450 cytochromes in the hepatocytes of sheep by monepantel has a role in rapidly metabolizing monepantel or other anthelmintics administered in combination (or subsequently), with postulated effects on efficacy and reproductive safety. Monepantel, as the marketed product, Zolvix[®] is well established in the key sheep production regions of the world. The efficacy and safety of Zolvix[®] has been confirmed in many published studies. Specifically, the blood levels of monepantel and the major metabolite, monepantel sulphone, were not altered after repeat treatments at clinically relevant treatment intervals. Additionally, clinical and reproductive safety was demonstrated in ewes, rams, and lamb progeny and at elevated doses. When monepantel was formulated in a combination with abamectin, pharmacokinetic bioequivalence of monepantel, its sulphone and abamectin was clearly demonstrated with the combined formulation compared to individual administration of one drug alone. Additionally, this combination was shown to have a superior efficacy (synergistic effect) in animal studies and is uniformly effective and safe in field studies that have been completed in all major sheep production regions of the world. It is concluded that if cytochrome induction is present in sheep after administration of monepantel alone or in combination with abamectin, that effect is not clinically relevant.

Oral poster session 9: "Bits and pieces" (cells, molecules and genes)

Thursday 20 August
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Ref: 0190

Poster Number: P2I353

Detection and quantification of *Cystoisospora suis* in infected IPEC-J2 cells

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Neonatal porcine cystoisosporosis is a widespread infection of suckling piglets caused by the apicomplexan parasite *Cystoisospora suis* (*C. suis*). Infected piglet shows watery diarrhea, wasting and weight loss leading to unequal weaning growth as well as huge economic losses in pig industry. Up-to-date, toltrazuril alongside with hygiene measures are the only effective metaphylactic medication used, but concerns about drug residues and resistance are emerging. Previously we have demonstrated that intestinal porcine epithelial cells (IPEC-J2) are a promising in-vitro culture system substituting the use of live animals and permitting to study and evaluate host-parasite interactions. The aim of the current study is to establish a new real-time quantitative PCR assay (RT-qPCR) based on TaqMan chemistry for the detection of the 18S ribosomal RNA gene (18S rRNA) of *C. suis* in infected IPEC-J2 cells. Moreover, the established assay will be used to evaluate the possible effect of various substances on the invasion / inhibition mechanisms of *C. suis* in infected IPEC-J2 cells. For this purpose, specific primers and probe were designed for the detection of the *C. suis* 18S rRNA. The specificity of the primers and probe sequences was tested using the nucleotide basic local alignment search tool (BLAST; blastn) homology search. The primers and probe were found to be specific for *C. suis*. Standard curve analysis of the developed RT-qPCR showed 98% efficiency. Furthermore, we are investigating the role of serum of infected animals as well as different chemical inhibitors in the invasion / inhibition mechanisms of *C. suis*.

Oral poster session 9: "Bits and pieces" (cells, molecules and genes)

Thursday 20 August
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Poster Number: P2D422

Vaccine efficacy of oligomannose-coated liposomes against *Toxoplasma* and *Plasmodium* infection

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The design and development of an effective vaccine against the infection with protozoan parasite presents a great challenge. To control the infection with intracellular parasites, *Toxoplasma gondii* and pre-erythrocytic-stages of *Plasmodium*, induction of the parasite-specific cellular immunity will be important. Oligomannose-coated liposomes (OML) are a novel adjuvant capable of inducing Th1 immune responses and cytotoxic T lymphocytes specific for the encased antigen. Therefore, vaccine efficacy of OML against *Toxoplasma* and *Plasmodium* infection was evaluated based on mouse model. *T. gondii* profilin (TgPF) is a potential immunodominant antigen for a candidate vaccine. Vaccination with TgPF entrapped with OML (TgPF-OML) increased the survival of the mice and reduced the parasite burden in their brains after *T. gondii* infection. Furthermore, immunization with circumsporozoite protein entrapped with OML (PbCSP-OML) showed substantial protection against challenge infections with sporozoite of *P. berghei*. Immunization with TgPF-OML and PbCSP-OML elicited the antigen-specific humoral and cellular responses. Our results represent the successful application of an OML-based vaccine against *T. gondii* and pre-erythrocytic stage malaria infection. This approach may offer a promising new vaccination strategy against protozoan diseases.

Oral poster session 9: “Bits and pieces” (cells, molecules and genes)

Thursday 20 August
12:00-13:00

Ref: 0251

Poster Number: P1F241

The analysis of stage differentiation mechanisms of *T. congolense* by RNA-binding protein-RNA interaction

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It is known that gene expression in trypanosomes is regulated post-transcriptionally. Several previous studies have shown that stage-specific gene expression in trypanosomes is regulated by *cis*-elements located at the 3' untranslated region (UTR) of each mRNA and also by RNA-binding proteins (RBPs). However, lack of *in vitro* culture systems for epimastigote (EMF) and metacyclic (MCF) stages of *Trypanosoma brucei* makes the study of these two stages very difficult. Unlike *T. brucei*, all the developmental stages of *T. congolense* IL3000 strain can be cultivated to study the mechanisms of life cycle development of African trypanosome. In this study, RBPs, which are expressed in a stage-specific manner or highly expressed in particular stage, were identified by RNA sequencing (RNA-seq). In addition, the expression profiles of some of RBPs were examined by Northern blot, real-time RT PCR, Western blot and indirect fluorescence antibody test (IFAT). Total RNA samples, which were extracted from four stages (Bloodstream stage, Procyclic stage, EMF and MCF) of *T. congolense* IL3000 *in vitro* culture, were subjected to transcriptome analysis using RNA-seq. Based on RNA-seq. analysis, 304 RBP candidates from total *T. congolense* genes were identified, and 15 RBPs were two times and more higher expressed in EMF than other stages. Since the TcRBP6 orthologous gene in *T. brucei* was reported as a key RBP for stage differentiation from PCF to EMF (Nikolay G. K. *et al.*, 2012), higher expression of the *T. congolense* RBP6 orthologue (TcRBP6) was further confirmed by mRNA and protein levels. Currently, we are investigating how the TcRBP6 and related RBPs regulates differentiation from PCF to EMF.

Oral poster session 9: “Bits and pieces” (cells, molecules and genes)

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Poster Number: P1F242

Strategies for the transfection of *Theileria annulata*

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Theileriosis caused by *Theileria annulata* is an important protozoan disease affecting cross bred cattle and buffalo species worldwide including India and around 10 million crossbred cattle are at risk for tropical theileriosis with an annual economic loss of US \$800 million. Because of shortcomings in disease control measures, therapy and tick control (for theilerial transmission), there is a demand for effective vaccines against this disease. Existing attenuated vaccines for *Theileria* suffer from the drawback of reverting to virulence in several cases. Genetically modified/attenuated vaccines can overcome these drawbacks, but have not been tried so far, due to the lack of a suitable method to manipulate/modify theilerial genome. In this study we have attempted REMI as a strategy for generating stable transformants of *Theileria annulata*. Transfer vectors for the study were constructed with available eukaryotic promoters with and without theileria specific promoters on the upstream of eGFP marker gene and their functionality was confirmed in mammalian cell line. Appropriate transfer vectors were selected for further transfection in *Theileria*. Transfection methods like lipofection and electroporation were attempted in different stages of theileria development and a suitable methodology was standardized. To attain a stably transfected theileria infected lymphocyte line we have attempted REMI and the selection, proliferation of the transformed parasite is under progress.

Oral poster session 9: "Bits and pieces" (cells, molecules and genes)

Thursday 20 August
12:00-13:00

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Poster Number: P1A198

Analysis of the prevalence of genes for benzimidazole resistance in *Nematodirus battus* populations in the UK

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Benzimidazole (BZ) resistance has been identified in a number of key ovine gastrointestinal nematode (GIN) species and is now widespread in the UK and globally. *Nematodirus battus* was believed to be refractory to developing resistance to BZ compounds as efficacy has remained high in this species. Due to the high safety index and efficacy of BZ compounds, these drugs are commonly administered early in the UK grazing season to control *N. battus* infections in lambs. The first case of BZ-resistance in this species was identified in a valley near Kendal in Northern England in 2010. Samples were submitted to APHA following treatment failure and anthelmintic resistance was confirmed by controlled dose and slaughter test. Further investigation of the isolate identified the F200Y SNP to be highly important in conferring resistance in this species, similar to other GIN. A *N. battus*-specific pyrosequencing assay for genotyping of β -tubulin P200 SNP has been developed. A genotyping survey was conducted to investigate the prevalence of BZ-resistance in *N. battus* populations. Faecal samples were obtained from 76 flocks from around the UK. Twenty nine samples were analysed from Scotland and 36 samples from England and Wales. Results show an overall low prevalence ($\pm$ SEM) of the resistant allele (r-allele) in all regions; $6.28\% \pm 1.80$. The r-allele was identified in 26 of the 76 farm samples collected, frequency ranging from 1.7-70.4%. Scottish farms were found to have a lower r-allele frequency than English and Welsh farms tested; $1.77\% \pm 0.69$ and $9.05\% \pm 2.82$ respectively. Samples collected from within the valley in which the primary BZ-resistant population was isolated possessed higher r-allele frequencies; $34.33\% \pm 8.25$ compared with the average allele frequency for England and Wales. Factors associated with the development of resistance in this species and the localisation of resistance is yet to be characterised in detail.

Oral poster session 9: "Bits and pieces" (cells, molecules and genes)

Thursday 20 August
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Ref: 0508

Poster Number: P1A203

Not just GluCl_s (Glutamate-gated Chloride channels): Abamectin has potent effects on nicotinic acetylcholine receptors of nematode parasites

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Introduction: Abamectin and derquantel (Startect[®]) is a combination of drugs showing synergistic anthelmintic effects against nematode parasites. We have observed in contraction and electrophysiological experiments that: 1) derquantel is a potent selective antagonist of muscle nicotinic receptors of *Ascaris suum*; and 2) abamectin is an inhibitor of the same nicotinic receptors (Puttachary et al., 2013). The purpose of our experiments was to examine the mechanism of the inhibitory effects of abamectin further.

Methods: We cloned muscle nicotinic acetylcholine receptor subunits of *O. dentatum*, expressed them in *Xenopus* oocytes and tested, under voltage-clamp, the effects of abamectin on a group of nicotinic receptors activated by pyrantel to determine examine the inhibitory effects of abamectin (Methods: Buxton et al., 2014).

Results: We observed that expression of *Ode-UNC-63:Ode-UNC-29:Ode-UNC-38* produced receptors that were selectively activated by pyrantel rather than levamisole. These receptors were antagonized by 0.03 μ M abamectin in a non-competitive manner (abamectin reduced the R_{max} with little change in EC_{50}). As anticipated, the non-competitive antagonism was greater with 0.1 μ M abamectin. Interestingly, when we increased the concentration of abamectin to 0.3 μ M, we found that the antagonism decreased and was less than with 0.1 μ M abamectin. The bi-phasic effects of abamectin suggest two allosteric sites of action: one causing antagonism, one causing potentiation. The site of action of abamectin on the receptors may be in the outer lipid phase of the membrane (Martin and Kusel, 1992) between M1 transmembrane domain of one receptor subunit and the M3 domain of the adjacent receptor subunit. Since the receptor channel is composed of heterogeneous subunits, binding between one pair of subunits may stabilize closing (negative allosteric modulator) while binding between another pair of subunits may stabilize opening (positive allosteric modulator).

Conclusions : Our observations confirm antagonistic effects of abamectin on nematode nicotinic receptors in addition to GluCl effects, and our observations relate to the use of combination therapies and development of molecular tests for anthelmintic resistance.

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Oral poster session 9: "Bits and pieces" (cells, molecules and genes)

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Evidence for promiscuity in the ligand selectivity of bovine MHC I proteins: Implications for development of *Theileria parva* CD8⁺ T-cell epitope-based vaccines

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Theileria parva is an apicomplexan protozoan parasite of wild and domestic ruminants transmitted transstadially by ixodid ticks and is the causal agent of East Coast fever (ECF), a frequently fatal disease of cattle resulting from the cancer-like immortalization of bovine lymphocytes. The development of a broadly cross-protective recombinant vaccine that mimics the intra-lymphocytic schizonts specific cellular responses induced by live vaccination for control of ECF is constrained by the fact that the response is strongly dependent on the class I MHC phenotype of the bovine host. Indeed, in many alleles in the class I MHC locus in cattle, polymorphisms are concentrated primarily among amino acid positions that determine specificity for peptide binding and different cattle breeds often carry distinct alleles. In the study described herein, we utilized the depth and multiplexing capabilities of high-throughput sequencing to catalogue the complement of class I MHC transcripts of distinct African and European taurines from the *T. parva* endemic regions of Eastern Africa. In sum, whilst differences are observed when comparing the raw peptide binding domain sequences of these taurine breeds, prediction of peptide binding specificity defines functional clusters that are invariably present in both breeds. These findings lend credence to the contention that functionally relevant cattle class I MHC allele groupings can be targeted for prediction of promiscuous ligands without compromising the coverage of *T. parva* CD8⁺ T-cell epitope-based vaccines. A complementary study, implemented in parallel, aims to determine whether the African buffalos (*Syncerus caffer*) class I MHC ligand repertoire can serve as an 'immune filter' for incorporation of candidate vaccines into cattle immunogenicity studies. Available data suggests that CD8⁺ T-cells in the peripheral circulation of *T. parva* infected buffalos are capable of killing targets infected with different stocks of both cattle and buffalo derived parasites. Such responses would be relevant with reference to cattle T cell responses only if bovine and buffalo MHC I proteins shared peptide binding motif. It is on this logic that efforts are underway to characterize buffalo class I MHC genotypes with a view to determine their antigen specificities.

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Oral poster session 9: “Bits and pieces” (cells, molecules and genes)

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12:00-13:00

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Poster Number: P2J246

The genetic architecture of the Major Histocompatibility Complex (MHC) class II in sheep and its role in resistance to nematode infection

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Selective breeding of sheep for resistance to nematode infection is one of the more attractive options for control of parasites. Identification of the genes underlying nematode resistance and fine mapping the causal mutations could facilitate selective breeding. The important role of the Major Histocompatibility Complex (MHC) gene in nematode resistance has been established in several studies. There is very high linkage disequilibrium in the sheep MHC, and consequently, identifying causal mutations has been challenging. In this study, the genetic architecture of the MHC class II region will be presented together with association analyses of haplotypes in Scottish Blackface and Texel breeds. The results indicate that both the Ovar-DRB1 and DQB2 are associated with resistance to nematode infection. These results make it possible to optimise diversity at the MHC and maximise its contribution to disease resistance in domestic sheep.

Oral poster session 10: Love me do

Thursday 20 August
12:00-13:00

Ref: 0066

Poster Number: P1H135

Evaluation of two strategies for the prevention of *Thelazia callipaeda* infection in naturally exposed dogs

Charlotte Lechat¹, Noémie Siméon², Olivier Pennant³, Loic Desquilbet¹, Sabine Chahory¹, Christophe Le Sueur⁴, Jacques Guillot¹

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The growing number of cases of canine thelaziosis in Europe is probably a consequence of the increased mobility of dogs and the absence of reliable preventive measures in pet carnivores. The aim of the present study was to determine whether application of the slow-release insecticide collar containing 10% imidacloprid and 4.5% flumethrin could reduce *T. callipaeda* transmission in dogs living in a region of France (Dordogne) where thelaziosis is enzootic. The prophylactic activity of a spot-on formulation containing moxidectin was also evaluated over the 9 month-study period (from May 2012 to January 2013). Ninety-six privately-owned dogs were enrolled in the multicentre, controlled study. Before summer (the period of transmission by fruit flies), naturally exposed dogs were allocated to one of three groups: Group A (n = 36)- treated once with a collar containing 10% imidacloprid and 4.5% flumethrin; Group B (n = 33)- treated every month for 8 months with a spot-on containing imidacloprid 10% and moxidectin 2.5%; and Group C (n = 27)- untreated control animals. Dogs were regularly subjected to ocular examination in order to assess *T. callipaeda* infection. During the trial, nematodes were detected in 12 (33%) collared dogs (group A) whereas no eyeworm could be found in dogs who received a monthly spot on application of moxidectin (group B). In the control group, 8 (30%) dogs became infected.

Oral poster session 10: Love me do

Thursday 20 August
12:00-13:00

Ref: 0073

Poster Number: P2E391

The first case of canine leishmaniasis in St. Kitts, West Indies

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Leishmaniasis is a neglected parasitic disease with an estimated two million new human cases diagnosed annually throughout four continents, with most cases reported in Asia, South America, and Africa. The disease has occasionally been reported in the Caribbean. In many endemic regions, dogs serve as a reservoir for human infection. Here we report the first case of canine leishmaniasis in St. Kitts, West Indies. The dog was a six-year old mixed breed born and raised in St. Kitts with no travel history beyond the island. The dog had a cutaneous lesion of 2 cm x 2 cm in size on the carpal pad of the right forelimb, which was indurated, raised, and centrally ulcerated. Antibodies to *Leishmania* spp. were detected by IFA at 1:256. Quantitative PCR using *Leishmania infantum chagasi* as a positive control yielded positive results in amplification of kDNAs and the *MAG* gene from DNA samples isolated from skin, bone marrow and kidney. Collectively these data confirmed the diagnosis of *Leishmania* spp. infection in this dog, which is the first autochthonous case reported in St. Kitts.

Oral poster session 10: Love me do

Thursday 20 August
12:00-13:00

Ref: 0131

Poster Number: P1H136

Combined serological detection of circulating *Angiostrongylus vasorum* antigen and parasite-specific antibodies in dogs from Hungary

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The occurrence of the nematode *Angiostrongylus vasorum*, also known as the French heartworm, is increasingly reported from various European countries. The adults of this parasite species live in the pulmonary arteries and right cardiac ventricle of wild canids and domestic dogs. Larval stages and eggs in the lungs induce inflammatory verminous pneumonia, causing severe respiratory disease in dogs; however, haematological and neurological signs up to death may occur. In Hungary, *A. vasorum* has been identified in red foxes, golden jackals and in two asymptomatic dogs and some slugs. In this first large scale survey, 1247 sera from pet dogs were collected and tested by an ELISA for the detection of circulating antigen of *A. vasorum* and by a separate ELISA detecting specific antibodies against the parasite. A total of 1.36% (n=17, 95% Confidence Intervals, CI: 0.80-2.17%) of the animals were positive in both ELISAs, while 1.76% (n=22, CI: 1.11-2.66%) of the tested dogs were antigen-positive only and 2.73% (n=34, CI: 1.90-3.79%) were positive for specific antibodies only. Regions with antigen- and antibody-positive animals were overlapping and distributed over nearly the whole sampled areas of the country. A considerable number of dogs positive for antibody and/or antigen detection was observed in Budapest and also in the southern part of the country bordering to Croatia (11 and 15, respectively), while in the most eastern parts bordering to Ukraine no positive samples were detected. These results confirm the endemic occurrence of *A. vasorum* in dogs originating from different parts of Hungary and the significant advantages of *A. vasorum* serology in epidemiological studies.

Oral poster session 10: Love me do

Thursday 20 August
12:00-13:00

Ref: 0309

Poster Number: P1H137

Parasitological, pathological and therapeutical findings in a case of feline leishmaniosis

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Leishmaniosis in cats is sporadically reported worldwide, usually from the same areas where the disease occurs in dogs and humans. Although infected cats have been speculated as possible secondary reservoir hosts, little data is available on leishmaniosis in cats as compared to dogs. Here we report diagnostic and clinical findings in a case of feline leishmaniosis in southern Italy (Sicily). A 6-year-old neutered female cat was referred to a private veterinary clinic with dermal, oral and ocular alterations. Clinical examination revealed areas of scaly dermatitis on the trunk, bilateral nodular blepharitis, conjunctivitis, mucocutaneous ulcerative lesions on the mouth and lymph node enlargement.

The cat was moderately anaemic and hyperglobulinemic at the complete blood count and serum protein electrophoresis, respectively, and tested negative for feline leukaemia virus and feline immunodeficiency virus. Fine needle aspirates of dermal and mucocutaneous lesions showed the presence of numerous macrophages containing intracytoplasmic amastigote forms of *Leishmania*. The disease was further confirmed by indirect immunofluorescence test (1:640) and by PCR on blood and conjunctival swabs. The cat was treated with antibiotics for 10 days and long-term administration of allopurinol (20 mg/kg q24h). While the treatment was clinically effective, the cat was persistently positive for *L. infantum* at both serology and PCR on blood and conjunctival swabs at the three months follow-up. This report confirms the susceptibility of cats to *L. infantum* infection. Veterinarians practicing in *Leishmania*-endemic areas should be aware of this disease and should include leishmaniosis in the differential diagnosis of ocular, oral and skin disorders.

Oral poster session 10: Love me do

Thursday 20 August
12:00-13:00

Ref: 0400

Poster Number: P1B068

Canine and feline dirofilariosis in a shelter: first report of feline dirofilariosis in Greece

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Dirofilariosis (heartworm disease), caused by the nematode parasite *Dirofilaria immitis*, is endemic in Greece with high prevalence in northern and sporadic cases in southern country. Although prevention is often applied to the owned dogs, there is a high number of stray dogs that do not receive any kind of preventives, representing an important reservoir of the parasite in endemic areas. The aim of the present study was to investigate the prevalence of dirofilariosis among stray dogs and cats, kept in a shelter, in a hyperendemic area of Greece. For that purpose, the animals of a shelter, located in Northern Greece, were examined. More precisely, blood samples from 148 dogs and 27 cats were examined by the modified Knott's test and by serology, i.e. antigen detection in the dog and antigen/ antibody detection in the cat samples. Thirty seven (25%) of the dogs and 3 (11.11%) of the cats were found positive, by at least one of the tests applied. In two of the positive cats, the adult worms were detected during echocardiography. One of these cats died suddenly one year after diagnosis and during necropsy two decomposing *D. immitis* worms were found in the right ventricle and pulmonary artery, while in the second cat, dirofilariosis was eliminated, as evidenced by serology and echocardiography, 3 months after the initial diagnosis and the administration of doxycycline. In the third cat, only specific antibodies were detected, while the dirofilaria antigen test was negative. This is the first report of feline dirofilariosis in Greece. The high prevalence of infection revealed in the present study emphasizes the important role of the stray animals as reservoirs of *D. immitis* in endemic areas. Moreover, the relatively high prevalence of infection in the cat population confirms that this animal species is in high risk of infection in hyperendemic areas and stresses the importance of preventive treatment for heartworm infection in cats as well as in dogs.

Oral poster session 10: Love me do

Thursday 20 August
12:00-13:00

Ref: 0418

Poster Number: P1H138

Prevention of lactogenic *Toxocara cati* infections in kittens by application of an emodepside/praziquantel spot-on (Profender®, Bayer) to the pregnant queen

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This study aimed to evaluate the efficacy of an emodepside 2.1% (w/v)/praziquantel 8.6% (w/v) topical solution (Profender® spot-on for cats) in the prevention of lactogenic *Toxocara cati* infections. A controlled test was performed with two groups of 8 cats with confirmed pregnancy. All cats were infected with daily doses of 2000 *T. cati* eggs for 10 consecutive days starting 50 days post conception to produce an acute infection. Treatment was performed 60 days post conception. Queens in the treatment group received the emodepside/praziquantel solution at the minimum therapeutic dose (3 mg/kg emodepside and 12 mg/kg praziquantel), while the control group was treated with a placebo spot-on. Efficacy was evaluated 56 days post partum by necropsy of one randomly selected kitten of each litter and comparison of the worm burdens between the study groups. Additionally the necropsy results were supported by quantification of worms expelled with the faeces after deworming of the remaining kittens and all queens. The treatment in late pregnancy resulted in an efficacy of 98.7% ($p < 0.0001$). All necropsied control kittens were infected (geometric mean 30.6). Seven of 8 kittens from treated mothers were free of *T. cati* (geometric mean 0.4). Worm counts after deworming reflected the results obtained at necropsy. No side effects of the treatment were observed. It is concluded that treatment with an emodepside/praziquantel spot-on solution during late pregnancy effectively prevents lactogenic transmission of *T. cati* to the offspring. The study design facilitated the generation of reliable data while at the same time a minimum number of animals was sacrificed.

Oral poster session 10: Love me do

Thursday 20 August
12:00-13:00

Ref: 0488

Poster Number: P1H139

Rapid and complete flea control in cats using a Dinotefuran-based flea spray

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Rapid and complete treatment of existing flea infestations and prevention of new infestations is very important in feline boarding or hospitalization situations in order to prevent environmental infestation by incoming animals and subsequent infestation of other pets sharing that environment. The objective of this work was to demonstrate the rapidity and adulticidal efficacy against fleas (*Ctenocephalides felis*) of a dinotefuran spray (Vectra[®] Pro Flea Spray) in cats.

Sixteen cats were used in this randomized, negative control study. Cats were allocated to two groups of eight animals based upon flea carrying capacity demonstrated by infestation and 24 hour flea counts conducted prior to the study. Cats were infested with 100 (± 3) viable unfed adult fleas on Day -1. Vectra[®] Pro Flea Spray was applied on Day 0 at a rate of 100 mg/m² and control cats were sham treated. The time after treatment that the first dead flea fell off each animal and the number of dead fleas recovered from a catch pan under each cage at 15, 30, 120, and 240 minutes after treatment were recorded. Live fleas were counted and removed at 4 hours after treatment. Cats were re-infested with 100 (± 3) viable unfed adult fleas on Day 3 and live fleas were counted and removed 48 hours after infestation on Day 5.

No dead fleas dropped from any of the control animals during the 4-hour observation period whereas dead fleas began to drop off the cats treated with Vectra[®] Pro Flea Spray within 15 minutes and the numbers of dead fleas recovered increased progressively. The percent efficacy at four hours (calculated on the live flea counts using Abbotts formula and geometric means) was 98.6% and the percent efficacy on Day 5 was 98.3%. All results were significantly different than controls. There were no abnormalities or adverse events noted in any of the cats.

These results demonstrate that Vectra[®] Pro Flea Spray is safe for use in cats and it is rapidly efficacious against fleas: it starts killing fleas within 15 minutes of application, is effective for treatment of existing infestations by four hours after treatment, and is effective at prevention of re-infestation for at least 5 days after treatment.

Oral poster session 10: Love me do

Thursday 20 August
12:00-13:00

Ref: 0548

Poster Number: P1H140

Novel foci of *Dermacentor reticulatus* ticks infected with *Babesia canis* and *Babesia caballi* in the Netherlands and in Belgium

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Autochthonous populations of *Dermacentor reticulatus* ticks in the Netherlands were discovered after fatal cases of babesiosis occurred in resident dogs in 2004. The presence of *D. reticulatus* in the Netherlands has also linked with the emergence of piroplasmosis in the resident horse population. The aim of this study was to put together results of continued surveillance of field sites and hosts for this tick in the Netherlands and also in Belgium and determine their infection status for *Babesia* and *Theileria* species.

Ticks were collected from the vegetation at 11 locations between 2011 and 2013. *D. reticulatus* ticks were also collected from different hosts between 2007 and 2013. Ticks were screened by PCR and reverse line blot (RLB).

A total of 1368 *D. reticulatus* ticks were collected from 4 previously known field locations and from 5 new locations in the Netherlands and from 2 sites in Belgium (one old and one new location). A total of 855 ticks collected from 8 locations in the Netherlands and 2 locations in Belgium were tested. Fourteen ticks (1,64%) collected at 4 field locations (Dintelse Gorzen, Rozenburg, Slikken van de Heen and St. Philipsland) were positive for *Babesia canis*, whereas two ticks were positive for *Babesia caballi*, one tick in the Dintelse Gorzen in the Netherlands and one tick was found positive in De Panne in Belgium. A further 1092 *D. reticulatus* ticks were collected between 2007 and 2013 from 40 dogs (132 ticks), two ticks from two humans, 51 ticks from 15 horses, two ticks from two cats, one tick from a roe deer, whereas most ticks (904) were collected from cattle (n=25). Ticks were found throughout the year on dogs in nearly all provinces of the Netherlands. None of the ticks collected from these hosts were infected.

D. reticulatus is continuing its spread into novel areas. The finding that some autochthonous ticks are infected with *B. canis* and *B. caballi* poses a threat to the resident dog and horse population and justifies year-round tick control measures.

Oral poster session 10: Love me do

Thursday 20 August
12:00-13:00

Ref: 0567

Poster Number: P2D425

rROP2 protein from *Toxoplasma gondii* by nasal route as a potential vaccine against oocyst shedding in domestic cats

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Toxoplasma gondii is a protozoan parasite that is able to infect birds, mammals and human beings. Domestic cats are the most important definitive host because of their strict contact with humans and ability to shed millions of oocysts by feces. The aim of the present study was to evaluate oocyst shedding in cats immunized by nasal route with rROP2 of *T. gondii*. Twelve short hair cats were divided in three groups G1, G2 and G3 (n=4). Animals from G1, G2, and G3 received 100µg of rROP2 proteins plus 20µg of Quil-A, 100µg of bovine serum albumin (BSA) plus 20µg of Quil-A and saline solution (control group), respectively. All treatments were done by intranasal route at days 0, 21, 42, and 63. The challenge was performed in all groups on day 70 with ≈ 800 tissue cysts of ME-49 strain by oral route. Animals from groups had their feces examined and the number of oocysts determined for 20 days after challenge. Animals from G1 excreted lesser oocysts than G2 and G3. ELISA was used to detect anti-*T. gondii* IgG and IgA against rROP2, however, there were no correlation between number of oocyst shedding and antibody levels. The present work showed that rROP2 was able to reduce oocyst shedding in domestic cats immunized by nasal route.

Oral poster session 10: Love me do

Thursday 20 August
12:00-13:00

Ref: 0645

Poster Number: P1H141

Prevalence of endoparasites in Swedish healthy cats

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No recent epidemiological data is available in Sweden about endoparasites and lungworms in healthy cats. Also, prevalence figures of feline zoonotic infections (*Toxocara cati*) can provide information useful to establish risk levels for the human population.

205 healthy cats were included in the study. A maximum of two cats per household could be sampled and only households with a maximum of three cats could participate. Other inclusion criteria were: outdoor access and no antiparasitic medication within the last 3 months. Three samples from three different occasions were collected and sent to the National Veterinary Institute (SVA).

Endoparasites were detected with a flotation technique (saturated NaCl plus sugar 500g/l; density 1.28) able to detect also heavier eggs (i.e. *Capillaria* spp.). Presence of lungworms was assessed with a modified Baermann test.

Flotation showed that 27% of the samples were positive for parasite eggs. *T. cati* was diagnosed in 20% of the samples, followed by *Taenia taeniaeformis* (4%), *Isospora felis*/I. *revoluta* and *Capillaria* sp (both 1%). One cat was positive for *Toxoplasma gondii* oocysts and in one case lungworm larvae (*Aelurostrongylus abstrusus*) were observed.

The recorded prevalence of endoparasites was quite high, however lower than in studies from other countries. The cosmopolitan distribution of *T. cati* as well as the circulation of *T. taeniaeformis* in cats having access to the outdoors were confirmed. Infection with lungworms was documented for the first time in recent times in Sweden, demonstrating that this parasite should be considered in cats with respiratory problems.

Poster Presentations

A: Drugs and drug resistance

Ref: P1A142/0011

Efficacy of Albendazole against ancylostomosis in Nigerian

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Faecal egg count reduction test (FECRT) and Egg hatch assay (EHA) were used to evaluate the efficacy of albendazole (ALB) in the treatment of canine ancylostomosis in 30 naturally infected Nigerian local dogs. The dogs were evenly assigned into four groups, namely, A, B, C and D on the basis of their pre-treatment FEC (PFEC). Groups, A (n = 8), B (n = 8) and C (n = 8) were given one, two or three days treatment with 2.5% ALB respectively while group, D (n = 6) served as the uninfected control. Faecal egg counts (FEC) were performed on samples obtained from the dogs prior to treatments with the ALB to determine their pre-treatment FEC. Another sampling was carried out on the same dogs at exactly 8 days post-treatment (PT) with the ALB to determine the PT FEC. The percentage FECR was calculated using the formula: $FECR = 100 \times (1 - [T2/T1][C1/C2])$. Egg hatch Assay was conducted on *Ancylostoma caninum* eggs harvested from pooled faecal samples obtained from group A dogs before and 8 day PT with ALB. The results of the FECRT showed that ALB produced less than 95%, 97 - 100% and 100% reduction of the pre-treatment FEC of dogs in groups A, B and C respectively. The result of the EHA showed that ALB had LC_{50} values of 0.343 and 1.36 $\mu\text{g/mL}$ on eggs collected before and 10 days PT. The efficacy of ALB and their resistance status in the dog population in the study area were discussed.

A: Drugs and drug resistance

Ref: P1A143/0016

Synergistic Anthelmintic effect of *Citrus aurantifolia* seeds and Mebendazole in Egyptian dogs infected with *Ancylostoma caninum* and *Toxocara canis*

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Synergistic combination have been described in human and veterinary medicine as to solve the anthelmintics resistance and overcome it. The objective of the present study is to evaluate the anthelmintic efficacy of the combination of herbal plant; *C. aurantifolia* seeds extract and mebendazole against *A. caninum* and *T. canis* parasites infection. Material and Method: ethanolic crude extract of *C. aurantifolia* seeds was prepared. A total number of 20 local Egyptian dogs that were naturally infected with *A. caninum* and *T. canis* parasites was used in the experiment. Dogs were randomly divided into 4 groups, 5 dogs each; A, B, C and D. Group A animals were untreated, group B, C and D were treated with mebendazole, *C. aurantifolia* seeds extract and combination of mebendazole and herbal seed extract, respectively. Additional non-infected non-treated animal group E (5 dogs) was added in the experiment. The treatment extends 2 weeks and administered orally where each dog received 3 consecutive days per week. The daily therapeutic dosage of mebendazole and *C. aurantifolia* seeds extract is 50 mg and 14.5 mg/body weight, respectively in divided doses for 3 consecutive days. Two weeks after treatment, blood and faecal samples were collected to evaluate haematological values and faecal egg counts, respectively. Results: show that the treatment of infected dogs with citrus extract and the combination of the extract and mebendazole produced significant increase in PCV, RBC, WBC and lymphocytosis and significant reduction in faecal egg counts. Conclusion: the synergistic combination treatment with crude extract of *C. aurantifolia* seeds and mebendazole could be served as anthelmintic compound.

A: Drugs and drug resistance

Ref: P1A144/0020

Ectoparasiticides and Anthelmintics used in Production Animals, Dogs and Cats in Spanish-speaking Latin America and Spain. A market survey (2015)

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Antiparasitic brands for cattle, sheep, swine, poultry, dogs and cats from 155 different multinational and local Animal Health companies in 12 Spanish-speaking countries were recorded and analyzed in 2012 and in 2015 in the corresponding corporate websites or veterinary vademecums.

The number of brands recorded was 1765 in 2012, and 2004 in 2015. From the brands recorded in 2015 most are approved for use on cattle (1166), followed by sheep (767), dogs (649), swine (574), cats (332) and poultry (158).

For production animals ivermectin was the active ingredient used in the largest number of brands (365) followed by cypermethrin (187), levamisole (152), albendazole (149) and fenbendazole (136).

For dogs and cats, the active ingredient used in most brands was praziquantel (176) followed by pyrantel (embonate or pamoate; 135), permethrin (76), ivermectin (65), and cypermethrin (63).

Between 2012 (1765 brands recorded) and 2015 a total of 239 new brands were introduced. Ivermectin is also the active ingredient used in most new brands (54), followed by praziquantel (32), permethrin (25), fenbendazole (19) and imidacloprid (19). Most new brands were introduced in companies based in Argentina (69), Colombia (35) and Spain (31).

Only four brands contained patent-protected parasitocidal active ingredients, three for use on dogs (afoxolaner, fluralaner, pyriprole, so far available in Spain but not yet in most Latin American countries), and one for use on sheep (monepantel). Consequently, all the recorded brands for use on cattle, swine, poultry and cats contained active ingredients that have already lost patent protection.

A: Drugs and drug resistance

Ref: P1A145/0030

Controlled test of anthelmintic efficacy of albendazole sulphoxide against a multispecies ivermectin-resistant isolate in cattle

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Ivermectin (IVM) and doramectin are the most commonly used anthelmintics in cattle in Brazil in the last three decades and there are several reports of resistance to these two avermectins, even in populations of helminthes that still are susceptible to moxidectin, levamisole and albendazole sulphoxide (ABZSO). However, one factor that must be considered when using the faecal egg count reduction as a method of diagnosis for resistance is the influence of the suppression effect on egg oviposition caused by the benzimidazoles. Therefore, the objective of this study was to evaluate the efficacy of ABZSO against a multispecies ivermectin-resistant isolate in an anthelmintic controlled test. Eighteen naturally infected calves were selected and divided into three groups based on the fecal egg counts: Placebo, IVM (200mcg/kg) or ABZSO (2,5mg/kg), both subcutaneous administration. The parasitological necropsy was performed seven days after treatment. IVM showed efficacy above 95% only against *C. punctata* and *Trichuris discolor* but showed efficacy below 95% against *Haemonchus placei*, *C. pectinata* and *Oesophagostomum radiatum* and no significant difference ($P > 0.05$) to the control group. ABZSO significantly ($P < 0.05$) reduced the number of parasites of all species and also showed efficacy above 99%, except against *O. radiatum*. This study confirms the results of several faecal egg count reduction tests performed in Brazil, showing that ABZSO still can be used against IVM-resistant isolates.

A: Drugs and drug resistance

Ref: P1A146/0042

Treatment of lice infestation in horses using a 10 mg/mL deltamethrin topical application

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This study evaluated the efficacy and the tolerance of a 10 mg/mL deltamethrin-based pour-on solution (Deltanil®; Virbac, Carros, France) in 11 horses with natural *Damalinia equi* infestation. Seven horses received 10 mL of the solution from the mane to the tail head on D0. Four horses, living separately, served as non-treated controls. Lice burden was recorded by parting the coat bilaterally, over the neck, shoulder, withers, barrel, buttock and quarter, and counting the number of live parasites. Lesional score was based on alopecia, crusts, papules/pustules, nodules/plaques, scales and wounds, each assessed on a 0-3 scale on the same regions. Evaluation was performed on D0 and subsequently weekly until D56 in treated horses and on D0 and D56 in control horses. The response to treatment was assessed as the change from baseline to each examination day for parasite and lesional scores. No adverse event was recorded. In control horses, parasite and lesional scores were maintained on D56, mean [SD]: D0 46 [8.5], 3 [0] and D56 51 [15.6], 3 [0]; respectively. Parasite scores of the treated horses were reduced by 98% on D7 and 100% from D15 to D56 (mean [SD]: D0 44 [58.4]). Lesional score in treated horses was reduced by 24%, 82%, 47%, 91%, 96%, 93%, 93% and 100% on D7, 15, 21, 28, 35, 42, 50 and 56, respectively (mean [SD]: D0 3.1 [1.8]).

A single application of the 10mg/mL deltamethrin preparation was effective and safe in the treatment of lice infestation in these horses.

A: Drugs and drug resistance

Ref: P1A147/0100

Absence of detectable benzimidazole resistance alleles in *Haemonchus placei* in cattle in Nigeria revealed by pyrosequencing of β -tubulin isotype 1

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Trichostrongyles are gastrointestinal parasites that occur globally and can cause subclinical to severe, sometime life threatening infections in ruminants, particularly young animals. Benzimidazoles (BZ) are commonly used for the treatment of gastrointestinal parasites in ruminants. Increasing spread of worm populations with anthelmintics resistance has been reported and is considered a consequence of highly frequent and longstanding use of anthelmintics. To obtain initial information regarding occurrence of putatively BZ-resistant Nigerian *Haemonchus* populations, screening based on the molecular analysis of BZ-resistance associated β -tubulin isotype 1 gene sequence polymorphisms was undertaken. Genomic DNA was isolated from pooled adult *Haemonchus* sp. from 35 animals from each of the 6 states of South-Western Nigeria. Sequencing of internal transcribed (ITS-2) and external (ETS) spacer regions was used to determine the *Haemonchus* species. Pyrosequencing assays were used for detection of single nucleotide polymorphisms (SNPs) in the β -tubulin isotype 1 genes of the worms at codons 200 and 167 (TTC/TAC) or 198 (GAA/GCA). Exclusively *Haemonchus placei* was detected and allele frequencies obtained at all three positions showed no evidence for the presence of resistance-related alleles. For Lagos State, pools of 10 worms from 30 different animals were analyzed separately for the codon 200 SNP successfully excluding presence of resistance associated SNPs very low frequencies. These positive findings, showing absence of elevated frequencies of BZ-resistance associated β -tubulin alleles, has considerable significance since it suggests that farmers can still rely on the efficacy of this important drug class when used for controlling trichostrongyle infections in cattle in Nigeria.

A: Drugs and drug resistance

Ref: P1A148/0108

Factors likely to influence the adoption of targeted selective treatment strategies by sheep farmers in Western Australia

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The targeted selective treatment (TST) concept is likely to prove challenging to many sheep farmers, but little is known about its likely uptake in areas where the most important nematode species are *Teladorsagia circumcincta*, *Trichostrongylus* spp. and *Nematodirus* spp. An investigation was conducted in Western Australia into farmers' current understanding and adoption of putative TST strategies, with participants derived from four farming regions where sheep enterprises are economically significant. Sixty-five percent of participants were aware of the TST concept and 25% had implemented it in some form. Farmers were more likely to adopt TST strategies where sheep were a major part of the farming enterprise; drench resistance was perceived to be a major constraint on production; sustainable worm control was actively utilised; and professional sources were used for worm control advice. Respondents that sought advice chiefly from rural merchandisers were considerably less likely to utilise these management tools or to be aware of TST approaches. This indicates that the adoption of TST strategies will require greater use of professional advisers for worm control advice, and that advisers are conversant with TST concepts. As the participants represented a range of environments and sheep management situations, it is likely that these results will apply in other locations throughout Australia and other countries where similar nematode genera predominate.

A: Drugs and drug resistance

Ref: P1A149/0111

In vitro evaluation of ivermectin associated with rutin or hesperedin activity against an IVM-resistant field isolate of *Haemonchus placei*

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Flavonoids are molecules produced by plants, characterized by several pharmacological properties such as their antioxidant action. To date, more than 8,000 compounds have been discovered and categorized into 11 classes. The class of quercetin in particular is a potential P-glycoprotein modulator, inducing the anthelmintic effect of ivermectin (IVM) against resistant-parasite species. Therefore, the aim of this study was to detect and evaluate the in vitro effect of rutin and hesperidin, two different quercetins structurally related to flavonoids. Furthermore, their nematicide activity against an IVM-resistant field isolate of *Haemonchus placei* was separately estimated in ten concentrations. Additionally, six concentrations of IVM were assessed in a larval migration inhibition test in order to determine the IC₅₀. Lastly, IVM IC₅₀ was associated with 13 different concentrations of rutin and hesperidin, respectively. Results indicated that IC₅₀ of IVM was $1.748 \times 10^{-5} \text{M}$ (95% CI: 7.91×10^{-6} to $3.864 \times 10^{-5} \text{M}$) when tested alone, 3.658×10^{-7} (95%IC 4.882×10^{-8} to 2.740×10^{-6}) when evaluated with rutin, and 2.367×10^{-10} (95%IC 1.343×10^{-10} to 4.172×10^{-6}) when tested with hesperedin. The two flavonoids showed no nematicidal effect ($p > 0.05$) and did not reduce the IVM IC₅₀ ($p > 0.05$).

A: Drugs and drug resistance

Ref: P1A150/0141

Repurposing human health drug leads for Animal African Trypanosomosis (AAT)

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African Animal Trypanosomosis (AAT - Nagana) is a protozoal disease of cattle and other livestock caused primarily by the trypanosomes, *T. congolense* and *T. vivax*, transmitted by tsetse and other biting flies. The resultant anaemia, productivity loss and death and the widespread incidence of AAT in sub-Saharan Africa make this disease one of the major causes of economic loss to livestock keepers, many of whom are amongst the worlds' poorest and least able to cope with this loss of their major assets. Despite the importance of AAT, there are no vaccines available and no new trypanocides have been introduced for 50 years. Moreover, there is increasing resistance to the existing therapeutic and prophylactic agents, diminazene and isometamidium.

Recent years have seen a resurgence in research into drugs for Human African Trypanosomiasis (HAT, cause by *T. brucei* spp) as well as Chaga's disease (*T. cruzi*) and other human NTD's and the pipeline of new drugs for HAT is looking very promising. Supported by DfID and BMGF, GALVmed has been leading a program to seek new control tools for AAT. As part of this work, an effort directed towards discovering new trypanocidal drugs has focused on leveraging the discoveries and leads from the HAT programs in both academic and industry laboratories.

This poster highlights the target product profiles for livestock trypanocides (therapeutic and/or prophylactic efficacy), which differ significantly from that for human trypanocides, and summarises the progress that has been made towards identifying potential drug candidates.

A: Drugs and drug resistance

Ref: P1A151/0172

Gastrointestinal nematodes of cattle resistant to ivermectin and doramectin in the state of Paraná, Brazil

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Anthelmintic effects of different chemical compounds, against cattle naturally infected by gastrointestinal nematodes (strongyles), were evaluated using the fecal egg count reduction technique (FECRT). Two experiments were conducted in different municipalities located at the State of Paraná (Umuarama and Douradina). In both experiments, bovines were randomized into four groups of 10 animals each, according to the average score of EPG on days -3, -2 and -1, as follows: T01, control (saline); T02, ivermectin 630 µg/kg (Ivomec Gold® - Merial Animal Health); T03, doramectin 200µg/kg (Dectomax® - Zoetis); T04, moxidectin 200µg/kg (Cydectin® - Zoetis), all administered by the subcutaneous route. Fecal samples were performed at 7 and 14 days post-treatment. On the property located in the municipality of Umuarama, ivermectin showed zero effectiveness (0%) on both count dates. Doramectin, in turn, reached 36.8% and 45.2% efficacies on the 7th and 14th DPT, respectively. On the other hand, moxidectin obtained efficacy indexes above 95% in two experimental dates cited above. Similar results were found with these same formulations in the property located in Douradina, where ivermectin showed zero effectiveness at days 7 and 14 post-treatment, doramectin obtained values inferior to 50% in these same dates, while moxidectin reached efficacies above 95% at 7 and 14 DPT. Based on these results, it is possible to conclude that different populations of helminths assessed in Paraná were considered resistant to both ivermectin and doramectin. Moreover, both were susceptible to moxidectin. It is the first report regarding nematodes of cattle resistant to avermectins in the Paraná State, Brazil.

A: Drugs and drug resistance

Ref: P1A152/0181

Reduced efficacy of two benzimidazole commercial formulations against a susceptible cyathostomin population

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Cases of low efficacy after anthelmintic treatment may not represent genuine cases of anthelmintic resistance and other causes in treatment failure should also be considered, including the drug:host:parasite interaction, errors in the drug administration route and dose, high challenge by parasites and the quality of the formulation. The aim of this study was to evaluate the efficacy of two commercial benzimidazole formulations against a population of small strongyles (Cyathostomes) previously characterized as susceptible to this anthelmintic class. Faecal samples from a naturally infected horse almost exclusively infected by small strongyles (Cyathostomes) were collected to perform the Egg Hatch Test with thiabendazole at 0.5, 0.3, 0.2, 0.1, 0.05, 0.025, 0.012, 0.005, 0.002 and 0.001 mcg/mL. 0.21% of hatchability was observed at the concentration of 0.1 mcg/mL, and no eggs hatching was observed at concentrations higher than this, demonstrating the susceptibility of this isolated to benzimidazole. Furthermore, three horses from the same herd were used in the faecal egg count reduction test (FECRT) to evaluate fenbendazole (40 mg / kg) efficacy, by comparing pre and post-treatment samples. On day zero, all the animals were weighed and sampled for faecal egg counts. Faecal samples were also collected 7 days after treatment. The second FECRT was performed three weeks later with the same animals to evaluate Mebendazole (10mg / kg). The efficacy of fenbendazole and mebendazole was 50.83% and 25.12%, respectively. These results show a situation when treatment failure was not due to resistance, but to low quality formulations.

A: Drugs and drug resistance

Ref: P1A153/0224

Failure of closantel treatment against *Fasciola hepatica* infection in beef cattle

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Anthelmintic control of liver fluke *Fasciola hepatica* in cattle is based on treatment using flukicides. However, triclabendazole and albendazole resistance against *F. hepatica* is emerging worldwide. In Sweden, a new drug combination closantel and ivermectin (Closamectin pour on) was registered in 2011. Closantel together with albendazole are currently the only available flukicides for Swedish beef cattle. The aim of the study was to evaluate efficacy of closantel against *F. hepatica* in naturally infected beef cattle using both coproantigen and faecal egg count reduction tests.

Faecal egg counts (FEC) and coproantigen ELISA examination were conducted in 3 beef cattle herds (A, B, C) in south-west Sweden. On each farm, 10 *F. hepatica* coproantigen positive animals were selected and treated with 20 mg closantel per kg bodyweight. Faecal samples were collected from selected animals on 0, 7 and 21 day after drug application.

Based on FEC, efficacy of closantel was 72% (95% CI 13.1%) and 97% (95% CI 1.6%) on farms A and B, respectively. No reduction of *F. hepatica* egg counts at all was observed on farm C. Totally 4, 1 and 4 animals remained coproantigen positive 21 days after treatment on farms A, B and C, respectively.

Closantel treatment failure was confirmed on two Swedish farms. Development of resistance to closantel and/or lower efficacy of 'pour on' application in comparison to injectable form are likely responsible for poor efficacy of closantel in the study.

This study was supported by the Swedish Foundation for Agricultural Research (contract no. H1350023).

A: Drugs and drug resistance

Ref: P1A254/0249

Moxidectin resistance of *Parascaris equorum* in an equine farm from Mexico.

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Moxidectin (MOX) is currently registered for gastrointestinal nematodes (GIN) control in horses. MOX have showed high efficacy on *Parascaris equorum*. However, in equine from USA and Europe have been reported resistance from *Parascaris equorum* to MOX. In Mexico there are no studies on MOX resistance of *P. equorum* in equine. Therefore, the aim of this study was to determine *Parascaris equorum* resistance to MOX in an equine farm from Campeche, Mexico. One equine farm was surveyed with frequent use MOX ≥ 4 times per year. Fecal egg count reduction test (FECRT) was used to determine the resistance to MOX. Treated group was dewormed with Equest® (0.4 mg/kg of weight-Day 1). Fecal samples were collected (Day 0 and 14 post-treatment). Two times was made this procedure to confirm resistance. 100% (18/18) of the fecal samples were positive to GIN (eggs) pretreatment. The genera involved were *P. equorum* (88.7%) and *Oxyuris equi* (11.3%). The FECRT showed 13% of reduction (reduction % from 0 to 77) suggesting resistant to MOX. Posttreatment the GIN identified was *P. equorum*. In conclusion equine farms that use MOX ≥ 4 times per year seemed to promote resistance to MOX. The resistant (to MOX) GIN genera found in Campeche, Mexico was *P. equorum*. This is the first report of MOX resistant of *P. equorum* in equine from Mexico.

A: Drugs and drug resistance

Ref: P1A155/0285

Evaluation of ivermectin oral and injectable formulations against susceptible and resistant gastrointestinal nematodes in cattle

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It is well known that ivermectin (IVM) is highly efficacious against susceptible gastrointestinal (GI) nematodes following both oral and subcutaneous (SC) treatments. However, enhanced efficacy has been reported for the oral route in the presence of resistant nematodes. The current study evaluated the comparative pharmacokinetic (PK) behaviour and clinical efficacy against susceptible and resistant nematodes observed after IVM administration by the oral and SC routes to cattle. The trial was conducted in two commercial farms. Farm A with predominance of an IVM-susceptible nematode population and Farm B with well established IVM-resistant nematode populations. Forty five (45) male calves (from each Farm) naturally infected with GI nematodes susceptible/resistant to IVM were randomly allocated into three groups (n= 15): untreated (control) and IVM treated by the SC and oral route at 0.2 mg/kg. Eight (8) animals from each treated group (Farm A) were randomly selected to perform the PK study. Plasma and faecal samples were taken from those animals up-to 25 days post-treatment. Drug concentrations were measured by HPLC. The clinical efficacy was determined at 16 days after treatment by Faecal Egg Count Reduction Test (FECRT). The FECRT in Farm A were 92.8% (SC treatment) and 91.3% (oral treatment) confirming that both routes of administration reached equivalent efficacy against susceptible nematodes. The IVM systemic availability (estimated as the area under the plasma concentration vs time curve) obtained after its SC administration (421.02 ± 70.25 ng.d/ml) was higher ($P < 0.05$) compared to that measured the oral treatment (121.48 ± 32.15 ng.d/ml). However, markedly higher IVM concentration profiles were observed in faeces collected from orally treated calves. This is in agreement with the enhanced abomasal/intestinal drug concentrations recovered from orally treated sheep, compared to the SC treatment, in a previous study. The clinical efficacy against IVM-resistant nematodes (Farm B) was significantly ($P < 0.05$) greater after the oral (74%) compared to the SC (54.1%) IVM treatment. *Haemonchus* and *Cooperia* spp. were the genus that survived the IVM treatment in Farm B. In conclusion, the increased GI nematodes exposure to the drug after the oral treatment should be considered when dealing with IVM resistant parasites in ruminants.

A: Drugs and drug resistance

Ref: P1A156/0294

Pharmacological evaluation of a 5-fold incremented dosage of the ivermectin+fenbendazole combination against highly resistant *Haemonchus contortus* in lambs

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The combination of nematocidal compounds from different chemical groups has been proposed as a strategy to delay the development of resistance. Previous studies involving ivermectin (IVM) and albendazole demonstrated that the enhanced systemic exposure achieved after their administration at high doses correlated to a significant increment in drug efficacy against resistant *Haemonchus contortus*. The aims of the current trial were: a) to characterise the plasma pharmacokinetic (PK) behaviour of IVM and fenbendazole (FBZ) administered either alone or co-administered to lambs (therapeutic dose x5) and, b) to compare the clinical efficacy of IVM and FBZ given separately (therapeutic dose x5) or co-administered to lambs naturally infected with gastrointestinal nematodes resistant to both molecules. Forty (40) lambs naturally infected with resistant nematodes were allocated into four groups (n=10) and orally treated either with IVM (1 mg/kg), FBZ (25 mg/kg) or with a combined formulation of IVM+FBZ (at the same x5 dose rate). An untreated control group (n=10) was included to assess the comparative clinical anthelmintic efficacy (14 days post-treatment) of the different treatments by the faecal egg count reduction test (FECRT). Additionally, faecal samples were cultured to determine nematode genus/species. The Kruskal-Wallis test was applied for the statistical comparison ($P < 0.05$). The presence of IVM did not change the systemic availability of oxfendazole, main FBZ plasma metabolite, after the combined administration of IVM+FBZ. However, the IVM plasma systemic exposure (expressed as AUC) obtained after its oral co-administration with FBZ was 50% higher compared to that observed after the IVM alone administration. FECRT values were 55% (IVM), 41% (FBZ) and 67% (IVM+FBZ). No differences ($P > 0.05$) were observed on faecal egg counts among treated animals. The faecal cultures showed *H. contortus* as the main nematode specie surviving all treatments. The combined administration of IVM+FBZ at 5 times the therapeutic dose did not improve the clinical efficacy against a highly resistant *H. contortus* strain.

A: Drugs and drug resistance

Ref: P1A157/0317

SCREENING OF ANTHELMINTIC RESISTANCE TO TRICLABENDAZOLE IN *Fasciola hepatica* ISOLATES FROM SHEEP BY MEANS OF AN EGG HATCH ASSAY

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In the present study, the detection of anthelmintic resistance to triclabendazole (TCBZ) in sheep infected by *Fasciola hepatica* was studied using an egg hatch assay (EHA). *F. hepatica* eggs were recovered from bile and faeces of infected animals by isolates with different grade of anthelmintic resistance to TCBZ: i) a resistant isolate (RT); ii) a susceptible isolate (ST); iii) naturally infected sheep by a susceptible field strain (FST). In the EHA the percentage of hatched eggs were calculated according to the following concentrations of TCBZ diluted in dimethyl-sulfoxide (DMSO): 0.05, 0.2, 1, 5, and 25 µg/ml.

In relation to the EHAs carried out with the eggs from bile of sheep infected by ST, differences were found in the percentage of hatched eggs between the control well, only with DMSO, and the two highest concentrations of TCBZ (5 and 25 µg/m) ($p < 0.05$). However, when we tested the drug with the eggs from bile of sheep infected by RT, the percentage of hatched eggs was similar among all concentrations. Since the range of hatching varied between isolates, we calculated the ratio of the results of each concentration to its control value confirming the higher hatching in RT than in ST.

Whit the purpose to avoid the slaughter of sheep, we developed an EHA with eggs recovered from faeces. The results of the EHAs with the isolate ST showed differences in the percentage of hatching between the highest concentration (25 µg/ml) and the control well ($p < 0.05$); however these differences were not confirmed under field conditions with the strain FST. In conclusion, the ovicidal effect of TCBZ in *F. hepatica* eggs from bile was shown using a commercial formulation diluted in DMSO with a minimum concentration of 5 µg/ml. However, in eggs recovered from faeces the results are not conclusive. The cleaning of eggs recovered from faeces is an important issue that should be reviewed and standardized before comparing results between susceptible and resistant isolates in this kind of EHA.

Study funded by the Spanish INIA (RTA2010-00094-C03-02).

A: Drugs and drug resistance

Ref: P1A158/0347

Comparison of two different long acting pre-lamb treatment options on the productivity of poor conditioned twin bearing ewes.

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This study was a single site, negative control comparative productivity study conducted under New Zealand farming conditions. The measure of productivity was based on the body weight and condition score (CS) of poor conditioned twin bearing ewes and the body weight of their lambs. The aim of the study was to determine if there was any benefit from treating the ewes with long acting anthelmintic products pre-lamb and to ascertain if there was any difference between the two treatment options.

A total of 599 mixed age twin bearing ewes (Average CS 2) were randomly drafted into 3 equal groups. The negative control group received no anthelmintic treatment pre-lamb. The other two groups received either a Bionic Capsule® (Merial) or an injection of Cydectin® Long Acting Injection for Sheep (Zoetis) four weeks prior to the start of lambing. All ewe's were body condition scored (0-5 scale) and weighed at pre-lambing, docking (65 days after treatment) and at weaning (127 days after treatment). Faecal egg counts and larval cultures were taken at these three times. The lambs were matched to the ewes at weaning and weighed.

At weaning the treated ewes were significantly heavier (Ave= 3.5kg, $p<0.0001$) than the non-treated ewes. There was no significant difference in weight between the treated groups. The lambs from the treated groups were also significantly heavier (Ave= 2.6kg, $p<0.0001$) at weaning. There was no significant difference in the weight of the lambs between the treatment groups.

A: Drugs and drug resistance

Ref: P1A159/0355

Comparative efficacy of ivermectin and fenbendazole against strongyle in naturally infected horses in Thailand

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This study was designed to compare efficacy between ivermectin and fenbendazole against strongyle nematodes in naturally infected horse at Queen Saovabha Memorial Institute, Prachuap Khiri Khan province, Thailand. The results of a survey in this farm by fecal examination from 131 samples showed that strongyle infection rate was up to 76.34%. Then, 30 horses with a strongyle fecal egg count (FEC) >200 eggs per gram (EPG) were randomly divided into three groups of ten horses each based on pre-treatment EPG counts and treatments were randomized among the groups. Group 1 was untreated control. Group 2 and 3 were given oral route with recommend dose of ivermectin and fenbendazole, respectively. FEC were performed pre and post-treatment for scrutinizing these drugs efficacy at day 0, 7 and 14. The re-infection period was monitored at day 21, 28, 56 and 84. The efficacy was compared using fecal egg count reduction tests (FECRT). Statistical analysis was using ANOVA ($P < 0.05$) for testing a significant among groups. Mean fecal egg count reduction at Day 14 post treatment of group 2 and 3 were decreased significantly compare to the control group. In addition, Group 2 showed most efficacy (100%) accordance with Group 3 (86.74%). Comparing the efficacy of ivermectin and fenbendazole, there was significant difference ($p < 0.05$). This present study showed that Group 2 had longer re-infection period than Group 3; egg reappearance periods were 3 and 1 weeks after ivermectin and fenbendazole treatment, respectively.

A: Drugs and drug resistance

Ref: P1A160/0356

Efficacy of pour-on and oral formulations of moxidectin against strongyle nematodes naturally infected in horses

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The objective of this study was to evaluate the comparative efficacy of moxidectin administered by different routes, against natural infection of strongyle nematodes in horses. The efficacy was compared using fecal egg count reduction tests (FECRT). 30 horses in Prachuap Khiri Khan province, Thailand with a strongyle fecal egg count (FEC) >200 eggs per gram (EPG) were randomly allocated to one of three groups; untreated control (Group 1), pour-on (Group 2) and *per os* (Group 3). On Day 0, the bovine pour-on and equine paste of moxidectin were administered topically and orally at a dose rate of 0.5 mg/kg body weight and 0.4mg/kg body weight to Group 2 and Group 3, respectively. Fecal egg counts were performed pre and post-treatment at day 0, 7 and 14 to examine the anthelmintics efficacy. At the recommended dosage, moxidectin showed efficacy levels of 100% in oral and 0.2% in pour on formulation. Statistical analyses was using ANOVA ($P<0.05$) of significance, the reduction in fecal egg count was significantly greater after post treatment with moxidectin paste than moxidectin pour on and control group. Comparing the efficacy of moxidectin paste and moxidectin pour on, there was statistically significant difference ($p<0.05$). The results demonstrated that pour on preparation in cattle is not suitable apply to use in horses.

A: Drugs and drug resistance

Ref: P1A161/0369

Deltamethrin tolerance in the cattle chewing lice *Bovicola bovis* in the UK

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The chewing louse *Bovicola bovis* is the most common and clinically important species of louse found on cattle in the UK. It causes significant welfare problems, and economic losses related to the parasite are estimated to be up to £20 million per annum.

B. bovis lice were collected from cattle populations in North Wales and Scotland. Samples were obtained from holdings where there had been a history of failure of various chemicals to control chewing lice on good condition cattle every winter for a number of years. In laboratory bioassays, lice were exposed to filter papers that had been dosed with ethanolic dilutions of a deltamethrin-based backline pour-on product containing 1 per cent (w/v) deltamethrin in a caprylic triglyceride excipient. Deltamethrin concentrations of 1, 0.5, 0.25, 0.1 and 0.01 per cent (w/v) were compared to ethanol and tea tree oil (2.5 per cent v/v) which were used as negative and positive controls, respectively.

The results provide evidence of pyrethroid tolerance in *B. bovis* in the UK. For both the Scottish and Welsh *B. bovis* populations sampled, 2.5 per cent (v/v) tea tree oil gave 100 per cent mortality after one hour of contact. However, exposure to concentrations of deltamethrin below 1 per cent (w/v) resulted in only low levels of mortality, which were not significantly higher than after exposure to the ethanol-only negative control.

Any apparent mortality following exposure to undiluted 1 per cent (w/v) deltamethrin was probably a result of mechanical suffocation or osmotic stress caused by contact with the hydrophobic excipient, caprylic triglyceride. The minimum concentration of deltamethrin required to kill fully susceptible *Bovicola ovis* is considered to be 0.5 parts per million (ppm) but here concentrations 10,000 times greater than that failed to result in significant levels of *B. bovis* mortality.

It is highly likely, given a number of similar, but as yet unverified, reports of treatment breakdown, that pyrethroid tolerance may exist in a large proportion of the UK herd.

A: Drugs and drug resistance

Ref: P1A162/0377

Characterisation of monepantel resistance in *Teladorsagia circumcincta* isolates

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Monepantel (Zolvix®) was the first new drug family of anthelmintic to appear on the livestock healthcare market for over 25 years in 2009. Although efforts have been made to protect the compound, the first cases of resistance have been reported only two years after its launch. The first case of resistance has been reported in sheep and goats in New Zealand and another case has been reported on a goat farm in New South Wales, Australia. All reports have found that *Teladorsagia circumcincta* was one of the parasites surviving treatment. The aims of this study were to characterise the monepantel resistance phenotype and genotype of three *T. circumcincta* isolates that had been artificially generated for monepantel (MPTL) resistance (named MTci2-11, MTci5-13 and MTci7-12) from previously phenotypically characterised parental isolates (MTci2, MTci5 and MTci7 respectively). Treatment efficacies based on the percentage reduction in average estimated total worm burden between treated and untreated groups were 48%, 28% and 9% for MTci2-11, MTci7-12 and MTci5-13 respectively confirming resistance to MPTL.

Two areas have been examined – firstly the generation of sequencing data for the MPTL-1 gene to look for the changes in the acetylcholine receptors of the DEG-3 subfamily that have been shown to impact on MPTL sensitivity in *Haemonchus contortus*. Secondly to characterise the impact of MPTL and monepantel sulfone on the development and hatching of eggs in vitro in hope of developing a tool to assess MPTL efficacy in the field.

Direct sequencing from pools of larvae resulted in 1400-1686bp of coding sequence for MPTL-1 in all isolates with the exception of MTci7-12 where no amplification was observed. Initial results do not show any of the anomalies seen in *H. contortus* MPTL-1 gene. The egg hatch test showed a decrease in sensitivity to MPTL and MPTL-sulfone in MTci2-11 and MTci7-12 compared to their unselected parental isolates, MTci5-13 was not examined. To conclude, as yet no genetic markers of resistance have been found in the *T. circumcincta* MPTL-1 gene. The egg hatch test did show a dose dependent response in its activity against parental and selected isolates. ABC transporters such as PgPs would be another key area for investigation to see if they play a role in MPTL resistance for the future.

A: Drugs and drug resistance

Ref: P1A163/0379

The efficacy of a single treatment with Cydectin 10% Long Acting Injection at housing in the control of psoroptic mange in Belgian beef cattle

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Bovine mange may be caused by several mite species. *Psoroptes ovis* affects a large proportion of the Belgian Blue beef industry. Mange can be managed by two consecutive injections with macrocyclic lactones at 0.2 mg/kg bodyweight, 7 to 10 days apart. Failure to comply with this schedule or under-dosing have been major challenges to this approach, leading to chronic mange infections in many Belgian beef herds. Furthermore, on farms with a high and continuous infestation pressure repeated treatment cycles throughout the housing period are often necessary. This study aimed to evaluate the benefit of a single treatment with the Cydectin 10% Long Acting Injection (CLA: 1 mg moxidectin/kg bodyweight) early in the housing period, combined with re-treatment using Cydectin 1% Injection (two treatments 7 to 10 days apart) of those animals that were positive for live mites at any follow-up visit, in order to manage the infestation and clinical disease at group level. Two farms were selected and on each farm, 15 animals, positive for live mites, were enrolled. Animals were treated at housing (Day 0) and were examined for the presence of live mites at fortnightly intervals throughout the housing period. In addition, an animal based Clinical Index (CI) and a group based Scratching Index (SI) were recorded at each of these time-points. Before treatment the average mite count on farm 1 was 465 (range 1 - 3156), with an average CI of 10.2 (range 2.3 - 18.3) and a SI of 3.2. On Farm 2, the average mite count was 953 (6 - 5590), with an average CI of 10.3 (3.1 - 36.6) and a SI of 3.7. On Day 14, 8 and 11 out of the 15 animals still had positive mite counts on Farm 1 and 2 respectively. The average mite count in these positive animals was 26 and 102 and the CI had decreased to 4.2 and 6.2, on Farm 1 and 2 respectively. These positive animals were re-treated. In the follow-up, 2 animals were still positive on Farm 1 and treated again. On Farm 2, 6 and 3 animals were positive and treated at subsequent time-points. On Farm 1, all animals were negative by Day 42 and on Farm 2 by Day 56. All animals remained negative for mite counts thereafter with low CI and SI. The current study demonstrates that a single administration of CLA provides a rapid decrease in number of mites and clinical symptoms, requiring only targeted follow-up treatments to provide adequate control of psoroptic mange in Belgian Blue cattle.

A: Drugs and drug resistance

Ref: P1A164/0380

The benefit of a treatment with Cydectin 10% Long Acting Injection at turn-out on the growth of beef calves compared to Cydectin Pour-on treatment in France

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The persistent efficacy of Cydectin Pour-On for Cattle (CPO: 0.5 mg moxidectin / kg bodyweight) is five weeks against gastro-intestinal (GI) nematodes. In order to protect first-grazing season calves against GI nematodes during the entire grazing season, two treatments in the early grazing season are required, usually administered at turn-out and eight to ten weeks later. The detrimental effect of GI nematode infections may be substantial if farmers fail to give the second treatment. Cydectin 10% Long Acting Injection (CLA: 1 mg moxidectin / kg bodyweight) has a persistent efficacy of 3 to 3.5 months against GI nematodes. In this study, the effects of a treatment at turn-out with CLA or CPO were compared. Twenty first grazing season beef calves were allocated to one of two treatment groups based on body weight on 3 farms (FR01-FR03) in Burgundy (France). Animals were weighed, treated and turned-out in April to a separate pasture plot for each treatment group. Weight was measured again mid-season (July) and at housing (November). Samples for pasture larval counts were collected at turn-out and again at housing. Blood samples for pepsinogen analysis were collected at housing. The average daily weight gain (ADG) on FR01 was 0.63 kg for CPO and 0.77 kg for CLA. The average daily weight gain on FR02 and FR03 were 0.49 kg and 0.62 kg, and 0.52 kg and 0.65 kg, respectively. Over the three farms, the average daily weight gain was 0.54 kg (± 0.05) for CPO and 0.68 kg (± 0.05) for CLA. Due to variability between farms, the overall analysis did not indicate a significant difference ($P = 0.119$) and therefore separate analysis for each farm was not performed. On each of the three farms, the percentage of *Ostertagia* larvae increased and the percentage *Cooperia* larvae decreased during the grazing season on those pastures grazed by CPO treated animals. In contrast, the percentage of larvae of the pathogenic abomasal nematode *Ostertagia* decreased on pastures grazed by CLA treated animals. Nevertheless, the pepsinogen levels indicated exposure to *Ostertagia* in CLA and CPO treated animals. The current study indicates that a single treatment with CLA resulted in an average of 0.14 kg higher ADG and in a better control of *Ostertagia* compared to a single treatment with CPO, while allowing the development of immunity.

A: Drugs and drug resistance

Ref: P1A165/0392

Molecular evaluation of benzimidazole resistance of *Haemonchus contortus* from Ceará State, Brazil

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Haemonchus contortus is the most prevalent nematode in tropical areas and is controlled with the use of anthelmintics, which may lead to increased resistance levels. Benzimidazole resistance is associated with SNPs F200Y, F167Y and E198A in the β -tubulin isotype 1 gene. Our objective was to characterize BZ resistance using real time PCR in eggs of *H. contortus* isolates from different locations in the Ceará State, Northeast Brazil. Feces were collected from 19 sheep farms from different counties in Ceará State. Quantitative PCR assays were done using DNA from pools of 10,000 eggs per farm. Eggs obtained from the ISE isolate were used as reference of susceptibility. Allele frequencies from egg pools were similar to frequencies from adults (both males and females) and larvae even in cases of mixed infections. Quantitative PCR results showed resistant allele frequencies that varied from 22.75 to 72.38% for the SNP F200Y, and 19.67 to 80.51% for the SNP F167Y. Resistance at SNP E198A was detected at very low levels in five isolates. Our results suggest that the SNPs F167Y and F200Y are both important for benzimidazole resistance in the studied populations. In addition, DNA obtained from eggs can be used for allele frequency determination for the β -tubulin isotype 1 gene of *H. contortus*. In the future, we hope that this test may be used as part of a resistance monitoring service that may generate geographically indexed information accessible for interested farmers.

A: Drugs and drug resistance

Ref: P1A166/0410

First report of anthelmintic resistance in nematodes of horses in Cuba

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The recognition of anthelmintic resistance has become increasingly important in the veterinary field during recent years and this is particularly true for equine the medicine. Benzimidazole resistance is ubiquitous worldwide and reports of ivermectin /milbemycin resistance is also beginning to emerge. The objective of this work was to evaluate the efficacy of albendazole (ABZ) and ivermectin (IVM) in the control of strongyle parasites of horses in the municipality of Camaguey, Cuba. The faecal egg count reduction test of strongyle eggs was carried out for both drugs in nine herds. The study involved 148 naturally infected horses with gastrointestinal nematodes. The obtained data were processed according to Coles et al. (1992). The gastrointestinal helminth larvae were identified as: *Cyathostomins*, *Posteriosomum* sp. and *Gyalocephalus* sp. (small strongyles); *Strongylus vulgaris*, *S. edentatus*, *Triodontophorus* sp. and *Oesophagodontus robustus* (large strongyles) and *Trichostrongylus axei*, *Strongyloide westeri*, *Oxyuris equi* and *Parascaris equorum*. We observed that five of the nine herd in study presented resistance to the ABZ, two were susceptible and only two presented the expected efficacy (>99%). The efficacy found in the resistant herd was of 41.7; 42.9; 81.2; 85 and 87.5% in Nadales, La Esperanza, Saratoga, Equitacion and Aranda, respectively. Larvae identification was done post-treatment from a pool of faeces and in all cases revealed the presence of cyathostomins. IVM was 100% effective in all studied herd. It was observed that more and more, farmers and horseman people are using IVM as the main compound for routine use in horses making it a concern for the selection of resistant parasite populations. In conclusion, anthelmintic resistance is present in horses in Camaguey, where parasite treatments should be examined routinely to determine its effectiveness. This is the first report of drug failure in the treatment of horse parasites in Cuba.

A: Drugs and drug resistance

Ref: P1A167/0470

The increase of the efficacy of benzimidazole anthelmintics by mechanochemical technology and Drug Delivery System

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The influence of mechanochemical technology and Drug Delivery System on the efficacy of benzimidazole anthelmintics was studied on 45 sheep which were spontaneously infected by *Nematodirus* and other species of gastrointestinal nematodes suborder Strongylata. The supramolecular complexes of albendazole and fenbendazole with arabinogalactan polymer produced by mechanochemical modification of substance in activator of percussive-grating type were used in tests. Sheep of different groups of 8–9 animals each received per os supramolecular complexes of albendazole and fenbendazole at a dosage rate of 1,0 mg/kg of active substance (AS) in comparison with the basic substances of albendazole and fenbendazole which were administered orally at dosage rates of 1,0 and 10 mg/kg. The control group was untreated. The efficacy of drugs was determined on the basis of the results of coproscopic study of feces by flotation methods before and 18 days after treatment. The efficacy of anthelmintics was determined by the control test with calculating middle amount of nematode eggs in 1 gram of feces. The supramolecular complex of albendazole at a dose of 1,0 mg/kg showed 88,9% efficacy against *Nematodirus* and 100 % against other gastrointestinal nematodes. Albendazole (substance) at doses of 1,0 and 10 mg/kg showed 7,1 and 100 % efficacy respectively. The supramolecular complex of fenbendazole has showed 95,6 % efficacy against *Nematodirus* spp. at a dose of 1,0 mg/kg of active substance and 100 % efficacy against other species of gastrointestinal nematodes. Basic drug – fenbendazole (substance) has showed 96,39 % efficacy respectively at a dose of 10 mg/kg and 5,13 % at a dose of 1,0 mg/kg bw. The present study demonstrates that the efficacy of supramolecular complexes of albendazole and fenbendazole was 8,5–10 times higher compared with the efficacy of the basic drug.

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A: Drugs and drug resistance

Ref: P1A168/0477

Field evaluation of the effectiveness of an oral toltrazuril and iron combination (Baycox® Iron) in maintaining weaning weight by preventing coccidiosis and anaemia in neonatal piglets

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Effectiveness of an oral combination of toltrazuril and iron dextran (Baycox® Iron) to maintain weaning weight by preventing coccidiosis caused by *Isospora suis* and iron-deficiency anaemia in neonatal piglets was investigated on three commercial pig farms. Piglets were randomised within litter by bodyweight to treatment or control group. On SD 3 piglets allocated to the control group (CG) each received 1 mL Baycox®, containing 50mg/mL toltrazuril orally and commercially available iron (200 mg/piglet) by intramuscular injection. Piglets allocated to the treatment group (TG) each received 1 mL toltrazuril and iron combination orally (Baycox® Iron) containing 50 mg/mL toltrazuril and 228 mg iron as iron dextran. 6493 piglets completed the study. Bodyweight at weaning on SD 21 of piglets treated with the oral toltrazuril and iron combination was confirmed to be non-inferior to the control treatment with <1% difference between group mean body weights. Faecal samples from at least 10% of litters on SD 14 demonstrated control of coccidiosis. On SD 21 haemoglobin levels were above minimum levels to maintain health. There was no difference in mortality between the two groups. This large scale field evaluation clearly demonstrated the effectiveness of a combination of oral toltrazuril and iron (Baycox® Iron) in maintaining body weight at weaning compared to conventional treatment. The combination was effective in preventing coccidiosis and anaemia and thus provides a valuable alternative that reduces stressful events in neonatal piglets. The combination product was safe without product related adverse events.

A: Drugs and drug resistance

Ref: P1A169/0516

Efficacy of three treatment regimens of a deltamethrin solution (Deltanil®) in the management of *Hippobosca equinum* infestation in horses

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This randomised, blinded, controlled clinical trial assessed the efficacy of a deltamethrin solution (Deltanil®, Virbac, France) in the management of *Hippobosca equinum* infestation in horses. Seven days before the start of the trial (D -7), 56 resting horses from the same farm were randomly allocated to a treatment regimen group based on pasture location and a semi-quantitative evaluation of parasite number in the anal area (SQP score). Horses were treated with 10ml deltamethrin applied to the back area from the tail to the mane either every week (Group 1), every four weeks (Group 2), every two weeks (Group 4), or were left untreated (Group 3). SQP score and count of flies on pictures from four body regions (head, body, inguinal and anal areas) (P score) were performed on Day 1 (before treatment), and subsequently on Days 2, 3, 8, 15, 22, 29, 36. No side effects were observed in the 41 horses being administered the product. Twenty-four horses with an SQP score ≥ 1 on D -7 were included for efficacy evaluation. The mean SQP score and P score before treatment were 1.75 [1-3] and 5.5 [0-20] respectively. In the untreated control group SQP score remained above 1, and P score above 3 throughout the study. In the treatment groups, both scores were equal to 0 on all evaluation days. Deltanil® was well tolerated when applied from once a week to once a month, rapidly decreased (<24 hours) *H. equinum* infestation, and prevented further infestations for at least one month in resting horses.

A: Drugs and drug resistance

Ref: P1A170/0543

Significance of timing on effect of metaphylactic toltrazuril treatment against eimeriosis in calves

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In this multicentric, randomized, blinded and placebo-controlled field study effect of treatment with toltrazuril (Baycox® Bovis) on oocyst excretion, diarrhoea score and weight gain was studied in Danish dairy herds with confirmed history of eimeriosis (coccidiosis) and prevalence of *Eimeria bovis* and *Eimeria zuernii*. Three commercial herds and a total of 71 calves, aged 48-135 days, were included. Treatment with a single oral dose of toltrazuril (15 mg/kg) was given after relocation to common pens and one week before expected outbreak of eimeriosis. The effect of treatment was followed by weekly faecal sampling and weighing initially and at the end of a study period of 8 weeks. In Herd 2 and 3 toltrazuril treated calves gained on average 7.95 kg more than placebo treated calves ($p=0.007$), and both oocyst excretion and prevalence of *Eimeria* spp. were significantly reduced the first weeks post treatment. In Herd 3, by contrast, the farmer made some unforeseen changes in the management which entailed relocation to large deep-litter pens 3-6 weeks post treatment. In addition, many calves were not treated metaphylactically while few calves excreted oocysts when the trial was initiated. Thus, no significant difference in weight gain was found between toltrazuril and placebo treated calves ($p=0.523$), and the oocyst excretion of toltrazuril treated calves was significantly higher during week 7 and 8. Significant differences in faecal scores were observed between the herds ($p<0.002$) but not between treatment groups in any of the herds. In conclusion, timing of treatment is crucial for optimal effect of metaphylactic toltrazuril treatment on weight gain and oocyst excretion.

A: Drugs and drug resistance

Ref: P1A171/0556

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 one 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, 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 the wettest month of 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 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 blood 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.

A: Drugs and drug resistance

Ref: P1A172/0560

A survey on anthelmintic resistance in grazing cattle of Argentina

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Anthelmintic resistance (AR) in nematodes parasiting domestic ruminants is an increasing phenomenon over the world. A large scale field study was carried out to assess the current situation in cattle of Argentina. Thirty-one beef farms from the central and northeastern areas of Argentina were selected, in which beef production was based on pasture grazing and the use of different drugs at several times to control trichostrongyle infections. Faecal egg count reduction tests (FECRT) were performed to determine the clinical efficacy of ivermectin (IVM 1%, 0.2 mg/kgbw), ricobendazole (RBZ 15%, sc, 3.75 mg/kgbw) and levamisole (LVM, Phosphate 18.8%, sc, 7.5 mg/kgbw). Weaned calves of 7-10 months old, weighing 180 kg in average, which had not been dewormed for at least 60 days before the survey, were distributed into 4 comparable groups of 15 animals each according to individual nematode eggs counts (epg), and assigned to one of the treatments as above and an untreated control group. Faecal samples were taken at treatment (day 0) and at day 14. Coprocultures to establish the proportional contribution of each species to worm infection were also performed. Efficacy of each drug was determined by comparing the mean epg of each group at day 0 and day 14. Resistance was established when FECRT revealed an efficacy < 90%. To establish the drug classes and frequency of use through the years a basic questionnaire was completed by farmers. Results show that 93.5% of the farms presented AR to any of the drug classes. Resistance to IVM was present in 28 farms (90.3%), mean efficacy of 60.9% (range 0-88.5%). Resistance to RBZ was diagnosed in 9 farms (29%), mean efficacy of 69.9% (range 13.9-88.1%). Multiple-resistance to IVM and RBZ was detected in 8 farms (25.8%). Resistance to LVM was not found in any farm, its mean efficacy was 97.7% (range 90.9-100%). Mainly *Cooperia* spp. but also *Haemonchus* spp. and *Trichostrongylus* spp. were the genera identified in those farms with resistance to IVM. *Cooperia* spp. was predominant in the farms with resistance to RBZ but also *Ostertagia* spp. and *Haemonchus* spp. in lower proportions. The high levels of resistance to IVM are in consonance with its intensive use in the last two decades. The increasing resistance to benzimidazoles and the multiple-resistance to IVM and RBZ should be also noticed. The relatively scarce use of LVM may account for its high efficacy in this study.

A: Drugs and drug resistance

Ref: P1A173/0616

Adaptation of the Larval Migration Inhibition Assay for cyathostomins

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Resistance to the Macrocyclic Lactone drug family among important equine parasites, like cyathostomins, is an emerging global problem and there is an urgent need for both quality surveillance of resistance and the development of better tools for detection. The Faecal Egg Count Reduction Test (FECRT), which is considered the gold standard for measuring resistance, lacks sensitivity and is complicated by various factors as it is applied to horses and so attention has recently been directed toward the potential use of in vitro techniques.

The Larval Migration Inhibition Assay (LMIA) has been used successfully to measure differences in drug susceptibility between isolates of *Haemonchus contortus*, an important gastrointestinal nematode of sheep, however, it has not been sufficiently validated for use with cyathostomins. A modification of the 96-well plate LMIA format, developed by Kotze and co-workers (2006), was tested on various cyathostomin populations in our laboratory. IC₅₀ values of individual populations showed a high level of repeatability between assays on the same day, however showed some inconsistency across time, indicating an effect of pre-assay storage time. The LMIA was able to demonstrate a significant difference in Ivermectin sensitivity between two cyathostomin isolates, which was not detectable via FECRT. The results from this work lend support for the potential use of the LMIA as a useful tool for the early detection of resistance in cyathostomin populations.

A: Drugs and drug resistance

Ref: P1A174/0632

Failure of ivermectin to control nematodes on cattle farms in England

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The efficacy of anthelmintics was investigated on 36 cattle farms in England, a self-selecting group of respondents to a postal offer of free testing made to 1300 farms. Individual faecal samples were taken by the farmers on the day of treatment and 14 days later, and sent to the laboratory for faecal worm egg counts (FEC) using the FLOTAC method set to a detection limit of 2 eggs per gram (epg). Macrocytic lactone efficacy was assessed on 17 farms, with starting FEC between 22 and 355 epg. Reduction in FEC ranged from 0 to 100%, and was less than 95% in 10/11 groups treated with ivermectin pour-on (median FECR 57%, quartiles 1-3, Q1-3 = 9, 81), 1/3 groups treated with moxidectin pour-on (FECR 100, 97, 91%) and 2/3 groups treated with injectable ivermectin (FECR 100, 89, 43%). Treatment by levamisole injection was evaluated on three farms (FECR 84, 75, <0%). Composite or pooled FEC was used to evaluate efficacy on 12 further farms: 10 using ivermectin pour-on (median FECR 46%, Q1-3 = 15-57, maximum FECR 65%), one using moxidectin pour-on (FECR 97%) and one using benzimidazole bolus (FECR 100%). The efficacy of ivermectin pour-on and injectable formulations was compared on three additional farms: percent FECR (pour-on/injectable) was 70/41, 7/53 and 0/69. Third-stage larvae were cultured from faeces collected 14 days after treatment with macrocytic lactone efficacy on six farms, and on two farms *Ostertagia ostertagi* was identified. On one further farm, calves that were coughing were treated with ivermectin pour-on to little clinical effect, and then randomly assigned injectable levamisole and oral albendazole treatment. Growth rate was reduced in the group treated with albendazole relative to that given levamisole, and numerous *Dictyocaulus viviparus* larvae were identified in the faeces. Results suggest that efficacy of anthelmintics on cattle farms in the UK is commonly poor. While this might in part be due to factors other than drug resistance, especially for pour-on formulations, follow-up tests indicate serious cause for concern, especially given survival of *O. ostertagi* and *D. viviparus* after treatment. Wider and more representative surveys of anthelmintic resistance in cattle are warranted. Novel FEC methods with a lower detection limit (= increased analytic sensitivity) provide a means to overcome low starting FEC, a common barrier to meaningful FECR data in cattle.

A: Drugs and drug resistance

Ref: P1A175/0633

Development of pyrosequencing assays for analysis of three *Trichostrongylus colubriformis* β -tubulin single nucleotide polymorphisms associated with benzimidazole resistance

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Resistance to benzimidazoles (BZs) in trichostrongylids is a worldwide problem, particularly for small ruminant livestock production. Sensitive, reliable and cost effective methods are required to assess the resistance status of at least the most important species. Besides currently available *in vivo* and *in vitro* methods for the detection of BZ resistance molecular tools are becoming increasingly important. Single nucleotide polymorphisms (SNPs) in the β -tubulin isotype 1 gene of various nematode species have been shown to correlate with BZ-resistance. Methods such as conventional PCRs, real time PCRs as well as RFLP methods and pyrosequencing assays, which have the advantage of enabling the analysis of pooled larval samples, have been described. While PCR-based methods have been reported for the three most important trichostrongylid nematode species *Trichostrongylus*, *Haemonchus* and *Teladorsagia*, pyrosequencing assays are so far only available for the latter two. This led to the design and evaluation of a pyrosequencing assay for both β -tubulin genes, isotype 1 and 2 of *T. colubriformis*. For both alleles respective PCR-fragments were combined in defined ratios to evaluate the assay and the correlation between the calculated and the measured allele frequencies was very high (> 0.97) for all three SNPs (Codon 167, 198 and 200). Defined isolates of known BZ-resistance status have been used to evaluate occurrence of SNPs in both β -tubulin genes. The susceptible reference isolate displayed no increased SNP frequency above technical background, while for a phenotypically resistant isolate increased SNP frequencies were observed in codon 200 in the β -tubulin isotype 1 gene.

A: Drugs and drug resistance

Ref: P1A176/0637

Are wild deer that carry anthelmintic-resistant nematodes reservoirs or refugia for livestock?

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Anthelmintic resistance in gastrointestinal nematodes of grazing ruminants is an emerging and potentially catastrophic problem for livestock farming. Wild and farmed red, roe and fallow deer from southern England (n=48) were investigated for the presence of anthelmintic-resistant nematodes, by sampling of adult nematodes in the abomasum and recovery of nematode eggs from rectal faeces. Alleles for benzimidazole resistance were identified in *Haemonchus contortus* from roe deer. Larvae cultured from the faeces were used to infect a calf and two lambs, demonstrating cross-infectivity and providing material for *in vitro* tests of anthelmintic efficacy. Phenotypic resistance to thiabendazole was confirmed by egg hatch test (EC50 = 0.149 ug/ml), and by the persistence of faecal egg counts and adult nematodes in lambs following treatment. The roe deer sampled occupied a mosaic mixed-use landscape, pasture being interspersed with fragmented woodland, which provides ample opportunity for shared grazing between deer and livestock. Preliminary predictive epidemiological modelling suggests that patterns of cross-infection are likely to be strongly influenced by livestock grazing patterns and spatio-seasonal habitat use by the deer, affording some opportunity to manipulate cross-transmission. While it is possible that wild deer act to transfer alleles for anthelmintic resistance between livestock farms, they might equally play a role in buffering such transfer by diluting resistance alleles and acting as natural refugia for susceptible genotypes. Further population-level studies are needed to provide evidence for either mechanism, an indication of their importance in the development of resistance in livestock, and justification for specific management actions.

A: Drugs and drug resistance

Ref: P1A177/0692

Parasite Control on Thoroughbred Studs

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Intestinal nematode infections can result in a substantial impact on health, welfare and performance of Thoroughbreds (TB). Life-long nematode control is required in horses, which is primarily achieved by anthelmintic administration. Decades of intensive anthelmintic use appears to have reduced prevalence of *Strongylus vulgaris*, but there is now widespread anthelmintic resistance in two other important parasites; the Cyathostominae and *Parascaris equorum*. The aim of this project is to identify current parasite control practices on TB studs within the UK, assess their clinical impact and determine drug efficacy on a subset of TB studs.

To date, six TB studs have been recruited for drug efficacy testing based on their history of anthelmintic use, either: a) frequent use of drugs at defined intervals (interval) or b) targeted by faecal worm egg count (targeted). Two studs reported clinical concerns over the control of *P. equorum* and the tapeworm, *Anoplocephala perfoliata*. On all studs the centrifugal flotation technique, sensitive to 1 egg per gram (epg), was used to detect strongyle-type and *P. equorum* eggs by faecal egg count (FEC) in baseline sampling of both adults and youngstock. On studs where >10 animals showed >50epg faecal egg count reduction tests (FECRT) and egg reappearance periods (ERP) were used to assess efficacy of two anthelmintics, pyrantel (PYR) and ivermectin (IVM). Larval culture and subsequent morphological identification of strongyle third-stage larvae was performed from pre- and post-treatment faecal samples. On stud E, double centrifugal sugar flotation and centrifugal saturated salt flotation faecal and antibody detection ELISA tests were used to detect infection with *A. perfoliata*. Preliminary analysis of FECs in youngstock and adults under both interval and targeted regimes show negative binomial distribution in adults and right shifted negative binomial distribution in youngstock on all studs. Baseline (pre-treatment) and drug efficacy testing and analysis work is ongoing and a questionnaire study to capture current practice across a larger cohort of studs is underway.

A: Drugs and drug resistance

Ref: P1A178/0701

The Effect of Cryopreservation on Infective Stage *Cooperia oncophora* Survival and their Infectivity in the Definitive Host

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Repeated passage of a strain through a donor animal has been shown to affect factors such as resistance to anthelmintics, decreasing the resistance profile of the strain when passaged outside of the presence of the resisted drug. Strains of parasitic nematodes isolated from the field are valuable for a number of purposes including efficacy studies. Maintenance of these strains in the laboratory requires regular passage through donor animals, however repeated passaging will inevitably lead to a change in genetic and phenotypic characteristics of the strain. Cryopreservation of nematode larvae is the gold standard for long term preservation of parasitic nematodes, and has been subject to a significant amount of study. It permits the possibility of efficient storage of large numbers of nematodes and maintains viability, pathogenicity and genetic stability, ensuring full functional and developmental potential. Some studies have suggested that invasive surgical implantation of larvae is necessary after cryopreservation. This study details an efficient method for storage of *Cooperia oncophora* in liquid nitrogen using a slow cooling technique on larvae which have been desheathed in 10% NaOCl, allowing for desiccation of the larvae and subsequent prevention of internal ice crystal formation. There was no significant decrease in recovery rates over a 12 week period. The mean recovery rate after 12 weeks in cryostorage was 87%. Larvae which were recovered after 12 weeks in cryostorage were used to orally challenge 2 calves. Faecal egg counts showed that the cryopreserved larvae were sufficiently viable to induce infection in the definitive host by oral challenge, suggesting surgical inoculation to be unnecessary to induce a patent infection in ruminants using desheathed *C. oncophora* larvae. This study shows cryopreservation of *Cooperia* sp. larvae in liquid nitrogen to be of value in the maintenance of laboratory strains, obviating the need for repeated passage through donor animals; a labour and resource intensive exercise which may alter the viability and genetic characteristics of the strain. Moreover, its use contributes to efforts to reduce the number of animals used in parasite maintenance whilst refining the method of larval administration.

A: Drugs and drug resistance

Ref: P1A179/0702

Production of the recombinant protein GST-TOG1/2 for *Haemonchus contortus* tubulins one step purification and target- based assay development.

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In the search for new anthelmintics able to overcome the resistance problem, we have recently reported the synthesis of novel valerolactam-benzimidazole hybrids *in vitro* and *in vivo* bioactive compounds using *Nippostrongylus brasiliensis* rat model integrating physiology- based assays and *ex vivo* diffusion studies (Munguía *et al.*, 2013, 2015). The present work is underway on the development of a target- based assay, using *Haemonchus contortus* β - tubulin, in order to study the involvement of this mechanism of action for the new hybrid compounds. By means of comparative studies using an anthelmintic- susceptible isolate of *H. contortus* adult parasites (*Hc S*) and a benzimidazole- resistant isolate (*Hc R*), the ability to inhibit tubulins polimerization will be assessed. For this purpose, the tubulins extraction from parasite homogenates will be conducted by affinity chromatography, based on the capacity of TOG (tumor over expressed gene) 1/2 domain in MAPs (microtubule associated proteins) to bind tubulins. In order to obtain the fusion protein in sufficient amounts to build affinity columns, we first expressed in *Escherichia coli* the TOG1/2 domain of the protein Stu2 from *Saccharomyces cerevisiae*, fused to GST (glutathione S- transferase) tag, according to Widlund *et al.*, 2012. The fusion protein GST-TOG1/2 was cloned into the pGEX-6P-1 vector (pGEX-6P-1 Stu2 1-590, Addgene plasmid #38314), and used to transform *E. coli* BL21, RosettaBlue DE3 and BL21 Codon Plus pLys strains. The growth and expression were carried out in the ZYM 5052 autoinduction media at different culture temperatures. The fusion protein was purified by glutathione sepharose, characterized by SDS-PAGE and anti-GST western blotting. The expression in BL21 strain gave rise to a large number of truncated proteins, while this problem was not observed with the other two strains. RosettaBlue produced the fusion protein (mainly present in the soluble fraction) with the highest recovery growing at 33 °C overnight. Finally, GST-TOG1/2 purified protein, was immobilized on N-hydroxysuccinimide activated sepharose to perform the affinity chromatography of *H. contortus* tubulins.

Acknowledgments: ANII Uruguay, for funding support

A: Drugs and drug resistance

Ref: P1A180/0706

Molecular confirmation and drug efficacy assessment against *Toxascaris leonina* infection in Asiatic lions (*Panthera leo persica*)

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The ascarid eggs recovered from Asiatic lions were delineated as *Toxascaris leonina* eggs based on morphometric and molecular studies (polymerase chain reaction targeting internal transcribed spacer regions). Therapeutic management with fenbendazole @ 10 mg/ kg body weight, singly for three days proved ineffective with maximum faecal egg count reduction on day 3 post treatments (69.35%), which further reduced to 51.61% at day 21 post treatments. Therapeutic intervention with extended period dose schedule (5 days) with fenbendazole (@10 mg/ kg body weight) although proved effective and showed a maximum faecal egg count reduction of 95.34% at day 7 post treatments. But, when ivermectin (@ 100µg/ kg body weight) was given on three alternate days, it proved effective as a faecal egg reduction of 95.74% was recorded at day 7 post treatments. Thus, the present study highlighted the molecular confirmation of *T. leonina* and first case of fenbendazole resistance against *T. leonina* in Asiatic lions.

A: Drugs and drug resistance

Ref: P1A181/0730

Evidence of anthelmintic resistance in a horse farm in Spain based on FERT and lower egg reappearance period

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The present study was done in a stud farm in the north of Spain. 41 animals were selected and were divided in four groups of 10 animals at random. All animals were excreting eggs before the treatment and faecal cultures identified only cyathostomines. Each group was treated with different anthelmintic products: Strongid® (pyrantel pamoate-PYR), Panacur® (fenbendazole-BZ), Equalan® (ivermectin-ML) y Equest® (moxidectin-ML).

The *Faecal Egg Count Reduction Test* (FERT) was the selected technique to test the products' efficacy and *Egg Reappearance Period* (ERP) was evaluated at 30, 37, 45, 60, 67 and 75 days post-treatment.

In horses, there is a lack of consensus on study design, data analysis and data to evaluate AR based on a FEC reduction test. In the present study, susceptibility was defined as a reduction of FEC higher than 90%. When considering this value of reduction in the elimination of eggs in faeces as the minimum for an effective treatment our result show ivermectin fulfilled in 80% of cases, moxidectin in 81.82% (the most effective), fenbendazole in 20% (the less effective) and pyrantel pamoate in 70%. About ERP, after 30 days of treatment 65% of animals were positive (included animals with no effective treatment) and the 55% of this eliminated more than 200 epg.

Although the best result were obtained with macrocyclic lactones, the observed reduction of the egg reappearance period can be considered as a first sign of resistance in cyathostomines. Recent studies have also provided evidence of reduced ERPs of less than 5 weeks probably due to apparent resistance on the luminal L4 stage.

A: Drugs and drug resistance

Ref: P1A182/0731

Field test screening for detecting resistant varroa mites in Spain

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Recently a lack of efficacy of the approved veterinary products against *Varroa destructor* has been verbally reported in Spain. One of the factors that may be involved is the development of mite resistant to the active ingredients used to this purpose, mainly pyrethroids. They are frequently used as the control of *Varroa destructor* in Spain is ruled by the RD 608/2006, which establishes one compulsory treatment with an approved veterinary medicine, although usually two treatments are needed.

During 2014 and 2015 Spanish beekeepers associations were contacted in order to determine possible mite resistances by Pettis Test-Detecting Varroa Mite Resistance, including a control group for higher reliance as a modification.

A total (up to April 2015) of 29 apiaries from different locations were checked using the commercial strips Checkmite® (Coumaphos), Bayvarol® (Flumethrine), Apistan® (Fluvalinate), Apivar® /Apitraz® (Amitraz). Due to methodology conditions, nine apiaries were discarded due to a lack of mites while seven more had not enough mites for reliable data. The inclusion of a control group jar has shown to be necessary for the final data analysis.

Our results indicate the presence of mites resistant to coumaphos in three colonies and to fluvalinate in one more. To confirm those results, the mites are being studied by molecular biology with already published resistance sequences. After inspection and questionnaire analysis, reduced efficacy problems currently reported seem to be more related with wrong apiary management, cross infection between apiaries, inadequate posology or a multifactorial status than with the presence of highly resistant mites.

A: Pharmacokinetics

Ref: P1A213/0211

Plasma disposition, milk excretion and efficacy of mebendazole in lactating donkeys naturally infected by Cyathostominae

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Therapeutics are administered to donkeys based on dosage and intervals recommended for horses because few drugs have donkey-specific indications. Although, mebendazole (MBZ) has been licensed for use in horses there are no data available regarding its pharmacokinetic disposition and effectiveness in donkeys. The plasma disposition, milk excretion and efficacy of a paste formulation of MBZ in donkeys were examined in a field trial. Two groups of six donkeys received MBZ paste at horse dosage 10 mg/kg body weight (MBZ 1) and at double horse dosage 20 mg/kg body weight (MBZ 2) in animals naturally infected by Cyathostominae. Heparinised blood and milk samples were collected at times between 1 and 120 h post treatment. The samples were analysed by high performance liquid chromatography. Individual FECs (Faecal Egg Count) were performed on each animal before the treatment (day -3) and weekly from day 7 until day 56 post treatment using a modified McMaster technique with a sensitivity of 10 EPG. The results of FECRT for both MBZ dosages were efficient (>95% efficacy) until day 28. The plasma concentrations and systemic exposure of MBZ in donkeys were relatively lower compared with the other benzimidazoles. Dose-dependent plasma dispositions were observed at the increased dose (10 vs 20 mg/kg). This study indicated that oral administration of MBZ has a minimal disposition rates into the milk (ratio of AUC milk/plasma was 0.18 ± 0.02) and may be used safely in lactating donkeys with zero milk-withdrawal period. Therefore, horse dose of MBZ could be used for parasite control in donkeys.

A: Pharmacokinetics

Ref: P1A214/0239

Use of eprinomectin against *Rhipicephalus (Boophilus) microplus* in cattle: measurement of tick exposure to the drug

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Eprinomectin (EPM) is a macrocyclic lactone used against endo and ectoparasites without withdrawal time in milk and meat after its pour-on administration to cattle at 0.5 mg/kg. Previous studies evaluated EPM efficacy against ticks. The current work investigated the relationship between plasma disposition and EPM concentration profiles achieved within ticks collected from infected cattle (parasite exposure). Furthermore, EPM efficacy against *Rhipicephalus (Boophilus) microplus* after its administration at two different dose rates was evaluated. Eighteen Braford calves naturally infected with *R. (B.) microplus* were divided in three experimental groups with a similar level of infestation (Kruskal-Wallis test, $P > 0.05$): control untreated group and two (2) EPM pour-on treated groups (1 and 1.5 mg/kg). Blood samples from the host and ticks at different life stages were collected between 0 and 21 days post-administration to measure EPM concentrations by HPLC. Female ticks (4.5-8 mm) remaining on treated animals were counted between 1 and 21 days post-treatment to evaluate EPM efficacy. EPM was recovered both in plasma and in collected *R. (B.) microplus* ticks. EPM systemic availability in treated cows was linearly related to the administered dosage level. Peak plasma concentrations were in a range between 13.8 and 90 ng/ml which may assure a post-treatment milk residual concentration below the permitted EPM maximum residue level. High EPM concentrations were measured in collected ticks. Parasite exposure to the drug had a good correlation to the EPM dose rate received by the experimental infected animals. EPM concentrations accumulated within *R. (B.) microplus* well reflected those measured in the host bloodstream ($r = 0.84$; $P = 0.0002$). EPM efficacy estimated by the Henderson-Tilton formula reached 92.9 % (at 1 mg/kg) and 98.9 % (at 1.5 mg/kg) on day 21 post-administration. The described preliminary data show that EPM topically administered may be a valid therapeutic tool contributing to tick control in dairy cattle, a serious growing inconvenient in many countries around the world.

A: Pharmacokinetics

Ref: P1A215/0514

Ivermectin Faecal excretion in horses given Equimax® - safety implications for dogs

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Equimax® (Virbac, Carros, France) is a widely used combination anthelmintic for horses containing ivermectin (IVM) and praziquantel (PZQ). IVM is also widely used for heartworm prevention in dogs with a very large safety index: normal dosage 6µg/kg, normal toxicity threshold >2500µg/kg. Dogs with ABCB1 mutations (commonly collies and associated breeds) have increased risk: the toxic threshold can be >100µg/kg. It has been suggested that dogs eating the faeces of treated horses could be at risk. It was therefore decided to assess the likely level of risk for dogs after treatment of horses using Equimax®. Six horses were given Equimax® at a standard anthelmintic dose (0.2mg/kg IVM). Faecal IVM concentrations were measured at 0,8,12,24,30,36,48,60,72 hours and at 5,7,9,11,13 and 15 days post-treatment. IVM excretion peaked within 36hrs post-treatment, falling to extremely low concentrations by 48hrs (mean 281 µg/kg faeces). The highest peak was in a single animal at 12hrs (7660 µg/kg faeces in relatively dry faeces). Another peaked at 12hrs, but with only 2760 µg/kg faeces. The remaining four peaked between 24hrs and 30hrs, with 1520-3600 µg/kg faeces. The volume of faeces required to reach toxic levels will normally be greater than the dog's stomach capacity, even at the peak of excretion. In extremely sensitive dogs, there is a theoretical risk at the time of maximal excretion (12-36hrs post-dosing, and only if the dog eats a significant volume of faeces. For the most susceptible animals, avoiding access to faeces for 2 days post-treatment is sufficient to limit all risk.

A: Pharmacokinetics

Ref: P1A216/0519

The intravenous and oral pharmacokinetics of sarolaner: a chewable antiparasitic for dogs

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Sarolaner is a member of one of the newest classes of antiparasitic agents, known as isoxazolines. The pharmacokinetics of sarolaner in dogs have been thoroughly investigated and well characterized in a series of laboratory studies. The pharmacokinetic properties of sarolaner were investigated in an absolute oral bioavailability study and a ¹⁴C distribution study. Sarolaner was determined to have low clearance (0.12 mL/min/kg) and a moderate volume of distribution (2.81 L/kg). Following oral administration to fasted dogs, t_{max} occurred at 3 hours post dose. Half-life was comparable for the intravenous and oral routes at 11 and 12 days, respectively. Bioavailability was high at >85%. The primary route of elimination was biliary excretion of parent molecule, with elimination through the feces. Plasma protein binding was determined in vitro and calculated at ≥99.9%. The pharmacokinetic properties of sarolaner are ideal for an orally administered product due to the high oral bioavailability, the rapid absorption, and the 11 – 12 day half-life, which support a rapid onset of action while ensuring efficacy throughout the dosing interval.

B: Climate change, epidemiology and modelling

Ref: P1B001/0022

Pomegranate (*Punica granatum*) juice possesses potent antioxidant activity against Cutaneous *Leishmaniasis* induced Oxidative Stress in Female BALB/c Mice.

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Leishmaniasis is one of the neglected disease created by intracellular parasites of *leishmania*. This disease is observed in three main forms of cutaneous, visceral and mucocutaneous leishmaniasis.

Cutaneous leishmaniasis (CL) is a parasitic disease characterized by single or multiple ulcerations. Lesions caused by *L. major* heal spontaneously after about 18weeks, but it is reasonable to treat the lesions since they may persist for a long time leaving unsightly scars on healing. Exposure of macrophages to *Leishmania* lead to the formation of (ROS) & (RNS) which contribute the regulation of the inflammatory response and controlled by the cellular antioxidant defence system.

Pomegranate Juice is effective *in vivo* against (CL) and it was demonstrated efficacy in Female BALB/c mice model via the subcutaneous route. As it was shown, Pomegranate has a better effect on reduction of size of lesion compared to control.

In the present study, the possible protective role of PJ (0.8 ul /ml) on the free radical damage in serum of the liver and kidney caused by (CL) which produced severe injury, as demonstrated by dramatic elevation of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST). In addition, (CL) caused oxidative stress in Mice, as evidenced by a remarkable reduction in catalase (CAT) and superoxide dismutase (SOD), to investigate the protective role of PJ as a beneficial to reduce Oxidative Stress and downregulation of antioxidant gene expression. It is therefore suggested that PJ can provide a protective effect against chronic injury caused by (CL) in BALB/c mice, which may mainly be associated with its antioxidative effect. The results suggest that pomegranate might be a promising approach for developing new anti-Leishmanial drugs.

B: Climate change, epidemiology and modelling

Ref: P1B002/0056

An experimental study of *Toxocara canis* eggs dispersion by chickens into the environment

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Toxocariasis is an important worldwide zoonosis. It is considered a primarily soil-transmitted disease, but food-borne transmission has been associated with the consumption either of raw or undercooked meat of paratenic hosts, including birds. Despite the number of experimental studies carried out to evaluate the behavior of *Toxocara* spp. larvae in birds, their role in the dispersion of eggs into the environment remains unclear. Thus, this study aimed to evaluate the potential of broiler chickens to release *Toxocara canis* eggs into the environment. Forty commercial broiler chickens, aged 60 days, were randomly distributed into three groups. Groups 1 (n= 16) and 2 (n= 16) were orally infected with 5000 embryonated and 5000 non-embryonated *T. canis* eggs, respectively. Group 3 (n= 8) served as control. Following infection, fecal samples of the chickens were examined using a centrifuge-sedimentation technique. At 24 h, 72 h and 7 days post-infection (PI), four chickens each from the G1 and G2 groups, and two from the G3 group were killed. After euthanasia, the intestinal content and liver were collected for recovery of *T. canis* larvae. According to the results, broiler disperse both embryonated and non-embryonated *T. canis* eggs, following 2-6 h PI. In addition, the eggs shed into the feces of the G2 birds, after incubation in laboratorial conditions, were infective when they were tested in a bioassay using mice. In conclusion, broiler chickens have the potential of releasing *T. canis* eggs and may be considered a good indicator of environmental contamination by *Toxocara* spp.

B: Climate change, epidemiology and modelling

Ref: P1B003/0067

An epidemiological study on caprine coccidiosis in Portugal

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Caprine coccidiosis represents a major health problem of goats worldwide causing high economic losses in caprine industry, mostly due to poor weight gains, costs of treatments and even death in kids. In Portugal, the goat industry rears approximately 400.000 animals/year, the majority of them being allocated in the Southern region. In the present study, we aimed to analyze the occurrence of caprine coccidiosis in the province Alentejo by probing 37 goat farms. In total, 382 animals were monitored from May 2011 to April 2013. Faecal samples from five kids and five dairy goats of each farm were rectally collected and examined by modified McMaster technique for OPG analyses. Additionally, an inquiry was made about husbandry conditions and anti-parasitic treatments. Furthermore, a GIS analysis was conducted. Overall, *Eimeria* spp. oocysts were detected in 99% of the animals and in 100% of the herds. From the nine *Eimeria* species identified, *Eimeria arloingi* (34.91%) and *E. ninakohlyakimovae* (29.90%) revealed as the most prevalent ones in both, the kid and dairy goat groups, followed by *E. alijvi* (22.73%). Other species identified were *E. caprovina* (5.25%), *E. hirci* (2.01%), *E. jolchijevi* (1.85%), *E. christenseni* (1.43%), *E. caprina* (1.43%) and *E. aspheronica* (0.50%). As expected, the number of OPGs was significantly higher ($P = 0.0307$) in kids (6980 ± 36133 OPG) than in dairy goats (1509 ± 2243 OPG). The high *Eimeria* spp. prevalence of Portuguese goat herds and the presence of the most pathogenic species emphasize the importance of coccidiosis for the Portuguese caprine industry.

B: Climate change, epidemiology and modelling

Ref: P1B004/0081

Toxoplasmosis in goat; age specificity and dynamics of antibodies in kids born from naturally infected does

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Background and Objectives: *Toxoplasma gondii* is a zoonotic protozoan parasite that infects humans and domestic animals. In this study, age specific seroprevalence and dynamics of antibodies in goat was investigated to determine the transmission strategy of *T. gondii*.

Methodology: The serum samples collected from goats of a dozen domestic farms of Rajshahi, Bangladesh were examined by latex agglutination test. The serum of kids born from eight seropositive female goats were also tested before and after sucking of colostrum to determine the transplacental-infection.

Significant Findings: Among 300 goat serum tested, 111 (37%) were seropositive ($\geq 1:32$) for the *T. gondii*. The seroprevalences among less than 1 year old, 1-2 years old, 2-3 years old and 3 years or more old goats were 17.30 %, 53.26 %, 65.85 % and 72.72 %, respectively. Young goats (<one year old) showed significantly lower prevalence than adult goats (>one year old). The eleven kids, born from naturally infected does, showed seronegative before suckling colostrum whereas they were seropositive after suckling. It strongly suggested that trans-placental infection rarely occurs in female goat which got infection before pregnancy.

Conclusion: The ingestion of oocyst plays more important role than endogenous trans-placental infection for sustenance of *T. gondii* among goats in Bangladesh.

B: Climate change, epidemiology and modelling

Ref: P1B005/0087

Detection of parasites in *Chanos chanos* (Milkfish) and *Oreochromis niloticus* (Nile tilapia) from Laguna De Bay

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This research determined the presence of parasites in 15 *Chanos chanos* (Milkfish) and 15 *Oreochromis niloticus* (Nile tilapia) collected from Laguna de Bay covering San Pedro, Laguna via purposive sampling. Standard protocol was used in the process of euthanasia and the extraction of the fish's organs. Compression method was applied in each fish tissue stained with methylene blue before observing under a compound microscope. Overall, 26.67% of *Chanos chanos* (Milkfish) and 73.33% of *Oreochromis niloticus* (Nile tilapia) examined were positive for parasites with *Dactylogyrus* sp. being the predominant parasite species in both fishes. This study concluded that *Oreochromis niloticus* (Nile tilapia) was more susceptible to parasitic infections than *Chanos chanos* (Milkfish).

B: Climate change, epidemiology and modelling

Ref: P1B006/0112

Gastrointestinal parasite burden and body condition score. Is there a correlation in adult horses?

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Correlation between the number of eggs per gram (EPG) and the body condition score (BCS) of adult horses, extensively raised, was evaluated in the present study. The 15 Pantaneiro mares (3 to 20 years old) participating in the study were characterized by good nutritional status. Faecal samples of all animals were collected and examined at constant intervals of 28 days in the period between April 2011 to March 2012. The BCS was additionally recorded and classified on a scale from 1 to 10. Faecal examination revealed an almost exclusive infection of small strongyles (*Cyathostomes*). Statistical analysis using Spearman's rank correlation coefficient (r) was performed for 148 paired data however; no correlation was found between these two variables ($r = 0.12$; $P = 0.13$). The obtained results of the microscopical examination were further divided into 8 different categories according to the calculated EPGs. Their comparison with the average BCS reinforced the initial hypothesis of no correlation between these variables. In particular the results obtained were the following: EPG zero ($n=50$) = 7.24 (mean BCS), EPG 50-200 ($n=26$) = 7.5, EPG 250-500 ($n=15$) = 8.2, EPG 550-1000 ($n=24$) = 7.74, EPG 1050-2000 ($n=17$) = 7.82, EPG 2050-3000 ($n=7$) = 7.71, EPG 3050-4000 ($n=4$) = 6.75, 4050-5000 ($n=5$) = 6.8. Absence of correlation between EPGs and BSC was additionally observed in samples, where 20% of the investigated animals, presenting negative EPG, showed a BSC ranging from 4 to 6, whereas 81.81% of the mares with EPG higher than 1050 indicated a BSC over 7.

B: Climate change, epidemiology and modelling

Ref: P1B007/0124

Prevalence of gastrointestinal parasites in adults and young sheep backyard in Oaxaca, Mexico

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Gastrointestinal diseases caused by parasites are one important cause of economic losses in sheep. The aim of this study was to estimate the infection caused by helminthes and protozoans in young lamb and adults.

Fecal samples from 1-year-old (n=70) and 2.5-year-old (n=109) sheep in Oaxaca, Mexico were examined using coprological techniques to detect and count coccidia oocysts as well as eggs of gastrointestinal nematodes and cestodes. Fecal cultures of *Eimeria* and strongylid-positive samples were performed to identify species and third-stage larvae, respectively. The Spearman correlation coefficient (rs) was used to evaluate the relationship

Forty-five percent of young sheep were positive to *Eimeria* spp., and the most prevalent species was *E. pallida* (50%). The infection rates of *Strongyloides papillosus*, *Haemonchus contortus*, *Trichostrongylus axei*, *Cooperia* spp., *Chabertia ovina* and *Oesophagostomun* spp. were 23.60%, 25.84%, 13.48%, 15.73%, 11.24% and 10.11%, respectively. The animals were infected with *Trichuris ovis* (8.57%) and *Moniezia* spp. (34.29%). Twenty-three adult sheep were positive to *Eimeria* spp., and the most prevalent species was *E. ovinoidalis* (41.62%). The infection rates of *Haemonchus contortus*, *Trichostrongylus axei*, *Cooperia* spp., *Chabertia ovina* and *Oesophagostomun* were 30.19%, 11.32%, 16.98, 22.64% and 18.87% respectively. The animals were infected with *Trichuris ovis* (3%) and *Moniezia* spp. (29%). A negative and significant ($p \leq 0.05$) correlation was found between parasite burden and age.

B: Climate change, epidemiology and modelling

Ref: P1B008/0150

Population dynamics of *Rhipicephalus (Boophilus) microplus* (Canestrini, 1887) in crossbred cattle, raised in pasture of *Brachiaria* in Formiga, Minas Gerais, Brazil

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Considering the importance of *Rhipicephalus (Boophilus) microplus* in cattle, the present study aimed to evaluate the seasonality of this arthropod in cattle maintained in *Brachiaria* spp. However, we investigated the population dynamics for 12 months in the city of Formiga, Minas Gerais, Brazil. Twenty-four cattle with 8-12 months of age were subjected to natural infestation by ticks. These animals were divided into two replicates of 12 animals each. Female counts 4.5 to 8.0mm were performed on days 0, 3, 7, 14, 21, 28 and every 14 days for 12 months. Tactical treatments were performed, topically (spray) with a formulation containing 15% Cypermethrin + 25% + Chlorpyrifos + 15% Piperonyl butoxide + 1% Citronella in all the dates on which mean tick exceeded 30 females per repetition/group. The results showed that the level of tick infestation presented parasitism peaks with the highest concentration in the rainy season. The infestation levels by *R. (B.) microplus*, was influenced by climate variables. The lower parasite load occurred in winter. In early spring (October) there was a peak of ticks, followed by another in late spring / early summer (December) and other lower in summer (February). From the autumn the parasite load naturally declined to record a peak in autumn (April), and another in winter (July). So, depending on the year, there is a presence of five generations of ticks for the study region, which differs from other regions of Brazil.

B: Climate change, epidemiology and modelling

Ref: P1B009/0154

Gastrointestinal Parasites with reference to sex, breed and age in Sheep and Goats in Gwagwalada Area Council, Federal Capital Territory, Abuja, North Central Nigeria

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A total of 100 fecal samples of sheep and goats of different breeds consisting of 56 goats and 44 sheep were examined. These samples were processed and screened by direct smear method for the detection of parasitic ova. Ova of parasites were identified through their morphological features using the available keys. The prevalence rate for goat was (37.5%) while for sheep was (43.1%). The gastrointestinal parasite encountered for goats were *Trichostongylus spp* (16.1%), *Fasciola spp* (7.1%), *Strongyloides spp* (3.6%), *Trichuris spp* (3.6%) *Eimeria spp* (1.8%), *Haemonchus spp* (1.8%), *Fascioloides spp* (1.8%) and *Skrjabinema spp* (1.8%) while for sheep were *Fasciola spp* (18.1%) and *Trichostongylus spp* (9.1%) *Eimeria spp* (2.3%) *Haemonchus spp* (4.5%), *Trichuris spp* (4.5%) and *Paramphistomun spp* (4.5%). Sex wise prevalence of gastrointestinal parasites was higher in female goats (26.8%), than male goats (10.7%) ($P < 0.05$). Goat breeds of West Africa Dwarf (WAD) and Kano brown had (14.3%) and (23.2%) infection rates respectively ($P > 0.05$). The older goats were more infected: (32.1%); compared to the younger goats: (5.4%) ($P > 0.05$). Sex wise prevalence of gastrointestinal parasites was higher in (ram) male (31.8%), than (ewe) female (11.4 %) ($P > 0.05$). Among sheep breeds the Yankasa, Uda and Balami had (13.6%), (15.9%) and (13.6%) infection rates respectively ($P > 0.05$). The Adult sheep were more infected: (27.2%) compared to the younger sheep: (15.9%) ($P > 0.05$). In conclusion, the data obtained in this study suggest that the age, sex, and breed are important factors which influence the prevalence of gastrointestinal parasites

B: Climate change, epidemiology and modelling

Ref: P1B010/0169

Helminth fauna of cattle from the microregion of Jaboticabal, São Paulo state, Brazil, reevaluated after fifteen years

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In 1999, the helminth fauna of 43 bovines from the Jaboticabal region was evaluated. Fifteen years later, such work was repeated with 65 animals. In this second stage, a questionnaire of anthelmintic use was carried out among owners, in order to associate the use of chemical classes with the possible difference in percentages of parasitism by some species. Necropsy results obtained in 1999 showed that the percent distribution of genres was: 77.9% *Cooperia* (*C. punctata*, *C. pectinata* and *C. spatulata*), 18.5% *Haemonchus* (*H. placei* and *H. similis*), 2.01% *Oesophagostomum* (*O. radiatum*), 1.13% *Trichostrongylus* (*T. axei* and *T. colubriformis*) and less than 1% for other helminths. In the present study, conducted in the same region 15 years later, the percentage distribution of these helminths was: 64.9% *Cooperia* (*C. punctata*, *C. pectinata* and *C. spatulata*), 23.6% *Haemonchus* (*H. placei* and *H. similis*), 7.3% *Oesophagostomum* (*O. radiatum*) and 3.7% *Trichostrongylus* (*T. axei* and *T. colubriformis*). The parasite with lower distribution percentage in both studies was *Moniezia benedeni*. According to animal owners, chemical compounds used for controlling endo and ectoparasites in this region, for the last 15 years, were ivermectin, abamectin or doramectin. Analyzing these results, it is clear to note that a reversal in the average intensity of infestation occurred, with a decrease in the number of *Cooperia* and an increase in *Haemonchus* and *Oesophagostomum*. This may be associated with recent reports of the difficulty in eliminating *Haemonchus* and *Oesophagostomum*, with the use of avermectins in Brazil.

B: Climate change, epidemiology and modelling

Ref: P1B011/0171

Effect of simultaneous grazing between cattle and sheep on endoparasites of sheep

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This experiment was conducted at the State University of Maringa, Umuarama Campus, Paraná state, Brazil, on a 10 hectare *Brachiaria brizantha* pasture. This area was divided into four equal parts, and two treatments were evaluated, with two replications each: T01 - sheep grazing alone, T02 - cattle and sheep grazing simultaneously, T03 - sheep grazing alone and T04 - cattle and sheep grazing simultaneously. Each paddock contained 14 sheep and eight heifers, and the study lasted 150 days. Counts of eggs per gram of feces (EPG) and fecal cultures were performed to estimate parasitism in sheep. Additionally, an ovine tracer was used in each paddock, every 30 days, to assess the number of larvae in pastures. Considering the region where the study was conducted, it appears that there was a significant ($p \leq 0.05$) decrease in EPG counts of sheep, after 90 days of grazing simultaneously with cattle. Evaluating sheep tracers used in this study, it is noted that among helminth species diagnosed, there was a significant reduction in the amount of L3 of *Haemonchus contortus* and *Oesophagostomum columbianum* from the 90th day of simultaneous grazing, while levels of *Trichostrongylus colubriformis* and *Cooperia curticei* did not change during the 150 days of grazing set between sheep and cattle. New studies are needed, but these results obtained in this region indicate that when using simultaneous grazing of sheep and cattle, special attention should be given to *T. colubriformis* and *C. curticei* species parasitizing sheep.

B: Climate change, epidemiology and modelling

Ref: P1B012/0204

Parasitological and molecular detection of *Hepatozoon* spp. in wild rodents from São Paulo, Brazil

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Species of *Hepatozoon* are apicomplexan parasites that infect a wide range of animals. The life cycle is heteroxenous, with tissue merogony and gametogony in the vertebrate host and sporogony in the invertebrate host. Wild rodents have been recognized as potential reservoirs of a variety of vector-borne pathogens and data on the occurrence of *Hepatozoon* spp. in these animals are still scarce. Here, we assessed the presence of *Hepatozoon* spp. in 67 wild-trapped rodents from Botucatu, São Paulo, Brazil, using histological and molecular methods. Blood and animal tissues were submitted to PCR targeting an 18S rRNA fragment of the genus *Hepatozoon*. PCR revealed *Hepatozoon* infection in 37 out of 67 wild rodents (55.22%). The 37 positive specimens were assigned to five species (*Akodon* cf. *montensis*, *Necromys lasiurus*, *Oligoryzomys nigripes*, *Oligoryzomys flavescens* and *Sooretamys angouya*). The 18S rRNA DNA sequences obtained by sequencing were blasted in GenBank and all of them showed a similarity of 99% to *Hepatozoon* spp. DNA isolates. Histopathology revealed *Hepatozoon* meronts in various stages of maturity exclusively in the livers. Additionally, monozyotic cysts were found in the lung, kidney and spleen. Typical elongated *Hepatozoon* gametocytes were also found in the peripheral blood. *Hepatozoon* infection was found to be highly prevalent in wild rodents from Brazil. Moreover, wild rats can be either obligate intermediate host or a facultative (paratenic) host, harboring cystozoite stages in tissues. This finding emphasizes the role of predation as a potential mode of transmission for mammalian hepatozoa.

B: Climate change, epidemiology and modelling

Ref: P1B013/0219

Some Studies on *Eimeria* spp of domestic rabbits(*Oryctolagus cuniculus*) in Delta region Egypt

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The present study aimed to determine the prevalence of different *Eimeria* species in rabbits in Egypt.

Eight *Eimeria* species were identified, the first one was the hepatic (i.e. *E. stiedae*) and seven intestinal *Eimeria* species. The intestinal *Eimeria* were identified as *E. exigua*, *E. perforans* , *E. media*, *E. intestinalis*, *E. flavescens*, *E. magna* and *E. irresidua*.

The prevalence of *E. stiedae* was (6.44%&7.7%), *E. exigua* was (10.85%&8.29%), *E. perforans*(45.34.%&39%), *E. media*(37.4%&32.5%), *E. intestinalis* (34.8%&0.0%) , *E. flavescens* (6.8%&1.9%), *E. magna*(28.3%&23.2%) and *E. irresidua* (7.6%&7.1%) in rabbits (2-3) and in (3-4) months respectively.

The prevalence of infection of pure hepatic coccidiosis was (3.22% &2.42%), intestinal coccidiosis was (47.97%&38.46%) while mixed both hepatic and intestinal coccidiosis were (3.82%&5.23%) rabbits (2-3) and (3-4) months respectively.

The seasonal dynamics of coccidiosis in rabbits also investigated, coccidiosis maximum values were recorded in Winter recording (74.8% & 58%), then it decreased in Spring (63%&52.35%) and continue to decreased in Summer (45.7%&34.3%) reaching its lower value in Autumn (22.9% &18.2%) in rabbits (2-3) and (3-4) months respectively .

The current study proved a high prevalence of both hepatic and intestinal coccidiosis of rabbits in Egypt and recommend further investigations on this problem.

Key words: Rabbits, *Eimeria*, Coccidiosis, Hepatic, Intestinal, Prevalence, Egypt

B: Climate change, epidemiology and modelling

Ref: P1B014/0231

Some aspects of *Trichinella* epidemiology on the arctic shores of the Russian Federation

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The survey of mammals hunted along the coasts of Chukotka Autonomous region and Sakha-Jakutia Republic for the presence of *Trichinella* juveniles was performed in 2004-2010. An important part of autochthonous population is living along the shores with only source of income from fishing and hunting. Cooking and other thermal methods of food preparation are not common and preservation and consumption of meat products are performed in traditional style. Jerked, frozen or fermented meat products constitute important part of the natives' diet. Immunological survey of local natives in 2010 from the cohort of people actively involved into hunting and consumption of marine and terrestrial wild mammals revealed that 24% of these demonstrated high level of antibodies against *T.nativa* antigens. During the survey genomic DNA was extracted from living juveniles of isolates originating from the tissues of polar foxes, wolves, brown bears, Arctic bears, wolverines, foxes, ringed seals, draught and stray dogs and a stray cat. Partial *cytb*-sequences of mitochondrial genome were obtained for recovered *Trichinella* isolates. BLAST analysis proved that all isolates belong to *T.nativa*. Samples of muscular tissues of carnivorous mammals with *T.nativa* juveniles detected were frozen and kept under -18 °C for several years. It was shown that *Trichinella* juveniles from such tissues of original hosts were still invasive after 4 years of freezing. Single passage of these *Trichinella* juveniles on experimental hosts (rabbits, guinea-pigs, mice) resulted in the irreversible loss of such ability for long survival under deep freezing. Juveniles of the generation obtained after passage on experimental hosts were turning inactive after 2 months under -18°C. Aborigines of Chukotka and Sakha-Jakutia are widely using 'copalchen' technology (meat fermentation under pressure with consecutive freezing of slices). Examination of "copalchen" content demonstrated that significant part of *Trichinella* juveniles were still active after 53 months of "copalchen" storage. One golden hamster from three inoculated acquired the *Trichinella* infection with resulting intensity of infestation on the level of 7.4 juvenile per g⁻¹. Such findings demonstrate that traditional culinary practices of aborigines of Russian Arctic play important role in the epidemiology of trichinellosis in this area. This study was supported by the grant 14-16-00026 from Russian Scientific Foundation.

B: Climate change, epidemiology and modelling

Ref: P1B015/0236

Clinical and serological dynamics of *Besnoitia besnoiti* infection in three endemically infected beef cattle herds

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The dynamics of bovine besnoitiosis was studied in an area where the disease is endemic in three beef cattle infected herds. During 4 years, four-six visits per herd were carried out and clinical and serological prevalence rates, as well as incidence rates were estimated. Clinical inspection was conducted at the beginning and the end of the study. Serological followed up was initially carried out by ELISA. Seronegative animals with clinical signs and seropositive animals with RIPC values lower than 30 along the period of study were further checked by western blot. Clinical prevalence rates were slightly higher (62%) than seroprevalence rates (50%) and tissue cysts located in *vestibulum vaginae* and sclera were the most frequently detected clinical signs. Seropositive animals with clinical signs varied from 16% to 73% among herds and 33 animals with clinical signs proved to be seronegative. As average 22% serological incidence rate was also reported together with clinical incidence rates that varied from 12 to 16. Parasitemia was also investigated in the most affected herd and only one PCR positive sample was detected. Thus, the role that blood may play in vector borne parasite transmission need to be further investigated. According to the results in the absence of control measures over time, infected herds remained with both high prevalence and incidence rates. These herds keep a high number of parasite carriers and there is a high risk of new cases in entries. Finally, reproductive and productive losses should be quantified in order to implement a cost-benefit control program.

This study was funded by AGL2010-20561

B: Climate change, epidemiology and modelling

Ref: P1B016/0267

The prevalence and identity of paramphistomes in cattle and deer in New Caledonia suggests transmission of *Calicophoron calicophorum* between host species

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An abattoir survey was performed in the French Melanesian archipelago of New Caledonia in May-June and October-November 2013 to determine the prevalence of paramphistomes in cattle and deer. Four hundred and eighty four calves and 387 adult cattle were inspected from 102 farms. The majority of herds (68.6%) were found to be parasitized (81.5% of adult batches and 54.5% of calf batches). Within-herd prevalence was similar to between herd prevalence, at 80.6% for adult cattle and 53.1% for calves. The calves' infestation was seasonal, with more calves parasitized in May-June than in October-November (74.7% and 35.8%, respectively, $P < 0.001$). In addition, animals from 7 deer farms were inspected at slaughter. Two farms had infected animals, with intra-herd prevalence of 41.4% (29/70) and 47.1% (33/70), respectively. Using ITS-2 sequencing, 3 species of paramphistomes were identified, i.e. *Calicophoron calicophorum*, *Fischoederius elongatus* and *Orthocoelium streptocoelium*. All three species were detected in cattle as well as deer, suggesting transmission between host species. This is supported by heterogeneity within the mitochondrial DNA encoding transfer RNA (tRNA-Thr/Cox1) region of New Caledonian *C. calicophorum*, i.e. there is no association between specific subtypes and host species. We discuss potential origins of the respective rumen fluke species, based on historic data, and discuss the possible implications of (co-)infection for livestock production on the island.

B: Climate change, epidemiology and modelling

Ref: P1B017/0270

Risk factors for gastrointestinal nematode infections in Dutch dairy herds.

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Ostertagia ostertagi (O. ostertagi) is the most important gastrointestinal parasite in cattle, with a wide distribution in temperate climate areas. Gastrointestinal nematode infections in dairy cattle are of high economic importance, due to milk production decreases and body condition losses. In the Netherlands, surveillance of gastrointestinal nematode infections in dairy cattle is based on the detection of O. ostertagi antibodies in bulk milk, using an ELISA. The relative amount of specific antibodies in bulk milk is related to the severity of clinical symptoms and milk production losses in a herd. Therefore, bulk milk ELISA results of GD Animal Health (The Netherlands) are combined with advices on further diagnostics and farm management measures. Results of the GD bulk milk ELISA revealed that gastrointestinal nematode infections had occurred less frequently on Dutch farms in 2012 as compared to 2011.

This study intended to identify management practices that were associated with an improved bulk milk result for O. ostertagi antibodies in 2012 as compared to the previous year. For this purpose, a questionnaire survey was conducted among dairy farmers in a case-control design. Farms were considered as cases (n = 53) when their bulk milk ELISA test results were positive in both 2011 and 2012 and as controls (n = 18) when their bulk milk antibody levels were reduced in 2012. The questionnaire included questions about general farm characteristics, as well as more specific questions about symptoms and levels of nematode infections on the farm and management factors regarding animal health, pasture, grazing and anthelmintic treatment.

Exact logistic regression analysis showed that unrestricted pasture management and remembrance of the information brochure (that accompanied the test result), were associated with an increased odds to retain a positive bulk milk status for O. ostertagi antibodies.

B: Climate change, epidemiology and modelling

Ref: P1B018/0275

Identification of species of *Anaplasma* in domestic animals at Northwest México.

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There are many factors that cause the heterogeneity of species of *Anaplasma*; geographical conditions and the host to which they adapt. That is why several species of *Anaplasma* have the ability to infect different species of animals, therefore this makes the control very difficult. *Anaplasma spp.* is an obligate intracellular bacteria in the order of the Rickettsiales, family Anaplasmataceae, includes the species *A. marginale*, *A. bovis*, *A. ovis*, *A. phagocytophilum* which are transmitted by various vectors. The objective of this study was to identify by PCR the species of *Anaplasma* in domestic animals. We obtained blood from bovine, equine and ovine of Culiacan, Sinaloa. Isolated DNA was used to PCR of 16S rRNA gene of *Anaplasma*, positive samples for this gene, we realized nested PCR, for cattle: *A. marginale*, *A. bovis*, *A. ovis* y *A. phagocytophilum*, ovine: *A. ovis* and *A. phagocytophilum*, equine: *A. phagocytophilum*. From 120 samples of bovine, 50 were positive to *Anaplasma spp.* from which 48 are *A. marginale* y 2 *A. bovis*. Of the 138 equine analyzed 21 were positive to *Anaplasma spp.*, 13 to *A. phagocytophilum* and 8 to other species. Of 48 ovine 25 were positive *Anaplasma spp.*, 19 *A. ovis* and the rest to other species, is the first report of identification of *Anaplasma* in domestic animals, which reveals endemicity in the northwest Mexico area, there was also finding of other *Anaplasma* species in ovine and equine indicating the adaptation and infectivity in different hosts.

B: Climate change, epidemiology and modelling

Ref: P1B019/0276

A coprological survey of the principal endoparasitic infections in donkeys in Italy

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In Italy despite the increasing use of donkeys in leisure activities, onotherapy and milk production, there are no epidemiological data regarding the parasites affecting this species. A survey was conducted to determine the prevalence of the principal endoparasitic infections in 50 donkey farms. Between March 2012-2015, faecal samples were collected from 961 donkeys and examined individually using a modified McMaster technique. In addition a centrifugation/flotation technique (Proudman test) and a sedimentation technique were used for the diagnosis of Anoplocephalidae and *Fasciola hepatica* eggs, respectively. On each farm pooled coprocultures were made. Strongyles were the most common parasitic species found, with a prevalence of 88.5%. 11.4% of donkeys were not infected and infection intensity was lower than the donkey cut-off selective therapy of 300 eggs per gram (epg) in 33.4% of animals. The other epg values were 300–500 in 14.3%, 501–1,000 in 26.0%, 1,001-2,000 in 12.8% and > 2,000 in 2.1% of donkeys. In all tested farms, coprocultures revealed the presence of Cyathostomes (100%) followed by *Strongylus vulgaris* in 13 farms (26%), *Poteriostomum* spp. (24%), *Triodontophorus* spp. (6%) and *S. edentatus* (4%). Other parasites found in studied donkeys were: *Oxyuris equi* (7.8%), *Dictyocaulus arnfieldi* (4.4%), *Parascaris equorum* (2.8%), *Anoplocephala perfoliata* (1.1%) *Eimeria leuckarti* (0.6%) and *Strongyloides westeri* (0.1%). No positivity was detected for *F. hepatica*. This study represents the first large-scale survey in Italy and added data to the parasitological scenario of donkey population.

B: Climate change, epidemiology and modelling

Ref: P1B020/0287

***Aelurostrongylus abstrusus* (Railliet, 1898) infection of domestic and wild felines in South Brazil**

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Aelurostrongylus abstrusus is a helminth parasite of respiratory tract of felines, with biological cycle that requires the involvement of intermediaries or parathenic hosts. This study evaluated the infection by *A. abstrusus* in domestic and wild cats in the state of Santa Catarina, southern Brazil. Fecal samples from 97 domestic (*Felis catus*) and seven wild cat (*Puma concolor*, *P. yagouaroundi* and *Leopardus tigrinus*) were collected that were submitted to three parasitological methods: simple sedimentation, flotation with zinc sulfate solution and flotation with supersaturated sodium chloride solution. The prevalence of L1 larvae of *A. abstrusus* was 2.1% (2/97) in *Felis catus*. Among the wild felines, *Puma yagouaroundi* was the only positive. Access to the larvae was made possible by sedimentation method only. Infected domestic cats have, respectively, six months and four years old. No data on the age of wild felines. The occurrence, although low in this region, serves as a warning about the presence of intermediate hosts and / or infected parathenic and the importance of this disease for felidae, even in the absence of respiratory clinical signs.

B: Climate change, epidemiology and modelling

Ref: P1B021/0293

Potential economic impact of parasites on the cattle industry of Mexico

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Parasitic diseases remain an important factor affecting the productivity of cattle in Mexico. Economic losses caused by cattle parasites in Mexico were estimated on an annual basis considering the total number of animals at risk and the potential detrimental effects of parasitism on milk production, weight gain, and impact according to federal regulations on livestock byproducts. Estimates in US dollars (US\$) were based on reported yield losses among untreated animals reflecting the major effects of six parasitic diseases on cattle in Mexico. The potential economic impact of parasites in millions of US\$ included: gastrointestinal nematodes US\$ 445.10, coccidia (*Eimeria* spp.) US\$ 23.78, liver fluke (*Fasciola hepatica*) US\$ 130.91, cattle tick (*Rhipicephalus microplus*) US\$ 573.61, horn fly (*Haematobia irritans*) US\$ 231.67, and stable fly (*Stomoxys calcitrans*) US\$ 6.79. The annual potential economic losses due to the six major endo- and ectoparasites of cattle in Mexico total US\$ 1.41 billion. Considering the national herd registered in 2013 that includes 32.40 million head of cattle, the estimated yearly loss per head was US\$ 43.57. Despite the limitations of some of the baseline studies used to derive our estimates, particularly when extrapolated from local situations to a national scale, the general picture obtained from the present effort demonstrates the magnitude and importance of cattle parasitism in Mexico. This situation emphasizes the impact on profitability for the livestock industry without proper parasite control. Integrated parasite control that is based on an agroecosystems approach, and adapted for each of the major ecological regions is recommended to reduce the impact of ecto- and endo-parasites on the cattle industry in Mexico.

B: Climate change, epidemiology and modelling

Ref: P1B022/0326

Dynamics of goat warble fly infection by *Przhevalskiana silenus* (Diptera: Oestridae) in Italy

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Among factors that negatively affect the livestock, parasite infections continue to represent a serious challenge to the health, welfare, productivity and reproduction. In Mediterranean countries for example the maggots of *Przhevalskiana silenus* cause a myiasis known as goat warble fly infection that could induce significant economic losses.

In this study the carcasses of 580 goats (551 females and 29 males), regularly slaughtered in southern Italy, were examined for *P. silenus* prevalence and dynamics across two years. For each carcass, the skin and subcutaneous tissues were examined for instars of *P. silenus* and larvae found were counted and classified according to their developmental stage, i.e. first (L1), second (L2) and third (L3) larval instars. Overall, *P. silenus* larvae were detected in 42 (87.5%) out of 48 farms and in 285 (49.1%) out of 580 goats. The number of larvae ranged from 1 to 38 per infested goat, with a mean intensity of 4.5 larvae. A total of 1,209 larvae were retrieved (i.e., L1=385, L2= 394 and L3=430). The prevalence rate was significantly higher in young goats compared to older animals.

Results of this study showed as *P. silenus* infection is widespread in farms from the study area. However, as in other Mediterranean countries, *P. silenus* infection is still an overlooked disease. A better awareness on this infection as well as adequate control strategies should be advocated. Indeed, control programmes including the administration of active antiparasitic drugs in late summer, when infection is mainly sustained by L1, should be recommended to avoid more severe damages caused by the further stages of development.

B: Climate change, epidemiology and modelling

Ref: P1B023/0339

Prevalences of Endoparasites in Austria from 2004-2014

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To get an overview on parasite prevalences in Austria we analysed the results of parasitological diagnostic from 2004 to 2014. Faecal samples were examined by standard coproscopy. *Giardia* was the most prevalent endoparasite of dogs (n=8715) and cats (n=3174) (13.5% and 15.3%, respectively); *Cystoisospora* was present in 13.5% and 5.5%; *Cryptosporidium* in 2.58% and 0.22% of the samples, respectively; *Toxocara* was found in 5.5% (dogs) and 6.0% (cats). Oocysts of the *Toxoplasma/Hammondia*-type were found in 1.3% of the cat samples. Co-infections with *Giardia* was most frequent in 19.5% of *Cryptosporidium*, 6.8% of *Cystoisospora* and 14.3% of *Toxocara* positive cases in cats and in 26.5% of *Cystoisospora* and 26.0% of *Toxocara* positive cases in dogs. In horses strongyles were seen in 41.2%; *Parascaris*, *Anoplocephala* and *Oxyuris* in 2.8%, 3.6% and 0.9% of samples, respectively (n=7587). In cattle, *Eimeria* was the most prevalent parasite with 51.8%, strongyles were present in 30.4% of samples; *Fasciola* in 2.8%. In small ruminants (n=2364) strongyles were the most prevalent parasites with 70.8% followed by *Eimeria* with 40.0%. *Dicrocoelium* was present in 5.1% of the samples. With their consistent abundance potentially zoonotic parasites remain an important issue in companion animals. Equine parasites were on a steady level with strongyles being the most common. In cattle farming we consider infections with *Eimeria* spp. as one of the predominant parasitological problems, while in small ruminants strongyles have high relevance especially due to the development of anthelmintic resistance.

B: Climate change, epidemiology and modelling

Ref: P1B024/0342

Detection of *angiostrongylus vasorum* in the bronchoalveolar lavage fluid or faeces from coughing and healthy dogs in Belgium

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Recent studies highlight the current spreading of canine Angiostrongylosis from long-standing endemic areas. Despite newly-available sensitive tests as qPCR, presence of *Angiostrongylus vasorum* in healthy and coughing dogs in Belgium has not been investigated. This study describes angiostrongylosis in a clinical recent autochthonous series and investigates on its prevalence in a retrospective series of coughing and healthy dogs using qPCR on bronchoalveolar lavage fluid (BALF) and Baermann analysis. From 2008 to 2014, dogs with pulmonary angiostrongylosis were diagnosed by positive qPCR on BALF and/or positive Baermann analysis. qPCR analysis was performed on BALF samples from 65 dogs, including 55 coughing dogs with various diagnosis and 10 healthy dogs; faecal Baermann analysis was available in 33 dogs. Seven young adult dogs living in Southeastern Belgium and presented for daily cough and/or moderate-severe exercise intolerance and dyspnea were diagnosed with angiostrongylosis between March 2013 and April 2014. Radiographic pattern included bronchointerstitial or alveolointerstitial changes; tracheobronchial congestion with bloody secretions were classical bronchoscopic observations. In 5 dogs, BALF and faeces were obtained with positive qPCR on BALF in 5/5 and positive Baermann in 2/5. In the two remaining dogs, only Baermann analysis was available and positive. Among the 65 coughing and healthy dogs recruited between 2008 and 2014, only one BALF from a dog originally diagnosed in 2011 with eosinophilic bronchopneumopathy was qPCR-positive. Canine angiostrongylosis used to be a very unfrequent disease until 2013 in Belgium. The disease should now be considered as an emerging condition and presently, canine angiostrongylosis needs to be included in differential diagnosis of young coughing Belgian dogs. qPCR detection of *A. vasorum* in BALF, if available, seems to be an adequate and reliable diagnostic technique in coughing dogs.

B: Climate change, epidemiology and modelling

Ref: PB025/0343

Gastrointestinal nematode infection in meat sheep from northwestern Spain. Risk factors

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In Galicia (North-west of Spain) there are around 24,000 sheep flocks with a mean of 11.5 animals, contributing to the economic and environmental sustainability of the whole farm. It seems that generalized anthelmintic treatment to control GI in commercial flocks has not reduced the prevalence of gastrointestinal nematodes (GI). The main objective of this study was to detect and classify determinant factors on sheep GI infection to improve the control measures applied in flocks maintained under semi-extensive system. 1,914 faecal samples from 74 commercial meat ovine flocks were collected and examined to detect GI infection. The risk of being infected was evaluated with a mixed-effects logistic regression algorithm. The dependent variable was the positiveness (0-1) to GI. Flock was introduced as a random factor to control its effect over factors. Factors from management, treatment and other infections were introduced in a backward conditional method and removed from the model one by one on the basis of the highest p-value until the best model was built. These statistical analyses were performed with glmer() function from lme4 package in the R statistical package (R v.3.1.2). 1,233 out of 1,914 faecal samples examined were positive for GI infection (64.4%; 95% CI 62.2-66.6). The factors detected as determinant in GI infection were climatic area, introduction of new animals in the farm, pasture rotation, *Dictyocaulus filaria* infection and *Calicophoron daubneyi* infection, with negative correlation in the latter. All these variables must be introduced to enhance the efficacy of control measures over GI infection in sheep under semi-extensive system in northwestern Spain.

B: Climate change, epidemiology and modelling

Ref: P1B026/0358

Effect of natural aflatoxin exposure on the immune response in Holstein cattle naturally infected with *N. caninum*

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The aim of the study was to relate the effect of natural exposure to aflatoxins on the immune response during the first pregnancy Holstein cattle naturally infected with *N. caninum*. Two dairies were selected, one use mycotoxin binder and another that did not. In each dairy were included 10 seropositive and 10 seronegative heifers to *N. caninum*, which were monitored during pregnancy. Monthly blood samples were taken for analysis by ELISA specific to *N. caninum* (IgG, IgG1, IgG2, SAG4 and GRA7). Aflatoxins were quantified and hepatic function tests were carried out in all cases. The results showed that the immune response was behave according the serostatus of each group, although a high seroconversion was presented at the dairy that not use mycotoxin binder; no detected presence of immunosuppression. The concentration of aflatoxin in food was in range of 3-19 µg/kg; values hepatic function tests were within reference values. We could not identify any relationship between ingestion of aflatoxins and immune response in animals under study.

B: Climate change, epidemiology and modelling

Ref: P1B027/0363

***Ancylostoma* spp. in sand of beaches of south zone of Sinaloa, México.**

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Ancylostoma spp. can disseminated in the soil and sand of public areas through eggs and larvae that resist the environment conditions, capable of surviving for many months. Humans and pets can infected when have been in contact with soil and sand of beaches contaminated with filariform larvae, and that penetrate the skin with most species; the larvae cannot mature in the human host, and can migrate aimlessly within the epidermis, causing cutaneous larva migrans (also known as creeping eruption), representing a serious health problem for animals and humans. The objective was determine the presence of *Ancylostoma* spp in sand of beaches of south zone of Sinaloa, México; for it were determined representative composite samples of sand of beaches, by the technique described of Thrusfield (2005), using: $n = [t \cdot SD / L]^2$. 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 (%); the total of composite sample of sand was 485, taken of surface moist sand 100 grams of sand for each sample; transferred to the laboratory of Parasitology of the FMVZ-UAS to be analyzed by the sedimentation technique. Resulting 63 (12.9 %) of the 485 composite samples of sand were positive to *Ancylostoma* spp. Concluding that the sand of beaches of south zone of Sinaloa, México contaminated with *Ancylostoma* spp. can represent a risk for the health of pets and public general that visit these recreation places.

B: Climate change, epidemiology and modelling

Ref: P1B028/0367

Agent-based modelling of *Cryptosporidium parvum* transmission in cattle

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Cryptosporidium parvum is an important pathogen of calves, with high economic impact on the cattle farming industry. With no treatment available, a better understanding of the biology and transmission of the parasite is essential to control it. One of the features that makes *C. parvum* so successful is the production of huge numbers (10^7 per gram faeces) of highly resistant transmissive stages (oocysts), which, it is generally assumed, have a long lifetime under a wide range of environmental conditions. Here we present an agent-based model of *Cryptosporidium* biology in which the fate of realistic numbers of oocysts can be simulated. The model was parameterized based on reported data of *C. parvum* prevalence in calves of different age, oocyst output from infected animals and most common practices of cattle farming, and was used to estimate oocyst survivorship necessary for transmission to occur in the cattle farm environment. The model captures the phenomenon of cryptosporidiosis outbreaks amongst calves and makes it clear that oocyst survival in the environment must be relatively poor, despite the resistance of individual oocysts to physico-chemical stress. On the basis of the model we suggest that a very large proportion of the oocysts must be removed from the environment in relatively short time after shedding.

B: Climate change, epidemiology and modelling

Ref: P1B029/0373

New data on prevalence of eye worm *Thelazia callipaeda* in wild animals in Russian Far East with remarks on its morphology

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Thelazia callipaeda is a spirurid nematode selectively parasitic in eyes of carnivores and humans. It usually localizes in a conjunctival sac causing medium to severe inflammation of conjunctiva such as conjunctivitis, keratitis and ulcers of cornea. Within the Russian Federation, the infection of *T. callipaeda* was reported in dogs, cats, foxes and racoon dogs in the Russian Far East. Wild animals sampled in Primorskiy Krai were examined posthumously in the period from the winter 2012 to the summer 2014. Conjunctival sacs of the animals including the space under a nictitating membrane were examined for the presence of thelazids. Totally, 544 animals were examined including 492 specimens of sables, 25 Siberian weasels, 11 racoon dogs, 4 American minks, 3 Amur leopard cats, 3 foxes, 2 yellow-throated martens and one specimen each of lynx, European badger, brown bear and Asian black bear. Morphological study of helminthes and their species identification were performed under light microscope. Totally, 6,8% (37 out of 544) of examined animals were found infected by *T. callipaeda*. Helminths were detected in 28 sables from 492 examined, in 5 racoon dogs from 11 in 2 foxes from 3 in one lynx and Asian black bear examined. Sable, Asian black bear and lynx were registered as hosts of *T. callipaeda* for the first time (until present, the only record of *T. callipaeda* infection in the lynx was related to the zoo). Morphological study of helminthes and their species identification were performed under light microscope. Morphometric data for the nematodes originating from sables are presented. The patterns of sensory structures of the male posterior end were examined, since descriptions of these structures in literature are contradictive. Thus, different authors report from 6 up to 12 of precloacal papillae in *T. callipaeda*, and from 2 up to 5 postcloacal ones. Our observations demonstrated extreme variability of the papillae count and allocation in *T. callipaeda*. It is also impossible to distinguish unequivocally between pre- and postcloacal papillae. The most typical pattern is 7 pairs of precloacal papillae; 2 pairs of paracloacal ones and 4 pairs of postcloacal ones. It was concluded that such variability in the number and distribution of papillae prevents the use of this feature in accurate species diagnostic in the genus *Thelazia*. Supported by the grant 14-16-00026 from Russian Scientific Foundation.

B: Climate change, epidemiology and modelling

Ref: P1B030/0443

Prevalence and genetic characterization of *Toxoplasma gondii* in free-range chickens raised in Bahia, Brazil

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Toxoplasma gondii is a coccidian protozoan that has a clonal population structure consisting of three strains, designated Type I, II and III. However genotypes isolated in Brazil are more diverse, having many atypical or recombinant alleles. Chickens are *T. gondii* infection source for humans and are considered good indicators of environmental contamination. The aim of this study was to determine the prevalence of anti-*T. gondii* antibodies and genetic characterization of strains isolated from free-range chickens in the State of Bahia, Brazil. For that, 286 blood samples, brain and heart were collected at chicken slaughter. To verify the presence of anti-*T. gondii* antibodies, Modified Agglutination Test (MAT) was used. From the first 20 seropositive samples, a biological assay using brain and heart were made. The genetic characterization was performed by PCR-RFLP technique using eight genetic markers (SAG1, SAG2, SAG3, BTUB, C22-8, PK1, GRA6, and L358). The positivity in serology was 31.8% (91/286). From the 20 samples inoculated in mice, 14 isolates were obtained and they were all different from each other and divergent from genotypes of clonal type I, II and III. The result confirms that the strains of *T. gondii* isolated in Bahia, Brazil, have a high genetic diversity.

B: Climate change, epidemiology and modelling

Ref: P1B031/0466

Structure of the community of gastrointestinal nematodes goats from Paraíba semi-arid region, Brazil.

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Gastrointestinal nematodes of goats cause economic losses from delayed growth, decrease in meat and milk production and high mortality. This study was designed to evaluate the prevalence and intensity of gastrointestinal nematodes, aiming in the future to establish a strategic control for the semi-arid region with savanna vegetation; Patos County, Paraíba State, Northeastern Brazil. The local altitude is 142m, 72001'25 'S latitude and 37016'41 "W longitude. During twelve months, a total of 60 crossbred adult goats (*Capra hircus*) born and raised in semi-intensive system, from Patos Public Slaughterhouse, were analyzed. Of the five goats slaughtered per month, were removed abomasum, small intestine and large intestine and were collected all helminths. The nematodes were recovered fixed and stored in alcohol 70°GL in jars identified, clarified with lactophenol of Aman and mounted between slide and cover slip for identification. Six species were identified and the most prevalent were *Haemonchu contortus* (96.6%), *Oesophagostomum columbianum* (87.0), *Trichostrongylus colubriformis* (80.0); higher mean intensity and mean abundance for *H. contortus* (146.2 ± 204.5), *T. colubriformis* (1158.2 ± 205.3) and *Cooperia pectinata*. The greater dispersion index (DI = 3862.38) and aggregation (GI = 0.06) was *T. colubriformis*, and the lowest was (ID = 7.2466) for *Trichuris ovis*. More studies are needed to know more deeply the parasite community of goats between the different regions of the world.

B: Climate change, epidemiology and modelling

Ref: P1B032/0475

Distribution of *Fasciola* sp in an endemic area of the Mexican tropics

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The objective of this study was to determine the distribution and frequency of condemned cattle livers as a result of *Fasciola* spp affection in an endemic area of tropical Mexico. A prospective observational study was conducted from January 2014 to January 2015. All slaughtered cattle during this period were included. Livers were inspected for lesions and presence of adult parasites. A total of 1,138 cattle from 31 communities belonging to the municipality of Jalapa were inspected and data recorded. Results: A total of 268 livers were condemned (24.5%) as a result of the veterinary inspection. The frequency of livers' condemnation due to fasciola related varied throughout the year. The month with the highest frequency of liver retention was February (34.7%), followed by October (30.8%). Out of 268 livers confiscated, 97.5%, proceed from only 10 communities, in which fasciolasis prevalence ranges from 13 to 40.2%. These results are essential to expanding the knowledge of distribution of the fluke in endemic areas by a none-invasive strategy. This information turns useful to locate specific regions and farms in problematic zones in order to design regional plans for prevention and control of fasciola in cattle. This information is also useful to prepare plans of strategic deworm control, snail surveillance and contingency strategies to manage fasciola spillover to human populations in hiperendemic locations of Mexico.

B: Climate change, epidemiology and modelling

Ref: P1B033/0505

Snails and trematode infections in cattle in highland and lowland areas of Iringa district, Tanzania.

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The effect of altitude on the occurrence of trematode infections in cattle was investigated in Iringa District in the southern highlands of Tanzania to be able to give better advice on their control. 450 faecal samples were collected from cattle that were traditionally managed with communal grazing in 15 villages ranging from 696 to 1,800 m above sea level. Samples were analysed using the Fluke Finder® (Richard Dixon, ID, US) method. Infection rates in cattle were *Fasciola gigantica* 28.2%, paramphistomes 62.8% and *Schistosoma bovis* 4.8%. Infection rates were significantly higher in the highland (>1,500 m) areas for *F.gigantica* and paramphistomes but not *S.bovis*. In the highlands where rainfall was in the range 500 – 2,700 mm per year 75% of water bodies frequented by cattle harboured snails but only 36% of water bodies in the lowland area with rainfall <600mm. Snails collected included *Lymnaea natalensis*, *Bulinus africanus*, *Bulinus tropicus*, *Bulinus forskali* and *Biomphalaria pfeifferi*. *Melanoides tuberculata* and *Bellamya constricta* were restricted to the lowland areas. Gymnocephalous cercariae were shed by *Lymnaea natalensis* and *B.africanus* while mammalian *Schistosoma* and amphistome cercariae were shed by *B.africanus*, *B.forskali* and *B.pfeifferi* respectively. Altitude is clearly an important factor in the transmission of trematodes and suggests a greater emphasis on control of *Fasciola* and paramphistomes is required in the highlands rather than the lowlands. The work was supported by the Leverhulme-Royal Society African Award Scheme.

B: Climate change, epidemiology and modelling

Ref: P1B034/0520

Prevalence and parasite identification in the gastrointestinal tract of Magellanic penguins (*Spheniscus magellanicus*) from the coast of Brazil

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Spheniscus magellanicus (Magellanic penguin) is the most commonly penguin found in temperate areas. This species of penguin is the most frequent also on the Brazilian coast, among the 17 species cataloged in 2009. These errant birds arrive in the South of Brazil carried out by cold currents looking for places with greater availability of food (fish and other aquatic organisms) and often show signs of weakness due to their long journey. In 2002, the Federal University of Parana, UFPR created the PROAMAR Project (Marine Animals Project) in the coastal city of Pontal do Sul, PR with the objective to treat and rehabilitate penguins and other marine animals before their return to nature. It is known that among other factors that can influence the weakness of these animals, parasite infections are of great importance. However, articles regarding Magellanic penguin parasites are scarce in Brazil. This research aimed to identify and quantify the endoparasites that inhabit the gastrointestinal tract (GIT) of 40 penguins through the necropsy of 33 animals that died in the PROAMAR Rehabilitation Centre. We also searched parasite eggs in the faeces through the Willis-Mollay technique in 07 live animals. GIT parasites were found in 90% of the birds and in 70% of the faecal material. The prevalence of parasite species found in the GIT was *Contracaecum pelagicum* (91%), *Cardiocephaloides physalis* (73%) and *Tetrabothrius lutzi* (64%). At inspection no clinical sings were evidenced. Although the animals face a very difficult task once in the shoreline - knowing that the return to their original place is quite unlikely, this research brings new information for the parasite literature regarding *S. magellanicus* and their errant occurrence in Brazil, serving of substantial assistance for Veterinary personnel and the care of these animals.

B: Climate change, epidemiology and modelling

Ref: P1B035/0521

Lymphocytic enteritis associated with *Cardiocephaloides physalis* infection in Magellanic penguins (*Spheniscus magellanicus*)

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Little is known about parasites from the Magellanic penguins (*Spheniscus magellanicus*) that arrive in the coast of Brazil. There is no information about tissue damage caused by endoparasites attached to the gut or any histological images. The aim of this work was to determine histologically possible changes caused by the *Cardiocephaloides physalis* trematode in the intestinal mucosa of *S. magellanicus*. Previously, we have determined at necropsy that 73% of these birds harbored this species. For this, samples from parasitized small intestine were obtained from two freshly dead penguins necropsied in 2014 at the Marine Study Centre, CEM, of the Federal University of Paraná, UFPR, Brazil. The samples were fixed in 10% buffered formalin for 48h and processed in automatic tissue processor, embedded in paraffin (58-62°C) and cut within 3-5 µm thick. Then, the histological slides were stained with hematoxylin-eosin technique. The procedures were performed at the Veterinary Pathology Laboratory and the Parasitic Diseases Laboratory, UFPR. We have evaluated 10 histological sections from 3 different areas (30 slides) of the small intestine with the presence of parasites. We were able to describe an inflammatory infiltrate, predominantly lymphocytic with a low number of heterophils and rare eosinophils in the lamina propria. The adhesion points of the parasite in the intestinal mucosa had a mild coagulative necrosis of the epithelium. The morphological diagnosis was a slight to moderate lymphocytic enteritis associated with cross-sections of *C. physalis* intralesional. This work complements the information about trematodes that parasitize *S. magellanicus* that are found erratically in the Brazilian coast.

B: Climate change, epidemiology and modelling

Ref: P1B036/0525

New data on the spatial distribution of the tick *Dermacentor reticulatus* in Romania

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Dermacentor (D.) reticulatus (Fabricius, 1794), also known as the marsh tick or ornate dog tick is next to *Ixodes ricinus* the second most significant vector of tick-borne diseases in Europe. It is the vector of different pathogens among them protozoa, rickettsiae and viruses. However, so far only very limited information on the distribution of *D. reticulatus* in Romania is available. Few surveys on the geographical distribution of ticks in Romania were conducted in the 1960s and then again in the 2000s. None of the 2000s' studies have brought new data regarding the presence of *D. reticulatus* after Feider's early study from 1965. A field survey monitoring the geographical distribution and periodic appearance of *D. reticulatus* in Romania was carried out in 17 counties from 2012 to 2014 in order to gain current data on the distribution and activity of *D. reticulatus* in Romania compared to former studies. Ticks were collected from dogs, foxes, sheep, cattle and wild boar which were available for other investigations. Part of the ticks was also flagged in 3 counties, in ecological areas suitable for this species. Altogether, 894 adult *D. reticulatus* ticks were collected, 442 of which were collected by flagging (231 females and 211 males) and 452 from animals: 380 from dogs (239 females and 141 males), 10 from cattle (6 females and 4 males), 1 from sheep (a female), 60 from wild animals: 52 from foxes, (19 females and 33 males), 8 males from a wild boar, and one female feeding on a human. In the current study, *D. reticulatus* was detected in 17 counties of which in 15 for the first time at all. This is in contrast to Feider's classical work from 1965 where he described this tick species only in three counties. The present survey provides evidence that this tick species has largely extended its geographical range since 1965 or that it was not correctly identified in the past. Furthermore, new tick-host association of *D. reticulatus* is reported by the current study, showing new hosts and new data on the periodic activity of this tick species.

B: Climate change, epidemiology and modelling

Ref: P1B037/0540

Seroprevalence of *Neospora caninum* in cattle in Bangladesh

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Neosporosis in cows causes abortion during 5 to 6 months of pregnancy, stillbirth, weak calves and a decrease in milk production. Neosporosis could spread easily by contamination of feed with faecal oocysts discharged from dogs. A number of studies in Bangladesh indicated that abortion in cows is one of the reasons of production loss in dairy farming. In this study, bulk milk and blood serum samples from cattle were collected in Chittagong, Satkhira and Sirajgong districts. Antibodies against *N. caninum* were tested in 92 bulk milk and 184 serum samples, using an indirect Enzyme-Linked Immuno sorbent Assay (ELISA), a commercially available kit (ID Screen® indirect ELISA kit, IDvet Laboratories, Inc., France) according to the manufacturer's instructions. Overall prevalence was found 1.81%. Among the tested bulk milk samples, 5.43% was found seropositive whereas no serum was found positive. Prevalence in Chittagong was high 4.35% which was followed by 1.09% in Satkhira. The investigation on neosporosis showing that *N. caninum* possibly distributed throughout the country. Considering that *N. caninum* seropositive cows are thought to be more likely to abort, substantial fetal losses may be induced by *N. caninum* infection in Bangladesh. This is the first study that reporting the seroprevalence of *N. caninum* in cattle in Bangladesh.

B: Climate change, epidemiology and modelling

Ref: P1B038/0549

Internal parasitism of domestic ruminants and its control in Arkhangai (Mongolia): current status and suggestions for a more rational management

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A questionnaire was used for the interview of 132 herders of domestic ruminants (cattle, yak, sheep, goat) to identify the most frequent parasitosis and the methods of parasite control in Arkhangai, a mountainous province of Mongolia. Among Cestodes, larval stages of *Cœnurus cerebralis* infest most herds (66 %), all species of ruminants being affected. Imaginal cestodes are frequently observed (91 % of herds) but do not seem to cause diarrhoea. Regarding Nematodes, clinical signs possibly linked to gastrointestinal nematodes (GIN) seem to occur only in spring, during the likely periparturient rise. This particularity could be due to the very cold and continental climate of Arkhangai, associated with semi-nomadism. Indeed, this context is not in favour of the ingestion of high numbers of nematode larvae even in summer, whereas in spring, when animals are underfed, weak and giving birth at the same time, the resumption of hypobiotic larvae may be very harmful. Among Trematodes, rumen flukes (Paramphistomatidae) are present in Arkhangai but rarely or never observed by 95 % of herders (liver flukes are not described in Mongolia). Parasite control is mainly performed through systematic treatment campaigns, using albendazole and / or ivermectin, during which all animals of the same species and age class (adults and young <1 yr for small ruminants or <2 yr for bovines) are treated. While adult bovines do not receive any treatment in 56 % of herds, calves, lambs and adult sheep are treated from 3 to 5 times a year in respectively 26 %, 40 % and 53 % of herds. Caprines are systematically treated like sheep in 63 % of herds and less or not treated at all in the remaining herds. The number of systematic campaigns can be considered as too high, especially for sheep, according to the estimated low risk of strongylosis. Unfortunately, to our knowledge, no visual indicator of level of GIN infection has been tested in Mongolia for targeting the treatment to the heaviest infected animals. On the other hand, performing coproscopies is not possible because of the absence of laboratory facilities. At least, systematic treatments could be limited, by focusing on 1 and 2 year-old ruminants, breeding ewes and female goats, in autumn, with ivermectin. Regarding cœnurosis, dog deworming should be generalized and the source of infection for dogs could be dried up by burning infected organs.

B: Climate change, epidemiology and modelling

Ref: P1B039/0565

Molecular identification of *Lutzomyia migonei* (Diptera: Psychodidae) as a potential vector of *Leishmania infantum* (Kinetoplastida: Trypanosomatidae)

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Visceral Leishmaniasis (VL) is caused by the protozoan *Leishmania infantum*. The parasites are transmitted by the bite of an insect vector, a phlebotomine. In Fortaleza, the capital of the State of Ceará, Brazil it was detected a simultaneous occurrence of *Lutzomyia migonei* and *Lutzomyia longipalpis* in the localities of VL transmission. Studies from La Banda, Argentina, have correlated the absence of *L. longipalpis* and the presence of *L. migonei* with autochthonous VL cases. A similar pattern has been demonstrated in San Vicente Férrer, State of Pernambuco, in northeastern Brazil. The purpose of this study was to identify other probable vector of VL in one of the main foci of the disease in northeastern Brazil by molecular detection and to quantify *L. infantum* in *L. migonei* using qPCR. The city of Fortaleza was divided in 6 regions and in each region 3 neighborhoods were examined. Using a CDC trap we performed phlebotomine capture during one night a month for a year, from July 2013 to June 2014. The identification of the *Leishmania* species was done using, primers targeting genomic DNA of *L. infantum* (MTHD). Phlebotomines were identified using primers targeting genomic DNA of *Lutzomyia* spp. (cacophony), *L. migonei* (period gene) and *L. longipalpis* (V-ATP). We collected approximately 2,436 *L. migonei*, 537 females and 1,899 males. From July to November 2013 the population of *L. migonei* reached the lowest levels in Fortaleza. *L. migonei* prevalence was higher in the rainy season during the period of January to June 2014. The total number of female *L. migonei* examined was 104; 91; 71; 91; 96 and 84, originated from the regions 1 to 6, respectively. Natural infection of *L. infantum* was detected in a sample of *L. migonei* (1/537) and infection rate was 0.18%. The parasite load was 32,492 *L. infantum*. The finding of *L. migonei* naturally infected with *L. infantum* strongly suggests its putative role in transmitting this parasite species in Fortaleza, Brazil. Moreover, determining *Leishmania* load in sandflies is an important tool for epidemiological studies of Leishmaniasis and vector competence.

B: Climate change, epidemiology and modelling

Ref: P1B040/0576

Intermediate snail host dynamics on rumen fluke positive farms in west Wales.

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The prevalence of rumen fluke (*Calicophoron daubneyi*) has increased significantly in the UK over the past decade. The lymnaeidae snail is believed to play a crucial role in the lifecycle of *C. daubneyi* in addition to that of liver fluke (*Fasciola hepatica*). However, there are unanswered questions regarding which species of lymnaeidae hosts *C. daubneyi*, and the type of habitats where its cercariae are shed and its metacercariae are consumed by cattle and sheep.

From spring 2015, the dynamics of the lymnaeidae population on lowland and upland farms positive for rumen fluke will be recorded. PCR and sequencing analyses will be used in conjunction with Geographical Information Software to record the prevalence of *C. daubneyi* and *F. hepatica* infection within host species, the identity of these host species and the associated habitat type. These indicators will be related to on-farm livestock infection as measured by faecal egg counting.

The results will increase our understanding of the transmission of *C. daubneyi* and its lifecycle in Wales. Understanding which lymnaeidae species, habitats and environmental preferences can support the *C. daubneyi* lifecycle will contribute to an understanding on why this parasites threat has increased over recent years, and which areas and farms are at risk of current or future infection. Interactions between *C. daubneyi* and *F. hepatica* may also be detected if it is found that both parasites share the same intermediate host snail species.

B: Climate change, epidemiology and modelling

Ref: P1B041/0577

Molecular characterization and occurrence of *Cryptosporidium* spp from fecal samples of chickens (*Gallus gallus domesticus* Linnaeus, 1758) raised under free-range system in Brazil

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Cryptosporidium species infect humans and a wide variety of vertebrate animals, and can be acquired through several routes: person-to-person contact, contact with companion and farm animals, ingestion of contaminated food, drinking water and recreational water. In birds, the infection has been diagnosed in different species, including chicken. The aim of this study was to determine the prevalence of *Cryptosporidium* species and genotypes in chickens raised under free-range system in Brazil. A total of 351 samples from 20 properties were collected. The fecal samples were obtained from the large intestine of slaughtered chicken, and stored at freezer temperature until DNA extraction. Molecular analyses were performed using nested-PCR for amplification of fragments of the 18S subunit of rRNA gene. Sequencing was performed to determine the species. Forward and reverse tapes were sequenced. Nucleotide sequences were compared to standard sequences of *Cryptosporidium* deposited in Genbank using BLAST system and manual alignment by BioEdit program. An epidemiological survey was applied to all properties to determine the risk factors associated with *Cryptosporidium* infection. It was observed amplification for *Cryptosporidium* DNA fragments in 90 (25,74%) samples. Sequencing of amplified fragments allowed the identification of two species that infect birds: *C. baileyi* in 15 (4,27%) samples and *C. meleagridis* in 60 (17,09%). Além disso, we identified two species that infect mammal: *C. parvum* in 4 (1,14%) samples and *C. bovis* in 3 (0,85%).

B: Climate change, epidemiology and modelling

Ref: P1B042/0578

Seroprevalence, isolation and genotyping of *Toxoplasma gondii* in chickens raised under free range system in southern Brazil

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Toxoplasma gondii is a cosmopolitan intracellular parasite that can infect all warm-blooded animals including man. The prevalence of infection in free-range chickens is a good indicator of the presence of this parasite in the environment. Thus, this study had aimed to evaluate the prevalence of *Toxoplasma gondii* antibodies in free-range chickens grown in the northern of the Brazilian state of Paraná, isolate and obtain its genotypical characterization. Indirect Fluorescent Antibody Test (IFAT) and Modified Agglutination Test (MAT) were used to detect antibodies to *T. gondii*. Titres ≥ 16 for both serological tests were considered positives. Blood and tissues (brain, heart, lung and liver) samples were obtained from 386 chickens from 24 different farms. The birds that had positive reaction in at least one of the serological tests had its tissues used for isolate the parasite through the bioassay in mice. The isolates were characterized through the PCR-RFLP by using 11 genetical markers (SAG1, 5'-3'SAG2, alt.SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 e Apico). Results of genotyping were classified according to the *Toxoplasma* Genomics Resource (ToxoDB) database. A survey made in closed question format was filled out by the producers to evaluate the environmental or management variables associated to *T. gondii* infection. Among 386 samples, 119 (30.8%) were positive at least in one test, 102 (26.4%) in IFAT and 64 (16.6%) in MAT. Among 24 farms analyzed, 21 (87.5%) had positive animals. A modest Kappa concordance index (0.45) was observed among the serological tests. The results have shown a higher specificity of MAT when compared to IFAT. The variables: presence of cats, water source protection and fence free rearing system were related to *T. gondii* infection. The bioassay showed 38 positives results with 18 isolates: 2 were from brain, 7 from tissues digestion and 9 from both of them. Genotypical characterization revealed the genotypes: #6, #19, #21, #111, #152, #175 and four not described before. Results show the genetic diversity of *T. gondii* in the north of Paraná.

B: Climate change, epidemiology and modelling

Ref: P1B043/0580

***Toxoplasma gondii* in cattle: seroprevalence and isolation from beef**

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The objective of the present study was to evaluate the serooccurrence of anti- *T.gondii* antibodies from slaughtered cattle and to isolate the parasite from meat and beef from Londrina, southern Brazil. Samples were collected from masseter, tongue, heart, diaphragm, liver and blood (subscapularis artery) from 400 cattle in a slaughterhouse. The sera of the animals were tested for the presence of anti-*T.gondii* IgG by Immunofluorescence Assay (IFA) and Modified Agglutination test (MAT). The animals were considered positive when titers were ≥ 64 in both tests. A total of 8.5% (34/400) of samples were seropositive, with 4.5% (18/400) positive results by IFAT and 4.8% (19/400) by MAT. Three samples were found positive for both tests. The positive samples were subjected to mouse bioassay. In contrast, none available tissue cysts were found when the bioassay was performed. Then, a total of 100 samples of beef (50 from rump and 50 from skirt steak) were collected in five different regions of Londrina, with a total of 50 supermarkets. Eighty grams of each sample were used to perform mouse bioassay and ELISA from meat juice for detection of anti-*T.gondii* IgG. The positive and negative control serum came from previously infected and uninfected cattle, respectively. The ELISA cut-off was OD=0.47. In conclusion, in the condition as the study was done, there was a low level of anti-*T.gondii* antibodies from cattle from Londrina, what could represent a low potential risk of *T. gondii* transmission to humans, since there were no beef detected as positives either in ELISA or mouse bioassay.

B: Climate change, epidemiology and modelling

Ref: P1B044/0587

Occurrence and dynamics of *Toxoplasma gondii* infection in Swiss finisher pigs

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Finisher pigs represent the main pig category involved in the transmission of *Toxoplasma gondii* infection to humans, as meat cuts derived from finishers are more prone to be consumed either raw or undercooked than those from older pigs. Therefore it is important to focus the surveillance and diagnostic efforts on this pig category. In order to determine the occurrence and dynamics of *T. gondii* infections in Swiss pig fattening farms, 20 farms situated in the main pig production area in Switzerland were sampled at the beginning and at the end of the fattening period. Blood samples were obtained from a total of 1,149 pigs when they entered the fattening units (age ~10-12 weeks; weight 23-28 kg), and again after ~3 months (weight ~110 kg) just before slaughtering. Sera were tested for antibodies against *T. gondii* by ELISA (PrioCHECK Toxoplasma Ab porcine, Prionics AG, Switzerland). At the beginning of the fattening period, specific antibodies were detected in pigs from 14 of 20 farms, with frequencies between 1.2 and 36.1% (mean: 10.8% $\pm$ 10.9). At the animal level, 73 out of 1,149 (6.4%) sampled pigs presented antibodies against *T. gondii*. It was assumed that maternal antibodies accounted for seropositivity in some animals, which became negative by the 2nd sampling. At slaughter, infection frequencies between 1.1 and 91.5% (mean: 12.2% $\pm$ 25.2) were observed in 12 of 20 farms, with 1.1 to 72.3% of the cases representing new infections. Specific antibodies were found in a total of 72 of 1,149 (6.3 %) slaughter animals; in at least 57 (79.2%) animals, the infection was assumed to have occurred during the fattening period. In 8 (40%) of the farms no seropositive animals were detected. According to the type of housing, infections occurred in 4 of 7 (57.1%, CI 95%: 29.6-90.8) farms and in 6 of 315 (1.9%, CI 95%: 0.8-4.2) animals with exclusive indoor housing and in 8 of 13 (61.5%, CI 95%: 35.4-82.4) farms and in 51 of 834 (6.1% CI 95%: 4.7-8.0) animals with indoor housing and outdoor yard. These differences are significant (p 0.002) only if the total number of animals infected in each farm type are considered. The frequency of *T. gondii* infections in Swiss conventional finisher pigs was frequently low (0-5%; $n=16$); however, in some cases it was moderate (6.3-12%; $n=3$) and also unusually high (91.5%; $n=1$). Special control measures would be needed if *Toxoplasma*-free meat production had to be guaranteed.

B: Climate change, epidemiology and modelling

Ref: P1B045/0590

Prevalence of gastrointestinal parasites in adult and young goats in Oaxaca, Mexico

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Gastrointestinal parasitic infections are one of the most important causes of production losses in ruminants. The aim of this study was to estimate helminth and protozoan infections in adult goats, kids and dams with kids during the preweaning period.

Fecal samples from 40 goats (2.5-year-old), seven kids (6-month-old), and 7 dams with kids in San Mateo Macuilxóchitl, Oaxaca, Mexico were examined using coprological techniques to detect and count coccidian oocysts and eggs of gastrointestinal nematodes and cestodes. Afterwards, *Eimeria*-positive samples were cultured to identify species. Fecal cultures were carried out to identify strongylid larvae. The Spearman correlation coefficient (rs) was used to evaluate the relationship between parasite burden and age.

Twenty percent of adult female goats were found to be passing coccidian, being *Eimeria alijevi* the most frequent species (40%) whereas *E. caprina* was only detected in 2% of these animals. The frequency of *Haemonchus contortus*, *Bunostomum* spp., *Chabertia ovina*, *Cooperia* spp., *Oesophagostomum* spp., and *Trichostrongylus* spp. were 26.38%, 18.05%, 18.05%, 16.66%, 12.5%, and 8.33%, respectively. *Trichuris ovis* was detected in 2.5% of adult goats, and 20% of them were infected with *Moniezia* spp. Kids were positive to *Eimeria*, and the most prevalent species was *E. caprina* (20%). The infection rates of *Haemonchus contortus*, *Strongyloides*, *Bunostomum*, *Cooperia*, *Oesophagostomum*, and *Chabertia* in kids were 22.52%, 22.52%, 17.11%, 16.21%, 13.51%, and 8.1%, respectively. Dams with preweaned kids harbored 50% of *Eimeria alijevi* as the most found species. These same adult goats hosted an infection consisting of 53.16% *Strongyloides*, 31.64% *Haemonchus contortus*, and 15.18% *Cooperia* spp. A negative and significant ($p \leq 0.05$) correlation was found between parasite burden and age.

The present study demonstrated that gastrointestinal parasitic infections occur frequently in young and adult goats around Oaxaca, Mexico, especially coccidian infections. Parasite burden and age are negatively correlated and this could be perhaps due to a stronger immunity in older animals.

B: Climate change, epidemiology and modelling

Ref: P1B046/0593

Analysis of Prevalence of Hepatic Disposal Fasciolosis Livers of Beef Cattle in Slaughterhouse in the State of Santa Catarina, Brazil

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The State of Santa Catarina is located in southern Brazil and is divided into five mesoregions, with significant differences in geographical and climatic aspects. Among the major diseases that affect the states beef cattle is liver fluke. This illness is caused by *Fasciola hepatica* helminth who parasites the biliary tract from animals, causing liver condemnation on infected cattle. This research consists of data collection held in a slaughterhouse located in São João do Itaperiú, Santa Catarina. The data was collected and analyzed from slaughters occurring since February 2011 to December 2014, to identify most affected state regions and quantify economic losses caused by the worm. The number of animals affected by fluke presented continuous elevation where as, in 2011 of 21.847 animals slaughtered, 644 (2,95%) had liver condemnation due to fascioliasis; In 2012, 1.130 cattle (4,51%) of the 25.039 were affected; In 2013 there was a more significant increase in numbers making it go to 2.860 (9,44%) in 30.286 slaughters. Finally in 2014, 5.040 livers in 40.871 slaughters were condemned, totaling it a 12,33% of affected cattle. Throughout four years in this study, the Florianopolis region condemned 1.454 livers (15,03%); North State region 1.337 (13,82%); West State region 1.349 (13,94%); Serrana region 932 (9,63%); South State region 1.134 (11,72%); and the Itajaí valley livers 3.509 (36,27%). With the analysis, it was possible to identify that the parasite is present in already all five mesoregions. The relationship between mesoregion-fasciolosis occurs due to the presence *Lymnaea* genre snail which makes the complete helminth cycle. The snail mainly inhabits wetlands and does not survive at low temperatures, making the Itajaí valley the most suitable place for their proliferation. Performing animal traceability on the regions of Santa Catarina state that do not have intermediate host ideal habitat, it was found that the snail is also adapting into those areas, overrunning existing physical and climatic barriers.

B: Climate change, epidemiology and modelling

Ref: P1B047/0598

Identification of prolactin-like receptors in larvae from *toxocara canis*

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Toxocariosis is a zoonotic disease in which the definitive hosts are dogs, mainly puppies. In adult dogs, the larvae of *Toxocara canis* is encysted in different tissues, during pregnancy, these larvae are activated and transmitted by transplacental and lactogenic way to the breed. Physiologically, one of the most important events in this stage is the hormonal variation, so it has been proposed that these variations are related to the reactivation of the larvae. This would imply that the larvae are able to recognize these hormonal variations and use them for its development. Because of this, the objective of this study was to identify the presence of prolactin binding proteins resembling receptors in larvae of *T. canis*. The larvae of *T. canis* were cultured in RPMI-1640 in the presence of 40 ng/mL of prolactin during 20 days. Cells of larvae were disaggregated before being marked with the antibody which recognizes the prolactin receptor. As secondary antibody was used anti-mouse Ig-G coupled with APC and they were analysed by flow cytometry. A group of larvae were incubated with the same antibodies (staining *in toto*) and fluorescence was detected by confocal microscopy. The percentage of positive cells to prolactin-like receptor by flow cytometry was (13%). Confocal microscopy showed the presence of prolactin-like receptors in the intestine of the larvae. These results suggest that cells of larvae of *T. canis* have prolactin-like receptors.

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B: Climate change, epidemiology and modelling

Ref: P1B048/0601

The cockroach *Blatella germanica* as a potential vector of infective *Toxocara canis* eggs.

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It has been proposed that some insects can disseminate *Toxocara canis*, but the potential the most common cockroaches (*Blatella germanica*) worldwide have to transmit it has not been determined. The aim of this study was to evaluate the ability of *Blatella germanica* cockroaches as *T. canis* disseminators.

Six groups (n=10) of cockroaches were infected orally with 500 *T. canis* embrionated eggs (TcEE). The feces from infected cockroaches were collected daily (10 days) and the egg number per gram of feces were counted. After, 7 groups of rats (n=5) were inoculated orally with 5mg of cockroach feces obtained at days 1, 2, 3, 6 and 10 P. I; two groups were used as positive and negative controls. All the rats were euthanized 21 days P. I. and lungs, kidneys, liver and brain were collected. The half of each sample was digested and the number of larvae found counted. Sera samples were also collected at days 0, 7, 14 and 21 P. I. and anti-*T. canis* antibodies were measured through ELISA test.

Infected cockroaches released more TcEE the first day P. I. (183±) and continued releasing until the sixth day P. I. Rats which received feces from infected cockroaches, developed larvae in their tissues. The organs where more larvae were found were lungs and brain (15-35 larvae). Sera from rats infected with cockroach feces from days 1 and 2 P. I, had higher antibody levels compared with the other groups. The results found show that the evaluated cockroaches are able to release and disseminate infective TcEE in the feces to other host.

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B: Climate change, epidemiology and modelling

Ref: P1B049/0602

Canine leishmaniosis in Paraguay, management by the National Programme of Zoonoses Control and Rabies National Center, Ministry of Public Health and Social Welfare, 2014

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Visceral leishmaniosis is a chronic parasitic disease caused in the Americas by *Leishmania infantum*, transmitted by infected sandflies that affects humans and its urban reservoirs dogs. The purpose of this work was to determine the seroprevalence of canine leishmaniosis by immunochromatographic rK39 test in serum samples obtained for routine exam requested by dog's owners and veterinarians, active surveillance in areas of silence transmissions and control of human cases notified for the National Service of Eradication of vector-borne diseases (SENEPA) to the National Programme of Zoonoses Control and Rabies National Center (PNCZyCAN) in 2014. A total of 7520 blood samples were analyzed by immunochromatographic rK39 (Biocan Diagnostics Inc, Canada), in the laboratory of Leishmaniosis of the PNCZyCAN, from 13/17 departments of the country. The results were analyzed by Epiinfo 7 and SigmaPlot11. The 3907 canine blood samples proceeding for routine exam from Asunción (capital city), Concepción, San Pedro, Cordillera, Central, Paraguari, Caazapá, Misiones, Alto Paraná, Canindeyú and Presidente Hayes departments, showed 1746 positive serum samples with a prevalence of (44.7%) of canine leishmaniosis. The active surveillance performed in Asunción, Central, Misiones, Caaguazú and Ñeembucú departments showed that 230 out of 1152 samples had a positive result with a prevalence of (19.9%) of canine leishmaniosis. From the 30 focus of human visceral leishmaniosis performed in Asunción, Central, Cordillera and Caazapá departments was observed that 410 out of 2461 samples were positive with a prevalence of (16.7%). It was observed a global seroprevalence of 31.7% of canine leishmaniosis. Euthanasia procedures were performed in 119 positive dogs (51.7%) proceeding from focus of human visceral leishmaniosis and 256 positive dogs (62.4%) from active surveillance in areas of silence transmission of visceral leishmaniosis. The high canine visceral leishmaniosis shows the urgent need to continue a strict epidemiological surveillance, sanitary education and community participation by the Ministry of Public Health and Social Welfare in the control of this disease in Paraguay.

B: Climate change, epidemiology and modelling

Ref: P1B050/0603

Three-dimensional organotypic models of hepatocytes to study the early development of *Echinococcus multilocularis* metacestodes

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A three-dimensional (3D) hepatic model was developed from co-cultured hepatocytes and mesenchymal stem cells (MSCs) by using a 3D collagen scaffold to study the development of *Echinococcus multilocularis* metacestodes. This model simulates the organotropism of the parasite toward the liver of the intermediate host. The suitability of the 3D hepatic as an infection model was first examined by immunohistochemistry, albumin secretion analysis, EROD and urea synthesis assay, and further RT-PCR detection. Then in vitro culture of protoscolices was performed by using this 3D hepatic model, and the early development of protoscolices was studied. Immunohistochemical characterization of the 3D hepatic model showed expression of hepatocytes and MSCs specific markers CK8 and CD44, respectively. Additionally, the model showed the advantages of the production of albumin secretion, EROD assay and urea synthesis, and high expression of hepatocytes functional gene Cldn-3, Bsep, AFP, G6P and α 1-AT, compared to their monolayer counterparts. In the presence of 3D hepatic models, 81.2% of the protoscolices fast developed into cysts which were composed of single layer of germinal layer, within 6 weeks. And their infectivity was completely maintained. In contrast, without the 3D models, 48% of the protoscolices degenerated after cultured for 6 weeks. The transcriptome analyses showed 807 differentially expressed genes ($p < 0.05$, $q < 0.05$) between the cysts (diameter > 3 mm) and protoscolices were revealed. Gene ontology and pathway analysis demonstrated that these differently expressed genes were enriched in up regulated of cyclic nucleotide biosynthetic and metabolic process, gluconate and aldinate transmembrane transport, phosphorus-oxygen lyase activity, sulfuric ester hydrolase activity, and lyase activity ($p < 0.05$). Meanwhile, they were also enriched in down regulated in response to stimulus, homophilic cell adhesion, cell communication, transmembrane signaling receptor activity, ion transmembrane and passive transmembrane transporter activity, and so on ($p < 0.05$). This indicates that, compare with protoscolices, the cysts appears to possess a higher metabolic activity, stronger tolerance ability, and more sealed to against the environment. This study provides the first 3D in vitro model of alveolar echinococcosis and will be a model for future studies on host-parasite interactions of this important zoonosis.

B: Climate change, epidemiology and modelling

Ref: P1B051/0605

Prolactin like-receptors in larvae-3 cells from *haemonchus contortus*

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Haemonchus contortus is the most important round worm of sheep worldwide. The *H. contortus* larvae can stop its development and stay in abomasal walls (true stomach). These larvae can be reactivated during the last two weeks of pregnancy. It has been suggested that larval reactivation is due to ewe's hormonal changes. The aim of this work was to identify prolactin-like receptors by flow cytometry, direct PCR, and in vivo prolactin stimuli in larvae-3 cells from *Haemonchus contortus*. Larvae 3 were obtained through fecal culture. For flow cytometry, larvae 3 were disaggregated and the intracellular labeling was made with a primary antibody which recognizes the prolactin receptor (Anti-PR) and a secondary coupled with Alexa 647 (anti-mouse IgG Molecular, Probes®, USA). For direct-PCR the CaPRL primers were used which recognize a conserved prolactin gene domain in mammals. Finally to prolactin stimulating process four doses were tested 20, 80, 200, 400 ng/ML, approximately a 1000 larvae-3 were added into individual wells (24-well microtitre plates) filled with 1mL of RPMI medium culture, after five days of prolactin exposure the hormonal effect was evaluated through morphological parameters (length and width larvae-3 measures). The flow cytometry analysis showed that 4% of the larvae-3 cells were positive to prolactin like-receptor. In direct-PCR a 500-bp band was found in *H. contortus* DNA in concordance with the control band. Finally larvae-3 stimulated with prolactin showed a decrease in length when compared with control group, however no significances ($p < 0.05$) were found among groups. No changes were observed measuring the larvae width. These results suggest that larvae-3 from *H. contortus* have prolactin-like receptor or a molecule which has the ability to bind this hormone.

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B: Climate change, epidemiology and modelling

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Piroplasmids infection in small mammals of north-eastern Brasil

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The aim of this study was to evaluate the molecular occurrence of hemoparasites of the piroplasmida order in small mammals of two environment conservation units in the municipality of Natal city, Rio grande do Norte, Brazil. Sampling and field studies were carried out between the months of October 2012, February and August 2013. Tissue samples (blood, spleen and lung) were processed for molecular detection assays of nucleotid sequences of 18S rRNA gene common in *Babesia*, *Theileria* e *Cythauxzoon* genus. Molecular analysis were performed in 26 tissue samples of six *Didelphis albiventris*, three *Monodelphis domestica*, two *Necromys lasiurus*, one *Thrichomys apereoides*, and one *Rattus rattus* captured during the field trips. Five of those samples (spleen of three *D. albiventris*, and sleep and lung o fone *N. lasiurus*) amplified in the PCR test. Phylogenetic analysis of full lenght 18S rRNA suggested a new species of *Babesia* closely related to two species of avian *Babesia* found in the pacific region: *Babesia poelea*(DQ200887), parasite of Brown boobies (*Sula leucogaster*), and another species of *Babesia* sp. (FJ717705), still unamed, found in common murrees (*Uria aalge*).

B: Climate change, epidemiology and modelling

Ref: P1B053/0609

Frequency of gastrointestinal parasites in horses in Sinaloa, Mexico

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Gastrointestinal parasites damages the health of animals, this result decrease in work then there are economic losses. The aim was to record the frequency and mean intensity of gastrointestinal parasites of equines in Culiacan, Sinaloa, Mexico. The study was conducted in 2 blocks in Culiacan, Sinaloa. 45 equines healthy with free grazing were analyzed. Faecal samples were collected individually from the rectum in plastic bags, then were sent to the laboratory de Parasitology, FMVZ-UNAM, and were maintained to 4°C until processing. McMaster technique was performed, gastrointestinal parasites were identified and counted. The frequency (%) and the mean intensity were obtained (eggs per gram: epg). With positive samples, stool cultures were performed. Genera were identified. The horses of the block 1 which tested positive were dewormed (fenbendazole), samples were taken the day of treatment (day 0) and day seven post-treatment (day 7), were processed as mentioned above. Block 1: 15% (116±115 epg) *Anoplocephala*, 50% (1375±1364 epg) strongilidos, 5% (50 epg) *Parascaris equorum* y 5% (50 epg) *Oxyurus equii*. The mean intensity of day 0 and 7 were 1600 epg and 83 epg, there was a reduction of 94.8% in nematodes and 100% of reduction in *Anoplocephala* spp. Block 2: 36% (700±1801 epg) strongilides and 4% (200h epg) *Parascaris equorum*. Genera identified for both blocks: *Strongylus vulgaris* (27%), *S. edentatus* (21%), *S. equinus* (6%), *Cyatostomum* spp. (25%), *Trichostrongylus axei* (16%) y *Strongyloides westerii* (5%). En block 1 there was greater parasite diversity than block 2. Deworming in block 1 there was a good reduction (94.8%) in epg reduction. So, we conclude that with the knowledge of the parasites, is possible to implement a strategic control program with good results to improve animal health and the economy of the producer.

B: Climate change, epidemiology and modelling

Ref: P1B054/0621

The isolation and identification of intestinal parasites of *Apodemus agrarius* in Korea

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In the isolation and identification of the intestinal parasites from *Apodemus agrarius* in Korea, ninety three of *Apodemus agrarius* were captured during 2012. It was detected several nematodes in the intestinal contents of 20 (21.5%) mice and in the feces of 1 (1.1%) mice. Ninety five of *Apodemus agrarius* were captured during 2013. It was detected many nematodes in the intestinal contents of 84 (88.4%) mice. It was detected some cestodes in the intestinal contents of 2 (2.1%) mice. And also, it was detected some trematodes in the intestinal contents of 3 (3.2%) mice. Thirty seven of *Apodemus agrarius* were captured during 2014. It was detected many nematodes in the intestinal contents of 30 (81.1%) mice, such as *Ascaris* sp (5.4%). It was detected some cestodes in the intestinal contents of 12 (32.4%) mice, they were all of *Hymenolepis* spp. And also, it was detected some trematodes in the intestinal contents of 3 (8.1%) mice, they were all of *Neodiplostomum seoulense*.

All kind of parasites will be identified by PCR and sequencing.

Key-words: intestinal parasites, *Apodemus agrarius*, *Ascaris* sp, *Hymenolepis* spp, *Neodiplostomum seoulense*

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B: Climate change, epidemiology and modelling

Ref: P1B055/0629

The influence of grazing management on seasonal activity of gastrointestinal parasites in goats

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The aim of performed study was to examine the influence of grazing management and other risk factors (age, treatment practices) on seasonal activity of gastrointestinal (GI) parasites in goats. Goat flocks naturally infected with GI parasites reared on four Lithuanian farms representing different management regimes were examined. On three farms the adult goats were grazed in different ways on open pastures (with or without supplementary feeding) or tethered. On one farm all animals were kept indoor (zero-grazing). On each farm, samples were collected at monthly intervals from 13 – 15 adult and 10 kids. The results showed that grazing of adult goats with feed supplementation or kept indoor, shed the lowest number of strongyle eggs when compared to those kept on pasture ($P < 0.05$). Delayed turnout and zero-grazing significantly reduced excretion of strongyle eggs but increased the output of oocysts when compared to those grazed on set-stocked pasture together with adult goats. The most prevalent genus on all farms and in both age groups of goats were *Teladorsagia* spp. This study demonstrates that goats are infected with mixed species of parasites, but proportions of these parasites differed in different grazing management systems. The grazing management, age and season were all major factors that had an impact on GI parasite infection.

B: Climate change, epidemiology and modelling

Ref: P1B056/0656

Impact of elevated environmental temperature on early parasitic gastroenteritis of sheep

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Background: As a consequence of climate change, weather conditions are expected to become warmer, with more severe and pronounced fluctuations in temperature and precipitations. This is expected to change epidemiological patterns of diseases, such as parasitic gastroenteritis (PGE) in small ruminants, or lead to different host responses to disease challenges. In this study, we investigate the combined impact of thermal challenge and gastrointestinal parasitism on parasitic traits during the early stages of PGE as well as the behavioural and performance changes of growing lambs.

Methods: 48 sheep balanced for sex and body weight were randomly allocated in 6 climate-controlled rooms. Rooms were maintained at either 25 or 15 °C (n=3). In each room, sheep were either trickle infected with 21,000 infective *Teladorsagia circumcincta* larvae per week, or sham-infected. Infection and temperature treatments were imposed for three weeks. On week 4 the temperature of all rooms was set at 15 °C. At the end of week 4, animals were euthanized for sample collection and worm counts. Animal activity and feed refusals were recorded daily. Live body weight and Faecal Egg Counts (FEC) were recorded on a weekly basis throughout.

Results: FEC, worm counts and female worm fecundity did not appear to be affected by environmental conditions during the early phase of infection. The combination of thermal load and infection resulted in greater penalties ($P<0.05$) on feed intake and final body weight gain compared to each challenge individually. Increased thermal load and infection resulted in a change of the activity patterns of the animals by increasing frequency of altering position.

Conclusions: The exacerbated effects of worm challenge and increased thermal load on the performance of lambs observed in our study, may be indicative of reduced production efficiency of parasitized animals under the projections for climate change.

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B: Climate change, epidemiology and modelling

Ref: P1B057/0663

Tick species on companion animals: high abundance of *Ixodes trianguliceps* on cats but not dogs.

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Epidemiological data about tick infestations in dogs and cats generally are rare, also in Switzerland. Following an outbreak of canine babesiosis in a small town surrounded by popular natural recreation areas in North Eastern Switzerland, tick occurrence on dogs and cats was investigated. To this end, dog owners were systematically invited to send ticks from their dogs and cats. In two consecutive years, beginning in April 2012, 3003 ticks were submitted for identification (93.5% adults, 4.5% nymphs, and 2% larvae) from 249 dogs (approximately 20% of the resident dog population) and from 117 cats. The overall infestation rates were 73.1% and 26.9% for dogs and cats, respectively. The castor bean tick *Ixodes ricinus* was the most abundant species, representing 96.8% (n = 2124) and 74.3% (n = 601) of the individual submitted specimens from dogs and cats, respectively. Abundances in dogs and cats, respectively, were 1.2% (n = 26) and 1% (n = 8) for the hedgehog tick *I. hexagonus* and 2% and 0.7% (44 and 6 specimens) for the European meadow tick *Dermacentor reticulatus*. This new focus of *D. reticulatus* in Eastern Switzerland was subsequently confirmed by collecting questing ticks by flagging the vegetation in areas where infested dogs are regularly walked. Further, *I. trianguliceps* (vole or shrew tick) was for the first time identified on cats (but not on dogs), representing 24.0% of the submitted specimens from cats (n = 194). This survey demonstrated that pet owners can contribute to a cost-effective tick surveillance, allowing to also identify new tick foci. The exclusive finding of *I. trianguliceps* on cats might be related to behavioural traits of the cats or to a more readily detection of these tiny ticks by the caressing owner.

B: Climate change, epidemiology and modelling

Ref: P1B068/0672

Isolation and biological characterization of *Neospora caninum* from naturally infected cow from Sao Paulo, Brazil (Nc-SP1)

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Neospora caninum is an apicomplexan parasite that is recognized as an important cause of abortion in cattle worldwide. Canids are the definitive hosts by shedding the oocysts in the environment. The isolation of viable *N. caninum* is difficult. There are few isolates throughout the world. This report describes the isolation and biological characterization of a new isolate of *N. caninum* from São Paulo, Brazil (NC-SP1). Approximately 400g of brain from a naturally infected cow with IgG antibodies against *N. caninum* (IFAT=200) was bioassayed in two-month old dogs (IFAT <50). DNA of *N. caninum* Nc-5 gene was detected in two aliquots of the cow brain. Shedding of *Neospora*-like oocysts was observed from 7 to 21 days after feeding (a total of 144,507 oocysts). The dog remained clinically healthy and did not developed IgG antibodies (IFAT <50) to *N. caninum* during the analyzed period (4 months). The oocysts were submitted to a nested PCR-RFLP to confirm that they were only from *N. caninum*. The number of oocysts was quantified each day of excretion, purified by standard sucrose flotation technique, mixed with 2% H₂SO₄ in Petri dishes for 5 days at 25°C and sporulated oocysts were stored at 4°C. A total of 100 unsporulated oocysts were examined by light microscopy and images were measured using Image-Pro ®Plus program ($11.19 \pm 0.45 \mu\text{m}$ by $11.46 \pm 0.47 \mu\text{m}$). To observe the viability of *N. caninum* oocysts, nine Mongolian gerbils (*Meriones unguates*) were orally inoculated with 10^1 , 10^2 and 10^3 oocysts. All animals remained clinically normal and seroconverted seven days post infection and during 4 months the IFAT titers of IgG antibodies to *N. caninum* were analyzed. The range titers were from IFAT 100 to 25,600, however the highest titers were observed after 28 days p.i. One gerbil that received 10^2 oocysts was euthanized 78 days p.i. and the brain was aseptically collected, homogenized, digested and part of it was inoculated onto a monolayer VERO cells. Tachyzoites were observed 24 days p.i.; 500 µl of the brain homogenate were collected and examined by light microscopy. A total of six tissue cysts were found and measured using Image-Pro® Plus program ($23.84 \pm 4.14 \mu\text{m}$ by $25.69 \pm 5.30 \mu\text{m}$). Finally, the future for the new isolate of *N. caninum* called NC-SP1 is to be completely described to become a reference for further studies.

B: Climate change, epidemiology and modelling

Ref: P1B069/0678

***Toxoplasma gondii* infection in a naturally infected caprine herd: antibody detection in sera and milk samples during lactation period.**

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Toxoplasma gondii is listed among the major pathogens causing reproductive failure in goats, with a considerable economic impact on caprine breeding. In a naturally infected herd, we investigated on the variation of immunity response both in sera and milk during lactation. Moreover, the possibility of using milk as an alternative for antibody detection was evaluated. From March to July 2013, blood and milk samples were collected for seven times from all 30 female goats bred in a family-run dairy farm in Northern Italy. A commercial ELISA (ID Screen® Toxoplasmosis Indirect Multi-Species, IDVET) was used to detect IgG anti-*T. gondii* in sera and in milk samples, after validation by using a panel of sera-milk pairs previously tested for *T. gondii* by IFAT (Camargo, 1974 Rev Bras Pat Clin 10:28). Antibodies were detected in nineteen goats (P=63.3%) both in sera and milk during the whole lactation. Results obtained in sera and milk showed a high agreement (Pearson's correlation=0.815), with the best value in the first fortnight after parturition (0.897). Different antibodies levels were registered in sera and milk: IgG level was high in the first fortnight after parturition, then in the second fortnight it further increased in sera and at the same time decreased in milk. Previous studies reported high IgG values in the first 24-36 hours after birth in which newborns acquire immunoglobulins from milk (Ferrer et al., 1997 Comp Immun Microbiol Infect Dis 20(3):253-260; Levieux et al., 2002 J Dairy Res 69:391-399, Mesquita et al., 2013 Vet Parasitol 196:327-333), but no data are available on immunoglobulins levels in goats' milk during the whole lactation. Another peak at the end of lactation was registered in both sera and milk, already described in cattle and due to the decrease of volume and consequent major milk protein and IgG concentrations (Schaes et al., 2004 Vet Parasitol 120:55-63). This is the first study in which the detection of IgG in milk has been used for the diagnosis of *T. gondii* infection in goats; serological tests have been adapted for domestic animals for the detection in milk of antibodies for many parasites (Sekiya et al., 2013 Irish Vet J 66:14). The choice of milk reduces costs and increase animal welfare having the same diagnostic performance of testing serum samples. Moreover, the analysis of IgG curves during lactation allowed deepens immunological dynamics of *T. gondii* infection in caprine model.

B: Climate change, epidemiology and modelling

Ref: P1B070/0684

***Babesia bigemina* in dairy and beef cattle in a region of southern Italy**

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Babesiosis caused by *Babesia bigemina* is an economically important infection in cattle as it may cause mortality, morbidity and decreased meat and milk production. Few studies have been conducted on bovine piroplasmoses in Italy, especially in southern Italy; therefore the aim of this study was to conduct a cross-sectional serological survey to evaluate the prevalence of *B. bigemina* in cattle farms in the Basilicata region of southern Italy. A geographical information system (GIS) was constructed in order to uniformly sample the bovine farms throughout the entire region; specifically, a grid of 10x10 km was overlaid on the region map. As a result, the territory of the Basilicata region was divided in 98 quadrants, the centroid of each quadrant was identified and among all farms present into the GIS database the farm closest to the centroid in each quadrant was selected. Only farms with more than 50 animals were included in the sample. Out of the total 98 quadrants, 73 were investigated (74.5%). Blood samples were collected from 6 autochthonous cows (total number of cattle sampled = 438) on each of the 73 cattle farms analyzed (36 dairy farms and 37 beef farms). Sera were collected and tested for antibodies to *B. bigemina* using an enzyme-linked immunosorbent assay test (SVANOVIR® *B. Bigemina*-Ab), following the manufacturer's recommended procedures. The farm prevalence was 95.9% (70/73; 95% Confidence Interval, 95%CI= 87.7-98.9), and the individual animal prevalence was 44.5% (195/438; 95%CI= 39.8-49.3). No statistically significant difference ($P > 0.05$) in the prevalence of *B. bigemina* was found between dairy and beef farms. The prevalence of *B. bigemina* found in this study was higher than those found in other similar studies conducted in cattle in Italy. The availability of geo-referenced point or areal data on bovine babesiosis and the construction of prevalence maps by GIS are suggested for dissemination of information to veterinarians on this infection in cattle. However further studies on the identification of the vectors of *B. bigemina* in Basilicata region are necessary.

B: Climate change, epidemiology and modelling

Ref: P1B071/0689

Variations of mosquito communities in Eastern Austria: potential effects of environmental factors

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Dispersal and establishment of indigenous and non-indigenous mosquito species as well as new emerging vector-borne diseases are supposed to be mainly driven by international trade and global change. Knowledge of mosquito species ecology and seasonal and spatial distribution patterns of mosquito communities is crucial to understand vector-pathogen dynamics in Europe. The mosquito species inventory of Austria consists so far of 46 species (including four alien species) in seven genera (*Aedes*, *Anopheles*, *Coquillettidia*, *Culex*, *Culiseta*, *Ochlerotatus* and *Uranotaenia*). However, information on its genetic diversity, seasonal and spatial distribution patterns of communities and single species in Austria is insufficient.

More than 10,500 Mosquitoes were collected at 27 permanent sampling sites, distributed across Eastern Austria (Vienna, Burgenland and Lower Austria). Female mosquitoes were sampled twice a month for 24 hours, using specific mosquito traps equipped with carbon dioxide as an attractant. In this study we aim to update current information on the mosquito species inventory in Eastern Austria, the prevalence of mosquito-borne pathogens (e.g., filarioid helminths and avian malaria) and terrestrial environmental factors determining seasonal and spatial distribution patterns of mosquito communities.

We will discuss preliminary results, including potential effects of environmental factors on spatial and seasonal variations of mosquito communities in Eastern Austria.

B: Climate change, epidemiology and modelling

Ref: P1B072/0698

The Modelling of African Animal Trypanosomiasis in KwaZulu-Natal, South Africa

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African animal trypanosomiasis (AAT), restricted to parts of the KwaZulu-Natal Province in South Africa, is a disease which contributes significantly to the disease burden of cattle. Drug resistance is a constraint and dipping of cattle using insecticides has proved to be unsustainable. Even though the incidence of AAT has increased, little is known about the epidemiology of the disease in the region. To better understand the dynamics of AAT, mathematical modelling was done to investigate the interactions between the cattle, tsetse flies and buffaloes which are considered to be the reservoir host. In addition, a statistical analysis of the data collected from three sites around the Hluhluwe-iMfolozi Game Park was done to assess the interactions between the variables.

A susceptible-infected (SI) model was constructed for the different classes of the population i.e. susceptible and infected cattle and tsetse flies and buffaloes. The basic reproduction number R_0 , a threshold determining whether the disease will die out or persist in the population, was derived using the next-generation matrix since we had two-hosts and one vector. R_0 was used to assess which interactions contribute to R_0 (i.e. transmission of AAT from the buffaloes and cattle to the tsetse flies or tsetse flies to the cattle and buffaloes).

Generalized linear models (GLMs) were used to analyse the data. The AAT prevalence data was modelled using a binomial GLM and the tsetse abundance data was modelled using Poisson GLMs, however the problem of overdispersion was evident and so alternative models were considered such as the zero-inflated and negative binomial models. The AAT prevalence data was fit to the mathematical model, and the input parameters were estimated and used to calculate R_0 again so that it is more site-specific.

B: Climate change, epidemiology and modelling

Ref: P1B073/0711

Influence of the gestational stage on the clinical course, lesional development and parasite distribution during experimental *Neospora caninum* infection in goats

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Herein we studied the consequences of experimental infection by *N. caninum* in goats at three gestation terms. Pregnant goats were distributed into three groups (7 animals per group) and IV inoculated with 10⁶ tachyzoites of the Nc-Spain7 isolate at 40 (GI), 90 (GII) and 120 (GIII) days of gestation. Infected goats from all groups had fever between 5 and 9 days post inoculation (dpi), with a peak at day 6. Foetal death occurred in GI and GII from 10 to 21dpi (7) and 27 to 35 dpi (4), respectively. Goats from GII also had stillbirth (1) and pre-colostral positive healthy kids (2) and GIII seronegative weak kids (7). Parasite dissemination in placentome showed a 100% of parasite detection by nested-PCR in GII and 87,5% in GIII. In group GI, parasite was only detected in placentomes (100%) from goats with foetal losses from 21th dpi. The parasite was detected in foetal/kid brain (> 87.5%) and liver samples (> 50%) of GII and GIII, and in fetuses from GI after 21th dpi. The highest parasite loads by qPCR were detected in placentomes of GI from 21 dpi and GII, and brain and liver foetal tissues in GI from 21 dpi, followed of GIII. Multifocal necrotic lesions were seen in the placenta of the three groups, but they were larger and more frequent in GI and GII. Similar lesions were observed in foetal liver and brain and they were more frequent in GIII. These findings suggest that as in cattle, clinical consequences of *N. caninum* in pregnant goats depend on the time of gestation. However, it is noted that different mechanisms could be involved in foetal loss during the first term and that infection in the third term causes the birth of kids with neurological signs.

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B: Climate change, epidemiology and modelling

Ref: P1B074/0713

Systemic and local immune responses in goats after primoinfection with *Neospora caninum* at the three gestational terms

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The aim of the present study was to investigate the immune responses in pregnant goats infected with *Neospora caninum* at the three terms of gestation. Pregnant goats distributed in three groups (7 animals per group) were inoculated IV with 10⁶ tachyzoites of the Nc-Spain7 isolate at 40 (GI), 90 (GII) and 120 (GIII) days of gestation. Foetal death occurred in GI and GII from 10 to 21 days post-inoculation (dpi) (7) and 27 to 35 dpi (4), respectively. Goats from GII also had stillbirth (1) and pre-colostral positive healthy kids (2) and GIII seronegative weak kids (7). Kinetics of IgG and IFN- γ cytokine levels were studied in sera samples collected twice a week. Significant IgG-specific levels were observed from 14dpi in all groups, remaining high until foetal death/birth of kids. A significant increase of IFN- γ levels was detected from 7 to 10 dpi in all groups, reaching a peak at 7 dpi. The highest IFN- γ levels were found in GI goats in which foetal death occurred at 10 dpi. The placentas were recovered for studying local expression of IL-4, IL-12, IL-10, IFN- γ and TNF- α by RT-qPCR. Cytokine expression significantly increased in placentomas from all groups with the exception of IL-12 in GIII. IFN- γ and IL-4 showed the highest fold change in expression, followed by IL-10, TNF- α and IL-12 (IFN- γ = IL-4 > IL-10 = TNF- α $\geq$ IL-12). The highest increase in cytokine expression was detected in goats from GII and those from GI that showed foetal losses from 21 dpi. High parasite burdens were also detected in these animals. IgG and IFN- γ responses and cytokine profile in placenta resembles that described in cattle, although some cytokine profile differences -limited IL-12 induction and increased IL-4 expression- were also detected.

Acknowledgements: This study was financed by the Brazilian National Science Foundation (CNPq), grant number 400405/2012-1.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C249/0026

Evaluation of the anthelmintic activity of the aqueous leaf extract of *Vernonia amygdalina* in Nigerian puppies

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Gastrointestinal nematodes remain a major economic consequence in domesticated livestock throughout the world. There has been an increasing interest in ethnomedical and ethnoveterinary practices across the world, hence *Vernonia amygdalina*, also known as bitter leaf, was used in this study to assess its anthelmintic potency.

Sixteen Nigerian puppies divided into four groups of four puppies per group were used where group A animals were uninfected and hence were untreated, whereas those in groups B, C, and D were naturally infected with helminths. Group B animals were untreated whereas group C animals were treated with a proprietary anthelmintic (Mebendazole). Group D animals were treated with aqueous crude extracts of *V. amygdalina* using the oral route. Two weeks after treatment, blood and fecal samples were collected to evaluate the hematologic values and fecal egg counts, respectively. Before the onset of this study, fecal egg counts showed that ascarids (*Toxocara canis*) and hookworm (*Ancylostoma caninum*) were present in the experimental animals. The results of this study showed that the aqueous crude extracts of *V. amygdalina* after its administration into local dogs produced significant changes ($p < 0.05$) in PCV, RBC, Hb concentration, and TWBC. The fecal egg counts also showed remarkable and significant reduction in the levels of the identified helminths. The effects of the plant extracts were broad spectrum in action. Because the aqueous crude extract of *V. amygdalina* significantly reduced the fecal egg count of the helminths, it could be a potential source of a new lead anthelmintic agent.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C250/0044

***Syphacia muris* infection delays the onset of hyperglycemia in WBN/Kob-*Lepr*^{fa} rats, a new type 2 diabetes mellitus model**

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Diabetes mellitus is one of the most common endocrine disorders and its continuous global increase is due to factors as population growth, urbanization, aging, and increasing prevalence of obesity and physical inactivity. The effect of pinworm infection on the development of hyperglycemia was examined in WBN/K-*Lepr*^{fa} (*fa/fa*) rats, a new model of the obese type 2 diabetes mellitus (T2DM) with pancreatitis. The rats were orally administered *Syphacia muris* eggs (infected group) and distilled water (control group). Hyperglycemia onset in the infected group was significantly delayed compared to the control group. Neither body weight nor intake of food and water were affected by *S. muris* infection. This study demonstrated that *S. muris* infection delayed the onset of T2DM in *fa/fa* rats and suggested that elucidation of the underlying mechanism and relevant pathways in the helminth-mediated protection may lead to the development of a new strategy to prevent diabetes mellitus.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C251/0059

Efficacy of oral afoxolaner plus milbemycin oxime chewables (NexGard Spectra®, Merial) against induced infestations with *Dermacentor reticulatus* ticks in dogs

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The novel combination of afoxolaner plus milbemycin oxime (AFX+MO), NexGard Spectra®, was evaluated against induced infestations of *Dermacentor reticulatus* in dogs in a negative control, blinded study in comparison to AFX single entity chewables (NexGard®, Merial).

Thirty Beagle or mongrel dogs were assigned to blocks of three animals each based on pre-allocation tick counts and were randomly allocated to one of three groups: untreated (control); treated with NexGard Spectra® to be as close as possible to the minimum recommended dose of AFX+MO (2.5 mg + 0.5 mg/kg); and treated with NexGard® to be as close as possible to the minimum recommended dose of AFX (2.5 mg/kg). Treatments were administered orally once. Starting two days before treatment, each dog was infested with ~50 ticks weekly for six consecutive weeks. Live ticks were counted at ~48 hours post-treatment (removal count), and at ~48 hours (in-situ counts) and ~72 hours (removal counts) following each post-treatment infestation.

Treatment with both products rapidly eliminated the existing tick infestations (100% efficacy) within two days following treatment. Weekly re-infestations were controlled for a minimum of five weeks with the efficacy ranging between 92.2% and >99% based on ~48 hours post-treatment in-situ counts and between 99% and 100% based on ~72 hours post-treatment removal counts ($p < 0.0001$ at each occasion).

This study demonstrated a high level of efficacy of both NexGard Spectra® and NexGard® against infestation of dogs with *D. reticulatus* ticks for at least five weeks and an excellent acceptability of the two oral treatments.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C252/0060

Field efficacy against nematode infections and safety of afoxolaner plus milbemycin oxime chewables (NexGard Spectra®, Merial) in domestic dogs in Europe

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The novel combination of afoxolaner plus milbemycin oxime, NexGard Spectra® chewables, was evaluated for safety and efficacy against naturally acquired nematode infections in dogs in a multi-centre, positive control, blinded field study.

A total of 408 dogs confirmed positive for intestinal nematodes were enrolled in ten countries. Pre-treatment fecal examination revealed Toxocara, Toxascaris, hookworm, Trichuris and/or Capillaria infections in 134, 30, 223, 155 or 14 dogs, respectively. Dogs were allocated randomly to Treatments in a 1 to 1 ratio: NexGard Spectra® or Milbemax® (Novartis), and treated according to the manufacturers' recommendation. For evaluation of efficacy, fecal samples collected prior to and once 9 to 21 days after treatment were examined using modified McMaster techniques.

No treatment related adverse events were observed throughout the study. For dogs treated with NexGard Spectra®, the efficacy was 99.7%, 99.7%, 97.2%, 99.7% and 99.7% against Toxocara, Toxascaris, hookworm, Trichuris and Capillaria, respectively; and the efficacy was 99.5%, 99.4%, 94.3%, 99.9% and 98.0%, respectively, for the positive control ($p \leq 0.002$ for all nematodes). For Toxocara, hookworm and Trichuris, non-inferiority analysis demonstrated that the efficacy of NexGard Spectra® was equal to or better than that of the positive control. In spite of both treatments provided $\geq 98\%$ efficacy against Toxascaris and Capillaria, hypothesis of non-inferiority for both genera could not be established due to the low numbers of dogs infected.

NexGard Spectra® was shown to be safe and demonstrated a high level of effectiveness when administered once to dogs a broad range of parasitic nematodes under field conditions.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C253/0061

Field efficacy against tick and flea infestations and safety of afoxolaner plus milbemycin oxime chewables (NexGard Spectra®, Merial) in domestic dogs in Europe

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The novel combination of afoxolaner plus milbemycin oxime, NexGard Spectra® chewables, was evaluated for safety and control of flea and tick infestations in dogs in a multi-centre, positive control, blinded field study.

Total 312 dogs, harbouring a minimum of five fleas and/or one tick, were evaluated in seven countries. Pre-treatment examination revealed infestation with fleas (103 dogs), ticks (119 dogs) or fleas plus ticks (90 dogs) representing three species of flea and five species of ticks. Dogs were allocated randomly to Treatments in a 1 : 1 ratio: NexGard Spectra® or Prac-Tic® (Novartis), and treated according to manufacturers' instruction. For evaluation of efficacy, dogs were examined at weekly intervals for one month following treatment. Counts per parasite type were compared to pre-treatment counts.

No treatment related adverse events were observed throughout the study. For fleas, efficacy as measured by arithmetic means was 97.8% at each post-treatment time point for NexGard Spectra®, while the efficacy ranged from 75.8% to 89.7% for the positive control. For ticks, efficacy as measured by arithmetic means was 95.2% at each post-treatment time point for NexGard Spectra®, while the efficacy ranged from 70.5% to 81.6% for the positive control. Analysis of the difference in the parasite counts of the two treatments demonstrated that NexGard Spectra® was superior to Prac-Tic® at each occasion for both fleas ($p < 0.01$) and ticks ($p < 0.05$).

NexGard Spectra® was shown to be safe and demonstrated a high level of effectiveness when administered once to dogs infested with fleas and/or ticks under field conditions.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C254/0062

Efficacy of topical fipronil, (S)-methoprene, eprinomectin and praziquantel (Broadline®, Merial) against induced infection with *Ancylostoma ceylanicum* in cats

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Ancylostoma ceylanicum is a hookworm distributed in Southeast Asia, China, Japan, Australia and the Arabian Peninsula with dogs, cats and humans serving as hosts.

To evaluate the efficacy of Broadline®, a combination of fipronil 8.3% w/v, (S)-methoprene 10% w/v, eprinomectin 0.4% w/v, and praziquantel 8.3% w/v against fourth-larval stage (L4) and adult *A. ceylanicum*, a blinded, induced infection study with 24 cats was conducted. Following oral inoculation with ~300 *A. ceylanicum* L3 larvae, cats were allocated randomly to three groups (eight cats each) based on bodyweight: Control (untreated), Broadline® treatment when hookworms reached L4 stage (five days post inoculation, dpi), or Broadline® treatment when hookworms were adults (25 dpi). Treatments were administered once topically at the minimum label dose of 0.12 mL/kg. Cat faeces were examined for hookworm eggs prior to treatment for adult infection (22 dpi) and when the cats were necropsied for *A. ceylanicum* recovery (35 dpi).

All cats accepted the treatment well and no health problems were observed throughout the study. Twenty-two dpi, all Control cats and the eight cats to be treated when hookworms had reached adult stage excreted hookworm eggs (range, 100-1000 eggs per gram). No eggs were recovered from the faeces of any Broadline®-treated cat at the time of necropsy. Based on comparison of nematode counts in the treated cats and untreated Controls, Broadline® treatment was 100% efficacious against both developing L4 and adult *A. ceylanicum* ($p < 0.001$): no hookworms were recovered from any Broadline®-treated cat while Controls harboured between eight and 35 *A. ceylanicum*.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C255/0063

Efficacy of Broadline® (Merial) against naturally acquired infections with *Joyeuxiella* spp. cestodes in cats

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The genus *Joyeuxiella* comprises its representatives : *J. pasqualei*, *J. echinorhynchoides* and *J. fuhrmanni*. Their main distribution is in subtropical and tropical countries and several species of carnivores may serve as definitive hosts. Their life cycle has, however not been described completely. Also, very few data are available on the treatment of these cestodes.

Broadline® (fipronil 8.3% w/v, (S)-methoprene 10% w/v, eprinomectin 0.4% w/v, praziquantel 8.3% w/v) has been previously shown to be efficacious against *D. caninum* and *Diplopylidium* spp. in cats. To evaluate its efficacy against *Joyeuxiella* species, a VICH compliant, blinded clinical efficacy study was conducted according to GCP including 26 naturally infected cats from the Dubai area, UAE. All cats were shedding dipylidid proglottids as confirmed by pre-treatment faecal examination. Cats were allocated randomly to two groups of 13 cats based on bodyweight: Control (untreated) and Broadline® at 0.12 mL/kg bodyweight (minimum effective dose) administered once topically.

No health problems were observed throughout the study. Based on comparison of helminth counts in the treated cats and untreated Controls seven days post administration, Broadline® treatment demonstrated 100% and 99.7% efficacy ($p < 0.01$) against mature *J. fuhrmanni* and *J. pasqualei*, respectively, with 11 and 13 of the Control cats harbouring 1 to 102 or 2 to 95 cestodes, respectively. Juvenile *Joyeuxiella* spp. were reduced by 69.8%. In addition, parasite counts indicated 95.9% efficacy against the rictularoid nematode *Pterygodermatites cahirensis*.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C256/0065

Efficacy of Broadline® (Merial) against induced infection with *Troglostrongylus brevior* (Nematoda, Metastrongyloidea) lungworms in cats

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In addition to *Aelurostrongylus abstrusus*, pulmonary *Troglostrongylus brevior* infection has been reported with increasing frequency in domestic cats from Mediterranean Europe during the last decade.

To evaluate the efficacy of Broadline®, (fipronil 8.3% w/v, (S)-methoprene 10% w/v, eprinomectin 0.4% w/v, praziquantel 8.3% w/v) against larval and adult *T. brevior*, a blinded, induced infection study was conducted. Following oral inoculation with ~100 third-stage *T. brevior* larvae, 24 purpose-bred cats were allocated randomly to three groups (eight cats each) based on body weight: Controls (untreated); Broadline® when *T. brevior* were developing larvae (six days post inoculation, dpi); and Broadline® when *T. brevior* were adults (28 dpi). Treatments were administered once topically at the minimum label dose of 0.12 mL/kg. Faeces were examined for lungworm larvae prior to treatment of adult *T. brevior* (28 dpi) and until necropsy of the cats for *T. brevior* recovery and count (48 dpi).

No health problems were observed throughout the study. Prior to 28 dpi, Controls and cats to be treated when *T. brevior* had reached the adult stage were demonstrated shedding *Troglostrongylus* larvae (range, 26.3-480 larvae per gram of faeces) while no larvae were recovered from the faeces of the cats which were treated at 6 dpi. Based on comparison of nematode counts in the treated cats and untreated Controls, Broadline® treatment was 100% efficacious against both developing larval and adult *T. brevior* lungworms ($p < 0.001$); no lungworms were recovered from any Broadline®-treated cat while Controls harboured from six to 52 *T. brevior*.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C257/0071

Vectorial ticks of cattle in Northeastern Nigeria: Breed and coat colour preferences

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Prevalence of Ixodid ticks of cattle in Borno and Yobe states, Northeastern Nigeria was studied from March to October, 2014. Of 3,150 cattle examined, 1,095 (34.8%) were infested with ticks. 57.1% of the infestation was among cattle in Borno, while 42.9% was observed in Yobe. Out of the total number of cattle examined, 2,175 (69.0) were males and 33.8% of them were infested with ticks. The number of females examined was 975 (31.0), out of which 36.9% had ticks. There was no significant difference ($p > 0.05$) in tick infestation between the male and female cattle. Ticks observed in both states were *Amblyomma variegatum* (43.8%), *Hyalomma* sp (24.7%), *Boophilus* sp (21.9%) and *Dermacentor* sp (9.6%). Overall, tick infestation occurred most on the White Fulani breed with a prevalence of 240 (21.9%), followed by N' dama and Red Bororo in which the prevalence of ticks was 180 (16.4%) for each of the breeds. The least (6.8%) infestation of ticks was among the Keteku breed. The preferred sites of attachment for ticks among cattle examined in Borno state were inner thigh, dew lap, abdomen, legs, udder, dorsum, ear and hump, in this order. Cattle examined in Yobe state were infested on their dorsum, inner thigh, legs, abdomen, udder and then on the dew lap, ear and hump. The ticks observed infesting cattle in these states are of veterinary importance, because they are vectors of economically important blood protozoan parasites. Adequate control measures should therefore be adopted to protect these animals.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C258/0078

Efficacy of oral afoxolaner plus milbemycin oxime chewable (NexGard Spectra®, Merial) to prevent heartworm disease in dogs after inoculation with third stage larvae of *Dirofilaria immitis*

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The novel combination of afoxolaner plus milbemycin oxime, NexGard Spectra®, was evaluated against the migrating *Dirofilaria immitis* larvae in beagle dogs in two negative control, blinded laboratory studies.

In each study, dogs were randomly allocated to an untreated control and a treated group of 10 dogs each. In each study, dogs were inoculated with third stage *D. immitis* larvae by subcutaneous injection (50 larvae in the first, 100 larvae in the second study). The larvae had grown into L3 stage in mosquitoes fed on blood obtained in the field from naturally infected dogs in Italy (Lombardy for the first, Sardinia for the second study). Thirty-one days after inoculation, each dog in the treated group was orally treated with NexGard Spectra® dosed as close as possible to the minimum effective dose (2.5 mg/kg Afoxolaner + 0.5 mg/kg milbemycin oxime). The control group remained untreated. All dogs were humanely euthanized and examined for adult *D. immitis* 146 or 147 days after inoculation. In the first study, seven of the ten untreated dogs were infected, harboring 1 to 5 adult *D. immitis*. In the second study, the ten untreated dogs were positive and harbored 22 to 46 adult *D. immitis*. In both studies all treated dogs remained free of heartworm infection as no *D. immitis* was recovered, demonstrating that the novel combination of afoxolaner plus milbemycin oxime was 100% effective in preventing heartworm disease in dogs.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C259/0084

Efficacy of afoxolaner chewables (NexGard[®], Merial) and afoxolaner plus milbemycin oxime chewables (NexGard[®] Spectra, Merial) against the Australian paralysis tick, *Ixodes holocyclus*, in dogs

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Ixodes holocyclus causes potentially fatal flaccid paralysis in dogs beginning 3 to 5 days after attachment. Accordingly, efficacy at 72 hours is the benchmark for regulatory assessment of acaricides against this parasite.

Two studies evaluated the efficacy of afoxolaner, NexGard[®] chewables; and the combination of afoxolaner plus milbemycin oxime, NexGard[®] Spectra chewables, against *I. holocyclus* in dogs.

In each study, 24 foxhounds were assigned to blocks of three animals based on pre-allocation tick counts, then randomly allocated to one of three groups: untreated (control); treated with NexGard[®]; or treated with NexGard[®] Spectra. Treatments were administered once on Day 0 either at the minimum dose (~2.5 mg/kg afoxolaner; Study 1) or according to commercial dosing recommendations (≥2.5 mg/kg afoxolaner; Study 2). Dogs were infested with 50 ticks weekly for five weeks commencing Day -1. Ticks were counted 24, 48 and 72 hours post-treatment or infestation.

In both studies, efficacy of both NexGard[®] and NexGard[®] Spectra was ≥94.7%, ≥99.2%, and 100% at 24, 48, and 72 hours after treatment, respectively. Efficacy of NexGard[®] in the two studies following weekly infestations ranged from 79.3% - 98.3% at 24, 98.7% - 100% at 48, and 99.2% - 100% at 72 hours to Day 38 (p≤0.0025). For NexGard[®] Spectra, corresponding efficacy was 87.5% - 98.0% at 24, 97.7% - 100% at 48, and 98.6% - 100% at 72 hours (p≤0.0019).

These studies demonstrated excellent therapeutic efficacy of both NexGard[®] and NexGard[®] Spectra against *I. holocyclus* in dogs and persistent activity for at least five weeks.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C260/0090

Efficacy of afoxolaner plus milbemycin oxime chewables (NexGard Spectra®, Merial) against intestinal nematode infections in dogs

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The efficacy of the novel combination of afoxolaner (AFX) plus milbemycin oxime (MO), NexGard Spectra® chewables, was tested against induced and naturally acquired intestinal nematode infections in 12 controlled, blinded studies (three studies each against *Toxocara canis* and *Toxascaris leonina*; and two studies each against *Ancylostoma braziliense*, *A. caninum* and *Trichuris vulpis*). All studies were conducted in compliance with GCP and relevant VICH guidelines to assess the efficacy of anthelmintics in dogs.

Dogs positive for the target parasite by pre-treatment fecal examination were included in the studies, assigned to blocks based on pre-treatment body weight and randomly allocated to one of two groups of 8 to 10 dogs each: Control (untreated); or treated once orally with NexGard Spectra® at a dose as close as possible to the minimum effective dose of AFX+MO (2.5 mg/kg + 0.5 mg/kg). Seven or eight days post treatment, dogs were necropsied for parasite recovery and count. Efficacy was evaluated based on geometric mean worm counts.

All dogs accepted the treatment well and no related adverse reactions were observed. All studies met the recommendations for adequacy-of-infection as per VICH. Efficacy of NexGard Spectra® was 97.8%, 98.0% and 100.0% against *T. canis*; 95.8%, 96.0% and 99.4% against *T. leonina*; 90.2% and 94.8% against *A. braziliense*; 90.9% and 99.7% against *A. caninum*; and 98.3% and 100% against *T. vulpis* (all $p < 0.01$).

NexGard Spectra® was shown to be safe and demonstrated a high level of effectiveness when administered to dogs infected with a range of common intestinal nematodes.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C261/0102

The effect of the diet and corticosteroid induced immune-suppression on the infection by *Haemonchus contortus* in lambs

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In order to test the hypothesis that changes in pH and concentrations of volatile short-chain fatty acids (SFA) in the rumen could impair larvae survival reducing worm establishment in the abomasum, one group of lambs was fed only with a grass diet, while another was supplemented with concentrate (amount corresponding to 3% of body weight). To minimize the influence of the acquired immune response in worm establishment, groups of lambs were immune-suppressed (IS) with prednisolone. The lambs were allocated to four groups of seven and treated as follows: Group 1 Basal Diet – Normal; Group 2 Basal Diet – IS; Group 3 Supplemented Diet – Normal and Group 4 Supplemented Diet – IS. Both diets offered to lambs proved to create distinct rumen environments with differences in pH and in the SFA concentrations. Such changes, however, did not have any apparent effect on larvae exsheathment and survival inside the rumen, with all groups presenting similar worm burdens after a single infection with 4,000 *Haemonchus contortus* larvae ($P>0.05$). Groups Supplemented presented stunted worms and females with fewer eggs inside uterus than animals fed with the Basal Diet ($P<0.05$). The immune-suppression caused a significant decrease in sera IgG anti-*H. contortus* and in the inflammatory cell numbers in the abomasal mucosa ($P<0.05$). In conclusion, the changes caused in the rumen contents by supplementation did not impair *H. contortus* establishment, however, the high nutritional plan enhanced the resistance indicating that some components related to innate immunity and mucosal maintenance were benefited by the improved nutrition.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C262/0116

Practices by veterinarians before and after anthelmintics became prescription only medicines for horses in the Netherlands

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Anthelmintics for horses became Prescription Only Medicines (POM) in the Netherlands in July 2008. This new regulation intended to strengthen the advisory role of veterinarians concerning parasite control. In support, several activities were undertaken by experts, notably providing guidelines for Good Veterinary Practice (GVP) with respect to the prudent use of anthelmintics. A questionnaire was used to evaluate the effect of the new legislation and GVP guidelines on the prescription of anthelmintics and the use of faecal diagnosis between 2008 and 2012. The response rate was 14% in 2008 (180 veterinarians) and 11% in 2012 (114 veterinarians). Results from 2012 showed that 95% of veterinary practices examined faecal samples against 77% in 2008 ($P<0.001$), 32% of the practices take faecal samples for monitoring purposes against 10% in 2008 ($P<0.001$), almost double the number of practices quantitatively examined faecal samples ($P<0.001$), and 78% examined pooled faecal samples versus 44% in 2008 ($P<0.001$). The number of veterinarians routinely examining a faecal sample when considering treatment increased by more than three-fold from 2008 to 2012 ($P<0.001$), whereas the percentage of veterinarians never examining a faecal sample before treatment declined from 28% in 2008 to 10% in 2012 ($P<0.001$). However, it was also clear that there is still room for further improvements. Specifically, continuing efforts need to be focused on increasing the number of quantitatively processed faecal samples and the interpretation of faecal diagnosis results.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C263/0117

Use of curcumin (*Curcuma longa*) as an alternative to control lamb coccidiosis

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Ovine coccidiosis is a disease that has great health and economic impact. It has been reported that it can cause a mortality of up to 20% in young animals. Currently, only three drugs are licensed to be used for the control of ovine coccidiosis: Decoquinate, Diclazuril and Toltrazuril. The latter should not be used in dairy or meat-producing animals due to its residual activity in tissues. It is therefore important to find alternatives for the treatment of coccidiosis. We propose the use of curcumin (*Curcuma longa*).

Twenty crossbred lambs aged 28-days-old, with an average weight of 12 kg were included in the study. Five groups (n=4) were formed as follows: the a control group (G1) placebo-treated (G2), one received 50 mg/kg (G3), 100 mg/kg (G4) and 200 mg/kg (G5) of the turmeric powder. Stool samples were obtained every other day and weighed on day 0 and 42. Statistically significant differences ($p < 0.05$) were observed between G4 and the untreated and placebo groups as from day seven. From day 21 until the end of the experiment, differences were observed between G1 and G2 with respect to the treated ones. Curcumin anticoccidial activity increased over time and reached a 100% efficacy at day 42. These results show that the administration of curcumin may reduce oocyst output of *Eimeria* spp results in lambs.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C264/0125

Assessment of the anti-*Eimeria* efficacy of curcumin in rabbits

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Coccidiosis in domestic rabbits (*Oryctolagus cuniculus*) is a major cause of morbidity and mortality mainly in young animals. Currently, sulphonamides, ionophores, quinolones, and other chemicals are used for treating coccidiosis. These drugs can cause selection pressure for anticoccidial resistance and are also found in edible tissues. Hence it is important to find alternatives for the treatment of coccidiosis, one of which is the use of curcumin (*Curcuma longa*).

Twenty-four New Zealand eight-month-old white rabbits with an average weight of 3 kg were orally given an aqueous extract of *Curcuma longa* (aeCl). 4 groups of 6 rabbits each were formed: The control group remained untreated, whereas groups 2, 3 and 4 received 10, 25 and 40 mg/kg of aeCl, respectively. During the experiment fecal samples were collected and weight gain was recorded every seven days.

Twenty-eight days after the beginning of the study, statistically significant differences ($p < 0.05$) in the log average number of oocysts per gram of feces in rabbits were observed between the control group and the groups receiving 25 and 40 mg/kg of aeCl, and between these two groups. At day 35, a statistically significant difference ($p < 0.05$) was observed between the control group as compared to the groups that received 25 and 40 mg/kg of aeCl. At day 42, the three treated groups differed significantly from the control group. It was therefore concluded that the aeCl is a good alternative to reduce the parasite load in rabbits infected with *Eimeria* spp at a dose of 40 mg/kg.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C265/0155

Effects of polyphenol removal methods on the *in vitro* anthelmintic activity of *Lysiloma latisiliquum* against *Haemonchus contortus*

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Tannin-rich forages have been recently investigated as a valuable alternative for the control of gastrointestinal nematodes in small ruminants. It has been suggested that the anthelmintic activity (AH) exhibited by these materials is related to their tannin content, particularly that of condensed tannins (CT). However, reports on the use of tropical fodders such as *Lysiloma latisiliquum* (Leguminosae), described some level of AH activity in extracts of such plant after the addition of tannin-blocking agents, e.g. PVPP or PEG. The latter raised the question whether at least part of the AH activity depends on the method of tannin blocking/removal or the detected AH is actually due to a different type of secondary metabolites. The aim of this study was to both compare the efficacy of different polyphenol removal methods and to evaluate their influence on the AH activity of *L. latisiliquum* extracts against *H. contortus*. The results showed that oven-drying of *L. latisiliquum* leaves, which seems to prevent CT extraction, yielded a crude extract with a higher AH activity than other acetone:water (70:30) extracts obtained from fresh or lyophilized leaves of such plant. These results suggests that CT are not essential for the AH activity. Treatment of the oven-dried leaves extract with polyamide C6, Sephadex, NaCl, and PVPP produced polyphenol-free extracts which were less active against *H. contortus*, indicating that some polyphenolic metabolites, different to tannins, are responsible for the expression of AH activity. HPLC analysis of the various extracts allowed the preliminary identification of phenolic metabolites such as gallic acid and quercetin that could be related to the biological activity against *H. contortus*.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C266/0183

Tannins Quantification and *In Vitro* Anthelmintic Efficacy of *Sesbania grandiflora* Leaves

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A range of tanniniferous plants have been studied before for their anthelmintic potential to control worm infection in ruminants worldwide. In this study, Folin-Ciocalteu method was conducted for measuring phenolic and tannins compound from *Sesbania grandiflora* leaves before butanol-HCL method was used to determine the condensed tannins. In 95% dry matter (DM) of this experimental plant, there is 4% of condensed tannins detected. Total aqueous extract then was used to evaluate the *in vitro* anthelmintic efficacy. Selected *in vitro* bioassays were tested on eggs and infective larvae (L3) as targeted stages. For Egg Hatch Assay (EHA), mixed parasites' eggs were used. While for the larvae stage, it was conducted based on Adult Motility Assay (LMA) and larvae motility was observed at the end of the trial to relate with the survivability concept. Three nematode species were chose namely *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Strongyloides papillosus*. For EHA, six increasing 2-fold concentrations ranged between 0.1 to 3.2 mg/ml showed significant effect on the egg hatching rates ($P < 0.01$) and the inhibition of egg hatching was determined as dose-dependent ($P < 0.05$). For larvae assay, four highest concentrations from EHA were used and it showed a dose and time-dependent ($P < 0.05$) with survivability rates were more than 50%. From the results, it can be suggested that *S. grandiflora* leaves could be a potential alternative to be used as an anthelmintic. However, further trials should be conducted through *in vivo* studies to strengthen the results.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C267/0197

In vitro, Efficacy of Herbal Extracts against *Eimeria tenella*

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The effect of organically grown plants, i.e., *Curcuma longa* (leaves), *Artemisia absinthium* (seeds) and *Saussurea lappa* (roots), was evaluated on the sporulation of *Eimeria tenella* under laboratory conditions. The oocysts used in this study were obtained by infecting 14-day-old broiler chicks with 75,000 sporulated oocysts of *E. tenella*. The harvested oocysts were exposed to various doses of 70% aqueous-methanolic herbal extracts (0.244 $\mu\text{g mL}^{-1}$ - 500 $\mu\text{g mL}^{-1}$). The oocysts were held at 27.5°C with 60-80% humidity and continuous aeration. The yield (w/w) of *C. longa*, *A. absinthium* and *S. lappa*, was 46.2, 45.9 and 47.5%, respectively. Inhibition of sporulation was the criterion for evaluation of efficacy. The herbal extracts exhibited graded dose responses on sporulation of *E. tenella* as evidenced by their lethal concentration (LC₅₀) values. LC₅₀ values of *C. longa*, *A. absinthium* and *S. lappa* were 173.4, 221.3 and 960.6 $\mu\text{g mL}^{-1}$, respectively. *C. longa* demonstrated the highest inhibitory effects on *E. tenella* oocyst sporulation.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C268/0206

Extracts of *Caesalpinifolia echinata*, *Leucaena leucocephala* cv. Cunningham, *Cratylia mollis*, *Gliricidia sepium* and *Calopogonium velutinum* on larval exsheathment of *Haemonchus contortus* in vitro

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A sustainable alternative to combat the gastrointestinal nematodes in sheep are tanniniferous plants, because of the use of anthelmintics on the market is exacerbated, nematodes developed resistance. The aim of this work was to evaluate the tanniniferous plants extracts on larval exsheathment assay (LEA) of *Haemonchus contortus*. The work was conducted at the Institute of Animal Science (IZ/APTA/SAA) Nova Odessa/São Paulo/Brazil. The leguminosae leaves of *Caesalpinifolia echinata*, *Leucaena leucocephala* cv. Cunningham, *Cratylia mollis*, *Gliricidia sepium* and *Calopogonium velutinum* were collected at agrostological field of the IZ/APTA/SAA, dried at 35°C and ground. Their actives ingredients were extracted by a solution of acetone:water (70:30), followed by dichloromethane and freeze-dried. The plants values found for total phenols (TP – grams of tannic acid/kg DM), total tannins (TT – grams of tannic acid/kg DM) and condensed tannins (CT – grams of leucocyanidin/kg DM) to *C. echinata* was 136.2, 95.9 and 59.7, respectively, to *L. leucocephala* cv. Cunningham was 89.9, 74.9 and 55.6, *C. mollis* (67.9, 47 and 95.7), *G. sepium* (27.3, 17.7 and 2) and *C. velutinum* (32.8, 23 and 0.53). The lyophilized extract was soluble in water at concentrations ranging from 25mg/mL to 0.19mg/mL to perform LEA. Data were analyzed by SAS/Probit to determine Lethal Concentration (LC₅₀) with independent variables (dose) transformed by natural logarithms (log-dose). The lowest LC₅₀ was found for *C. echinata* of 1.25mg/mL, followed by *L. leucocephala* cv. Cunningham of 1.58mg/mL, *C. mollis* of 1.94mg/mL, *G. sepium* of 3.03mg/mL and *C. velutinum* of 3.61mg/mL. Among the tested plants *C. echinata* shown to be more effective on larval exsheathment of *H. contortus*.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C269/0207

Larval exsheathment of *Haemonchus contortus* with extracts of *Arachis pintoi*, *Mimosa caesalpinifolia*, *Leucaena pulverulenta*, *Cajanus cajan* and *Neonotonia wightii* cv. *Cianova in vitro*

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Parasites are resistant to the main existing anthelmintic drugs on the market, so tanniniferous plants is a sustainable alternative against gastrointestinal nematodes. The aim of this work was to evaluate the tanniniferous plants extracts on larval exsheathment assay (LEA) of *Haemonchus contortus*. The work was conducted at the Institute of Animal Science (IZ/APTA/SAA) Nova Odessa/São Paulo/Brazil. The leguminosae leaves of *Arachis pintoi*, *Mimosa caesalpinifolia*, *Leucaena pulverulenta*, *Cajanus cajan* and *Neonotonia wightii* cv. *Cianova* were collected at agrostological field of the IZ/APTA/SAA, dried at 35°C and ground. Their active ingredients were extracted by a solution of acetone:water (70:30), followed by dichloromethane and freeze-dried. The plants values found for total phenols (TP – grams of tannic acid/kg DM), total tannins (TT – grams of tannic acid/kg DM) and condensed tannins (CT – grams of leucocyanidin/kg DM) to *A. pintoi* was 48.7, 32.8 and 59.4, respectively, to *M. caesalpinifolia* was 159.5, 108.7 and 100.8, *L. pulverulenta* (90.6, 75.2 and 69.6), *C. cajan* (135.6, 91.6 and 241.1) and *N. wightii* cv. *Cianova* (18.2, 11.1 and 0.30). The lyophilized extract was soluble in water at concentrations ranging from 25mg/mL to 0.19mg/mL to perform LEA. Data were analyzed by SAS/Probit to determine Lethal Concentration (LC₅₀) with independent variables (dose) transformed by natural logarithms (log-dose). The lowest LC₅₀ was found for *A. pintoi* of 1.04mg/mL, followed by *M. caesalpinifolia* of 2.11mg/mL, *L. pulverulenta* of 3.40mg/mL, *C. cajan* of 4.79mg/mL and *N. wightii* cv. *Cianova* of 9.60mg/mL. *A. pintoi* proved to be the most effective on larval exsheathment of *H. contortus*.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C270/0209

Use of tanniniferous plants extracts on larval exsheathment of *Haemonchus contortus*

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Anthelmintic drugs demonstrate resistance to major parasites. Studies with tanniniferous plants have been demonstrated their efficacy on gastrointestinal nematodes. The aim of this work was to evaluate the tanniniferous plants extracts on larval exsheathment assay (LEA) of *Haemonchus contortus*. The work was conducted at the Institute of Animal Science (IZ/APTA/SAA) Nova Odessa/São Paulo/Brazil. The leguminosae leaves of *Stylosanthes guianensis*, *Dipteryx alata*, *Peptadenia colubrina*, *Neonotonia wightii* cv. Tinaroo and *Canavalia ensiformis* were collected at agrostological field of the IZ/APTA/SAA, dried at 35°C and ground. Their actives ingredients were extracted by a solution of acetone:water (70:30), followed by dichloromethane and freeze-dried. The plants values found for total phenols (TP – grams of tannic acid/kg DM), total tannins (TT – grams of tannic acid/kg DM) and condensed tannins (CT – grams of leucocyanidin/kg DM) to *S. guianensis* was 34, 24.6 and 10, respectively, to *D. alata* was 147, 109 and 199.7, *P. colubrina* (143, 127.6 and 8.9), *N. wightii* cv. Tinaroo (18, 11 and 0.99) and *C. ensiformis* (20.2, 12.7 and 1). The lyophilized extract was soluble in water at concentrations ranging from 25mg/mL to 0.19mg/mL to perform LEA. Data were analyzed by SAS/Probit to determine Lethal Concentration (LC₅₀) with independent variables (dose) transformed by natural logarithms (log-dose). The lowest LC₅₀ was found for *S. guianensis* of 0.51mg/mL, followed by *D. alata* of 1,42mg/mL, *P. colubrina* of 2,11mg/mL, *N. wightii* cv. Tinaroo of 2,41mg/mL and *C. ensiformis* of 9,50mg/mL. Among the tested plants *S. guianensis* shown to be more effective on larval exsheathment of *H. contortus*, however the total phenolics, total tannins and condensed tannins were not in the highest concentration among the plants evaluated.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C271/0245

Prevention of heartworm disease in dogs with afoxolaner plus milbemycin oxime chewables (NexGard Spectra[®], Merial) under field conditions in Japan

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The novel combination of afoxolaner plus milbemycin oxime, NexGard Spectra[®] was evaluated for safety and prevention of canine heartworm disease in a GCP compliant, multi-centre, positive control, double-blinded field study in Japan.

Total 84 client-owned dogs, negative for heartworm infection based on antigen and microfilariae tests prior to first treatment and with no history of treatment with an oral heartworm preventive within four weeks or with an injectable heartworm preventive within six months were enrolled through 16 clinics located in Kinki, Chugoku, Shikoku and Kyushu regions, known endemic areas for *Dirofilaria immitis* infection. Dogs were allocated randomly to Treatments in a 1 ÷ 1 ratio: NexGard Spectra[®] or Moxidect[®] tablets (positive control product, PCP), administered per manufacturers' instruction at monthly intervals during the period of risk of heartworm infection (six to nine treatments per dog). Heartworm antigen and microfilariae tests were performed at the visit prior to first treatment (= Day 0), at Day 120, at the day of the last treatment and six months thereafter.

All dogs in both groups tested negative for heartworm antigen and for microfilariae at the day of the last treatment and six months after the last treatment (100% efficacy). Six and seven dogs treated with NexGard Spectra[®] or PCP, respectively, experienced one or more adverse event(s) during the study but none was considered to be related to the treatment.

In conclusion, the efficacy of NexGard Spectra[®] for prevention of heartworm disease and systemic safety for monthly treatments were excellent under field conditions in Japan.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C272/0246

Field efficacy against nematode infections and safety of afoxolaner plus milbemycin oxime chewables (NexGard Spectra[®], Merial) in domestic dogs in Japan

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The novel combination of afoxolaner plus milbemycin oxime, NexGard Spectra[®] chewables, was evaluated for safety and efficacy against naturally acquired nematode infections in dogs in a multi-centre, positive control, blinded field study.

Total 118 dogs, confirmed positive for intestinal nematodes, were enrolled through 8 veterinary clinics located in Kinki, Chugoku and Kyushu regions of Japan. Pre-treatment fecal examination revealed *Toxocara*, *Toxascaris*, hookworm and/or *Trichuris* infections in 27 (100-10,200 eggs per gram, EPG), 5 (200-1400 EPG), 60 (100-12,500 EPG) or 76 (100-26,800 EPG) dogs, respectively. Dogs were allocated randomly to Treatments in a 2 ÷ 1 ratio: NexGard Spectra[®] or Drontal[®] Plus (Bayer; positive control product), and treated according to the manufacturers' instruction. For evaluation of efficacy, fecal samples collected prior to and once 17±5 days after treatment were examined using a modified McMaster technique.

No treatment related adverse experiences were observed throughout the study. For dogs treated with NexGard Spectra[®], the efficacy was 100%, 100%, >99.9% and 99.9% for *Toxocara*, *Toxascaris*, hookworm and *Trichuris*, respectively; the efficacy was 99.5%, 100%, 100% and 99.6%, respectively, for the positive control product. For *Toxocara*, hookworm and *Trichuris*, non-inferiority analysis demonstrated that the efficacy of NexGard Spectra[®] was equal to or better than that of the positive control product. No non-inferiority analysis was performed for *Toxascaris* because of the insufficient number of infected dogs.

NexGard Spectra[®] was shown to be safe and demonstrated a high level of effectiveness when administered once to dogs infected with a broad range of parasitic nematodes under field conditions.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C273/0247

Efficacy of oral afoxolaner plus milbemycin oxime chewables (NexGard Spectra[®], Merial) against induced infestations with *Haemaphysalis longicornis* ticks in dogs

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Primarily a parasite of cattle, *Haemaphysalis longicornis* Neumann, 1901 readily infests a range of hosts in its area of geographic distribution including domestic dogs. In Japan and the Republic of Korea, it is one of the ticks found frequently associated with dogs.

The novel combination of afoxolaner plus milbemycin oxime (AFX+MO), NexGard Spectra[®] was evaluated against induced infestations of *H. longicornis* in dogs in a GCP compliant, negative control, blinded laboratory study.

Sixteen Beagle dogs were assigned to blocks of two animals based on pre-allocation tick counts and randomly allocated to two groups: untreated (control); or NexGard Spectra[®] at a dose as close as possible to the minimum recommended dose of AFX+MO (2.5 mg + 0.5 mg per kg). Treatment was administered orally once. Starting two days before treatment, each dog was infested with 50±5 female ticks (parthenogenetic population) with challenge continued weekly for six consecutive weeks. Total live ticks were counted at ~48 hours post-treatment (removal count), and at ~48 hours (in-situ counts) and ~72 hours (removal counts) following each post-treatment infestation.

Geometric mean tick counts in the control dogs ranged from 18.6 to 31.1 for the removal counts established following treatment and post-treatment infestations. Treatment with NexGard Spectra[®] rapidly eliminated the existing tick infestations (100% efficacy), and weekly re-infestations were controlled for minimum five weeks with 100% preventive efficacy based on both 48 hours counts and 72 hours counts (p<0.0001 at each occasion).

This study demonstrated an excellent acceptability and a high level efficacy of NexGard Spectra[®] against infestations with *H. longicornis* ticks for at least five weeks.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C274/0248

Spatial and temporal effect on acaricide activity of *petiveria alliacea* against *rhhipicephalus microplus* larvae resistant to acaricide

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To determine the spatial and temporal effects of *Petiveria alliacea* on *Rhipicephalus microplus* resistant tick, methanol extracts of leaves and stems collected in dry and rainy season from three municipalities of Yucatan, Mexico (Yaxcaba in the center, Hunucma in the west, and Colonia Yucatan in the northeast) were used. *R. microplus* adult ticks multiresistant to acaricides were also collected for progeny and bioassays. For testing the acaricidal activity of methanol extracts from *P. alliacea* the larval immersion technique (20%, 10%, 5% and 2.5% concentration) was used. Methanolic extracts of leaves and stems collected in dry season in the municipalities of Yaxcaba, Hunucma and Colonia Yucatan showed larvicidal activity with lethal concentration at 50% (LC₅₀) of 4.4% and 1.7%, 8.2% and 2.2%, 18.3% and 22.4%, respectively. Leaves and stems extracts from rainy season showed LC₅₀ of 7.2% and 6.6%, 15.8% and 7.5%, 10.6% and 38.7%, respectively. Results of LC₉₉ from leaves and stems collected in dry season in Yaxcaba, Hunucma and Colonia Yucatan showed 42.8% and 19.1, >100% and 21.8, >100% and >100%, respectively. Leaves and stems extracts from rainy season showed LC₉₉ of 44.4% and 61.6%; >100% and 40.0%; 92.6% and >100%, respectively. According to the obtained results it is possible to establish effect in the acaricidal activity due to season of year of *P. alliacea* collection. The plant material collected during dry season was better at controlling larvae for LC₅₀. Also was observed that the stem methanolic extract of *P. alliacea* performs better to control *R. microplus*. Complementary studies to determine the variability in active compounds concentrations are need. We are very grateful to CONACYT for supporting this project (CB-2012-01-178216).

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C275/0264

Evaluation of the adulticidal efficacy of imidacloprid 10%/moxidectin 2.5% spot-on (Advocate[®], Advantage[®] Multi, Bayer) against *Dirofilaria repens* in experimentally infected dogs

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This study aimed to evaluate the efficacy of imidacloprid 10%/moxidectin 2.5% spot-on (Advocate[®]/Advantage[®] Multi, Bayer) against adult *Dirofilaria (D.) repens* in a blinded, placebo controlled randomised laboratory study. 24 Beagle dogs were experimentally infected with approximately 75 infective *D. repens* larvae on study day (SD) 0. A modified Knott test was used in monthly intervals to monitor the onset of patency and to follow the course of microfilariemia after treatment. Treatment was initiated on SD 228 after patency had been confirmed in 21 dogs. 11 dogs received monthly treatments with imidacloprid/moxidectin at the minimum therapeutic dose for six consecutive months and 12 control dogs were treated with a placebo formulation. Approximately one month after the last treatment, all dogs were euthanized and necropsied for the detection of *D. repens* worms. 11 control dogs harbored live adult *D. repens* (range 2-11, geometric mean 5.44). 8 of 11 imidacloprid/moxidectin treated dogs were free of live worms. The live worm count was reduced by 96.2% (range 0-1, geometric mean 0.21). The majority of dead worms was encapsulated and degenerated. After the first treatment, Knott Tests were negative in all imidacloprid/moxidectin treated dogs and this status was maintained in 10 dogs until study end. One dog showed a low microfilariae count (1 and 4/ml) on four occasions but was also negative before necropsy. The treatment was well tolerated in all study animals. It is concluded that six consecutive monthly treatments with imidacloprid/moxidectin topical solution are effective and safe against adult *D. repens* and provide an option for the prevention of further spreading of this zoonotic parasite.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C276/0286

Strategic control of worms from beef cattle herd in Brazil: effects on parasite burden

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This study aimed to evaluate the effect of different schemes of anthelmintic treatment on parasite burden in beef cattle in Brazil. Two experimental cycles were performed from May 2013 to December 2014 and from May 2014 to December 2015, with 96 Nelore pre-weaned calves in each cycle. The animals were divided, based on the number of eggs per gram of faeces and live weight, in six experimental groups (n=16), with area and time repetition: T1 (control group); T2 (May – doramectin/ November – doramectin; T3 (May – doramectin/ July – levamisole/ September – doramectin); T4 (May – doramectin/ July – moxidectin/ September – doramectin); T5 (May – doramectin/ August – levamisole/ November – doramectin) and T6 (May – doramectin/ August – moxidectin/ November – doramectin). The dosages used were 700, 200 µg/Kg and 4,7 mg/Kg of doramectin, moxidectin and levamisole phosphate, respectively. Faecal samples were collected each 28 days. *Haemonchus*, *Cooperia*, *Oesophagostomum* and *Trichostrongylus* genus were predominant. The results were similar in each experimental cycle: the treatments with doramectin did not reduced ($P>0.05$) the Nematode eggs per gram (EPG) of faeces, when compared to the untreated group; none of the treatment strategies caused significant reduction on EPG burden until July ($P>0.05$). The EPG reduction was most evident ($P<0.05$) in T3, T5, T5 and T6 groups, respectively, on August, September, October and November. However, independently of the treatment, no significant differences ($P>0.05$) were observed between the groups from December up to the end of this study.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C277/0290

Effect of strategic gastrointestinal nematode control in cattle on *Trichostrongyle* larval populations on herbage in Brazil

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This study aimed to evaluate the effect of different anthelmintic treatment strategies on L3 population on the pasture in Brazil. Two experimental cycles were performed from May 2013 to December 2014 and from May 2014 to December 2015, with 96 Nelore pre-weaned calves in each cycle. The animals were divided in six experimental groups (n=16), according to EPG counts and live weight, and allocated in 12 paddocks (area repetition) composed by *Brachiaria brizantha*. All anthelmintics were administered subcutaneously in a single dose of 700µg/kg of doramectin (DRM), 200µg/kg of moxidectina (MOX) or 4,7mg/kg of levamisole phosphate (LEV) according to the following scheme: T1 (control group); T2 (May – DRM/ November – DRM); T3 (May – doramectin/ July – LEV/ September – DRM); T4 (May – doramectin/ July – MOX/ September – DRM); T5 (May – DRM/ August – LEV/ November – DRM) and T6 (May – DRM/ August – MOX/ November – DRM). Grass samples were taken every 28 days, in eight different points in each paddock, using a square of 0,5 m² to recover and quantifying the amount of L3 on the grass. L3 counts from pasture were logarithm transformed and its interactions with each treatment were evaluated using ANOVA, followed by a Duncan's multiple comparison procedure as a post hoc analysis. The highest amount of L3 on the paddocks where the untreated group was allocated was observed in July (116.03 L3/m²), during the dry period, and the lowest in September (11.31L3/m²). None of the strategic treatment schemes caused a significant (P>0.05) reduction in the amount of infective larvae on the pasture.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C278/0322

Evaluation of three copromicroscopic techniques for measuring digestive nematode egg output in adult cattle

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Infection with gastrointestinal nematodes (GIN) in adult grazing dairy cattle is highly prevalent and occurs almost exclusively at a subclinical level. Several studies indicate a possible impact of GIN on milk production for some herds or some cows within these herds. The routine tools for evaluating levels of infection with GIN in adult cattle are limited and their interpretation difficult. Concerning copromicroscopic examination, the level of nematode egg output is often very low and classical techniques fail to identify cows with high parasitic burden which could benefit from treatment. Highly sensitive techniques could be more efficient and reliable for that purpose. Thus, the objective of our study was to compare three copromicroscopic techniques for GIN in adult dairy cattle: the coproculture (larvae/g of faeces, LPG), the Cornell-Wisconsin technique and the FLOTAC technique (eggs/g of faeces, EPG). The comparison of the 3 techniques included the sensitivity, the "efficacy" i.e. the recovery rate, the repeatability and also practical considerations. Faecal sampling was performed in spring in 5 organic dairy cattle farms located in western France. All adult cows (n=238) were sampled once. The coproculture was made from a 50 g faecal sample mixed with vermiculite according to Gevrey et al. (1971). The Cornell-Wisconsin technique was described by Egwang and Slocombe (1982) and used a double centrifugation and sucrose as flotation liquid. The FLOTAC technique was described by Cringoli et al. (2010) and used a specific floating/reading device, a double centrifugation and, in our study, magnesium sulphate as flotation liquid. Mean intensities of nematode larval/egg output were 3.6 ± 6.2 LPG, 7.6 ± 14.8 EPG and 8.5 ± 14 EPG for coproculture, Cornell-Wisconsin and FLOTAC techniques, respectively. Decreasing sensitivities (number of positive results/number of examinations) were seen with coproculture (99%), Cornell-Wisconsin (93%) and FLOTAC (79%). The highest recovery rates (highest values in EPG/LPG) were observed with FLOTAC, followed by Cornell-Wisconsin and coproculture. Within a range of 10 to 14 EPG, the Cornell-Wisconsin technique was more repeatable than the FLOTAC technique. Regarding practical aspects, Cornell-Wisconsin and FLOTAC techniques showed specific advantages or drawbacks that have to be kept in mind before choosing a high sensitive technique for low-excreting animals as adult cattle.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C279/0360

Ewe worm control has greater effect on weaning weights of meat-breed lambs than lamb worm control

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This experiment determined the effect of ewe and lamb worm control on the live weight of meat-breed lambs up to weaning. The experiment was conducted with two mobs of 120 twin-bearing Border Leicester x Merino ewes on each of 3 farms over a period of 3 years in northern NSW, Australia. From within each mob, half were randomly allocated to be worm-suppressed (SUP) and remaining ewes were managed using regional WormBoss best practice (NSUP) strategies. SUP ewes were serially treated with persistent anthelmintics and an effective short-acting drench at 80–90 day intervals. NSUP strategy had low worm-risk lambing paddocks, strategic anthelmintic treatment of ewes at 2 weeks pre-lambing and tactical treatment at other times based on worm egg count (WEC). Five weeks post lambing, lambs (sired by Dorset rams) were identified to ewe group and then randomly allocated from within each ewe worm control treatment to receive SUP or NSUP strategy. SUP lambs were administered persistent and short-acting anthelmintics at 5 and 9 weeks of age, while NSUP lambs remained untreated. Both SUP and NSUP ewes and their lambs grazed together. The period from 2 weeks pre-lambing to weaning (at 18 weeks post lambing) is reported.

Mean pre-lambing WEC of SUP and NSUP ewes was 0 epg and 174 epg respectively. At 5 and 18 weeks post lambing, mean WEC was 4 epg (SUP) and 885 epg (NSUP) and 44 epg (SUP) and 827 epg (NSUP) respectively. *Haemonchus contortus* (56%) and *Trichostrongylus* spp. (31%) were the predominant worm species present in NSUP ewes. Mean WEC of lambs at 9 weeks and at weaning were 0 epg (SUP) and 1 epg (NSUP) and 2 epg (SUP) and 912 epg (NSUP) respectively. *H. contortus* (67%) and *T. spp.* (26%) were dominant in NSUP lambs at weaning. Live weights of lambs at weaning were higher ($P < 0.0001$) when reared by SUP ewes (32.9 ± 0.15 kg v's 31.7 ± 0.15 kg). SUP lambs were heavier at weaning but the effect was less than observed for ewe treatment (32.5 ± 0.15 v's 32.1 kg ± 0.15 , $p < 0.03$).

These results highlight the importance of controlling worm infections in peri-parturient and lactating ewes with 70% of this difference in lamb bodyweight at weaning due to ewe worm control.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C280/0378

Effect of Egyptian propolis on immunosuppressed rats-as animal model- experimentally infected with *Cryptosporidium* spp.

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This study aimed to assess the effectiveness of therapeutic and prophylactic treatment with ethanolic (EEP) and water (WEP) extracts of propolis against *Cryptosporidium* infection in dexamethasone-immunosuppressed rats. Nitazoxanide (NTZ) was used as a standard anticryptosporidial drug. One hundred and eighty adult male Sprague-Dawley rats of 190-220g BW were randomly divided into nine equal groups. Groups I, II and III were kept as normal, immunosuppressed and infected control groups, respectively. Groups IV to IX were administered orally with EEP, WEP and NTZ at a dose of 50, 50 and 100 mg/kg BW to infected rats with different regimes, respectively. At the 15th day post infection (dpi), blood and tissue specimens from intestine were collected from all groups for assessment of leukogram, serum proteins, total immunoglobulin G (IgG) and histopathological changes. Result revealed that the highest reductions of oocysts shedding (88% and 91%) in rats prophylactically treated with EEP and WEP at the 4th and the 6th dpi, respectively. Compared to the infected control group, the absolute neutrophils count and the levels of α_2 -, β -globulins and total IgG markedly increased in infected rats treated with EEP and WEP, while the absolute lymphocytes significantly decreased. Moreover, NTZ administration caused significant increase in β -globulin level. The sections of ileum showed histopathological changes resulted from administration of dexamethasone and *Cryptosporidium* infection. In conclusion, EEP and WEP have powerful efficacy against *Cryptosporidium* infection and modulate the immunity in dexamethasone-immunosuppressed rat as evidenced by reduced oocysts shedding.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C281/0417

The effect of short-term consumption of sainfoin (*Onobrychis viciifolia*) or extra proteins on strongyle infection in horses

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The expanding diffusion of anthelmintic resistance in horse small strongyle nematodes stresses the need to explore alternative solutions for a sustainable control of these parasites. The potential of secondary metabolites of bioactive plants or of improved nutrition for increasing host response to parasites has been documented in ruminants but little evidence exists for horses. Here, we investigated the efficacy of a short-term consumption of sainfoin (*Onobrychis viciifolia*), a tannin-rich plant, or of extra proteins in naturally infected horses. Thirty horses were divided into three groups of 10 individuals receiving for 18 days either i) a tannin-rich diet with 70% DM of sainfoin pellets and 30%DM of wheat straw (3.6%DM of condensed tannins in the diet) (SD), ii) a protein-rich diet with 52% DM of Italian rye-grass pellets, 18% DM of grinded linseed expeller and 30%DM of wheat straw (PD), or iii) a control diet with 45% DM barley, 25% DM cereal-based pellets and 30%DM of wheat straw (CD). The three diets were isoenergetic, covering on average 94% of animal energy requirements while the SD and PD diets provided extra proteins (227% of protein requirements vs. 93% for the CD diet). Comparing PD and CD thus allowed testing for the benefits of receiving extra proteins, while comparing SD and PD accounted for the effect of sainfoin secondary metabolites. Neither faecal egg counts, nor worm counts in the faeces of drenched horses at the end of the experiment did reveal any difference between the feeding regimes. Interestingly, routine coprocultures from the faeces collected in each group suggested a lower rate of strongyle larval development in the SD group (SD: 8.1%, PD: 30.5%, CD: 22.6%). This was confirmed by two tests performed *in vitro*: adding 29% of sainfoin extract in faeces reduced by 82% the development of strongyle eggs into infective larvae ($P<0.001$), and egg hatching was reduced by 37% ($P<0.05$) using solutions with a minimum sainfoin concentration of 7.5 mg.mL^{-1} . Our hypothesis that protein enrichment could increase the resistance of parasitized horses was however rejected maybe because proteins were not limited enough by the level of infection. Overall, our study reports for the first time that the short-term use of sainfoin in horse diet could constitute a promising means to reduce infectivity of pastures due to reduction of development of eggs into infective larvae.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C282/0426

First report of lambda-cyhalothrin resistance in *Rhipicephalus (Boophilus) annulatus*

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Background: *Rhipicephalus (Boophilus) annulatus* is a major tick of cattle in North of Iran. The use of pyrethroid insecticide is at the center of tick control strategy; however, acaricide resistance has led to operational failure. The aim of this study was to detect of susceptibility status in the cattle tick to common acaricides.

Methods: A multistage cluster random sampling method was used to examine 819 domestic animals (cow, sheep and goat) in three different geographical areas of plain/littoral, woodland and highland. After species identification using appropriate keys, the fully engorged ticks were kept in an insectary under 27±2°C temperature, 78±5% relative humidity and 12:12 photoperiod until they laid eggs in about 4-10 days. Twelve populations of *Rhipicephalus (Boophilus) annulatus* ticks were tested with lambda-cyhalothrin. The two-week old larvae were used for the Larval Packet Test (LPT) with filter paper (Whatman® Grade # 541) treated with the serial discriminating doses of lambda-cyhalothrin. The mortality of the treatment and control replicates was scored after 24 hours. Probit analysis was employed to calculate the LC₅₀ and LC₉₉ of the populations and ANOVA-Tukey test was also used to compare the Probit analysis data between populations.

Results: Population 25 showed a resistance ratio of 5.32 with lambda-cyhalothrin when compared to the most susceptible population 66 at the LC₉₉ level which is about 4-fold higher than the dose recommended by the formulating company. We documented for the first time a *Rhipicephalus (Boophilus) annulatus* population in Iran with resistance to lambda-cyhalothrin insecticide.

Conclusions: This is the first report of lambda-cyhalothrin resistance in *Rhipicephalus (Boophilus) annulatus*. The resistance ratio of insecticide tested confirms operational failure with lambda-cyhalothrin. Therefore monitoring the acaricide susceptibility status of the field populations is the key to manage insecticide resistance by implementing resistance management strategies in the long run and also choosing alternative acaricides for the short term.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C283/0433

Experimental infections of rabbits with tachyzoites and bradyzoites of *Besnoitia besnoiti*

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Cattle besnoitiosis due to *Besnoitia besnoiti* is spreading across Europe and is responsible for severe economic losses in newly infected herds. Experimentally speaking, rabbits have been found to be more or less susceptible to this parasite. The adaptation of *B. besnoiti* to rabbits may offer a new, easier and cheaper model of investigation for this disease. This experimental study compared the virulence between tachyzoites and bradyzoites of *B. besnoiti* in rabbits. Eighteen New Zealand rabbits were allocated into three groups of six animals each. The control group (group C) was inoculated subcutaneously with 66 µg of ovalbumin at the right flank. The rabbits from the "Tachyzoite" (group T) and "Bradyzoite" (group B) groups were subcutaneously injected in the right flank with 6.10^6 tachyzoites (125th passage on Vero cells) and 6.10^6 bradyzoites of *B. besnoiti*, respectively. Clinical follow-up and blood sampling for serological survey and qPCR were performed during ten weeks until euthanasia. qPCR and histological examination were performed on twenty-five samples of skin and organs per rabbit. Rabbits of group B exhibited a febrile condition with a temperature above 40°C from day 8 to day 11 following injection, with positive qPCR in blood. Cysts of *B. besnoiti* were found on skin samples and organs of rabbits from group B corresponding to tissue-positive qPCR results. These results suggest a high virulence of bradyzoites in rabbits. The proposed model could be used to assess the *in vivo* effectiveness of vaccine or drugs against cattle besnoitiosis.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C284/0450

Natural gastrointestinal nematode infection in goats do not modify the selection of tannin rich foliage resources in a cafeteria study

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This study estimated the effect of natural gastrointestinal nematode (GIN) infection on the selection of tannin rich foliage resources by Criollo goats. A cafeteria trial was implemented in individual concrete floor pens using adult goats (32 ± 2.3 kg LW, multiparous). Three groups were formed: Group A (n=4) with high faecal egg count (FEC) from 1450 to 2150, Group B (n=4) with low FEC from 592 to 1167 and Group C (n=4) control with no faecal egg excretion (dewormed with anthelmintic). In the first period, goats in groups A and B had a mixed natural GIN infection for 10 days. In the second period all animals were dewormed and remained free of GIN infection for 10 days. Balanced feed was offered in the morning at 1% LW (dry matter basis). Three hours later, fresh foliage of different trees were offered *ad libitum* using individual feeders (1 hour): *Viguiera dentata* with null level of polyphenols (PP), *Leucaena leucocephala* with mid level of PP, *Gymnopodium floribundum* and *Mimosa bahamensis* both with high PP content. Selection indexes for each experimental foliage were determined daily. The Jacobs' Selection Index (JSI) was similar ($P>0.05$) between groups A, B and C on both study periods. Plants positively selected were *M. bahamensis* and *V. dentata*. Refused plants were *L. leucocephala* and *G. floribundum*. This cafeteria study showed that the natural GIN infection levels tested failed to modify the selection of foliage resources by Criollo goats.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C285/0451

Suppressing the natural gastrointestinal nematode infection in goats fails to modify condensed tannin intake while browsing a tropical deciduous forest

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This study determined the effect of suppressing the natural gastrointestinal nematode (GIN) infection in goats on their condensed tannin (CT) intake when browsing a tropical deciduous forest (TDF). The direct observation method was implemented with 12 Criollo goats (adults, non-pregnant, 34 ± 2 kg LW) browsing 4 h / day in the TDF of Mexico. Animals were observed three days every month from June to September (rainy season). First month observations were used as baseline information with all goats naturally infected. After that, goats were divided in a treated group (TG, n=6) receiving moxidectin every 28 days at 0.3 mg/kg LW s.c., and a naturally infected group (IG, n=6). With the monthly observations 3 faecal samples per animal were used to determine eggs per gram of faeces (EPG). Baseline EPG were similar between TG and IG groups (1081 vs. 953 EPG; $P > 0.05$). After baseline, the TG remained with 0 EPG and IG with a mean of 1300 EPG. Daily dry matter intake (DMI) of foliage was estimated from 79703 bites observed from all the goats. The TG and IG showed similar daily DMI ($P < 0.05$) from the same plant species. Thus, CT intake was also similar between TG and IG (0.822 ± 0.1 vs. 0.829 ± 0.3 g CT/d in log10; $P > 0.05$). For goats browsing the TDF, suppressing GIN infection fails to change CT consumption from the foliage consumed. The latter suggests that mixed GIN infection, at the level found in naturally infected goats, is not modulating consumption of tannin rich plants.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C286/0457

Worm control practices against gastro-intestinal parasites in French goat flocks

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The control of digestive strongyles is a major issue for goat farmers using pasture. As, in France, few molecules can be used in lactating goats and as BZD resistance is very prevalent, goat owners are prone to search for other ways to control these infections. A survey was set up to identify the worm control practices in western France. Thirty-three goat breeders were directly interviewed on their perceptions of strongyle infection and on the control methods they applied. A parasitic risk was calculated for each farm by combining data on grazing practices. Twenty farmers used allopathic treatments, mainly benzimidazoles or eprinomectin. Twenty-five owners proposed tannin-rich plants, medicinal plants in forages, commercial phyto-aroma products or essential oils to their goats. Farmers using complementary tannin-rich and medicinal plants used less than one allopathic treatment per year while others treated their goats one or two times a year. A discriminant analysis of the data defined three types of farmers. The first group comprised mainly dairy farmers, with rather large flocks and high producing goats, showing high parasitic risk and a predominant use of allopathic treatments. The second group had balanced practices: cheese-maker or dairy farmers, low parasitic risk and combined use of allopathic treatment, tannin-rich forages or medicinal plants. The last group comprised mainly organic producing farms and cheese-makers with low size flocks and low producing goats and a combined use of diverse alternative methods. Phytotherapy being widely used, the scientific knowledge about this medicine (efficacy, residues ...) needs to be improved.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C287/0461

Assessment of anti-Trypanosomal activity of the Egyptian herb *Xanthium strumarium* {Pathological and Clinicopathological Study}

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The present study investigated anti-trypanosomal activity of a locally growing herb *Xanthium strumarium* in comparison to the currently used anti-trypanosomal drug diminazene aceturate (Batrnil ®) in rats experimentally infected with *Trypanosoma evansi* (T.evansi). The rats were divided into 4 groups of 20 animals each. Group 1 was a –ve control group. Group 2 was a +ve control group injected intraperitoneally with 0.25 cc blood containing 4x10⁴ T.evansi. Treatment groups 3 and 4 received the same dose of trypanosomes. Group 3 received, on the 3rd, 6th and 10th days post infection, 8, 40 and 40 mg/kg body weight of diminazene acetate (Batrynil R) in 0.25 cc distilled water intraperitoneally. Group 4 received, from the 3rd day post infection, 0.25 cc distilled water containing 600 mg/kg body wt of 50% alcoholic extract of *Xanthium strumarium* intraperitoneally for five successive days.

The results revealed that both treatments reduced parasitaemia for about two weeks post treatment. Based upon parasitaemia profile, diminazene acetate appeared to be initially effective and the rats in group 3 showed complete clearance of T.evansi from the peripheral blood of all animals one day post treatment. Parasitaemia reappeared in 50% of the rats at 6 DPI (3 days post treatment), and after 3 days from the second dose (9th DPI). Although treatment with *Xanthium* extract didn't eliminate trypanosomes from the blood, it significantly reduced parasitaemia for about two weeks post treatment.

All infected rats developed a progressive increase in total leukocyte count and progressive macrocytic hypochromic anaemia with significant ($P < 0.05$) decline of PCV, Hb and RBCs and increase in MCV with normal MCH and decreased MCHC. Serum transaminases (AST, ALT) and alkaline phosphatase activities and total bilirubin were significantly increased. Blood urea, serum uric acid and creatinine levels were significantly lower in all treated rat groups than the infected non-treated rats.

Serum protein electrophoresis revealed increases in alpha and gamma globulin fractions in all infected groups in comparison to the non-infected control group. The diminazene acetate treated group also showed a prominent increase in beta globulin fraction. Some bands in alpha, beta, and gamma fractions disappeared in infected groups.

The results of pathological examinations emphasized significant progressive hepatosplenomegaly in all infected rats. Although the spleens in Groups 3 and 4 were significantly larger than the non-infected control group (Group 1), they were significantly smaller than the infected, non-treated group (Group 2).

Histopathological examination revealed congestion and active follicular hyperplasia in the spleen, with mitotic blast forms, tingible body macrophages and deposition of haemosiderin. In the extract-treated groups, appearance of the aforementioned changes was delayed, and was milder in severity and magnitude than in the infected, non-treated rats (Group 2).

The liver of all infected rats showed irregular arrangement of the hepatic cords and focal areas of necrosis with lymphocytic infiltration, mainly portal and periportal in distribution, often with haemosiderin deposition. Oval cell proliferation (fig.1) and bile duct hyperplasia with portal and periportal infiltration of mononuclear cells was also seen in all infected rats increasing with time.

Necrosis of the epithelium lining the bile duct was observed in some sections of the *Xanthium* treated group after 12 and 18 d post infection.

Lungs showed circulatory changes with intense macrophage and lymphocyte infiltrations in the alveolar walls of all infected rats. The lesions were mild in Group 4, and even milder in Group 3.

The kidneys revealed pronounced, progressive, necrobiotic changes. Lesions were particularly mild in Group 3 & 4 at early stages of the disease. At the later stage, the renal lesions were more pronounced in Group 4 milder in Group 2 and were mildest in Group 3. The heart showed focal myocardial degeneration and necrosis with variable interstitial oedema and mononuclear cells infiltration (Fig.2). These cardiac lesions increased in severity in the late stages of infection, and were particularly severe in Group 2, milder in Group 4 and mildest in Group 3.

Conclusion: The trypanosome induced lesions in lung, liver, kidney and heart were less severe in the *Xanthium* extracts and diminazene aceturate treated rats compared to infected non treated rats.

The recommended dose of diminazene aceturate was inefficient in controlling *T. evansi* infection in rats.

The results confirmed the anti-trypanosomal activity of the crude extracts of leaves and aerial parts of native *Xanthium strumarium* weeds. Comparison of the activity of the tested extract with the commercial drug diminazene aceturate showed that the drug is much more active than the crude extracts

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C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C288/0479

Dietary cinnamaldehyde enhances the acquisition of humoral immunity to *Ascaris suum* during a primary infection in pigs

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Cinnamaldehyde (CA) is a bioactive essential oil that is reported to have antimicrobial and immuno-stimulatory properties. However, whether CA has an anthelmintic effect and/or influences host immune responses to nematode infections have not yet been explored. Here, we investigated whether feeding CA to growing pigs could 1) reduce establishment of an *Ascaris suum* infection, and 2) enhance the acquisition of adaptive immunity to the parasite.

Two groups of 2-month-old *A. suum*-naïve pigs (n=5) were each infected with 5000 embryonated *A. suum* eggs, and received either a daily supplement of 1000 mg of CA or no supplement (control). The establishment of the infection was assessed 14 days post-infection by recovery of larvae from the small intestine. *A. suum*-specific antibodies in plasma were assessed by ELISA.

Recovery of larvae was similar between groups, indicating that CA did not have a direct *in vivo* anthelmintic effect. However, levels of *A. suum*-specific IgM and IgA were significantly higher in the pigs fed CA, and there was also evidence of faster acquisition of an *A. suum*-specific IgG₁ response in these animals.

In conclusion, dietary CA did not directly reduce the establishment of an *A. suum* infection in pigs. However, the acquisition of both IgM and class-switched parasite-specific antibodies was clearly enhanced by CA, which may lead to faster expulsion of the parasites and improved resistance to re-infection. On-going experiments are investigating the molecular mechanisms underlying this increased antibody response.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C289/0509

The extent of uptake by ticks determined using tick weight and coxal index in ticks from fluralaner (Bravecto™) treated and negative control dogs

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Bravecto™ chewable tablets are highly effective at controlling tick and flea infestations. Three laboratory studies were conducted to quantify the extent of uptake after tick attachment by comparison of ticks' weight and coxal index obtained from Bravecto™ treated and negative control dogs.

All studies included a treated (Bravecto™ chewable tablets, MSD Animal Health) and a negative control group and had a similar study design for assessing vitality and weighing of ticks. In one study the coxal index was additionally calculated as a ratio of tick's dorsal width of scutum and ventral coxal gap. The experimental tick infestations were conducted with either *Ixodes ricinus* (Ric) or *Ixodes scapularis* (Sca) (50 females/5-10 males) after oral administration of fluralaner according to label instructions. Tick weight data and coxal indices from Bravecto™ treated and negative-control groups were statistically analyzed.

Study 1+2: Infestations were conducted 1 week (Ric) or 1 day (Sca) after treatment. 1 day after infestation a maximum of 3 ticks per dog and species (Ric, Sca) were collected daily. This was repeated for 7 days. Ticks were assessed for vitality, weighed and photographed. Additionally, 20 live, non-infested (naïve) ticks were also weighed. Infestations (Sca) were also conducted 4, 8 and 12 weeks after treatment with efficacy assessments approximately 12 hours after each infestation. Study 3: Infestations (Ric) were conducted 2 days before treatment to assess immediate efficacy, and 4, 8 and 12 weeks after treatment to assess persistent efficacy (tick collections 48 hours post treatment/infestations).

Ticks collected from Bravecto™-treated dogs weighed significantly less ($p \leq 0.0108$) than ticks collected from negative control dogs, and their coxal index was also significantly lower ($p < 0.0001$). The difference in tick weights was demonstrated irrespective of the tick species investigated (*I. ricinus*, *I. scapularis*). At some assessments the mean tick weights of Bravecto™-treated dogs were even significantly lower than those of non-infested baseline-ticks. The demonstrated tick efficacy was in the range of 94.6 – 100%.

Tick weights and coxal indices confirm that even a minimal uptake results in ticks' exposure to fluralaner (Bravecto™) which induces a potent acaricidal effect.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C290/0511

Prevention of transmission of *Babesia canis* by *Dermacentor reticulatus* ticks to dogs treated orally with fluralaner chewable tablets (BRAVECTO™)

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The transmission of *Babesia canis* by infected *Dermacentor reticulatus* ticks can be prevented if ticks are killed before transmission occurs. In this study, the preventive efficacy of Bravecto™ chewable tablets was evaluated. Methods: Only dogs that had tested negative for *B. canis* by PCR and IFAT were included in the study. On day 0, dogs in the treatment group (n=8) were treated once orally with Bravecto™ according to label recommendations. Dogs in the control group (n=8) remained untreated. On days 2, 28, 56, 70 and 84, dogs were infested with *D. reticulatus* ticks with tick *in-situ* thumb counts 48 ± 4 h post-infestation. On day 90, ticks were counted and removed from all dogs. The *D. reticulatus* ticks were confirmed to harbour *B. canis* by PCR analysis prior to each infestation. The percentage tick efficacy was calculated for the treatment group at each assessment time point and significant differences to the untreated group were assessed. Each dog was physically examined in conjunction with blood collection for PCR analysis every 7 days, blood samples for IFAT were collected every 14 days and the dog's rectal body temperature was measured thrice weekly. Control dogs tested positive for *B. canis* by blood smear and PCR were rescue treated and replaced with a replacement dog. The preventive efficacy against *B. canis* transmission was evaluated by the comparison of the total number of infected dogs in the treated group with the total number of infected dogs in the untreated control group. Last blood samples for PCR analysis and IFAT were collected on day 112 after treatment. Results: All control dogs were tested positive by PCR and blood smear for *B. canis* and rescue treated before developing severe clinical signs. The diagnosis of babesiosis was also confirmed by the development of *B. canis* antibodies (IFAT). For each rescue-treated dog, a replacement dog was included before next tick infestation. None of the 8 Bravecto™-treated dogs became infected with *B. canis*, as IFAT and PCR analysis were negative throughout the study period. Therefore, single oral administration of Bravecto™ to dogs prevents the transmission of *B. canis* by infected *D. reticulatus* ticks by 100% over 12 weeks. A tick killing efficacy between 99.2-100% was achieved over the entire 12-week study duration and tick reduction was significant for all time points.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C291/0536

In vitro antihelmintic effect of *bocconia frutescens* extract and its fractions on excysted metacercariae of *fasciola hepatica*

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Fasciolosis due to *Fasciola hepatica* is the most important hepatic disease in veterinary medicine. Its relevance is important because of the major economical losses to the cattle industry such as: reduction in milk, meat and wool production; miscarriages, anemia, liver condemnation and occasionally deaths, are estimated in billions of dollars. The emergence of fluke resistance due to over or under dosing of fasciolides as well as environmental damage produced by the chemicals eliminated in field have stimulated the need for alternative methods to control *F. hepatica*. The aim of this study was to evaluate the *in vitro* anthelmintic effect of fifteen tropical plant extracts used in traditional medicine, on newly excysted flukes of *F. hepatica*. To determine the antihelmintic effect of *Bocconia frutescens* extracts on the mortality of excysted flukes a series of *in vitro* experiments were undertaken. The flukes were exposed in triplicate at a dose of 500, 400, 300, 200 and 100 mg/L to each *B. frutescens* extract and its fractions, using as a positive control a commercial product at a dose of 10 and 50 mg/L. The efficacy was assessed as the mortality rate based on the number of live and dead flukes after 24, 48 and 72 h post-exposure. The extracts and fractions were evaluated three more times in order to confirm the results, when an extract or fraction showed an *in vitro* efficacy greater than 90%, it was considered to possess fascioliscide activity. Three extracts out of fifteen showed high activity. The active extracts and fractions were subjected to phytochemical analysis to determine the presence of Secondary Metabolites groups following standard published protocols. A Kruskal-Wallis test, ($P < 0.05$) was used to determine significant differences. Of the three extracts selected, one showed promising *in vitro* fasciolicide efficacy 100% at all doses ($P < 0.05$) and from the fourteen fractions of the former only one showed promising activity 100% at doses of 200 to 500 mg/L ($P < 0.05$), thus indicating that they could possibly be strong candidates for further biological analyses aimed at demonstrating their potential as a flukicide in ruminants. Study supported thanks to the Project UNAM-DGAPA-PAPIIT IN220313.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

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Fascioliscide activity of the raw extract and fractions of estafiate (*artemisia ludoviciana* Nutt. Spp *mexicana*) in vitro

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The aim of the present study was to evaluate under in vitro conditions the fascioliscide activity of some extracts of Estafiate (*Artemisia ludoviciana* Nutt. spp *mexicana*) of different polarities, using different organic solvents in tropical plants from Veracruz, Mexico. The evaluation was carried out in newly artificially excysted *Fasciola hepatica* metacercariae, incubated at 37°C in sterile 24 well NUNC culture plates in a 5% CO₂ atmosphere. Each extract was tested in triplicate at concentrations of 500, 375, 250 y 125 mg/L, always including the corresponding non-treated controls. The efficacy was assessed as the mortality rate based on the number of live and dead flukes after 24, 48 and 72 h post-exposure compared with the non-treated control using an inverted microscope at 40X. All procedures were carried out in an airflow cabinet. The results obtained in the first bioassays indicated that after 24 hours the methanolic extracts and the ethyl acetate exerted an efficacy of 100% (p<0.05), while the hexanic extract showed an efficacy of 71%, reaching 100% efficacy after 72 hours of evaluation (p<0.05). After these preliminary results a rapid chromatography column was set up to obtain the extract fractions of the ethyl acetate. A total of 14 fractions were evaluated *in vitro*, using the same criteria before mentioned. The results indicated that fractions C and D exerted 100% efficacy at 48 hrs post-exposition (p<0.05), while fraction B showed 100% efficacy at 72 hours (p<0.05). It is concluded that all fractions obtained from the ethyl acetate of raw extract from *A. ludoviciana* showed a promising efficacy in vitro against newly excysted flukes, suggesting that further work should be undertaken to identify the secondary metabolites responsible for this fascioliscide activity. Study supported thanks to the Project UNAM-DGAPA-PAPIIT IN220313.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C293/0538

In vitro fasciolicidal effect of secondary metabolites of *bocconia frutescens* obtained by column and thin-layer chromatography

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The fasciolicidal efficacy of the secondary metabolites of the Gordolobo plant (*Bocconia frutescens*) obtained by thin-layer chromatography was tested in vitro. The plant under study was collected in Veracruz, Mexico. The extracts were obtained from dry leaves using solvents of different polarities. To evaluate the fasciolicidal effect of each extract, artificially newly excysted flukes were used. Each vegetal extract was tested in triplicate and those that showed higher activity were re-evaluated. Then each extract was monitored by thin-layer chromatography obtaining 50 ml. aliquots for each of the solvents of different polarities. Each aliquot was distilled with the aid of a Heidolph® rotaevaporator. All aliquots of equal extracts were united in only one fraction. The obtained fractions were tested by thin-layer chromatography to evaluate if they were equal on the united fractions. All fractions were submitted to the Dragendorff test to determine the presence of alkaloids. The positive fractions to alkaloids were again tested and the data were submitted to the Kruskal-Wallis test within 95% confidence limits. The results indicated that the methanolic extract showed 100% activity ($p < 0.05$). From this extract 215 aliquots were obtained. However, since they were mixed, they gave a total of 13 different fractions (A-M). From them, 8 fractions (F to M) were positive in the alkaloid test. The fractions F, G and H were evaluated in triplicate showing 100% efficacy ($p < 0.05$) at concentrations of 500 ppm, 400ppm, 300ppm, 200ppm and 100ppm; at 24 hrs post-exposition. Fraction I showed an efficacy of 100% ($p < 0.05$) at 48 hrs post-exposition in all concentrations as mentioned above. The remaining fractions are at present under evaluation. It can be suggested that the fasciolicidal effect exerted by the extract of *Bocconia frutescens* can be associated with the presence of alkaloids in 61.5% of the obtained fractions. It is concluded that the *Bocconia frutescens* extract seems to be a good candidate for the continuance of further evaluations aimed to demonstrating the real fasciolicidal potencial of this promising extract. Study supported thanks to the Project UNAM-DGAPA-PAPIIT IN220313.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C294/0539

Comparison of Two Pour-on Formulations of Ivermectin against Gastrointestinal worms, Fleas and Lice in Naturally Infected Stray Dogs

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The aim of the present study was to evaluate the efficacy of two commercial pour-on Ivermectin formulations against intestinal parasites (IP), fleas and lice in naturally infested stray dogs. Eighteen crossbreed dogs with eggs of IP as well as adult fleas and lice were included in the trial. On day 0, the dogs were randomly divided into 3 groups of 6 animals each: a group receiving a single pour-on treatment with 0.5% Ivermectin (500 mcg/kg), a group treated similarly with 0.2% Ivermectin (200 mg/kg), and a control group. Fecal and skin analyses were carried out on days 0, 7, 14, 21 and 28 to determine the reduction of eggs and the number of fleas and lice. Weight gain was also measured on day 28. On day 30, the dogs were humanely sacrificed in order to count adult IP. Efficacy was measured as the percentage of the reduction of eggs per gram fecal mass (EPGF), of adult IP, fleas and lice relative to the control group. For the eggs of IP, Ivermectin at 0.5% showed an efficacy of 100% against ascarids and 79% against *Ancylostoma caninum*. Ivermectin at 0.2% removed 90.2% of the ascarids and 50.4% of *A. caninum*. For adult IP, the efficacy of 0.5% Ivermectin against *Toxocara canis* and *A. caninum* was 100%, and for 0.2% Ivermectin it was 62.4% and 76.4% for *T. canis* and *A. caninum*, respectively. Both compounds were 100% effective against lice and 96% and 71.1% efficacious against fleas, respectively. However, neither treatment was effective against *Dypilidium caninum*. Weight gain in treated dogs was statistically different from that of the controls ($p < 0.05$). We conclude that 0.5% pour-on Ivermectin showed better efficacy than 0.2% pour-on Ivermectin in the reduction of eggs and adult intestinal parasites and fleas; it was similarly efficacious against lice.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C295/0541

Comparison of the efficacy of two commercial coccidicidal compounds on experimentally infected dogs

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The aim of the present study was to compare the coccidicidal efficacy of two commercial compounds for artificially infected dogs. Eighteen coccidia-free dogs, male and female aged between 2 and 4 months old, were infected each with 20,000 *Cystoisospora* oocysts cultured under laboratory conditions. When the puppies showed high counts of oocysts per gram (OPG) (McMaster method), they were divided into 3 groups (G). G1 was treated with a compound named One® (Lab. Bio Zoo) containing 150 mg of toltrazuril/tablet, administered daily/5 days dosing 1 tablet per 10 kg b.w. G2 received Giacoccide® (Pet's Pharma) containing 250 mg of sulfadimetoxine and 165 mg of dimetridazole dosing 1 tablet per 10 kg b.w. twice a day for 10 days. G3 served as an untreated control. The puppies were coprologically monitored on days 0, 3, 5, 10, 15, 20 and 25 to determine the percentage of OPG. Efficacy was measured based on the OPG reduction on treated animals relative to the untreated control. The results indicated an efficacy for compound One of 78.4, 100, 100, 100, 100 and 100%, for days 3, 5, 10, 15, 20 and 25, respectively and for Giacoccide 40.6, 45.5, 47.4, 65.9, 90.4 and 92.7%, for days 3, 5, 10, 15, 20 and 25, respectively. No statistical difference was observed on the weight of the treated puppies ($p < 0.764$), but the control group was statistically different to the treated ones ($p < 0.014$). It was concluded that compound One showed greater efficacy than Giacoccide for the treatment of canine coccidiosis in artificially infected dogs.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C296/0542

In vitro acaricide effect of tropical plants against *Rhipicephalus (Boophilus) microplus* larvae.

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Rhipicephalus (Boophilus) microplus is one of the most widespread tick species and constitutes a major problem for cattle production in the tropical and subtropical regions of the world. It causes blood loss, resulting in reduced weight gain and milk production, as well as the injection of toxins and pathogens. Chemical acaricides play an essential role in the control of this parasite. However, as a consequence of the extensive use of chemicals, this tick species has developed resistance to drugs. Commercial acaricides are also usually toxic to humans and leave residues in the environment. Therefore, new approaches have been explored involving the use of natural products, since bioactive compounds from plants have shown effectiveness against this and other parasites. In the present study the larvae of *Rhipicephalus (Boophilus) microplus* were exposed to 31 plant extracts (*Acacia cornígera*, *Acacia farnesiana*, *Arachis pintoii* 17434, *Arachis pintoii* 18744, *Arachis pintoii* 22159, *Arachis pintoii* 22160, *Artemisia absinthium*, *Artemisia mexicana*, *Bocconia frutescens*, *Bursera simaruba*, *Cajanus cajan*, *Carica papaya*, *Cordia* spp, *Cratylia argentea*, *Cratylia argentea* 22386, *Gliricidia sepium*, *Guazuma ulmifolia*, *Hibiscus rosa-sinensis*, *Lantana camara*, *Leucaena diversifolia*, *Mangifera indica*, *Melia azederach*, *Mentha* spp, *Ocimum basilicum*, *Persea americana*, *Petroselinum crispum*, *Piper auritum*, *Psidium guajava*, *Teloxys ambrosioides*, *Theobroma cacao* and *Struthanthus* spp). Using a larval packet test (LPT), they were exposed to 1%, 0.5%, 0.25%, 0.125% y 0.0625% concentrations of each extract. The efficacy was assessed according to the mortality rate which was based on the number of live and dead ticks after 24 hrs. post-exposure. When an extract showed an in vitro efficacy greater than 90%, it was considered to possess larvicidal activity. A Kruskal-Wallis test, ($P < 0.05$) was performed in order to validate the obtained results. From thirty-one plant extracts, the *Hibiscus rosa-sinensis* extract showed a larvicidal efficacy of 94.72% ($P < 0.05$). In conclusion, an intrinsic in vitro efficacy on this tropical plant was determined. Further *in vivo* toxicological and biological evaluation as well as the purification of bioactive compounds will determine their real potential.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C297/0545

Anti-tick activity of some methanol plant extracts indigenous in Saudi Arabia

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Ticks are economically important parasites all over the world. They are vectors of different animal and human infectious diseases. Tick control is globally based on chemical acaricides, but the developing tick resistance to most of the currently used synthetic acaricides encouraged to look for alternative ways to control ticks, especially natural products that are relatively environment friendly. An *in vitro* screening of the effects of some indigenous plants was carried out. Twenty- one plant species were extracted and tried to have effects on the most prevalent tick species; *Hyalomma drommederi* of camels. Fully engorged female ticks of the same weight, were immersed in the different extracts for 5,15,30 and 60 minutes. Treated ticks were revived and incubated with the control groups for daily observation of their activity and egg production. While some plant extracts have killing effects after 30 – 60 minute exposure (*Rhanterium epapposum*, *Achillea fragrantissima* and *Eragrostis poaeodes*) others have stopped oviposition (*Artemisia herba alba*, *Haloxylon salicornicum*, *Plantago coronopus*, *Moltkiopsis ciliate* and *Lasiurus hirsutus*). Most of the extracts have reducing effect on the egg mass. Marked reduction of the egg masses associated with delayed oviposition was recorded on exposure to *Lepidium sativum* seeds extract. The phytoconstituents were determined by a standard methods and further studies are on going to determine the bioactive components that may have the above mentioned acaricidal effects.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C298/0562

Trapping behaviour of the biological control agent *Duddingtonia flagrans* against gastrointestinal nematodes of sheep and cattle in the environment under spring conditions

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An experiment was carried out using the nematophagous fungi *Duddingtonia flagrans* as biological control agent of gastrointestinal nematodes of sheep and cattle, when exposed to the spring climatic conditions of central Buenos Aires province, Argentina. For each animal species, 40 artificially-formed faecal pats containing trichostrongyle eggs were deposited on pasture, 20 under direct sun and 20 under shadow. Half of the faecal pats contained 11,000 fungal chlamydospores/g of faeces and the other half remained as control. GraphPad Prism® v. 5.04 software was used for statistical analysis.

Coprocultures performed to assess the *in vitro* nematophagous effect of the fungus revealed that *D. flagrans* was capable of reducing the trichostrongyle larval population by 78.3% ($P<0.05$) in sheep faeces and 67.7% ($P<0.05$) in cattle faeces.

Grass samples collected around each faecal mass at day 15 and 28 post-deposition showed a different picture, after one month the fungus reduced the overall larval infectivity by 65.2% ($P<0.05$) in sheep and 72.5% ($P<0.01$) in cattle.

The faecal pats were collected at day 28 in order to extract the remaining infective larvae still inside them. This revealed that, overall, *D. flagrans* had a reduction effect of 97.4% ($P=0.0186$) in sheep faeces and 76.7% ($P=0.0244$) in cattle faeces.

Temperature measured throughout the experiment period inside faecal mass under direct sun had a large min-max range, with values of 2.6-42.6°C and 1.5-39.9°C for sheep and cattle faeces, respectively, while inside faecal mass deposited under constant shadow the temperature varied between 2.6-28.16°C for sheep and 2.9-26.2°C for cattle. At the end of the experiment the reduction effect of the fungus against gastrointestinal nematode L3 on pasture was shown to be 92.8% ($P<0.0001$) and 77.9% ($P=0.1404$) for sheep faeces under direct sun and under shadow, respectively. For cattle faeces the reduction in larval infectivity at the end of the experiment was 88.6% ($P=0.00011$) and 72.2% ($P=0.0265$) for pats deposited under direct sun and under shadow, respectively. Preliminary results seem to indicate that the fungus predatory efficacy could be intensified by high temperatures although the underlying reasons for it remain to be confirmed.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C299/0563

***In vitro* effects of carvacryl and thymol acetate on *Haemonchus contortus* and toxicity on mice**

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Essential oils has been described as an alternative to control gastrointestinal nematodes of small ruminants. Acetylation of compounds isolated from essential oils, especially carvacrol and thymol, can be an alternative to reduce the toxicity of these compounds and increase the solubility and thereby improve their biological effects. This study evaluated the effect of carvacryl and thymol acetate *in vitro* on *Haemonchus contortus* eggs and larvae and acute toxicity on mice. The egg hatch test (EHT) was performed using a suspension (250 µl) containing approximately 100 *H. contortus* fresh eggs incubated with 250 ml of carvacryl and thymol acetate at concentrations from 0.5 to 8 mg/ml and 0.25 to 4 mg/ml respectively; 1% Tween 80 and 0.025 mg/ml thiabendazole. After 48 h drops of Lugol's solution were added. The eggs and the first-stage larvae were counted under a microscope. On larval development test (LDT), 500µl of *H. contortus* larval suspension, and 500 µl of carvacryl and thymol acetate at concentrations from 2 to 0.125 mg/ml; 1% Tween 80 and 0.008 mg/ml ivermectin, were incubated with 1 g of faeces from a nematode-free sheep for 6 days at room temperature. Three repetitions with five replicates for each concentration and for each control were performed. In the acute toxicity test, the mice were divided into groups (n = 10) based on treatment: G1 to G4: 500, 1000, 1500 and 2000 mg/kg of carvacryl acetate, G5 to G8: 1000, 2000, 3000 and 4000 mg/kg thymol acetate; G9: 1% Tween 80 solution. The data were analysed using ANOVA and were compared using the Tukey test ($P < 0.05$). Carvacryl and thymol acetate exhibited a dose-dependent effect in EHT, inhibiting 89.2 ± 1.4 and $67.1 \pm 1.4\%$ of *H. contortus* larvae hatching, at a concentration of 8 and 4 mg/ml, respectively. The EC₅₀ of egg hatching was 1.7 (1.9-2.4) and 1.9 (1.6-2.4) mg/ml of carvacryl and thymol acetate, respectively. In LDT, carvacryl and thymol acetate at concentration of 2 mg/ml inhibited 100 and $99.3 \pm 0.2\%$ development of *H. contortus* larvae, presenting EC₅₀ values of 0.3 (0.1-0.7) and 0.4 (0.3-0.6) mg/ml, respectively. In the acute toxicity test, LD₅₀ of thymol and carvacryl acetate was 1,327 (1,012-1,736) and 4,146 (3,356-191,729) mg/kg, respectively. Carvacryl and thymol acetate has *in vitro* activity against *H. contortus* eggs and larvae and low toxicity. However their anthelmintic activity should be confirmed *in vivo*.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C300/0566

***In vitro* anthelmintic effect of *Acacia pennatula* or *Onobrychis viciifolia* acetone-water extracts on *Haemonchus contortus* of different geographical origins**

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This study determined the *in vitro* anthelmintic effect of acetone-water extracts of 2 tannin-containing plants, *Acacia pennatula* or *Onobrychis viciifolia* on *Haemonchus contortus* of different geographical origins. Ten *H. contortus* isolates from different latitudes of the world were evaluated using an egg hatch assay. Fresh eggs from each isolate were incubated in PBS with increasing concentrations of *A. pennatula* (tropical plant) or *O. viciifolia* (temperate plant) acetone:water extracts (from 0 to 8000 µg/ml). Effective concentration 50% values (EC50) and 95% confidence interval (95CI) were calculated for each isolate with both extracts. The lower EC50 of the most susceptible isolate for each extract was used to calculate resistance ratio (RR). Polyvinylpyrrolidone was used to confirm the role of polyphenols in the AH activity of acetone-water extracts. Although no ovicidal activity was detected for *A. pennatula* and *O. viciifolia* extracts, both blocked hatching of eggs into L1 larvae. However, the amount of extract to achieve the AH effect varied between extracts. The *A. pennatula* extract showed poor activity irrespective of parasite origin (EC50 from 4429.7 to 8360.6), thus the RR was low between different isolates (RR from 1.15 to 1.88). For *O. viciifolia*, wider variation was found with EC50 (from 104.6 to 4441.3). Also, the RR varied considerably from 3.66 to 42.47. There was no clear association between origin of isolates and their susceptibility to either of the plant extracts tested. The addition of polyvinylpyrrolidone on both extracts suggested that polyphenols are not involved in the AH activity observed (blocking egg eclosion).

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C301/0595

Efficacy of new ethyl carbamates on different parasitic instars of *Rhipicephalus microplus*

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For many years, the most common strategy for *Rhipicephalus microplus* control has been the use of chemical acaricides, however, the high selection pressure extended by the use of current commercial acaricides has led to acaricide resistance. Carbamates are currently used as antiparasitics and insecticides. The aim of this study was to evaluate the effect of new carbamates on *R. microplus* larvae, nymphs and adults on infested cattle.

A triple-resistant tick strain of *R. microplus* was used. Approximately 1000 larvae were collocated into hand-made cloth chambers on the back of bulls. The treatment with the new ethyl-carbamates at egg hatching inhibitory concentration (obtained previously *in vitro*) was applied on the different parasitic development instars (larvae, nymph or adult). The total engorged females by chamber, the percentage of females laying eggs, the egg laying inhibition percentage and the egg hatching percentage with *Ethyl-4-chlorophenyl* and *Etil-4-bromophenyl carbamate* were determined.

The number of engorged females by chamber decreased ($p < 0.001$) with the treatment in the three instars larvae, nymph and adult, compared with their control. The percentage of females laying eggs was not affected ($p > 0.05$); but compared to untreated females, eggs produced by treated females had a dark, dry, opaque appearance and were less adhered. The egg laying inhibition percentage increased ($p < 0.001$) with the treatment in the instars of nymph and adult compared with their control. Finally, the hatching percentage decreased ($p < 0.001$) in all instars compared with their control. The efficacy of these carbamates was calculated above 98%.

The study with these new ethyl-carbamates showed their good acaricide potencial, nevertheless, it is important to continue with further studies to consider them as a new pharmaceutical option for tick control.

This study was supported by PAPIIT/UNAM IN221515 and PAPIIT/UNAM IN215314.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C302/0597

Two new synthesized ethyl-carbamates, able to induce apoptosis in ovaric cells from engorged *Rhipicephalus microplus* females

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Development of new deworming drugs is an alternative way to fight resistant ticks. The new synthesized carbamates: ethyl-4-clorofenyl carbamate (E4CC) and ethyl-4-bromofenyl carbamate (E4BC) affect biological and reproductive parameters of *Rhipicephalus microplus* ticks independently of acetyl cholinesterase inhibition, so, it become necessary to know the way this two carbamates work. In this work, ovary cell apoptosis induction of tick treated with E4CC or E4BC, was evaluated using TUNEL test and transmission light microscopy (TLM) on histologic slides of ovaries from engorged female ticks obtained at 0, 1, 3, 5 y 7 post-treatment. Corion reduction, oocyte and ovary epithelium vacuolization, pyknotic cells, pedicel damage and oocyte development decrease were found on ovary histologic slides processed by TUNEL test and counterstained with hematoxylin, furthermore there was a higher number of apoptotic cells per mm² while compared with control group. TLM showed oocyte ultrastructural damage, vacuolization and alterations on plasmatic membrane. This ultrastructural damages are likely related to death cell and the results obtained through TUNEL test confirm apoptotic pathways in ovary cells due to E4CC and E4BC exposition.

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C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C303/0604

Effect of diet with *Acacia mangium* on TCD4+ and TCD8+ lymphocytes and infection of *Haemonchus contortus* and *Trichostrongylus colubriformis* in goats.

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Gastrointestinal nematodes are responsible for significant economic losses in goat production. Tanniniferous plants (CT) represent an alternative to control these parasites. One hypothesis of anthelmintic activity of CT is the improvement in the immune response of animals supplemented with tanniniferous plants. However, little is known about the activity of these secondary compounds in the immune system. The aim of this study was evaluate the profile of the immune response of goats supplemented with bark powder of *Acacia mangium*, rich in CT, correlating with the antiparasitic effect on *Haemonchus contortus* and *Trichostrongylus colubriformis*. Eighteen goats were divided into three groups: Control (n = 6), Acacia (n = 6) and Acacia + polyethylene glycol (PEG) (n = 6). Animals from Acacia and Acacia+PEG groups received in a diet the concentrate (88% corn and 12% soybean) with CT from *A. mangium* bark at the dose 100 mg/kg BW/animal/day. The Acacia+PEG group received the diet with 10 g of PEG/day. After 15 days of dietary supplementation with *A. mangium*, all animals were orally infected with 6600 L3 of *H. contortus* and 5400 L3 *T. colubriformis*. After 35 days of artificial infection, the animals were slaughter and gastrointestinal parasites burden was evaluated. Blood and faeces were collected for determination of immunological and parasitological parameters, respectively. The results revealed that supplementation with the Acacia induced changes in the immunophenotypic profile of goats, significantly increasing the number of TCD4+ and IL-4 in Acacia group (P <0.05); and IL-4 in TCD8+ and IFN-γ in TCD8+ in Acacia and Acacia+PEG groups compared to the control group (P <0.05). The increase of lymphocytes was correlated with the reduction *T. colubriformis* adults (r = -0.7746; P <0.0001). We conclude that *A. mangium* bark powder can modulate the immune response involved in the control of infections with gastrointestinal nematodes.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C304/0607

Failure of anthelmintic treatment with monepantel in sheep in Brazil

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The failure of the routine treatment with monepantel was reported in a farm with mixed production of sheep and goat in the Northeast of Brazil. The aim of this study was to evaluate the efficacy of monepantel to control sheep and goat nematodes in Brazil. A fecal egg count reduction test (FECRT) was performed with these species. Two groups of goats (n= 18, each group) and sheep (n= 12, each group) with fecal egg count level (FEC) ≥ 200 were selected, blocked by FEC and randomly assigned as following: 1) untreated control and 2) treated with monepantel (Zolvix®, Novartis Animal Health) at the dose of 2.5 mg/kg of body weight for sheep and 5.0 mg/kg for goats. Fecal samples were collected directly from the rectum from each animal on day 0 and on day 14 post-treatment and FEC and fecal culture were performed. The FECR was 93.0% (95% CI = 69.0 – 99.0) and 97.0% (95% CI = 87.0 – 99.0) for sheep and goats, respectively. The genus *Haemonchus* was the most prevalent. A confirmatory slaughter study is been conducted using 10 sheep/group. Here we show the first monepantel treatment failure in sheep in Brazil.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C305/0610

In vitro* activity of hydroalcoholic extracts of four strains of the edible mushroom *Pleurotus djamor* on infective larvae of the parasitic nematode *Haemonchus contortus

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Haemonchus contortus is a hematophagous and pathogenic abomasal nematode, causing one of the most important gastro-enteric diseases in sheep. The control of this nematode is based on the use of anthelmintics, however it has been reported at the global and national level the phenomenon of anthelmintic resistance. There is an urgent search for alternative methods. Edible mushrooms have a great etnomedicinal tradition because of their high potential to produce secondary metabolites with different therapeutic properties. In the present study it has been evaluated the *in vitro* activity of hydroalcoholic extracts of four strains of the edible mushroom *Pleurotus djamor* on infective larvae of *H. contortus*. Carpophores of four strains of *P. djamor* (ECS-0142, ECS-0143, ECS-0127 and ECS-0123) were produced on Pangola (*Digitaria decumbens*) grass following regular procedures. *H. contortus* were obtained from an artificially infected hair sheep. Hydroalcoholic extracts were obtained through the maceration process. The concentration of extract used was: 20, 40, 80 and 175 mg/mL. Bioassays were performed in 96 wells ELISA plates from. 80 µL of the mushroom extract and 20 µL of an aqueous solution containing 200 sheathed and unsheathed larvae of *H. contortus* were deposited. The control group commercial anthelmintic (1% Ivermectin) and control group distilled water was placed. Four repetitions were made per treatment. Larval motility was observed under a microscope at 24, 48 and 72 h post-confrontation. Data were analyzed by ANOVA test Tukey test was used for mean comparison. *In vitro* mortality (%) of the hydroalcoholic extract of *P. djamor* at different concentrations (20, 40, 80 and 175 mg/mL) at three times (24, 48 and 72 h post-confrontation) unsheathed and sheathed larvae of *H. contortus* (CV=sheathed larvae) (SV=unsheathed larvae). *P. djamor* (ECS-0123) showed 100% larval mortality at a concentration of 175 mg/mL at 72 h post-confrontation against unsheathed larvae and 99.9% on infective sheathed larvae of *H. contortus*. These results point to an inherent anthelmintic potential in *P. djamor* and warrants further investigation to identify the active moiety its mode of action. This study was funded by resources of the INIFAP (Project No. 9442232005).

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C306/0648

Anti-cryptosporidial activity of Egyptian propolis and garlic oil against *Cryptosporidium* oocysts *in vitro*

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The present study aimed to evaluate the efficacy of ethanolic extract of propolis (EEP), water extract of propolis (WEP) and garlic oil (GO) against *Cryptosporidium* spp. *in vitro*. *Cryptosporidium* oocysts were isolated from faecal samples of naturally infected neonatal calves and propagated in goat kids then purified and stored at 4°C. For infectivity test, seventy female BALB/c mice, weighing 18-20g, were randomly assigned into 10 equal groups and numbered individually. These mice were injected subcutaneously with 0.5ml of 4mg/ml dexamethasone every 48h for 8 days. On the day of the last injection, the groups from II to X were inoculated orally with 10⁶ *Cryptosporidium* oocysts pre-suspended in different concentrations (6.25, 25 and 50 mg/ml) of EEP, WEP and GO. Untreated control group I was inoculated with 10⁶ *Cryptosporidium* oocysts. The oocysts shedding were recorded daily from the 1st to the 9th day post infection. Faecal smears were stained by modified Ziehl-Neelsen and oocysts counts per 50 fields at 100× magnification were determined. The overall oocysts count was obtained by collecting the oocysts scores of all mice at all periods. The result revealed that all extracts with different levels reduced faecal oocysts shedding but marked reduction ($P<0.001$) was observed in mice infected with oocysts pre-treated with WEP at concentrations 6.25 and 50 mg/ml compared to untreated control group. Also, at a concentration of 25 mg/ml, faecal oocysts count significantly decreased ($P<0.001$) in mice infected with oocysts pre-treated with WEP than that EEP and garlic oil. In conclusion, Egyptian WEP has a powerful anti-cryptosporidial activity than the EEP and GO.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C307/0652

Effect of condensed tannins on adult nematodes

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Objectives: Condensed tannins (CTs) are polyphenolic compounds that are synthesised by many plants as secondary metabolites. It has been shown that CTs have anthelmintic (AH) activity against various life stages of parasitic nematodes [1, 2]. Here, we examined the relationship between CT molecular structures and AH activity by preparing a series of purified CTs with contrasting structural characteristics and using these to screen for AH activity against adult nematodes *in vitro*. For this purpose we applied the free-living nematode *Caenorhabditis elegans* which is commonly used as a model of parasitic gastrointestinal nematodes [3]. Use of the model avoided sacrifice of experimental animals and drawbacks related to *in vitro* culturing of parasitic adult stage. **Methods:** CTs from various plant materials (e.g. white clover, black currant, birch leaves, willow bark and *Tilia* inflorescence) were extracted with acetone/water and fractionated by Sephadex LH-20 chromatography. Characterisation of B-type CTs was performed by thiolysis, HPLC and LC-MS [1]. CT effects were tested *in vitro* by measuring motility inhibition of adults [3]. The EC₅₀ values were estimated using curve fitting to a range of dilutions. **Results:** A series of B-type CTs was isolated, which covered mean degrees of polymerisation (mDP) from 7.8 to 16.6 (i.e. average molecular size of 2364 to 5010 Da respectively), % procyanidins ranged from 1.2 to 99.1 and % *cis* flavan-3-ols from 11.1 to 95.6. The preliminary analysis showed weak correlations between *C. elegans* motility and mDP or *cis/trans* ratios. However, prodelphinidin-rich fractions appeared to have higher AH activity compared to procyanidin-rich (with correlation of R²=0.911). **Conclusions:** The *C. elegans* adult stage proved to be a suitable model for anthelmintic screening [3] of contrasting CTs. It is concluded that the procyanidin/prodelphinidin ratio was a key characteristic for predicting B-type CT activity against adult *C. elegans* nematodes. Further experiments are planned to verify this finding. **References:** [1] Williams A.R., Ropiak H.M., Fryganas C., Desrues O., Mueller-Harvey I., Thamsborg S.M., (2014) *Parasit Vectors*, **7**, 518. [2] Novobilský A., Mueller-Harvey I., Thamsborg S.M., (2011) *Vet Parasitol*, **182**, 213-220. [3] Katiki L.M., Ferreira J.F., Zajac A.M., Masler C., Lindsay D.S., Chagas A.C., Amarante A.F., (2011) *Vet Parasitol*, **182**, 264-268.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C308/0675

Effect of parasitic gastroenteritis on haematological markers, albumin and body condition in sheep and goats in the Czech Republic

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Parasite occurrence in sheep and goat farms is one of the main problems which the breeders and keepers have to deal with. Parasitic infection can be tolerated in low and middle intensity but when it raises more than the organism is able to accept it manifests by general health alterations. The degree of pathogenity often depends on immunity and nutrition quality.

This study focuses on the severity of parasitological gastroenteritis (PGE) in comparison with body condition (BSC), haematological and biochemical parameters in sheep and goats in the Czech Republic.

We examined 207 blood and faeces samples from 53 goats and 154 sheep during years 2013 and 2014. Faeces sampling was performed individually from rectum and blood was taken from the jugular vein. Faeces was tested by flotation, sedimentation and valued semi-quantitatively (+, ++, +++). EPG assessment was done with the Flotac method but only if the parasitic occurrence was higher than one +. In blood samples we analysed albumin and basic haematological markers.

The overall prevalence of gastrointestinal nematodes was 86 % in sheep and 87 % in goats often in low and middle intensity. Trichostrongylid nematodes including *Haemonchus contortus* were found the most frequently. Albumin values were in helminth infested animals under the physiological level in 65 % in goats and 48 % in sheep. Haematological parameters were measured bellow the physiological level in haematocrit in 52 % goats and 26 % sheep; haemoglobin in 45 % goats and 20 % sheep. Goats older than 3 years bear the situation considerably worse in contrast to sheep; the worst health alterations were in one-year-old and younger sheep.

BSC was not responsible to parasitic infection. 12 % negative goats and 7 % negative sheep were emaciated (BSC 1- 2).

Alterations of analysed parameters were clearly worse in farms where we noticed inadequate nutrition. Goats are more sensitive namely during milk production. Sufficient nutrition and adequate breeding and keeping conditions are necessary.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C309/0686

Paramphistomicidal potential and tegumental alterations in medicinal plant treated flukes

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Paramphistomiasis due to *Paramphistomum cervi* fluke which is the most important rumen and reticulum disease in veterinary medicine. Its relevance is important because of the major economical losses in the water buffalo industry such as: reduction in milk and meat, increase in anemic conditions, and occasionally deaths, are estimated in billions of dollars. The emergence of fluke resistance due to over or under dosing of conventional chemotherapies as well as environmental damage produced by the chemicals eliminated in field have stimulated the need for alternative methods to control flukes. In this scenario, researchers are exploring secondary metabolites of medicinal plants which can pave a new way in ethnoveterinary therapies. The aim of this study was to evaluate *in vitro* flukicidal effect of four native methanolic plant extracts of ethnopharmacologically importance by adult motility assay. The flukes were exposed in triplicate at 50, 25, 12, 6 and 3 mg/ml to each extract viz; seeds of *Eruca sativa*, leaves of *E. sativa*, peels of *Citrus sinensis* and leaves of *Convolvulus arvensis*. The efficacy was assessed as the mortality rate based on the number of live and dead flukes after every hour till 9hr post-exposure to calculate lethal concentrations at 50% (LC50). Across the whole series of tests carried out, methanolic extract concentrations of *E. sativa* seed ($p<0.05$), time of exposure ($p<0.05$) and their interactions ($p<0.05$) had statistically significant effects on the number of dead flukes (*Paramphistomum cervi*) as compared to *C. sinensis*, *C. arvensis*, *E. sativa* (leaves), respectively. Moreover, histological study was also carried out to study the effects and possible mode of action of selected plant extracts on tegument of tested flukes. Result revealed that *E. sativa* seed with $LC_{50}=7.208\text{mg/ml}$ cause severe erosion on fluke tegument as compared to positive control (Albendazole) and other medicinal plant treated flukes. It is concluded that said four plant extracts had promising anthelmintic effects against *P. cervi*. Further studies on toxicity and *in vivo* biological evaluation in ruminant models might help to determine the flukicidal potential of these plant extracts.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C310/0704

In vitro* anthelmintic activity of leaves and husk extracts of three *Theobroma cacao* varieties against *Haemonchus contortus

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This study assessed the *in vitro* anthelmintic (AH) effect of acetone-water extracts of leaves and husks of *Theobroma cacao* on egg hatching of *Haemonchus contortus*. Acetone:water (70:30) extracts were produced with husk and leaves of three varieties of *T. cacao* (Azt, Cal, Cey). Extracts were evaluated with the egg hatch assay. Respective *H. contortus* isolates (CENID and FMVZ) were obtained and incubated in PBS and different extract concentrations (0, 600, 1200, 2400 and 3600 ug / ml PBS). Effective concentrations 50% (EC50) were calculated for each extract in both isolates. Polyvinylpyrrolidone was used to determine the role of polyphenols in the AH activity. The EC50 obtained for leaf extracts of *T. cacao* on CENID were 1618.89 (Azt), 3316.65 (Cal) and 2558.73 (Cey). The EC50 of leaf extracts on FMVZ were 2282.89 (Azt), 3070.70 (Cal) and 2801.32 (Cey). In both cases the Azt extract had lower EC50 ($P > 0.05$). The EC50 obtained from fruit husk, showed that Cey variety had similar effect on both *H. contortus* isolates (1544.39 vs. 1304.99; $P > 0.05$). Meanwhile, the FMVZ isolate was more susceptible to Azt and Cal extracts, than CENID isolate ($P < 0.05$). The EC50 for FMVZ and CENID were 1533.53 vs 4174.37 (Azt) and 1223.66 vs 3378.45 (Cal). Contribution of polyphenols to the AH activity reported was low to nil. The AH activity of *T. cacao* leaves and husks against *H. contortus* eggs was not strong, however, it helped to perceive variation due to variety of *T. cacao* and by-product, as well as worm isolate.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C311/0716

Inactivation of *Ascaris suum* eggs in composting manure

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Introduction: *Ascaris suum* is the most common helminth in most pig production systems. The result is a high number of eggs in manure and slurry that may potentially contaminate agricultural land if not handled correctly. To inactivate the eggs under Danish conditions slurry should be stored for one year or more to inactivate depending on the temperature, but survival of eggs in composting manure has not previously been examined.

Methodology: Manure and straw bedding was obtained from a deep litter pen on an organic pig farm mid-July 2013. The material was arranged in three round stacks that were 1.2 m high and 2 m in diameter at the base. "Pie" sections of each stack were marked and three sealed nylon bags (20 µm mesh size) with 3000 unembryonated *A. suum* eggs (initial viability: 98%) isolated from fresh faeces were placed in close proximity at 15 cm from the centre and 20 cm from the surface in three layers (30, 60 and 90 cm from the ground). In addition, two nylon bags per section were placed within the outermost 5 cm of the stack. Each stack was covered by a tarpaulin. Every week for four weeks, the temperature was recorded and the bags recovered from one section. The eggs were isolated and washed in tap water before incubation in H₂SO₄ buffer pH1 at 20°C for six weeks to test viability.

Results and discussion: Temperatures increased rapidly and steam was emitted from the top of the stacks within 24 hours. Mean temperatures (week 1-4) in the stack surface, middle and center (irrespective of layer) were 43-31°C, 63-37°C and 67-56°C, respectively. Non-viable eggs mainly died before onset of embryonation. Viable eggs were only recovered from the surface of mainly one stack where temperatures were less severe. Overall, 89% of the surface eggs were dead, but viable eggs were still present week 4.

Conclusions: The vast majority (>98%) of *A. suum* eggs appeared to be inactivated within the first few days due to a rapid temperature increase due to microbial activity that was boosted when the manure and straw was aerated during stack construction. On-farm, the material should be aerated and stacks turned inside out when the inside of the stacks become too hot to touch (approx. 70°C) to ensure inactivation of even surface eggs.

C: Novel approaches to chemical and non-chemical control (endo and ecto-parasites)

Ref: P2C312/0719

Evaluation of the efficacy of Fluron® Gold Pour On in the treatment of cattle experimentally infested with *Rhipicephalus (Boophilus) microplus* in Brazil: Stall Test.

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The objective of this study was to evaluate the therapeutical efficacy of **Fluron® Gold Pour On**, a novel association of cypermethrin (5%), chlorpyrifos (7%), fluazuron (2.5%) and piperonyl butoxide (5%) in the treatment of cattle experimentally infested with *Rhipicephalus (B.) microplus* in a stall test. Twelve mixed breed (holstein) calves were used. All animals were infested on their back three times a week, specifically on their neck and tail with insertion of 5,000 larvae in alternate days from D-25 to D-1. After treatment they were infested once a week with 7,500 larvae. Animal's randomization in two groups was done considering engorged female ticks fall between days -3 and -1. Day 0 was considered the treatment day when the six animals from the treated group received a single topical administration of **Fluron® Gold Pour On** (pour on route) at a dose of 1mL/10 kg b.w. Animals from negative control group (6 animals) did not received any treatment. From D-3 until the end of the study, all engorged females, from each animal in both groups, were collected and counted. In order to evaluate reproduction rates, engorged female ticks belonging to each group were weighed and incubated. After incubation period, engorged female ticks were discarded and the eggs were weighed and placed within test tubes for posterior evaluation of hatching percentage. The mean percentage of efficacy against ticks on the animals was 94.32%, considering the period that the product was more effective (D+5 until D+23). Efficacy evaluations have shown also that **Fluron® Gold Pour On** was also effective in preventing animals against new infections with superior level of 95% up to 63 days after treatment. The reproductive fitness index (RFI) and the percentage of reproductive inhibition (reproductive viability) was also evaluated and the results showed a RFI of 93.85% considering the mean between D+1 to D+23 and a reproductive inhibition percentage of 99.88% in the post-treatment phase. The results have shown that an association among cypermethrin, chlorpyrifos, piperonyl butoxide and fluazuron conferred an immediate efficacy against ticks and a prophylactic effect up to 63 days.

D: Vaccines and immunology

Ref: P2D400/0023

The putative role of *Rhipicephalus microplus* and *Ixodes ovatus* cystatins in tick physiology

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Studies about cystatins in parasites have contributed to clarify their roles in blood digestion and modulation of host immune response during blood feeding. However, cystatin functions in ticks remain undetermined, especially. Here we report the inhibitory profile of two in *Rhipicephalus microplus* (BrBmcys2b and BrBmcys2c) and one *Ixodes ovatus* (Jplocys2a) cystatins. Also, a peptide from Jplocys2a was synthesized for rabbit immunization, which in turn afforded to obtain a serum that was used to analyze the cross-antigenicity between *R. microplus* and *I. ovatus* cystatins. The enzymatic inhibition profile of tick cystatins showed a distinct modulation for cathepsins related to tick blood digestion and evasion of host immune response. Furthermore, western blot showed that BrBmcys2b was detected in saliva and different tissues along tick stages, while BrBmcys2c was detected mainly in the gut from partially engorged *R. microplus* females, demonstrating a distinct pattern of cystatin expression, secretion, and traffic between tick tissues. Finally, cross-antigenicity assays revealed that antibodies against the Jplocys2a peptide recognize native and recombinant *R. microplus* cystatins. The presence of these proteins in different tissues and their ability to differentially inhibit cathepsins suggest distinct roles for Jplocys2a, BrBmcys2b, and BrBmcys2c in blood digestion, egg and larvae development, and modulation of host immune response in tick physiology. The cross-antigenicity between native and recombinant cystatins supports further experiments using Jplocys2a, BrBmcys2b, and BrBmcys2c as vaccine antigens.

D: Vaccines and immunology

Ref: P2D401/0024

***Babesia bovis* as a potential vector for an anti-tick vaccine**

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The tick *Rhipicephalus microplus* is a parasite vector for several pathogens, including the protozoan *Babesia bovis*. The control of this tick is based on chemical acaricides, but due to the risk of environment contamination and selection of drug-resistant populations, the use of acaricides should be replaced by other methods, such as vaccination. The *Haemaphysalis longicornis* glutathione-S transferase (HIGST) is a candidate for such anti-tick vaccines. Control of *B. bovis* can be achieved by eradicating ticks vectors, and through vaccination, using attenuated *B.bovis* strains. Thus, a possible platform for immunological control of ticks could be based on the use of a recombinant *B. bovis* expressing a protective tick gene, such as HIGST, as a live vector vaccine. The aim of this work was to develop transfected *B. bovis* expressing a tick protective antigen. Thus, the *B. bovis* T2Bo strain was stably transfected with a transfection plasmid containing the *bidirectional promoter of the B.bovis* 1.4 Kb *ef-1α* intergenic region responsible for controlling expression of the selectable marker GFP-BSD and HIGST genes. Single cell clones expressing HIGST and GFP were isolated via a FACS-based sorting method. Expression of HIGST by transfected parasites was demonstrated by fluorescence microscopy, RT-PCR and western blot. In both parasite lines the insertion of the transfected genes occurred in the targeted *ef-1α* locus. In conclusion, a clonal line ready for immunization purpose was obtained, reinforcing the potential use of transfected *B .bovis* in a live vector vaccine against *R. microplus*.

D: Vaccines and immunology

Ref: P2D402/0025

Three salivary serpins from the cattle tick *Rhipicephalus microplus*: insights into the tick-host relationship

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The cattle tick *Rhipicephalus microplus* is a hematophagous parasite and its saliva contains bioactive compounds that modulate host defense mechanisms. A proteomic analysis revealed that its saliva contains three serpins (RmS-3, RmS-6 and RmS-17) which could be involved regulating host defense mechanisms such as inflammation, complement activation and blood clotting. A characterization was performed to predict their physiological role in tick feeding. Three serpins coding cDNA sequences were cloned and expressed in *Pichia pastoris* system. The purified proteins were used to assay their inhibitory activity against enzymes related to host defense systems. Serum from animals infested with different tick species were used to check cross-reactivity against recombinant serpins. The three recombinant serpins are glycosylated and bind to serum from cattle infested with *R. microplus*, serum from rabbits infested with *Amblyomma americanum*, *R. sanguineus* and *Ixodes scapularis* ticks. These salivary serpins have distinct enzymatic inhibition profiles: rRmS-3 has anti-chymotrypsin and anti-cathepsin G activity; rRmS-6 has anti-trypsin, anti-plasmin and anti-factor Xa activity; and rRmS-17 has anti-chymotrypsin, anti-trypsin, anti-cathepsin-G and anti-plasmin activity. Furthermore, rRmS-3 and rRmS-17 inhibited platelet aggregation induced by cathepsin G. Since enzymes such as trypsin-like, chymotrypsin-like, cathepsin G, and plasmin are involved in processes that enhance inflammation and platelet aggregation, we suggest that these salivary serpins have a role as immunomodulatory and anticoagulant proteins in *R. microplus*.

D: Vaccines and immunology

Ref: P2D403/0036

***Cryptosporidium parvum* induces NET formation and modulates cytokine and chemokine gene transcription in bovine PMN**

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Cryptosporidium parvum is a zoonotic infection with worldwide distribution. Besides humans, cryptosporidiosis affects a wide range of animals leading to significant economic losses due to severe enteritis in neonatal livestock. Neutrophil extracellular trap (NET) formation has been demonstrated as an important host effector mechanism of PMN acting against several invading pathogens. In the present study, *C. parvum*-mediated NET formation was investigated *in vitro*. We here demonstrate that *C. parvum* sporozoites indeed trigger NET formation in a time-dependent manner. Thereby, the classical characteristics of NETs were demonstrated by co-localization of extracellular DNA with histones and myeloperoxidase (MPO). A significant reduction of NET formation was measured following treatments of PMN with NADPH oxidase-, NE- and MPO-inhibitors, confirming the key role of these enzymes in *C. parvum*-induced NETs. Additionally, sporozoite-triggered NETosis revealed as dependent on intracellular Ca⁺⁺ concentration and the ERK 1/2 and p38 MAPK-mediated signaling pathway as detected by inhibitor treatments. Moreover, sporozoite-triggered NET formation led to significant parasite entrapment since 15 % of the parasites were immobilized in NET structures. Consequently, PMN-pre-exposed sporozoites showed significantly reduced infectivity for epithelial host cells confirming the capability of NETs to prevent active parasite invasion. Besides NETs, we here show that *C. parvum* sporozoites also modulate the cytokine and chemokine gene transcription in PMN, since a significant upregulation of the mRNAs of CXCL8, IL6 and TNF α and of GM-CSF was observed upon sporozoite confrontation, indicating an overall important role of PMN in innate immune reactions against *C. parvum*.

D: Vaccines and immunology

Ref: P2D404/0069

***In vivo* evidence of *Eimeria arloingi*-induced "Neutrophil Extracellular Trap" formation**

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Coccidiosis caused by different *Eimeria* species is a globally spread and economically important disease in farm animals and related costs caused by treatments, poor performances and even death of heavily infected animals are generally high. Polymorphonuclear neutrophils (PMN) form the first line of defence against pathogens and usually represent the first leukocytes to be recruited to the site of infection. Besides phagocytosis and the production of antimicrobial molecules, PMN are able to attack pathogens via the release of so-called "Neutrophil Extracellular Traps" (NETs). Recent *in vitro* analyses on parasite-triggered NET formation have shown that PMN release NETs in response to *E. arloingi* sporozoites thereby immobilizing them and hampering them from host cell invasion. Here we sought to provide *in vivo* evidence for this mechanism. Therefore, gut samples of an *E. arloingi*-infected kid were analysed histologically for characteristics of NETs. Overall, histological analyses revealed the presence of NET-like structures in *E. arloingi*-infected gut mucosa in close proximity to parasitic stages. Classical characteristics of this mechanism were confirmed via immunofluorescence analyses showing the co-localization of extracellular DNA with nuclear histones and neutrophil granular proteins, such as neutrophil elastase. Interestingly, we observed different forms of NETs, i. e., outspread, diffused and aggregated NETs in infected mucosa. These findings clearly prove NETosis to occur *in vivo* during coccidiosis and suggest NETs to play an important role in the early innate host response against *E. arloingi* infection in goats.

D: Vaccines and immunology

Ref: P2D405 083/0077

Establishment of an ELISA for the detection of *Cystoisospora suis* antibodies in pigs

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Recent findings on protective effect of humoral immunity on *C. suis* infection encouraged us to establish a diagnostic tool for further research on this topic. We aimed to establish an ELISA to monitor the experimental infection of sows and piglets and the development of antibodies of different subclasses as markers of infection and protection. For this purpose soluble proteins of *C. suis* oocysts crude antigen were used. Infected piglets' blood serum, infected sows' blood serum and their colostrum/milk samples with pre-determined IFAT in a previous study were examined with selected anti-pig antibody subclasses. Correlations between recorded OD and IFAT results were analysed. Optimum amounts of antigen, dilution of sera and conjugates were determined performing checkerboard titration. Analyses showed strong correlations between ELISA and IFAT results. The current assay showed to be capable of detecting changes in antibody levels in tested samples in a more convenient and faster way compared to the existing IFAT. This research tool enables us to optimize planning of larger studies involving sows and their piglets for immunization studies.

D: Vaccines and immunology

Ref: P2D406/0094

Safety and potency of trivalent frozen live attenuated tick fever vaccine under experimental conditions in Thailand

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Tick borne diseases are caused by protozoal parasites, *B.bovis*, *B.bigemina* and rickettsial parasite, *A.marginale*. The diseases caused economic impact world-wide. To present, many forms of vaccine were produced for preventive purpose. The objective of this study is to find that the trivalent frozen live attenuated tick fever vaccine could protect cattle against local strains of tick fever parasitic agents in Thailand. Sixteen 3 months old female HF calves from a private dairy cattle farm were vaccinated under experimental condition. The purity test, only DNA of *B. bigemina*, *B. bovis* and *Anaplasma centrale* were detected whereas no DNA of *Brucella* spp. and bovine leukemia virus were detected by using the PCR. The safety test, two calves were observed for 8 weeks after vaccinated with double doses with no clinical sign. For potency test, fourteen calves were divided to two groups: ten for vaccinated and four for unvaccinated group. Sera was taken twice at day 0 and 8 weeks after vaccination to study protective level against *B. bigemina* and *A. marginale* using the iELISA. After vaccination antibody response against *B. bigemina* and *A. marginale* were increase. Eight weeks after vaccination, all calves were challenged with 7.6×10^7 infected RBC with *A. marginale* and 2.5×10^8 infected RBC with *B. bigemina* and observed for another 8 weeks. Unvaccinated group showed abnormal clinical sign: pale mucus membrane, anorexia, high body temperature, low haematocrit value. In conclusions, the vaccine could protect against local strains of tick fever parasitic agents in Thailand.

D: Vaccines and immunology

Ref: P2D407/0097

Metalloproteinases in *Echinococcus granulosus* and hydatid patients: Parasite-host relationship

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Echinococcus granulosus is among the most important parasite worldwide. This helminth engages a plethora of surface and secreted biomolecules to invade tissues and to modulate host immune response. It is accepted that the outer layer of hydatid cyst represented by the laminated layer (LL) plays a relevant role in parasite evasion. However, it's was also reported that LL is permeable to host molecules. In our present study, we aim to investigate the presence of matrix metalloproteinases (MMPs) in the soluble extract of LL (LLs) and sera of hydatid patients (HPS) by gelatin zymography. EDTA was used as an MMP inhibitor. MMPs were involved not only in the degradation of extracellular matrix, but also in the modulation of immune response. Our results show a strong proteolytic activity in the LLs and HPS. However, both exhibit a different electrophoretic pattern, with the presence of latent MMP9 and active MMP9 respectively in LLs and HPS. Interestingly, both share a band corresponding to active MMP2 (61kDa). Further studies are needed to investigate the parasitic or host origin of MMP in the LL and their role in host relationship.

D: Vaccines and immunology

Ref: P2D408/0191

***Cystoisospora suis* antigens: Analysis by SDS-PAGE and Western blot**

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Cystoisospora suis is a ubiquitous apicomplexan parasite in the intensified pig production system and is the causative agent of neonatal porcine coccidiosis. Oocysts isolated from the feces are currently only the source of antigens. The aim of the present study was to examine protein profiles of the early endogenous stages of *C. suis* namely sporozoites and merozoites. Sporozoites collected after excystation of sporulated oocysts and merozoites harvested after *in vitro* culture, were subjected to trichloroacetic acid (TCA) precipitation and/or SDS treatment. To obtain antigenic markers, proteins were separated by SDS-PAGE and probed with highly positive sera obtained from infected piglets in Western blot. SDS-PAGE fingerprint analysis showed that protein patterns of both sporozoites and merozoites ranged from 10 kDa to 250 kDa. Western blot analysis, however, revealed three predominant bands of 45, 70 and 75 kDa for both sporozoites and merozoites while 50 kDa and 105 kDa bands were specific to sporozoites only. The band patterns of TCA precipitated pellets of merozoites dissolved in either PBS or DIGE buffer matched, suggesting that these protein fractions mainly represent hydrophilic proteins. Further investigation of immunogenic proteins of *C. suis* may facilitate in the identification of possible candidates for diagnosis and vaccination against porcine cystoisosporosis.

D: Vaccines and immunology

Ref: P2D409/0196

Studying the lympho-proliferative responses against various *Fasciola hepatica* antigens: An approach for future vaccine development

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Fasciola hepatica, which can infect a wide variety of mammals, is able to survive within the bile ducts of their hosts for many years through its ability to suppress host immunity. In the present study, lympho-proliferative responses of hepatic hilar lymphocytes of infected sheep in response to different *F. hepatica* antigens and the suppressive effects of excretory/secretory (ES) and tegument (TEG) antigens were investigated. Fractionation of these antigens was carried out according to their molecular weights and the suppressive responses of these fractions were also investigated. Our results showed that when ES was added with soluble worm antigens (SWA), it caused a significant reduction ($P < 0.05$) in the proliferation and this reduction was greater with concentration of $10\mu\text{g/ml}$ (74% reduction). When TEG was added with SWA, it caused significant reduction ($P < 0.05$) in the proliferation response and this reduction was greater with higher concentrations, $5\mu\text{g/ml}$ and $10\mu\text{g/ml}$ (87% and 92% reduction, respectively). When lymphocytes were incubated with different fractions of ES with SWA, fraction 3 (MW >10000 - 30000) showed the greatest reduction of the proliferative effect of SWA (64% reduction). When HLN were incubated with different fractions of TEG with SWA, fraction 3 showed the greatest reduction of the proliferative effect of SWA (59% reduction), followed by fraction 4 (MW of ≤ 10000) with 38% reduction. The suppressive effects of fractions 3 of ES and TEG could be attributed to glutathione S transferase and/or cathepsin L which are important vaccine candidates. However, the suppressive effects of fractions 4 of TEG needs further investigation to identify the responsible agent(s) which could be potential vaccine candidate(s).

D: Vaccines and immunology

Ref: P2D410/0198

Proteomic analysis of the *Fasciola hepatica* secretome for vaccine development

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The liver fluke *Fasciola hepatica* is an economically important pathogen of livestock worldwide. Parasite control is reliant on the use of drugs, particularly triclabendazole (TCBZ), which is effective against multiple parasite stages. However, with the spread of parasites resistant to TCBZ discovering novel control strategies, such as vaccine development, is now a major focus for *F. hepatica* researchers. Our pursuit for a vaccine has focussed on understanding fluke biology and how this relates to survival in the host. We have specifically interrogated the excretory-secretory (ES) proteins that we believe act at the host-parasite interface and are central to infection, virulence and long-term survival within the definitive host.

Here, we report the generation of extensive proteomic datasets of the ES proteins for the *F. hepatica* lifecycle stages found within the mammalian host, including metacercariae, newly excysted juveniles (NEJ), liver-tissue juveniles and adult flukes. In particular, this analysis reports for the first time the NEJ secretomes covering the first 24 hours of infection, namely that of 1hr, 3hr and 24hr post-excystment NEJs, a prime target for vaccine development. This extensive dataset reveals the complexity of the ES proteins and the changes in the molecules that *F. hepatica* releases into the host during its lifecycle. Integrating this analysis with the newly reported *F. hepatica* draft genome and transcriptome reveals the complexity of important multi-copy gene families, for example the cathepsin cysteine B and L cysteine proteases, revealing the stage-specific and relative expression of each member, and pin-pointing those that are secreted and those that play house-keeping functions. These investigations are vital to our understanding of fluke/host interactive biology and will be exploited for vaccine development. Several proteins have been identified from this study for future characterisation and vaccine testing in ruminants.

D: Vaccines and immunology

Ref: P2D411/0205

Presence of CD4⁺ and CD8⁺ T lymphocytes and macrophages in the gut of dogs naturally infected with *Leishmania infantum*

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The aim of this work was a semi-quantitative analysis about the presence of T lymphocytes (CD4⁺ and CD8⁺) and macrophages in the intestinal wall of dogs naturally infected with *Leishmania infantum*. For this work, 10 positive dogs for canine visceral leishmaniasis (CVL) were euthanized in the Zoonosis Control Center, and three negative for CVL (control group) were dead in traffic accidents or in surgical procedures. The animals did not show any co-infection with other gastrointestinal parasites. The dogs were divided into three groups: group 01 (G1, n = 5) were CVL positive dogs with *L. infantum* amastigotes in the intestine; group 02 (G2, n = 5) were CVL positive dogs without intestinal amastigotes; and group 03 (G3, n = 3) were CVL negative dogs. The identification of the parasite amastigotes in the intestinal tissues and the cells was by immunohistochemistry or histochemistry. It was not observed significant statistical difference ($p \leq 0.05$) in numbers of CD4⁺ T lymphocytes among groups G1, G2 or G3. However, there was significant increase ($p \leq 0.05$) of CD8⁺ T lymphocytes and macrophages in the group G1 in relation to G2 and G3 groups, mainly in duodenum and colon, which were the most infected organs. The significant increase of CD8⁺ T cells and macrophages, but not CD4⁺ in the intestinal wall tissues of G1 dogs associated with the high parasitic intensity suggest a possible immune regulation by the parasite.

D: Vaccines and immunology

Ref: P2D412/0230

Uncovering immune suppressor molecules in exosome-like vesicles of liver fluke (*Fasciola hepatica*)

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Fasciola hepatica is known to regulate the host immune system. Whilst the Th1 immune response predominates during early infections, in chronic cases they are suppressed in favour of the Th2 immune response. While research has embarked upon the host innate immune response towards *F. hepatica*, few investigations have researched molecular interactions on a cellular level. With Fascioliasis control limited to unsustainable chemotherapy, current research effort is now focussed towards vaccine development with immune modulators as ideal candidates. Recently, exosome-like vesicles (EVs) have been of particular interest in many fields, including veterinary parasitology. EVs (30-100 nm), of endocytic origin, have been shown to function in host-parasite communication, the transfer of pathogenic proteins and the release of toxins and enzymes. Furthermore, EVs of *F. hepatica* have been shown to contain immune suppressor molecules (ISMs), such as FhGST-S1. Using transmission electron microscopy for EV confirmation, a proteomic lead approach (Gel based: 1D and 2D SDS-PAGE electrophoresis and Western blotting; Gel-Free: LC-MSMS) has been developed to identify likely *F. hepatica* ISMs. Proteins located both within *F. hepatica* EVs and upon the surface have been investigated to identify additional ISMs. Both newly identified ISMs and whole EVs will be examined for their interaction with host immune cells and in parasite invasion with host intestinal cells utilising *in vitro* cell cultures. The experimental results gained will lead to increased knowledge of novel host-parasite immune interactions and to identify protein targets suitable for vaccine formulation.

D: Vaccines and immunology

Ref: P2D413/0313

Harbour seal (*Phoca vitulina*) PMN and monocytes release extracellular traps to capture *Toxoplasma gondii*

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Extracellular traps (ETs) are composed of nuclear DNA as backbone adorned with histones and antimicrobial molecules which are released from a range of host immune cells in response to several invading pathogens. So far, this innate defense mechanism was not demonstrated in any marine mammal. Interactions of harbour seal (*Phoca vitulina*) PMN and monocytes with viable tachyzoites of *Toxoplasma gondii* were investigated in this respect *in vitro*. Fluorescence-based assays as well as scanning electron microscopy analyses (SEM) demonstrated PMN- and monocyte-promoted ET formation rapidly being induced by exposure to *T. gondii*-tachyzoites. The co-localization of extracellular DNA with histones (H3), neutrophil elastase (NE) and myeloperoxidase (MPO) in parasite entrapping structures confirmed the classical characteristics of PMN- and monocyte-promoted ETs. Harbour seal ETs were efficiently dissolved by DNase I treatment and were significantly reduced by PMN and monocyte treatment with the NADPH oxidase inhibitor diphenilane iodondium. Tachyzoites of *T. gondii* were firmly entrapped and immobilized within *P. vitulina*-ETs structures hampering them from host cell invasion. To our best knowledge, we here report for the first time on *T. gondii*-induced ET formation in harbour seal-PMN and -monocytes. Our results strongly indicate that PMN- and monocyte-triggered ETs may represent a relevant and ancient conserved effector mechanism of the pinniped innate immune system acting against the pathogenic protozoon *T. gondii* and probably also against other foreign pathogens occurring in the ocean environment.

D: Vaccines and immunology

Ref: P2D414/0314

Histological characterization of *Hypoderma lineatum* (Diptera: oestridae) warbles

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Hypoderma larvae are tissue invading parasites which spend several months migrating within the host tissues before completing their development in the sub-dermal tissues of the back. In order to elucidate the microscopical structure of the warbles, three hides from warbled cows were collected in a slaughterhouse in Lugo (NW, Spain) between March-May 2012. A total of 60 skin samples, including warbles at different phases of development, were chosen for histopathological. Microscopic lesions were classified into six groups, according to the predominance and distribution of different cell populations. In warbles containing living larvae (type 1), plasma cells were observed in high number as well as in those with dead larvae but with apparently preserved cuticle (type 2). However, macrophages and lymphocytes were the predominant cells in granulomas (types 3 to 5) formed in relation to remnants of the dead parasite, containing or not remains of the altered cuticle. Scars (type 6) were characterized by granulation tissue. Our results suggest the predominance of a humoral response inside the warble as long as larvae are intact. Once they are destroyed, cellular response occurred, isolating and destroying the remains of the larvae until healing process completes and scars with low numbers of inflammatory cells appear.

*Supported by the Research projects AGL-2009-08939 and R2014/005

D: Vaccines and immunology

Ref: P2D415/0334

Characterisation of the extracellular vesicles secreted by *Fasciola hepatica*

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Helminth parasites release a range of molecules that perform crucial roles for parasite survival including the acquisition of nutrients and modulation/avoidance of the host immune response. Molecules displayed on the parasite surface or those secreted via classical pathways into the local host environment have been long regarded as the major players in the host-parasite interaction. However, the recent discovery that exosome-like extracellular vesicles (EVs) mediate molecular cross-talk between parasite and host make these of particular interest for study. To understand how parasite EVs influence host cell function, we set out to identify the cargo of potential effector molecules that are packaged within them. We isolated EVs secreted by the trematode parasite *Fasciola hepatica*, and using sequential extraction methods, identified the component proteins associated with the EV surface, vesicular membrane and lumen by mass spectrometry. We identified ~150 *Fasciola* proteins (including several proteases, antioxidants, metabolic enzymes and membrane channels) many of which are known exosomal markers. A number of homologs of proteins that regulate exosome formation and release from mammalian cells were also identified which may provide insight into EV biogenesis in *Fasciola* and whether this secretory pathway could be targeted by novel anti-fluke therapies.

D: Vaccines and immunology

Ref: P2D416/0396

Phylogenetic variation of the isoform 4 of H11 protein from *Haemonchus contortus*

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Haemonchus contortus is the most prevalent parasite with a significant economical importance in the tropics. Vaccine development represents the best alternative for parasite control. Promising results were obtained with the characterization of *H. contortus* hidden antigens and when sheep were immunized with H11 native complex, present at the microvilli of the intestinal cells of the parasite. The objective of this work was to determine the phylogenetic variation of the isoform 4 of the H11 protein from *H. contortus*. Faecal samples were obtained from sheep from 20 farms from the State of São Paulo, Brazil. DNA from larvae was obtained with phenol-chloroform protocol. Specific primers were used to amplify a 713bp and a 550 bp fragments. After purification, the products were sequenced by capillary electrophoresis (ABI-3130; Myleus Biotechnology, MG). The sequences were aligned with the algorithm ClustalW and the Vector NTI Suite 7 software for the H11 regions. We observed a low degree of molecular diversity and genetic structure for H11 with an average of 95.5% of identity between the samples, including the reference gene FJ481146.1 from GenBank. We identify 21 SNPs' (*Single Nucleotide Polymorphism*-SNP) one in the coding region (exon), 6 in the non-coding region (intron) and 14 of low importance. It was observed 3 substitutions in the transition (1 between purine, 2 between pyrimidine) and 4 substitutions that are transversion (2 between A to C and 1 between G to T). The data suggests a low genetic variation including a low genetic migration in *H. contortus*. The findings may have a positive implication to the development of a recombinant vaccine using the isoform 4 of the H11 protein, which could have a good efficacy against *H. contortus* in Brazil, reversing the terrible multidrug resistant scenario, and bringing an adequate parasite control.

D: Vaccines and immunology

Ref: P2D417/0557

Horn fly feeding behavior is affected by cattle immunization against a horn fly salivary antigen

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The horn fly, *Haematobia irritans irritans* (Linnaeus) is a blood-sucking livestock ectoparasite. It has been suggested that the immune response against fly saliva could regulate the parasite feeding behavior. In this context, we investigated the parasite loads and its feeding behavior on 15 naturally infected bovines, over the fly season 2010–2011. Parasite burdens fluctuated during the trial, and the haemoglobin content per fly decreased after fly population increases. Antibodies against a newly described fly salivary antigen (hematobin, HTB) were detected in cattle upon natural exposure to the fly and relative high antibody levels observed at the beginning of the summer tended to decrease fly haemoglobin consumption ($p = 0.066$). We also assessed the effect of immunization against recombinant HTB in naturally infected bovines. The immunized animals ($n=4$) had lower fly loads than control animals ($n=4$), indicating that the antibody response may contribute to reduce the blood intake by the flies; that, in turn, leads to a decrease in the fly population. Taken together, our results highlight a putative role for the immune response against salivary antigens in the fluctuations of the fly population during infection that should be taken into account when considering the design of parasite control strategies.

D: Vaccines and immunology

Ref: P2D418/0561

Withdrawn

D: Vaccines and immunology

Ref: P2D419/0687

***In vivo* depletion of Lymphocytes T $\gamma\delta^+$ /WC1⁺ in resistant Canaria Hair Breed Sheep infected by *Haemonchus contortus*.**

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On the island of Gran Canaria there are two breeds of sheep with different susceptibility to *Haemonchus contortus*. Canaria Hair Breed (CHB) sheep are more resistant to infection compared to Canaria Sheep (CS) breeds and unlike other breeds of sheep, the target of the resistance in CHB sheep are the adult stage of the parasite (Vet Parasitol, 2008, 153:374-378). The study of the cellular immune response in CHB sheep revealed significant negative correlations between eosinophils and lymphocytes T $\gamma\delta^+$ /WC1⁺ with the fecundity of adult worms, suggesting that these cells could play a hitherto unknown role in fecundity (Vet Parasitol, 2011, 154:137-141). Furthermore, the study of the humoral immune response demonstrated mucosal IgA (specific to somatic antigens of *H. contortus* adult parasites) were negatively related to the length of the parasites. The aim of this study was to evaluate the effect of $\gamma\delta$ T cell depletion (via infusion of anti- $\gamma\delta$ T cell CC15 monoclonal antibodies) on the subsequent immune response of CHB sheep infected with *H. contortus*. Significantly lower $\gamma\delta$ T cell levels in both peripheral blood and abomasal tissues resulted in monoclonal antibody infused groups compared to placebo controls. In addition, in these animals the worms were significantly longer and prolific than in the control group. The antibody-treated groups, when compared to controls, also had no negative associations between abomasal mucosal IgA and eosinophils counts. Taken together, these results suggest that $\gamma\delta^+$ T cells play a critical role in fecundity (length and eggs in uterus) possibly involving mucosal IgA and eosinophils.

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D: Vaccines and immunology

Ref: P2D420/0712

Duration of immune response generated by recombinant-mutant *Fasciola hepatica* cathepsin L1 based vaccine in cattle

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Fasciolosis is a parasitic disease, which causes health and economic impact in several regions from all over the world. Reports of anthelmintic resistance are increasing in incidence in the last decade and, as the control still relies mainly on antiparasitic drugs, the development of alternative treatments is utterly necessary. Here we verified the duration of humoral immune response conferred by an experimental vaccine, previously tested in a smaller scale field trial. The vaccine consists of 200 µg of recombinant-mutant *Fasciola hepatica* cathepsin L1 in 2 ml of the commercial adjuvant Montanide ISA 70 VG. Fifteen calves from a commercial fluke-free farm received two subcutaneous doses of vaccine, three weeks apart and were compared to 15 control calves, which shared the same pasture. Vaccinates received a booster dose one year after the first vaccination. Immunological response was checked by periodical serology examinations (ELISA), for different subtypes of IgG. Faecal egg counts were performed during the trial to verify infection. Seronegativity of control animals and the absence of eggs in faeces from all experimental animals confirmed that animals were not exposed to liver fluke infection. Two vaccinated animals and two controls were slaughtered at different time points after the last vaccination and no signs of fluke infection were found in their livers. A high humoral response was detected in vaccinated animals throughout the first year and a strong boost in antibody production was achieved with the last vaccination.

E: One health

Ref: P2E360/0045

Lyme Disease Ecology in Texas: A One Health Evaluation of the Interplaying Factors of the Tick-Vector and Pathogen

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Lyme Disease (LD) is the most prevalent arthropod-borne zoonotic disease in the United States. It is caused by the bacterial pathogen *Borrelia burgdorferi* and transmitted by the tick-vector *Ixodes scapularis*.

The objective of this study is to examine the role of White-Tailed Deer (WTD), *Odocoileus virginianus*, (which maintain the adult stages of *Ixodes* populations), the tick-vector *Ixodes scapularis* and environmental variables in the ecology of LD in low endemic areas where there is a lack of understanding.

Therefore, 1411 WTD serum samples were obtained during Texas hunting seasons from 2001-2014. These samples were evaluated using indirect ELISA to determine the sero-reactivity to Bbss. Those samples with significantly higher reactivity values compared with the negative control group are being additionally tested using a standardized Immunoblot assay to determine their specificity and sensitivity, and to rule out false positives. Preliminary results show that 65 out of 1411 samples tested (4.6%), showed immune reactivity to Bbss, distributed across different eco-regions in Texas. Moreover, the high sero-reactive WTD samples represented graphically along the span of years studied were correlated with an increase in human LD cases in Texas. Future studies aim to analyze the positive samples using statistical and geographic techniques, representing the samples in space and time, taking into consideration different climate variables such as precipitation, isothermality and relative humidity, together with vector and reservoir distribution models available.

These studies will generate comprehensive distribution models to help in the management of this One Health, tick-transmitted, zoonotic pathogen in WTD populations in Texas.

E: One health

Ref: P2E361/0074

Effect of direct exposure of *Toxoplasma gondii* cysts to different degrees of water salinity

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T. gondii is an obligate intracellular protozoan parasite that can infect all warm blooded and marine aquatic animals forming tissue cysts. Shellfish and fish that feed by filtering seawater through their gills may then transmit *T. gondii* to marine mammals.

Dead bodies of infected animals which are thrown into water may play a role in transmission of the infection to aquatic animals and filter feeder fish. The ability of *T. gondii* cyst to survive in marine environment is not fully understood. This study aimed to examine the stability and infectivity of *T. gondii* cyst after direct exposure to sea water of different degrees of salinity, and to understand if it considered as a risk factor for transmission of the disease in the marine environment. In vitro cyst of *T. gondii* PLK/ DUAL directly exposed to different degrees of water salinity (sea water 33.5ppt, brackish water 16.75 ppt and fresh water). Cysts and tachyzoites were monitored for any morphological changes for 24 hours of direct exposure to sea water and images for pre and post exposure cysts and tachyzoites were taken. The cyst infectivity was examined by infecting new VERO cell cultures (12 hrs, 1 day, 2 days, 3 days, 4days and 5 days) post exposure to sea water. Direct exposure of cyst revealed reactivation of tachyzoites from 24 hours incubated cyst in sea water and brackish water but in contrast, none of the tachyzoites were reactivated from fresh water incubated cyst. The results showed tissue cysts can survive in seawater for 24 hours, 12 hours in fresh water and at least for 6 days in brackish water when incubated at 20°C. These results give the cysts another role in the transmission of toxoplasmosis in marine environment especially in places away from the land in the middle of seas or oceans where arrival of oocysts to this places by the action of waves is very low but animals in these places can be infected by toxoplasmosis, through eating tissue cysts from infected dead marine mammals and dead bodies of infected animals. Also, *T. gondii* cyst represent as a risk factor in areas of low salinity and in areas where fresh water met with the seas or the oceans

Toxoplasma gondii, cysts, marine environment, resistance, in vitro.

E: One health

Ref: P2E362/0085

Prevalence and chemotherapy of *balantidium coli* in sheep and goats in the River Ravi region, Lahore (Pakistan)

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Parasitism is not only a burden on small ruminant farmers nationwide but also a contributing factor in the reduction of per animal yield in Pakistan. Balantidiasis is an infectious and zoonotic disease of small ruminants throughout the world. The prevalence of *Balantidium coli* (*B. coli*) in sheep and goats from the River Ravi region of Lahore were examined and therapeutic trial was conducted to compare the efficacy of Secnidazole, Metronidazole and Oxytetracycline against *B. coli*. A total of 752 (n=376 sheep; n=376 goats) fecal samples were collected through random sampling strategy from sheep and goats and examined microscopically. Prevalence of *B. coli* was estimated by Pearson's chisquare test for significance whereas data on hematology was analyzed by Student's *t*-test. Data on chemotherapy was analyzed by one-way ANOVA, using SPSS. Total of 28 (3.72%) animals were positive for *B. coli* cyst whereas in sheep and goat, it was 15 (3.99%) and 13 (3.46%), respectively. Haemoglobin (Hb), pack cell volume (PCV) and total erythrocyte count (TEC) were significantly decreased ($P<0.05$), while the concentration of total leukocyte count (TLC), non-significantly increased ($P>0.05$) in sheep and goats. Efficacy of Secnidazole (86.66%) was higher as compared to Oxytetracycline (66.66%) and Metronidazole (43.75%) in sheep similarly in goats; efficacy of Secnidazole (93.75%) was higher as compared to Oxytetracycline (73.33%) and Metronidazole (50%), making secnidazole the most effective.

Key words: *Balantidium coli*, Small ruminants, Treatment, Prevalence, Hematology, Oxytetracycline, Metronidazole

E: One health

Ref: P2E363/0099

Seroprevalence and risk factors of *Toxoplasma gondii* infection among pregnant women in Ad-Dawadimi, Saudi Arabia

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The present study was conducted to investigate the seroprevalence of two antibodies (anti *T. gondii* IgG and IgM) among the pregnant women in Ad-Dawadimi, Saudi Arabia along with possible risk factors associated with *Toxoplasma* infection. A prospective study was carried out at Obstetric & Gynecology Clinic at Ad-Dawadimi General Hospital (Ministry of Health, Kingdom of Saudi Arabia) during the period from May 2013 to November 2013. A total of 461 samples of pregnant women was enrolled at any stage of pregnancy, either asymptomatic or having clinical signs and symptoms suggestive of toxoplasmosis (fever, lymphadenopathy, hepatomegaly, splenomegaly, and rash). A short face-to-face questionnaire interview for pregnant women was carried out to obtain information regarding *T. gondii* infection. Out of 461 samples tested, 189 samples (40.9%), were found seropositive for specific anti *T. gondii* IgG antibodies. None had positive IgM results. Seroprevalence of *T. gondii* antibodies was high among pregnant women and the prevalence showed a corresponding increase as the age of the pregnant women increases. Seroprevalence of *T. gondii* antibodies was high among pregnant women and the prevalence showed a corresponding increase as the age of the pregnant women increases. Therefore, awareness creation on the modes of transmission and prevention of *T. gondii* should be made to women of child bearing age in general and pregnant women in particular during their antenatal care follow up.

E: One health

Ref: P2E364/0135

Calcium kinetics in lambs infected with *Haemonchus contortus*

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The calcium is fundamental in various physiological functions including bone formation, muscle contraction, enzyme co-factor, stabilization of membrane potentials and blood coagulation. Net calcium balance depends not only on the absorption of this mineral in the gut, its retention or excretion by the kidneys and loss in the feces, but also on bone turnover. In addition, endoparasite infection can produce variation in mineral concentration. Nematode parasites still one of the major constraints to sheep health worldwide and can cause alterations on metabolism with a decrease in animal performance and, in extreme cases, death. It competes with the host for the absorption of nutrients and vitamins. In tropical and sub-tropical environment *Haemonchus* spp. are among the most important gastrointestinal parasites. Aiming to evaluate the kinetics of calcium metabolism in hair sheep infected with *Haemonchus contortus*, 18 lambs with an average live weight of 28.14 ± 3.36 kg were kept in individual metabolic cages, fed *Cynodon dactylon* hay *ad libitum* and a concentrate mix. The animals were divided into two treatments: infected with 4.000 L₃ *H. contortus* and a control. After 35 days of infection 7.4 MBq ⁴⁵Ca was injected into the jugular vein of the animals, followed by blood sampling after 5 minutes and then every 24 hours for seven days to determine the concentration of stable Ca by isotopic dilution technique using radiocalcium. The experimental design was fully randomized, with two treatments (healthy and infected) and nine repetitions. The stable calcium in plasma was unaffected ($P > 0.05$) by inducing endoparasite infection, with plasma values for healthy lambs (17.86 ± 0.35 mg/100mL) and infected lambs (18.36 ± 0.33 mg/100mL). Also, no significant difference ($P > 0.05$) was found on biological half-life of ⁴⁵Ca for healthy (49.98 ± 1.75 hours) and infected (50.49 ± 1.65 hours) lambs. Our study suggests *H. contortus* infection did not change the calcium level in plasma and biological half-life of ⁴⁵Ca, indicating similar Ca metabolism between healthy and infected lambs.

E: One health

Ref: P2E365/0142

Ensuring quality standards for Livestock Trypanocides in Sub-Saharan Africa

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African Animal Trypanosomosis (AAT) is a parasitic disease caused primarily by *T. congolense* and *T. vivax*, which inflict greater losses on livestock than any other disease in Africa. Transmitted primarily by tsetse and other biting flies, the disease is present in some 40 African countries and affects nearly all domestic animals. Overall economic losses attributable to AAT are estimated at USD 4.75 billion per annum. These losses are borne by those who can least afford it; small scale keepers and rural communities throughout sub-Saharan Africa who rely on their livestock for their livelihoods.

One of the tools that is extensively relied on currently are the trypanocidal drugs used for both treatment and prophylaxis. These drugs are widely available but were developed over 50 years ago and there are significant limitations in terms of safety and lack of efficacy against resistant trypanosome strains. Furthermore, the regulation of trypanocides is weak with high volumes of sub-standard and falsified drugs available in the market.

GALVmed, supported by DfID and BMGF and with support from other partners (FAO, International Federation of Animal Health, Strathclyde University and Manchester Metropolitan University (MMU), IAEA, AU PATTEC and TRYRAC), has developed monographs for the existing trypanocides and enabled two laboratories in Africa to carry out analysis of trypanocides collected in both formal and informal markets.

The analysis of these samples presented in the poster showed that between 40 – 70% of all samples are sub-standard or falsified.

E: One health

Ref: P2E366/0175

***Toxoplasma gondii* (Nicolle & Manceaux, 1909) research in semen, testicles and epididymis of primeinfected cats (*Felis catus*)**

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The present study aimed to investigate presence of *Toxoplasma gondii* in semen, testicles and epididymis of felines, experimentally infected by this coccidian. In order to achieve this, twelve male mixed breed cats were selected and distributed in three experimental groups: GI, animals inoculated with approximately 600 tissue cysts of *T. gondii*; GII, with cats inoculated with 2×10^5 tachyzoites; and GIII, non-inoculated felines (control group). On days -7, 0, 7, 14, 21, 28, 42, 56 and 70 post-inoculation (DPI), all animals were submitted to clinical and serological (IFA) exams. Semen was collected by means of electroejaculation at these same dates, with *T. gondii* research posteriorly conducted through nested PCR and bioassays in mice. At the 70th DPI, all twelve cats underwent orchiectomy, with testicles and epididymis being evaluated regarding presence of the parasite (nested PCR, bioassay in mice, histopathological and immunohistochemical analysis). All felines which were inoculated with *T. gondii* (GI and GII) seroconverted to toxoplasmic infection. *T. gondii* presence in seminal samples was not detected (bioassays and nested PCR) in any experimental dates on all groups (GI, GII and GIII). Analyzing testicles and epididymis collected from experimental felines, the parasite's presence was not detected as well, by any of the utilized diagnostic techniques (nested PCR, bioassays in mice, histopathological and immunohistochemical analysis). Such results allow us to infer that sexual transmission, in domestic cats, is not an important infection route on the dissemination of feline toxoplasmosis.

E: One health

Ref: P2E367/0210

Canine infection with *Borrelia burgdorferi*, *Dirofilaria immitis*, *Anaplasma* spp., and *Ehrlichia* spp. in Canada, 2013-2014.

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Canine test results generated by veterinarians throughout Canada from 2013–2014 were evaluated to assess the geographic distribution of canine infection with *Borrelia burgdorferi*, *Dirofilaria immitis*, *Ehrlichia* spp., and *Anaplasma* spp. The percent positive test results of 115,636 SNAP® 4Dx® Plus tests for *D. immitis* antigen and antibodies to *Ehrlichia* spp. and *Anaplasma* spp. were low, with less than 0.5% of dogs testing positive for any one of these three agents nationwide. In contrast, 2,844/115,636 (2.5%) of dogs tested positive for antibody to *B. burgdorferi*, and provincial seroprevalence ranged from 0.5% (Saskatchewan)–15.7% (Nova Scotia). The areas of highest percent positive test results were those that adjoin regions in the United States considered endemic for Lyme disease, and include Nova Scotia (15.7%) and eastern Ontario (5.1%). These high endemic foci, which had significantly higher percent positive test results than the rest of the nation ($P < 0.0001$), are surrounded by areas of moderate to low seroprevalence in New Brunswick (3.7%), Quebec (2.8%), and the rest of Ontario (0.9%), as well as northward and westward through Manitoba (2.4%) and Saskatchewan (0.5%). Insufficient results were available from the westernmost provinces, including Alberta and British Columbia, to allow analysis despite the fact that Lyme disease is considered endemic along the western coast. Increased surveillance of these vector-borne diseases, especially Lyme disease, is important as climate, vector range, and habitat continue to change throughout Canada. Using dogs as sentinels for these pathogens can aid in recognition of the public and veterinary health threat that each pose.

E: One health

Ref: P2E368/0291

Detection of *Borrelia burgdorferi sensu lato* in rodents from two rural communities of Yucatan, Mexico

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Lyme disease is a multisystemic zoonotic disease caused by *Borrelia burgdorferi sensu lato* (*B. burgdorferi* s.l.). This spirochete circulates in an enzootic cycle between the primary vertebrate reservoir and the ticks. Different species of rodents are identified to be efficient natural reservoirs for *B. burgdorferi* s.l. In this study, a molecular epidemiological survey was conducted to investigate the presence of *B. burgdorferi* s.l. in rodents from two rural communities (Opichen and Tixmehuac) of Yucatan, Mexico for the first time. A total of 123 rodents (94 *Mus musculus* and 29 *Rattus rattus*) were trapped, and ear and bladder samples were collected. The gene targets for PCR amplifications were the flagelin B (*flaB*) and the outer surface proteins, *ospC* and p66. The overall infection rates in rodents were 44.7% for *flaB* (55/123), 10.6% (13/123) for p66 and 1.6% (2/123) for *ospC*. From all examined tissue, 18.7% (23/123) of bladders, and 26.0% (32/123) of ears were infected with the spirochete. No statistical differences ($p > 0.05$) were found between the two tissue samples used for diagnosis. Results of this study confirmed that *B. burgdorferi* s.l. is present with high frequency in rodents from rural communities of Yucatan, Mexico. It is concluded that *M. musculus* and *R. rattus* are playing an important role on the maintenance of *B. burgdorferi* s.l. in rural communities of Yucatan, Mexico. Currently, sequencing analysis as well as ecological modeling is in progress and will determine the potential risk for human infection.

E: One health

Ref: P2E369/0333

Biochemical Profile of *Biomphalaria straminea* and *Biomphalaria tenagophila* (Mollusca, Gastropoda) Experimentally Infected with *Angiostrongylus cantonensis* (Nematoda, Metastrongylidae).

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Introduction: *Angiostrongylus cantonensis* has been considered the main agent responsible for human eosinophilic meningoencephalitis, this parasite has low specificity for mollusc hosts and it has caught attention for experimental models of infection. Changes in metabolic patterns of nitrogen excretion products reflect a sequence of events revealing the exploitative action of helminth larvae inside their hosts. However, few studies on the metabolic profile of *Biomphalaria* spp. infected by *A. cantonensis* have been conducted, although this nematode has great importance to public health.

Methodology: The urea, acid uric and the protein content and the enzyme activity of aminotransferase such as ALT (E.C. 2. 6.12) and AST (E. C 2. 6. 11) in the hemolymph of both snails experimentally infected with *A. cantonensis* were evaluated. The snails were dissected from 1 to 3 weeks after infection to collect the hemolymph and separate the tissues for histological observations.

Results: A huge decrease was observed in the urea content of both species at the first moment of infection. Also a marked increase was observed in the acid uric content. Changes in the aminotransferase behavior were observed where both, AST and ALT enzymes, presented high activity during the infection period. The protein content for *B. straminea* was remarkably decreased, but *B. tenagophila* showed a huge increase in that hemolymph content. The histological investigation showed a marked disorder in the tissue organization with granuloma area.

Conclusion: These host snails are able to change their metabolic profile suggesting a strategy to maintain the homeostatic balance.

E: One health

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***Dicrocoelium dendriticum* induces autophagic vacuoles accumulation in human hepatocarcinoma cells**

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It is estimated that over 20% of malignancies worldwide can be attributed to infectious agents, including parasites. This percentage may grossly underestimate the true size of infection-induced cancers. The relationship between helminths and cancer has been poorly investigated so far. The knowledge of the mechanisms by which parasites induce malignant transformation of host cells is unclear. The purpose of the present study was to investigate whether the trematode *Dicrocoelium dendriticum* - a liver fluke widespread in small and large ruminants of southern Europe - may be involved in the neoplasia onset. Livers from sheep naturally infected by *D. dendriticum* were collected at 3 slaughterhouses in southern Italy and adult *D. dendriticum* were removed and then used to prepare the antigenic extracts. Somatic antigens derived from adult flukes were used to evaluate the cell proliferation, cell death mechanisms and oxidative stress induction in hepatocellular carcinoma (HCC) cell lines (HepG2 and HuH7). Results showed that short time exposure to low concentration of somatic antigens from *D. dendriticum* caused slight proliferation in both HepG2 and HuH7 cells. However, high concentrations and long time exposure to *D. dendriticum* extracts caused a significant growth inhibition. This effect was, however, not paralleled by apoptosis but it occurred with a 40% increase of the formation of autophagic vacuoles. These data suggest the occurrence of an escape anti-apoptotic mechanism in HCC cells. In the same experimental conditions, a strong oxidative stress was recorded with a 100% increase of the intracellular O₂⁻. In conclusion, these results suggest a role for *D. dendriticum* in the chronic oxidative stress and in the regulation of transformation processes in HCC. Future research is necessary in order to clarify the complex mechanisms that regulate host-parasite interaction and to better define the involvement of *D. dendriticum* in the development and spreading of animal and human tumours.

E: One health

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Assessment of neurobehavioral alterations in mice during *Toxocara canis*- and *Toxocara cati*-induced neurotoxocarosis

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Infections with the zoonotic roundworm species *Toxocara canis* and *T. cati* may lead to severe symptoms in paratenic hosts due to somatic migration of larvae. One of the occurring forms is neurotoxocarosis induced by larvae migrating into the central nervous system (CNS). Neurotoxocarosis is mainly caused by *T. canis* larvae as these show a strong affinity to the CNS, whereas *T. cati* larvae prefer muscle tissue. Previous studies on neurotoxocarosis revealed severe abnormal neurobehavioral symptoms such as circling and spatial disorientation in *T. canis*-infected mice during the chronic stage of infection. Such obvious neurobehavioral alterations were less frequent and less pronounced in *T. cati*-infected mice. However, these were subjective impressions. Thus, the present study aimed to precisely characterize neurobehavioral changes as well as their onset and progression of severity during *T. canis*- and *T. cati*-infections by an objective test (S. Irwin, 1968, *Psychopharmacologica*), which allows systematic quantification of physiological status and neurobehavioral alterations in mice under standardized conditions. Therefore, C57Bl/6J mice were infected orally with 2000 embryonated *T. canis* or *T. cati* eggs, respectively, whereas the control group received tap water. Mice are screened weekly for general health as well as neurobehavioral symptoms by an adapted Irwin screen protocol, starting the day prior infection until 98 days post infection. In addition, brains of infected as well as control mice are histologically analyzed to correlate neurological disturbances with histopathological changes. Results of analyses (currently in progress) will be presented.

E: One health

Ref: P2E372/0385

Host preferences of Swiss mosquitoes assessed by animal-baited experiments and blood meal analysis of field-collected mosquitoes

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West Nile virus (WNV) which can cause zoonotic neuroinvasive diseases in horses and humans has spread in southern and eastern Europe over the last decade. Mosquitoes are the main biological vectors of WNV for which birds act as reservoirs. To assess the risk for autochthonous WNV transmission in Switzerland, a large project was initiated to determine (1) population dynamics, (2) host preferences, and (3) vector competence of Swiss mosquitoes. Host preferences were assessed with horse- and chicken-baited traps under field conditions and with blood meal analysis from blood-fed mosquitoes collected at the Zoo Zürich. The observed host preferences of mosquitoes were statistically corrected with Poisson rate ratios adjusting for different body weight, body surface and Kleiber's scaling factor of the bait species or by calculating 95% Poisson confidence intervals, respectively. Altogether 1068 mosquitoes belonging to 15 species were trapped in the animal bait experiment (horse: 1010; chicken: 58). Among the abundant mosquito species, *Aedes cinereus/geminus*, *Ae. japonicus*, *Ae. rusticus*, *Ae. sticticus* and *Culiseta annulata* showed no preference for either bird or horse after considering the correction factors. *Aedes cantans/annulipes* and *Ae. vexans* showed a preference for horse and *Culex pipiens* for chickens after abundance correction, though they were found in both baited traps. At the Zoo Zürich, 35 host species were identified in a total of 385 blood-fed females of nine different species. The three most abundant species (*Cx. pipiens/torrentium*, *Ae. vexans*, *Ae. japonicus*) had taken blood meals from both birds and mammals. Based on our results, *Cx. pipiens/torrentium*, *Ae. japonicus* and *Ae. vexans* are suggested best candidate bridge vectors for WNV in Switzerland.

E: One health

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Prevalence and risk factors associated with geohelminths contamination in public areas in Curitiba, Brazil

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The cats and dogs population in cities has an important epidemiological factor in the soil contamination of urban areas of infections of several endoparasites, such as *Ancylostoma* sp. and *Toxocara* spp. This study aimed to investigate the frequency of geohelminthic contamination of public parks and squares in Curitiba, Brazil, between August and December 2010. Five sand samples (50g each) were collected from separate points in the same area from 69 (66 squares and 3 recreational parks) public city places, totaling 345 samples. In the laboratory, all samples were homogenized, processed and analyzed by three different methodologies: Faust, Lutz and Baerman parasitological techniques. The risk factor estimate used for the associations was the odds ratio with a lower and upper interval of confidence. A total of 36% (124/345) samples were positive for helminths and 65.2% (45/69) of the areas were classified as contaminated in one or more samplings. Only 11.1% (5/45) of the positive samples occurred in neighborhoods located in the central region of the city. The most common parasite eggs identified were *Ancylostoma* sp. 14.5% (50/345); followed by *Toxocara* sp. 9.6% (33/345) and Strongyloidea superfamily (excluding hookworms) 2.3% (8/345). The most frequent association found was between hookworms and *Toxocara* spp. 2.0% (7/345). A significant association between soil contamination and the number of animal faeces (cat or dog) near the collection site was observed ($p < 0.0001$). The probability of positive samples was amplified when the number of risk factors increased with a crude odds ratio of 3.0270 (1.7823 to 5.1412) with the presence of one to four excrements and 7.8182 (4.0767 to 14.9936) with five or more. The analysis of the epidemiological risk factors also indicated that the presence of dogs and faeces in the sandboxes increased the chances of contamination of the site. In other hand, the use of fences had a protective positive impact reducing soil contamination. Health education programs should be applied within the community to protect the risk of human contact by the simple removal of dogs' faeces. The use of fencing in these areas is highly recommended to prevent or reduce the contact of the users to stray-animal excrements.

E: One health

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Survey of *Toxoplasma gondii* antibodies in Magellanic penguins (*Spheniscus magellanicus* Forster, 1781)

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Toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii* is a worldwide zoonosis. The disease has been reported in many species of birds, including penguins. Magellanic penguin (*Spheniscus magellanicus*) breeds on the Atlantic and Pacific coast of South America, and migrate north as far as Peru and Brazil, been the most common specie of penguin in the Brazilian coast. Serum samples (n=100), from Magellanic penguins from three zoos and two Rehabilitation Centres (RC) from Brazil, were analysed for the presence of antibodies to *T. gondii* by the modified agglutination test, with a cut-off ≥ 20 . The penguins were divided into young (≤ 4 years old) and adults (≥ 4 years old), and in male, female and non-gender identified (NI) and the occurrence of *T. gondii* antibodies were analysed using Chi-square test with p value ≤ 0.05 . The overall *T. gondii* antibody occurrence was 28%, with 25.8% of male, 27.8% of female and 30.3% of NI birds positive to *T. gondii* ($p > 0.05$), and 25.4% of young and 31.1% of adults presenting antibodies ($p > 0.05$). Statistical analyses found no difference between the occurrence value in the zoos and RC penguins ($p > 0.05$). This is the first description of the presence of *T. gondii* antibodies in Magellanic penguins (*Spheniscus magellanicus*) from the Brazilian coast.

E: One health

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Canine faecal contamination and parasitic risk assessment in Lisbon dog parks

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Dog parks fulfil the desire of dog owners to spend quality time with their dogs. These parks pose an exposure risk for zoonotic agents among dogs, humans and wildlife. Regarding the concept of One Health, it is important to characterize and assess the parasitic population in these places. However, in Lisbon, few studies investigating gastrointestinal infections in urban dogs have been performed and none in park-attending dogs.

In total, 369 fresh faecal samples were collected from three dog parks: 125 from Algés, 124 from Benfica and 120 from Campo Grande, from October to December 2014, every 15 days. Samples were analysed at the Laboratory of Parasitic Diseases (FMV-ULisboa) by Centrifugal Sedimentation/Flotation, McMaster (in the positives) and Modified Ziehl-Neelsen stained faecal smear techniques.

The overall prevalence for positive samples was 33% (122/369). Ancylostomatidae represent 17% (62/369), *Cryptosporidium* spp. 12% (44/369), *Giardia* spp. 11% (42/369), *Toxascaris leonina* and *Cystoisospora* spp. 1% (4/369) each, *Toxocara* spp. 0.5% (2/369) and *Sarcocystis* spp. 0.3% (1/369). Samples tested positive for more than one type of egg in 9.8% (36/369) mainly due to Ancylostomatidae and *Cryptosporidium* spp. Protozoa and nematode prevalence was, respectively, 24% (87/369) and 17% (62/369).

Approximately one third of these dog faecal samples were infected with parasitic agents, some of zoonotic concern, representing a menace to other animals and humans. These findings highlight the need to raise public awareness and increase the promotion of faecal removal control measures and effective deworming practices.

E: One health

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The anatomical distribution of *Toxoplasma gondii* tissue cysts in cattle

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The protozoan parasite *T. gondii* is a zoonotic pathogen that has the ability to infect all warm blooded animals including humans (Tenter *et al* 2000). Toxoplasmosis is a major opportunistic disease of immunocompromised patients. It also represents a serious threat during pregnancy, causing severe foetal abnormalities or potentially leads to problems in childhood or later adult life (Koppe *et al* 1986). Undercooked or raw meat containing infective tissue cysts are a significant source of human infection (Cook *et al* 2000). Compared to other livestock species, the predilection sites of *T. gondii* tissue cysts in cattle are not fully understood. This research aims to determine the dissemination of parasite tissue cysts to different tissues, organs and cuts of meat intended for the food chain following infection with *T. gondii* oocysts. Six seronegative calves were orally inoculated with 10^6 *T. gondii* oocysts. At 42 dpi, post mortem samples were collected and tested for infectivity using mouse bioassay from heart, brain, liver, diaphragm, masseter muscle, tongue and four edible tissues (sirloin, fore leg muscle, hind leg muscle, fillet). Following the six week bioassay, each mouse brain and the tissue homogenates used for inoculation of mice were tested for the presence of parasite DNA by qPCR. Blood was also collected for ELISA; weekly from all cattle and from all mice at the end of the experiment. Initial results show that the main predilection sites for the parasite in cattle are tongue, brain and diaphragm, although some tissues aimed for human consumption were also found to be infected, such as hind limb. Mice which showed signs of *T. gondii* infection were also seropositive and *T. gondii* DNA detected from their brains. In addition all cattle tested seropositive by 21dpi. A comparison between bioassay and detection of parasite DNA from the tissue homogenate will also be discussed. *The research was conducted by a consortium within the framework of project n° GA/EFSA/BIOHAZ/2013/01 entitled "Relationship between seroprevalence in the main livestock species and presence of *Toxoplasma gondii* in meat", grant agreement funded by the European Food Safety Authority (budget). This publication is based on the results obtained in the framework of this mentioned project and it is published under the sole responsibility of the authors, and shall not be considered as an EFSA output.

E: One health

Ref: P2E377/0506

Fascioliasis in schoolchildren in the inter andean valley of Cajamarca, Peru

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Fascioliasis, caused by *Fasciola hepatica*, is a public health problem in Peru, especially in schoolchildren. Prevalence rates in livestock are over 80% in dairy cattle reared in the Andean valley of Cajamarca, Peru. The present investigation aimed to determine the prevalence of *F. hepatica* infection in schoolchildren and the main risk factors involved in its presentation. Two hundred and seventy schoolchildren nine years old and over from primary public institutions from the district of Los Baños del Inca (Cajamarca) were included in the investigation. Questionnaires were applied to parents and children and fecal samples were taken and evaluated using the rapid sedimentation technique. Blood samples were also collected and analyzed. Seventeen fecal samples were positive to *F. hepatica* eggs, giving a prevalence of 6.3% (95% CI 3.21 - 9.38). Significant differences were found with origin of the child, history of intestinal parasitism, and the habit of chewing grass ($p < 0.05$). The rural origin (OR 4.8, 95% CI: 1.53-15.08) and the habit of chewing grass (OR 3.26, CI: 95% 1.07 - 9.96) were the most likely risk factors associated with the acquisition of infection. The leukocyte count of infected children varied between 3900 and 10580 cells /mm³ (mean $\pm$ SD = 6458.3 $\pm$ 2080.3). Thirty three percent of children positive to *F. hepatica* eggs presented eosinophilia. We conclude that the prevalence of human fascioliasis in the district of Los Baños del Inca is at the mesoendemic level and rurality provides conditions for acquiring the infection.

E: One health

Ref: P2E378/0524

***Alaria alata* infection on foxes and wild boars in Romania**

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Alaria alata (Goeze, 1792) is a trematode with zoonotic and emerging potential which is widely distributed in Eurasia. The existence of *Alaria alata* in Romania and its medical and veterinary importance have not been studied so far. From 2013 to 2014, 34 foxes from the rabies vaccination program and five wild boars during the hunting season were investigated at the Institute for Diagnostic and Animal Health (IDAH), Bucharest. The foxes' intestines were examined by sedimentation and counting technique for helminth eggs and adults. For identification of mesocercariae in wild boar meat the larvoscopic technique was used. The identity of the metacercariae and adult worms was confirmed using molecular techniques. 9/34 foxes (26.4%) were found positive for *Alaria alata*. In three foxes only adult trematodes were detected, in two foxes adult worms and eggs were found, and in four foxes only the eggs in the faeces were seen. Mesocercariae were collected from two of the five wild boars. Our results for the first time report the detection of *Alaria alata* mesocercariae in wild boars and of adults and eggs in foxes from Romania. *Alaria alata* trematodes exhibited high prevalence rates in wild carnivores (foxes) and omnivorous scavengers (wild boars). The veterinary and medical importance of these findings for Romania is unclear. The trematode *Alaria alata* is mostly unknown in this country and therefore needs further investigations in wild and farm animals and in humans with clinical symptoms compatible with *Alaria alata* infection.

E: One health

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***Onchocerca lupi*: A neglected and hidden parasitic infection in the South of Portugal.**

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Onchocerca lupi was firstly reported from a wolf in the Caucasian region in 1967 and its report as the agent of a zoonotic disease occurred in 2011. Since then seven human cases have been confirmed. In Portugal, after the first publication of a clinical case in a dog, several studies have reported the occurrence of *O. lupi* infection in autochthonous dogs from the south of Portugal. In this study, samples were obtained from the head frontal region of 54 dogs of different ages and breeds from the district of Faro (region of the Algarve). The hair was cut on the frontal region of the head in each animal before a skin biopsy was performed. The skin fragments were incubated in 0.9% NaCl at 37°C for 12 h. Twenty microliters of sediment was observed on a slide with a coverslip under a magnification of x100. Microfilariae of *O. lupi* were identified in seven dogs (13%), none of them presenting clinical signs of onchocercosis. This study confirms the presence of *O. lupi* in dogs from the study area, with apparent prevalence results being higher than the ones reported in publications from previous studies in Europe, and suggests a zoonotic potential of this parasite. As simuliids are possible vectors on the transmission of *O. lupi*, the simuliid fauna is also being studied in southern Portugal. *Simulium ornatum* (Complex) and *Simulium pseudequinum* are the most prevalent species and should be included in further entomological studies. In the present work, we alert for the possibility that *O. lupi* may be also infecting humans in Portugal, and suggest including this parasitosis on the list of differential diagnoses. The risk of confounding the present infection with orbital neoplasia, together with its direct or indirect ability to cause ocular pathology in man, should also be stressed.

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E: One health

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A retrospective serological survey on human babesiosis in Belgium

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Babesiosis is a tick-borne disease caused by protozoa of the genus *Babesia*. In humans, clinical symptoms that develop early or during a mild *Babesia* sp. infection can be confused with malaria, showing high fever, headaches and myalgia. In acute clinical infection, anaemia, icterus and haemoglobinuria are conspicuous and this condition can be fatal. In Europe, most clinical babesiosis cases have been attributed to *Babesia divergens* and *Babesia* sp. EU1. *Babesia microti* occurs mainly in the USA although a case of autochthonous *B. microti* infection and serological evidence of human infections in different European countries have been reported. Because of the recent first report of potentially zoonotic *Babesia* species in the *Ixodes ricinus* tick together with an older report of a human clinical case in Belgium, a retrospective serological survey was performed. IFAT was used to screen 200 sera from anonymous Belgian patients with history of tick bite and clinical symptoms compatible with a tick-borne disease. The serological screening detected positive reactivity in 9% (18), 38.5% (77) and 42% (84) of the samples against *B. microti*, *B. divergens* or *Babesia* sp. EU1, respectively. Presence of the three potentially zoonotic species of *Babesia* has been confirmed together with evidence of contact with human reported in Belgium. Preventive actions, such as improved awareness of physicians and development of better diagnostic tools should be helpful to prevent clinical cases and assess the true risk to public health.

E: One health

Ref: P2E381/0564

Withdrawn

E: One health

Ref: P2E382/0635

Distribution of *Cryptosporidium* spp. in pre-weaned lambs of Andhra Pradesh and Telangana states of India

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Cryptosporidium is an obligate intestinal coccidian parasite, which infects a wide range of hosts. This paper aims with an objective to investigate the distribution of *Cryptosporidium* infection in pre-weaned lambs of Andhra Pradesh and Telangana states of India. Modified Ziehl-Neelsen (mZN) staining method was followed for preliminary screening of faecal samples, subsequently mZN positive isolates were confirmed by nested PCR (nPCR). The nPCR amplicons were sequenced to determine the *Cryptosporidium* species. In mZN staining method, 74 (9.87%) *Cryptosporidium* positives were detected out of 750 faecal samples screened. Influencing risk factors viz., age, sex, breed, faecal consistency and rearing systems were studied. All mZN *Cryptosporidium* positives were subjected to nPCR targeting 18S rRNA gene for confirmation of *Cryptosporidium* DNA and was successful. Sequence analysis of randomly selected nPCR *Cryptosporidium* positive isolates revealed the presence of *C. ubiquitum* and *C. parvum* in the sheep farms of both Andhra Pradesh and Telangana states. In addition, *C. xiaoi* was also isolated from the sheep of Telangana state. In conclusion, *C. ubiquitum* was the major species recorded among the sheep flocks of Andhra Pradesh state. In Telangana state, *C. ubiquitum*, *C. parvum* and *C. xiaoi* were distributed. *Cryptosporidium* infection was prevalent and first time observed the species variation in the sheep of Andhra Pradesh and Telangana states of India.

E: One health

Ref: P2E383/0641

Tick-borne pathogen xenodiagnosis in ticks collected from ruminants in Maban County, South Sudan

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Ticks ($n=482$) collected from 33 cows and 1 camel in March 2013 in a refugee camp in Maban County, South Sudan were identified and subsequently screened for the presence of *Anaplasma*, *Babesia*, *Ehrlichia*, *Rickettsia* and *Theileria* species by Reverse Line Blot hybridization (RLB). Identified tick species included *Amblyomma lepidum*, *Hyalomma excavatum*, *Hyalomma rufipes*, *Rhipicephalus (Boophilus) annulatus*, *Rhipicephalus (Boophilus) decoloratus*, *Rhipicephalus muhsamae*, *Rhipicephalus preatextatus*, *Rhipicephalus sanguineus*, and *Rhipicephalus turanicus*. DNA of zoonotic rickettsial pathogens, including *Rickettsia africae*, *R. aeschlimannii* and *Rickettsia massilliae*, as well as DNA from uncharacterized *Anaplasma* species, *Anaplasma marginale*, *Ehrlichia ruminantium*, *Theileria mutans*, *Theileria separata* and *Theileria velifera* were detected by PCR/RLB. These results indicate that pathogens of veterinary and zoonotic relevance are circulating in the tick and ruminant population in Maban County and imply that the local population is at risk for tick-borne rickettsial diseases.

E: One health

Ref: P2E384/0658

Insights into the epidemiology of cystic echinococcosis in livestock in Romania

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Cystic echinococcosis (CE; hydatidosis), caused by the larval stages of *Echinococcus granulosus* sensu lato, is an important zoonotic infection in the world. The disease has a considerable impact on both human and animal health, with economic consequences arising out of the costs of medical treatment and morbidity for human cases and losses in animal productivity. In Romania, CE continues to pose significant problems of public health and causes important economic losses. The awareness of the socio-economic impact of the disease has stimulated an increased interest on knowledge of the epidemiology of this important zoonosis. Therefore, here we present updates on the CE based on epidemiological surveys conducted during of 2009 – 2013 period in slaughtered livestock from 23 counties in Romania. A total number of 6168 sheep, 4921 cattle, and 1355 pig carcasses were examined for CE by visual inspection, palpation, and serial cuts of the organs. Hydatid cysts were found in 3177 (51.5%) 1738 (35.32%), and 14 (1.03%) sheep, cattle and pigs, respectively. From a sample of 1072 cysts undergoing closer examination (474 from sheep, 556 from cattle, and 42 from pigs), the highest fertility rate (51.68%) was found in sheep cysts, while from the cattle only seven cysts (1.26%) were found to be fertile. No fertile cysts were found in pigs. Assessment of the genetic diversity of the *Echinococcus* strains on 111 cysts from sheep (n=40) and cattle (n=71) showed the presence of the *E. granulosus* sensu stricto complex and disclosed an apparent dominance of the G1 genotype within the G1–G3 complex. Furthermore, several mitochondrial variants were identified for the G1 and G2 genotypes. These findings, combined with human and animal epidemiological data, highlight the ongoing active transmission of *E. granulosus* in Romania and reveal high potential risks of zoonotic infection.

E: One health

Ref: P2E385/0695

Molecular detection of *Leishmania (Viannia) braziliensis* in domestic cats (*Felis catus*) from a cutaneous leishmaniasis endemic region

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The Cutaneous Leishmaniasis (CL) is a zoonosis caused by intracellular protozoan parasites of the genus *Leishmania*, which epidemiology involves the man and other domestic mammals and wild species, having different phlebotomine species as vectors. CL is the most frequently disease form found in domestic cats and it has been described in several countries, including Brazil, where the etiological agent is *Leishmania (V.) braziliensis*. Thus, studies show that domestic cats can act as reservoirs for the parasite, developing important infections source for other domestic animals and the man. Considering the importance of CL for public health, this work aims for the detection of natural occurrence of *L. (V.) braziliensis* in blood samples of 31 domestic cats from the Zoonosis Control Center in Bauru city, São Paulo state, Brazil; an endemic area for CL. The animals were anesthetized for blood sampling and submitted for clinical exam. Molecular diagnosis was done through PCR using the kDNA minicircle primers B1/B2 for *L. (V.) braziliensis* to verify the amplification of products of 750 bp. Seven cats (22.6%) were positive for *L. (V.) braziliensis* DNA in blood samples and presented literature clinical signs: skin lesions, snout lesions, ocular infections, alopecia, and lymphadenopathy. Hence, *L. (V.) braziliensis* DNA present in domestic cats with clinical signs suggests the possibility of its participation as reservoirs in the domestic cycle of CL demonstrating the importance of this zoonotic disease in public health, in view of the proximity of these animals to man.

E: One health

Ref: P2E386/0697

Tegumentar and visceral leishmaniasis in cattle (*Bos taurus*) captured on public area in the city of Bauru, São Paulo, Brazil

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Leishmaniasis are diseases caused by protozoan flagellates transmitted by sandflies in animals and humans. The main reservoir is the dog, but many other species can participate in the epidemiological cycle of this zoonosis, including the bovines. This study was done in order to evaluate the presence of tegumentar or visceral leishmaniasis in free bovines captured in public areas from Bauru, São Paulo state, located in the central-western Brazil, which is considered an endemic area for visceral leishmaniasis. The diagnostic tests performed were blood culture and ITS-PCR. Blood samples were harvested from 87 cattle and culture was performed in Liver Infusion Tryptose (LIT) medium. Out of 87 bovines, 3 (3.4%) presented great number of flagellar forms in culture. From positive cultures, PCR was performed using ITS (Internal Transcribed Spacer) region primers (ITS-PCR), which amplifies fragments of Trypanosomatidae family. After ITS-PCR, according to the pattern of electrophoresis, out of three cultures, two (66.7%) presented amplification of 300-350 pb, which varies according to the reference strains of *Leishmania* spp. (*Leishmania infantum* / *Leishmania braziliensis*). However, due the cross-reactivity between *Leishmania* spp and *Trypanosoma* spp, other molecular studies need to be conducted, in order to identify the species. Therefore, these preliminary results show the needs more attention to this important zoonosis in that area.

E: One health

Ref: P2E387/0707

***Dientamoeba fragilis* in household dogs.**

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While the presence of the flagellated protozoan *Dientamoeba fragilis* in the large intestine of humans, primarily in children, and primates has been associated with chronic gastro-intestinal complaints, fatigue, weight loss, and anorexia, its role as a primary causative agent is debated. Treatment failure is common and might be related to relapse of infection or to reinfection. It has been proposed that pet dogs or cats can serve as a possible source of (re-)infection, but to our knowledge infections with *D. fragilis* in these animals has never been reported.

The aim of this study was to estimate the prevalence of *D. fragilis* in domestic dogs.

Two populations of Dutch household dogs were screened, namely i: samples from dogs (n=127) with diarrhoea, which were submitted for routine diagnostics to the veterinary microbiological diagnostic centre of Utrecht University, and ii: samples from dogs without reported symptoms (n=190) enrolled in a screening project for helminth infections. One dog, without symptoms, was included from a household with a history of recurrent *D. fragilis* infections in a child. Real Time-PCR was used to detect *D. fragilis* DNA in faecal samples from these two populations.

Two samples tested positive for *D. fragilis*. This concerned one sample of a healthy dog and one clinical sample. Both ct-values were higher than 35 indicating that the amount of DNA in the faecal sample was low. The results of the dog from the household with *D. fragilis* problems were negative.

This is the first time that *D. fragilis* was detected in canine faeces. The estimated prevalence of *D. fragilis* in this tested population of household dogs was 0.6%. No association could be established with gastro-intestinal signs in dogs.

Further research is needed to elucidate the epidemiological role of dogs in recurrent human *Dientamoeba* infections. E.g. case-control studies can identify risk factors, but, given the low prevalence, the size of these studies must be large.

E: One health

Ref: P2E388/0720

Hookworms infection aggravates disorders associated with obesity

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Ancylostomiasis and obesity are considered public health problem worldwide. Hookworms are responsible for one of the most prevalent parasitic infections in the world, causing anemia and malnutrition in the affected individuals. Obesity accounts for an estimated 2.8 million deaths per year due to disorders caused by the inflammatory response characterized as Th1. Studies suggest that the increase in the eosinophils number caused by helminth infection in induced obese animal models, leads to decrease of glucose levels and insulin sensitivity increasing. Other studies claims that the Th2 response observed in helminth infection can interfere with the Th1 response observed in obese individuals decreasing the damage caused by this response. Considering that hookworms are highly prevalent in developing countries and obesity has also been growing significantly, the study of the association between obesity and this parasite becomes relevant. Therefore, this study aims to clarify the effects of infection by *Ancylostoma ceylanicum* using Diet Induced Obesity (DIO) experimental models. Hamsters were divided into 4 groups with 12 animals each: control normonourished (NN), infected normonourished (NNI), obese (OB) and infected obese (OBI). The NNI and OBI groups were orally infected with 50 L3 of *A. ceylanicum* 40 days after the beginning of the diet treatment. After 30 days from infection, blood was collected to perform complete blood cells count and biochemical tests. After infection the OBI group had a higher mortality, weight loss and dysentery. There were changes in glucose levels ($p = 0.019$), leukocytosis ($p = 0.0095$), total cholesterol ($p < 0.0001$) and triglycerides ($P < 0.01$) in the OBI group. This group also showed a decrease of the following parameters: low worm recovery ($p = 0.0002$), oviposition ($p = 0.0011$) and lower fertility rate ($p = 0.0002$). Therefore, it is concluded that *A. ceylanicum* infection have aggravated disorders associated with obesity.

F: Genes and genomics

Ref: P1F217/0021

Identification of *Anaplasma* species in sheep of khouzestan province by PCR

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The aim of this study was to determinate the variety of *Anaplasma* species among sheep of khouzestan province, Iran. From April 2013 to June 2013, a total of 200 blood samples were collected via the jugular vein from healthy sheep (100), randomly. The extracted DNA from blood cells were amplified by *Anaplasma*-all primers, which amplify an approximately 1468bp DNA fragment from region of 16S rRNA gene from various members of the genus *Anaplasma*. For raising the test sensivity, the PCR products were amplified with the primers, which were designed from the region flanked by the first primers. The amplified nested PCR product had an expected PCR product with 345 nucleotides in length. In 100 sheep blood samples, 7 samples were *Anaplasma* spp. positive by first PCR and nested PCR.. The results showed that 2 of total 100 blood samples (2%) were *A.phagocytophilum* positive by specific nested PCR based on 16S rRNA gene. The extracted DNA from positive *Anaplasma* spp. samples were amplified by *Anaplasma ovis* specific primers, which amplify an approximately 866bp DNA fragment from region of *msp4* gene. 5 out of 100 sheep blood samples (5%) were positive for *Anaplasma ovis*. This study is the first molecular detection of *A. ovis* and *A.phagocytophilum* from sheep in Iran.

F: Genes and genomics

Ref: P1F218/0046

Molecular and functional characterization of glucose transporter genes from *Echinococcus multilocularis*

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Alveolar echinococcosis (AE) is a zoonotic parasitosis caused by larval stage of fox tapeworm, *Echinococcus multilocularis* (Em). Em is distributed widely in northern hemisphere, causing serious health problems in various animals and humans. Em, like other cestodes, lacks digestive tract and absorbs essential nutrients including glucose across syncytial tegument on its external surface. Therefore, Em is supposed to use membrane transporters on its surface to gain glucose as previously reported for a closely-related species, *Taenia solium* (Rodríguez-Contreras, 1998). Based on this speculation, we tried to clone and characterize glucose transporter homologues from Em. As a result, we obtained full-length sequence of putative 2 glucose transporter genes from Em, designated as EmGLUT1 and EmGLUT2. *In silico* analysis revealed that these were both facilitated diffusion transporter classified in solute carrier family 2. Transcriptional expression of each transporter was confirmed by realtime RT-PCR both in larval and adult stages of the parasite. Functional expression analysis using *Xenopus* oocytes demonstrated clear uptake of 2-deoxy-D-glucose (2-DG) by EmGLUT1, but not by EmGLUT2 in this experimental system. Kinetic values of EmGLUT1 for 2-DG were calculated as follows: $K_m = 6.5 \pm 1.8 \text{ mM}$, $V_{max} = 41.8 \pm 5.8 \text{ mM}$, showing that EmGLUT1 has relatively high activity in glucose transport. Further analyses using the *Xenopus*-oocyte system revealed that 2-DG uptake of EmGLUT1 did not depend on presence/concentration of Na^+ nor H^+ , respectively. Based on above-mentioned findings, we have concluded that EmGLUT1 is a simple facilitated glucose transporter and possibly plays an important role in glucose uptake by Em throughout its life cycle.

F: Genes and genomics

Ref: P1F219/0070

Molecular detection and characterization of *Anaplasma* species in African buffalo (*Syncerus caffer*) and black wildebeest (*Connochaetes gnou*) populations in South Africa

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Bovine anaplasmosis, caused by *Anaplasma marginale* is the most prevalent tick-borne disease of cattle worldwide, and causes significant economic losses. *Anaplasma marginale* subsp. *centrale* provides protection against *A. marginale* and is used as a vaccine in many countries. Although it is well-known that the African buffalo is the natural reservoir host of various tick-borne haemoparasites, there is limited information about the susceptibility of buffalo and other wild ruminants to infection by *Anaplasma* species. In this study, the occurrence of *A. marginale* and *A. marginale* subsp. *centrale* in buffalo (n=226) and black wildebeest (n=42) from nine National Parks in South Africa was determined using reverse line blot (RLB) and duplex quantitative real-time PCR (qPCR) assays. The RLB and qPCR results revealed an overall higher prevalence of *A. marginale* subsp. *centrale* (39%) than *A. marginale* (29%) in buffalo samples. *A. marginale* subsp. *centrale* DNA was detected in 45% of the black wildebeest samples, and *A. marginale* in just 5% of the samples. The *groEL*, 16S rRNA and *msp4* genes were amplified from ten positive samples and sequenced. Phylogenetic analysis revealed separate *A. marginale* and *A. marginale* subsp. *centrale* clades suggesting that the two taxa are distinct. Our results show that *A. marginale* and *A. marginale* subsp. *centrale* are prevalent in African buffalo and black wildebeest populations in South Africa which could suggest that they are natural reservoirs for *Anaplasma* species. Identifying wildlife as a reservoir could indicate that these animals play an important role in the epidemiology and spread of anaplasmosis.

F: Genes and genomics

Ref: P1F220/0088

Morphological and molecular characterization of *Eurytrema cladorchis* parasitizing cattle (*Bos indicus*) in Bangladesh

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There is always controversy regarding identification of different species in the genus *Eurytrema*. Identification has been based mainly on morphology, which can be misleading and subject to differing interpretation among the scientists. Therefore, the aim of this study was to identify *Eurytrema* flukes both by morphology and molecular properties on the basis of 18S ribosomal RNA (18S rRNA) gene as well as internal transcribed spacer 2 (ITS2) to clarify their phylogenetic status. Among six different agro–ecological areas of Bangladesh, 22 *Eurytrema* flukes were recovered from the bile ducts of 22 cattle in Bandarban, a hill district. The flukes were identified as *Eurytrema cladorchis* through morphometric and morphological studies after staining with hematoxylin–carmine solution. The most notable morphological feature of the flukes is big testes, which are located symmetrically postero–lateral to the acetabulum and are heavily branched. Phylogenetic analyses were conducted by neighbor–joining phylogram inferred from both 18S rRNA (1784 bp) gene and ITS2 (229 bp) sequences. A monophyletic clade was constructed by the *E. cladorchis* from Bangladesh; however, the clade was distinct from those formed by *Eurytrema pancreaticum* and *Eurytrema coelomaticum*. From the final host selectivity, it is assumed that the flukes were primarily the parasites of wild ruminants and gradually become adapted to domestic ruminants. This study first described the existence of *E. cladorchis* from Bangladesh and may provide useful information for both morphological and molecular properties that may further help to clarify phylogenetic relationships within the genus *Eurytrema* and also for other digeneans.

F: Genes and genomics

Ref: P1F221/0119

Molecular characterization of autophagy-related genes from the cattle tick *Rhipicephalus (Boophilus) microplus*.

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The cattle tick *Rhipicephalus (Boophilus) microplus* is an obligate hematophagous arthropod that represents a major problem for cattle industry in tropical and subtropical areas, due to the economic losses that it causes. This tick also acts as pathogen vector, transmitting diseases such as babesiosis and anaplasmosis. The parasite exhibits remarkable adaptability to changing environmental conditions, as well as an exceptional ability to survive long-term starvation. Under favorable environmental conditions, *R. microplus* can complete its life cycle in 3_4 weeks. However, in the summer season, larvae can survive for as long as 3_4 months without feeding and, in cooler temperatures, may live without food for up to 6 months. This ability has been related with a process of intracellular protein degradation denominated autophagy. This process in ticks is to date poorly understood, and only few Autophagy-related (ATG) genes have been characterized. Therefore, the aim of the present study was to examine the *Boophilus microplus* gene index (BmiGI) database, in a search for ATG homologues. We predicted the following five putative ATG genes: ATG3; ATG4; ATG6 and two ATG8. Further characterization led to the identification of RmATG8a and RmATG8b, homologues of GABARAP and MAP1LC3, respectively, both of these belonging to the ATG8 family. PCR analyses showed that the expression level of RmATG8a and RmATG8b was higher in egg and larval stages when compared with ovary and midgut from adult ticks. This up-regulation coincides with the period during which ticks are in a starvation state, suggesting that autophagy is active in *R. microplus*.

F: Genes and genomics

Ref: P1F222/0121

Identification and molecular characterization of *Babesia* species in brown (*Parahyaena brunnea*), striped (*Hyaena hyaena*) and spotted hyaenas (*Crocuta crocuta*)

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Fifty-five blood and skin samples collected from three hyaena species were obtained from the biobanks of South African National Parks and the National Zoological Gardens of South Africa: 12 from brown hyaenas (*Parahyaena brunnea*), four from striped hyaenas (*Hyaena hyaena*) and 39 from spotted hyaenas (*Crocuta crocuta*). These were screened for the presence of *Babesia* and *Theileria* species using the Reverse Line Blot (RLB) hybridization technique. Samples were simultaneously screened for the presence of *Ehrlichia* and *Anaplasma* spp. The RLB results revealed that the PCR products of 74.5% of the samples hybridized only with the *Theileria/Babesia* genus-specific probe, and not with any of the species-specific probes, suggesting the presence of a novel species or variant of a species. No *Ehrlichia* and/or *Anaplasma* species DNA could be detected. The parasite 18S rRNA gene of five spotted and seven brown hyaena samples was subsequently amplified, cloned and the recombinants sequenced. Homologous sequence searches of databases that were performed indicated that the obtained sequences were most closely related to *B. lengau*, previously identified in cheetah in South Africa. The observed sequence similarities were subsequently confirmed by phylogenetic analyses which showed that the obtained hyaena sequences formed a monophyletic group with *B. lengau*, *B. conradae* and sequences previously isolated from humans and wildlife in the western USA. Within the *B. lengau* clade, the obtained sequences and the published *B. lengau* sequences grouped into four distinct groups, of which groups I, II and III each represented a novel *B. lengau* genotype. We suggest that these genotypes cannot be classified as new *Babesia* species, but rather as variants of *B. lengau*. The study confirms that hyaena are susceptible to infection by a *Babesia* sp similar to *B. lengau*, but also demonstrates that they are not clinically affected by the infection. Their role as carriers of this organism and their ability to carry this infection over to other species still needs elucidation.

F: Genes and genomics

Ref: P1F223/0215

Molecular phylogenetic analysis of *Fasciola* flukes from Eastern India

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Fasciola flukes from Eastern India were analyzed to infer their phylogenetic relationship with populations from neighboring countries using nuclear (ITS1) and mitochondrial (*nad1*) markers. Both *Fasciola gigantica* and aspermic *Fasciola* flukes were detected in Imphal, Kohima and Gantoku districts. The haplotypes of aspermic *Fasciola* flukes were identical or showed a single nucleotide substitution compared to those from populations in the neighboring countries, corroborating the previous reports that categorized them in the same lineage. However, the percentage of aspermic *Fasciola* flukes in Eastern India was lower than those in the neighboring countries, suggesting that they have not dispersed throughout Eastern India. In contrast, *F. gigantica* was predominant and well diversified, and the species was thought to be distributed in the area for a longer time than the aspermic *Fasciola* flukes. *Fasciola gigantica* populations from Eastern India were categorized into two distinct haplogroups A and B. The level of their genetic diversity suggests that the populations belonging to haplogroup A have dispersed from the west side of the Indian subcontinent to Eastern India with the artificial movement of domestic cattle, *Bos indicus*, whereas the populations belonging to haplogroup B might have spread from Myanmar to Eastern India with domestic buffaloes, *Bubalus bubalis*.

F: Genes and genomics

Ref: P1F224/0226

Molecular characterization of *Fasciola gigantica* from Nigeria: tracing the dispersal route of flukes within the African continent

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Fasciola gigantica is widely distributed in the tropical regions including the African continent. Recently, we have revealed the phylogenetic relationships between *F. gigantica* from Zambia (Ichikawa-Seki et al. submitting) and that from Egypt (Amer et al. 2011). In this study, we analysed *Fasciola* flukes from the three agroclimatic regions of Nigeria (forest, sahel and savannah) and characterized the phylogenetic relationships of *F. gigantica* within the African continent.

One hundred and seventy one *Fasciola* flukes were collected from cattle in the 28 locations. The flukes were fixed in 70% ethanol and shifted to the laboratory. Spermatogenic status of the flukes was examined by observing the presence or absence of self-produced sperm under an optical microscope after staining the seminal vesicles with hematoxylin-carmin. Nuclear ribosomal internal transcribed spacer 1 (ITS1) region, mitochondrial NADH dehydrogenase subunit 1 (*nad1*) and cytochrome oxidase subunit 1 (*cox1*) genes were amplified by PCRs. DNA types of ITS1 were distinguished by using a PCR-RFLP method whereas *nad1* and *cox1* haplotypes were determined by direct sequencing. Phylogenetic analyses were performed by using the mitochondrial DNA haplotypes together with those of *F. gigantica* from other African countries available in GenBank.

All of the Nigerian *Fasciola* flukes were identified as *F. gigantica* because they had abundant mature sperms in their seminal vesicles and displayed Fg type in ITS1 which is the identical band pattern for *F. gigantica*. The concatenated sequences of *nad1/cox1* detected from Nigerian *F. gigantica* were divided into the two haplogroups. However, *F. gigantica* from the three agroclimatic regions were almost equally categorized into both the haplogroups. Interestingly, one of the haplogroups detected in Nigeria had close relationship with the *F. gigantica* population from Egypt, and the other haplogroup was located at intermediate position between the Egyptian and Zambian *F. gigantica* populations. These findings may suggest the dispersal route of *F. gigantica* within the African continent.

F: Genes and genomics

Ref: P1F225/0233

Nucleotide differences at three loci of nuclear and mitochondrial genome of *Toxocara* nematodes

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The discrimination of two *Toxocara* species common in urban environment of Russia (*T. canis* and *T. cati*) is important for proper implementation of deworming procedures, as their infectivity for humans differ significantly. Primers LSU391 and LSU501 by Nadler *et al.*, 2006 amplify an approximately 1100 bp long fragment of large ribosomal subunit rDNA. Though only shorter sequences for *Toxocara* LSU rDNA are deposited in NCBI GenBank, it is possible to obtain about 700 bp long which demonstrate the difference in 21-22 bp between *Toxocara canis* and *T. cati*, what constitutes 2-3% of alignment total length. No intraspecific variability was discovered between *T. canis* populations from Russia and data from NCBI GenBank. Comparatively high level of intraspecific variability was found in *T. cati* – up to 6-8 bp (or about 1% total length of sequences under comparison). Several pairs of primers were tested for amplification of ITS region of *Toxocara* nematodes. It was found that combination of forward primer '18S' by Vrain *et al.*, 1991 and reverse primer AB28 proposed by J. Curran (see Joyce *et al.*, 1994) were the most efficient for amplification of entire ITS rDNA region (ITS1+5.8S+ITS2). These are mainly ITS1 rDNA sequences for *Toxocara* nematodes which can be found in NCBI GenBank. Total length of the alignment obtained for original and deposited sequences was about 450 bp. The level of intraspecific differences between *T. canis* and *T. cati* was on the level of 29-31 bp (6-7%). Intraspecific nucleotide differences were only evaluated for *T. canis* and found to be not exceeding 1-3 bp (below 0.6%). The most pronounced intraspecific differences between two *Toxocara* species were reported in the sequences of cytochrome oxidase I mitochondrial gene, reaching 48-49 bp (or about 12% of the total nucleotide count of 393 bp of the alignment). These sequences were obtained with standard LCO1490 and HCO2198 primers proposed by Folmer *et al.*, 1994, but very unstable and template quality dependent efficiency of amplification was observed. More stable amplification of cytochrome oxidase I fragment was obtained with primers and COI_F1 (CCT ACT ATG ATT GGT GGT TTT GGT AAT TG) and H7005 (CCGGAT CCA CAN CRT ATA NGT RTC RTG by Hafner *et al.*, 1994). Supported by the grant 14-16-00026 from Russian Scientific Foundation.

F: Genes and genomics

Ref: P1F226/0237

The regenerative effect of *Trichinella spiralis* on drug-induced myopathy in animal model.

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Myopathy is a muscle disease resulting in muscular weakness leading to impaired function in activities of daily life. The present study was designed to assess the regenerative effect of local injection of antigen, attenuated (irradiated) or living newborn larvae (NBL) of *T. spiralis* to overcome the experimentally drug-induced myopathy in rats. Experimental animals were divided into four groups; injected intramuscularly by *T. spiralis* antigen, irradiated NBL or living NBL either treated orally with mebendazole or not and a control group. Assessment of the motor function of the left quadriceps muscles, histopathological study of these muscles, determination of fibrinogen level and detection of Pax7 and MRF4 in the affected muscles were done. The results revealed that injection of *T. spiralis* antigen or living NBL then treated by mebendazole produced improvement and regression of muscle myopathy with better results with *T. spiralis* antigen injection. In conclusion, intramuscular injection of *T. spiralis* antigen or living NBL then treated by mebendazole have a role in amelioration the severity of drug induced myopathy. Further investigations regarding the identification of different *T. spiralis* molecules and their mechanisms of action in improvement of idiopathic myopathy are recommended.

F: Genes and genomics

Ref: P1F227/0320

Infections by *Cryptosporidium* spp in post-weaned and adult domestic ruminants

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Cryptosporidiosis is a zoonotic disease mainly affecting pre-weaned ruminants, which show the highest percentages of infection and oocyst-output. In order to provide data on the prevalence and *Cryptosporidium* species in domestic ruminants older than 2 months, faecal samples from 417 cattle, 229 sheep and 161 goats were collected in 96 farms from NW Spain. Two age groups were considered: post-weaned (2–12 months) and adults (>12 months). *Cryptosporidium* species were identified by SSU rRNA PCR-RFLP analysis.

Individual prevalences were higher in cattle (12.9%) than in sheep (7.4%) and goats (6.2%). Five *Cryptosporidium* species and one genotype were found. In cattle, *C. bovis* (56.4%) was predominant over *C. ryanae* (17.9%), *C. parvum* (10.3%), *C. andersoni* (5.1%) and a genotype similar to *Cryptosporidium suis-like* (10.3%). All species were more prevalent in post-weaned animals, with the exception of *C. andersoni* that was only identified in adults. In contrast, *C. parvum* was the most common species (63.2%) in both small ruminant species and in all age groups; infections by *C. xiaoi* (26.3%) were more prevalent in post-weaned goats, whereas *C. ubiquitum* (10.5%) was only identified in adult sheep. Our results revealed that post-weaned and adult small ruminants are carriers of *C. parvum* and therefore considered as an important risk for the appearance of neonatal diarrhoea outbreaks and a threat to Public Health.

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F: Genes and genomics

Ref: P1F228/0387

Multilocus analysis reveals further genetic differences between *Tritrichomonas foetus* and other tritrichomonads

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A degree of genetic heterogeneity in *T. foetus* isolates from feline and bovine origin has been observed using multiple genotyping techniques. However, the study of the diversity of tritrichomonads has been hampered by the lack of genetic information. In this study microsatellite genotyping and multilocus sequence typing were evaluated as candidate molecular markers. Approximately 5,004 *T. foetus* sequences deposited in GenBank were initially screened for microsatellite sequences using the TROLL software and subsequently the TRF programme. Ten putative markers were initially assessed and 5 of them, named TFMS3, TFMS5, TFMS6, TFMS 7 and TFMS10, were finally selected. In addition, the *gap2* gene (encoding for *T. foetus* GAPDH) and the TR7/TR8 variable-length DNA repeat sequences were also assessed along with the ITS region. A total of 17 field bovine isolates, 9 feline isolates of *T. foetus* and reference strains obtained from the American Type Culture Collection (*T. foetus*, *T. mobilensis*, *T. suis*, *Tetratrichomonas gallinarum* and *Pentatrichomonas hominis*) were analysed using these markers. The most polymorphic markers were found to be microsatellites MS10 and MS7. An 809 bp fragment of the *gap2* gene was successfully amplified from all the tritrichomonads included in the study. Sequence analysis showed differences between *T. suis*, *T. mobilensis*, and *T. foetus* feline with respect to the *T. foetus* ATCC reference strain. The amplification of the TR7/TR8 variable-length DNA repeat showed different patterns between feline and bovine isolates. Sequence analysis of a 217 bp band of this region revealed the existence of 3 SNPs (98.6% homology) between cat and cattle sequences. In order to develop a RFLP analysis, a search for sequences similar to the 217 bp was performed and a 503 bp region (named TF1) revealed the existence of two BbvI restriction sites that generated different length fragments for feline and bovine isolates. Overall, results obtained with the different markers were consistent. The neighbor-joining analyses of the multiple alignments performed with the sequences obtained in this study showed that *T. suis* clusters together with the bovine isolates of *T. foetus* whereas *T. mobilensis* and the feline isolates of *T. foetus* form a separate cluster.

F: Genes and genomics

Ref: P1F229/0390

Brain inflammation-related genes during canine visceral leishmaniasis

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Chronically infected dogs by *Leishmania infantum* may present neurological disorders, nevertheless, the pathogenesis of the cerebral form was not still elucidated. Toll-like receptors (TLRs), components of the innate immunity, recognize the lipophosphoglycan, present in the *Leishmania* parasite, mediating the proinflammatory cytokines production. Therefore, we aimed to evaluate the histopathological lesions and gene expression of TLRs and cytokines in the brain from fifteen infected dogs and four uninfected. Brain fragments and choroid plexi were collected in RNAlater and others samples were subjected to routine histological procedures and haematoxylin–eosin staining. RNA was extracted, reverse transcript to cDNA and submitted to RT-qPCR to quantify the gene expression of TLR-1, TLR-2, TLR-9, IL-1 β and TNF- α , using specific primers and Taqman probes. G3PDH was used as a reference gene. Meningitis and choroiditis were detected in the infected dogs. There was upregulation of IL-1 β (6.36-fold more; $p<0.001$) and TNF- α (4.31-fold more; $p=0.001$) and trend of upregulation of TLR-2 in the brain from infected dogs (2.69-fold more; $p=0.0799$). In the choroid plexus, TLR-2 was up-regulated (3.09-fold more; $p=0.047$), while the cytokines presented no differences between groups. Concerning TLR-1 and TLR-9, there was no differences between groups in the two areas evaluated. Therefore, these results indicate that TLR-2, IL-1 β and TNF- α may be being produced in the brain, and TLR-2 in choroid plexus, contributing to the development of the brain inflammatory process, contrasting to TLR-1 and TLR-9, that seem not to be related to the brain inflammation in dogs with visceral leishmaniasis.

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F: Genes and genomics

Ref: P1F230/0460

Search for genetic polymorphism in canine *Dirofilaria immitis*

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Dirofilaria immitis canine infections are worldwide prevented by the use of macrocyclic lactones (MLs). Resistance to ML in heartworm infection was brought into attention in 2005, when the first evidence of low ML efficacy was published. Since then, many studies have tried to identify the genetic basis for the development of drug resistance by the worms. In Brazil, the heartworm infection prevalence upward trend observed in the late 2000s raised the resistance issue locally. At the same time, the occurrence of single nucleotide polymorphisms (SNPs) in P-glycoprotein (*pgp*, positions 11 and 618) and β -tubulin (*tub*, position 561) genes were suggested as markers for ML resistance in *D. immitis*. Therefore, the present study aimed to identify those markers in microfilariae from twenty-six naturally infected dogs living at an endemic area (68,9% prevalence). All dogs were previously treated with four doses of ML prophylactic treatment as recommended by the manufactures. DNA samples of microfilariae pools were extracted from each dog and amplified with specific primers for the *pgp* and *tub* genes. PCR products were sequenced using 3730XL DNA analyser system (Macrogen) but did not reveal any SNP on the specific positions. Therefore, those resistance markers could not be found in *D. immitis* strains from an endemic coastal area of the State of Rio de Janeiro, Brazil, suggesting that markers for ML resistance may differ regionally.

F: Genes and genomics

Ref: P1F231/0572

Molecular identification of Paramphistomes from sheep in Cajamarca, Peru

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Paramphistomosis is a trematode disease affecting domestic and wild ruminant species in different parts of the world. The disease is an emerging problem in Cajamarca, Peru. The flukes that cause the disease are morphologically difficult to identify. Therefore, the purpose of the present study was to characterize using molecular techniques the species of paramphistomes present in sheep in Cajamarca. Specimens of parasites were collected individually from the rumen of thirty sheep slaughtered at the Municipal Abattoir of Cajamarca. The parasites were subjected to PCR-RFLP analysis for the ITS2+ fragment of the rDNA. DNA previously characterized as *Calicophoron microbothrioides* from local cattle was used as control. All samples amplified a band of ~500bp. Amplicons were then restricted by endonucleases: *Hinf*I, which gave three bands of 130, 170 and 200 bp; *Hha*I two bands of 220 and 280 bp; and *Tru*1I two bands of 480 and 20 bp. The restriction pattern found with the three enzymes matched that for *C. microbothrioides* previously found in cattle. Two amplicons were sequenced and compared with those reported at GenBank. The sequence of the 480 bp band was 100% homologous to *C. microbothrioides* (ABO56570 and KF791030) and with three *Paramphistomun cervi* reports (KJ459938.1, KJ459937.1, KJ459934.1). However, the *P. cervi* reports did not include a complete morphological description. It is concluded that *C. microbothrioides* is the main paramphistome found in sheep from Cajamarca, Peru.

F: Genes and genomics

Ref: P1F232/0574

Canine babesiosis in Lithuania: Detection and molecular characterisation of causative agent

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Canine babesiosis have recently shown expansion in their distribution in Europe. During the last decade, canine babesiosis has been continuously spread in Lithuania. It has become quite frequent in the last few years, and an increasing number of cases with a wide variety of clinical signs have been recorded throughout the country. In Lithuania identification of the disease agent in veterinarian clinics is based on microscopic analysis of size and morphology. The aim of the present work was to investigate the presence of causative agent in dogs clinically suspected of babesiosis using light microscopy and PCR for diagnosis, and to characterize detected *Babesia* parasites based on partial sequencing of 18S rRNA gene. A total of 123 blood samples from dogs showing clinical signs of babesiosis on the basis of veterinary inspection were tested for the presence of babesial parasites. *Babesia* isolated from dogs were detected and characterized by nested-PCR and sequence analysis of a fragment of the 18S rRNA gene. According to standard identification methods, *Babesia* parasites were detected in blood smears of 94 dogs (76.4%). Molecular analysis revealed the presence of *B. canis* in 108 dogs (87.8%). Sequence analysis showed that *B. canis* isolates from dogs were heterogenic. Two genotypes were distinguished based on two nucleotides (GA→AG) substitutions observed in 18S rRNA gene sequences. The results of present study confirmed presence of *B. canis* in Lithuania, and provide knowledge on distribution of *Babesia* species and genotypes in dogs in central part of Lithuania, and show necessity of using molecular analysis for the precise diagnosis of canine babesiosis.

F: Genes and genomics

Ref: P1F233/0575

Molecular characterization of *Cryptosporidium* isolates from pre-weaned cattle calves and lambs in Jammu and Kashmir, North India

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Faecal samples of 248 pre weaned cattle calves (135 diarrhoeic and 113 non diarrhoeic) and 180 lambs (112 diarrhoeic and 68 non diarrhoeic), aged 1-30 day-old were collected from government and privately owned dairy and sheep farms of Jammu and Kashmir, North India. Microscopical screening of faecal samples after modified Zeihl-Neelsen stain revealed that *Cryptosporidium* infection was significantly ($p < 0.05$) higher among diarrhoeic animals (62.22% calves and 31.25% lambs) than the non diarrhoeic animals (29.20% calves and 17.64% lambs). Molecular characterization by a nested PCR of the small subunit rRNA gene (18S) followed by restriction fragment polymorphism analysis with SspI, VspI and MboII restriction enzymes showed that all the cattle calves were infected with *Cryptosporidium parvum*. Of the 47 samples of lambs subjected to PCR-RFLP, 30 (63.82%) could be classified as *C. parvum* and 17 (36.17%) as *Cryptosporidium ubiquitum*. The results were also confirmed by sequencing of 18 *Cryptosporidium* DNA samples. Three sequences with accession numbers KC569975, KC569976 and KM225275 have been submitted to gene bank. This is the first report of *C. ubiquitum* in any host species in India. Our results conclude that identification of *Cryptosporidium* in livestock warrants better care of farm animals to avoid contamination and illness in vulnerable population. Moreover, both the species detected in the present study are zoonotic and suggest that these animals may serve as reservoir for human infections.

F: Genes and genomics

Ref: P1F234/0600

Genetic barcoding of fleas from urban-dwelling dogs in Paraguay

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Fleas are the most common ectoparasite infesting cats and dogs world-wide and contribute significant dermatological pathology in these animals. Fleas of the genus *Ctenocephalides* and in particular the cat flea, *Ctenocephalides felis* predominate flea populations of both cats and dogs. These fleas can be vectors for zoonotic pathogens such as *Bartonella* spp. and *Rickettsia* spp. and are capable of transmitting disease in family homes and veterinary clinics. Despite the global economic and public health significance of fleas of the genus *Ctenocephalides*, there is limited understanding of the genetic diversity and phylogenetic relationships of global populations, including in South America. Genetic 'barcoding' using the mtDNA *cox1* gene is an effective method within the integrative taxonomy toolkit of taxonomic confirmation following morphological classification of arthropod species. The marker also provides informative phylogenetic data. It is an especially significant tool for fleas of the genus *Ctenocephalides*, which are notoriously difficult to classify notwithstanding current contention in the literature regarding the existence of subspecies. In this study we investigate the flea species and their genetic diversity affecting dogs in Paraguay. We collected fleas from dogs in urban regions across Paraguay including the surrounding suburbs of the capital city Asunción and from the Minga Guazú district and performed amplification of the mitochondrial DNA *cox1* gene and sequencing on a cohort of fleas. We confirm the effectiveness of the *cox1* marker for 'barcode' species identification and phylogenetic analysis. Resolving the genetic diversity of fleas in Paraguay will provide essential baseline data to aid understanding of how to control these fleas and assess the associated zoonotic disease risks in Paraguay.

F: Genes and genomics

Ref: P1F235/0611

Overexpression of the *tcap1* endonuclease and a negative dominant in the viability of *trypanosoma cruzi* y strain submitted to oxidative stress

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Trypanosoma cruzi, the causative agent of Chagas Disease, survives to DNA damage generated by ROS/RNS inside the insect vector and the mammalian host. In recent eukaryotes, oxidative DNA damage is repaired mainly by Base Excision Repair (BER) pathway, being essential apurinic/apyrimidinic endonuclease activity of APE1. Nucleotide sequences that encode TcAP1 of *T. cruzi* (ortholog of human APE1) and a putative negative dominant (DN) of both TcAP1 (TcAP1DN) and APE1 (APE1DN), obtained by site-directed mutagenesis, were amplified and inserted into the expression vector pTREX-GFP. Constructs were transfected in epimastigotes of Y strain. Parasites that overexpress all endonucleases were submitted to *in vitro* metacyclogenesis to obtain trypomastigotes and amastigotes that express TcAP1, TcAP1DN and APE1DN. Protein expression was verified by direct fluorescence microscopy, immunofluorescence assays and western blot. TcAP1 and both DN were purified in native conditions from transfected epimastigotes. AP endonuclease activity of TcAP1 was determined using a modified oligonucleotide labeled with P³². TcAP1DN and APE1DN showed a low AP endonuclease activity and maybe both act as negative dominant over TcAP1. Epimastigotes that overexpress TcAP1 increase its viability when are exposed to increasing concentrations of H₂O₂ for 30 minutes, while those parasites overexpressing a DN do not showed differences in its viability. However, trypomastigotes that overexpress a DN showed a lower viability when were treated in oxidative conditions. Our results confirm that BER pathway is involved in the resistance of *T. cruzi* against oxidative DNA damage and suggests their participation in the persistence of the parasite in their hosts.

F: Genes and genomics

Ref: P1F236/0612

Molecular systematics unravelled: the case study of the Piroplasms.

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Piroplasms are ubiquitous tick-borne protozoan parasites that infect mammals and birds. The order Piroplasmida includes three accepted genera (*Babesia*, *Theileria* and *Cytauxzoon*) comprising some highly pathogenic species, of significant medical and economic importance.

Over the last decades, molecular studies have strengthened morphological classifications, allowed the development of diagnostic assays and the investigation of evolutionary relationships among the piroplasms. The small ribosomal subunit RNA gene (18S rDNA) is the most frequently used gene in phylogenetic studies and over 3,000 sequences are available in GenBank. Despite this, the current molecular systematics is still confused and there is continuing *debate* over the number of valid genera and inter-generic relationships.

In the present study we evaluated the reliability and robustness of different phylogenetic reconstruction methods and assessed their effect on piroplasms molecular classification.

A comprehensive set of available 18S rDNA sequences was processed in parallel, using different combinations of alignment algorithms (ClustalW, PAGAN, PRANK, MUSCLE, MAFFT), alignment curation options and tree-building methods (Bayesian inference, Neighbour Joining, Maximum Likelihood).

The comparison of the outcomes of each workflow clearly demonstrates that each process has the potential to produce plausible tree topologies, characterized by strongly supported clades. On the other hand, critical topological discrepancies were obtained, when different phylogenetic reconstruction criteria were adopted.

The present analysis clearly shows that many of the literature disagreements about the molecular phylogeny of the piroplasms can be explained by alternative alignment algorithms, alignment curation options and tree-building methods. The comparison of the outcomes allowed the identification of the most robust workflow and a novel systematics for the order is presented. Finally, the potential application of the analytical approach implemented during the present study, to other genes and organisms is discussed.

F: Genes and genomics

Ref: P1F237/0613

A putative novel species of *Theileria* from the boodie (Marsupialia, Potoroidae, *Bettongia lesueur*)

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Piroplasms are tick-borne apicomplexan haemoparasites of global socio-economic importance. The order Piroplasmida comprises the genera *Theileria*, *Babesia* and *Cytauxzoon*, and some species highly pathogenic to humans, companion- and production-animals. Despite this, the overall molecular systematics of the group is still confused and under debate.

Conservation studies on Australian marsupial hosts have focussed primarily on parasite prevalence, transmission and pathogenicity. DNA sequence data obtained from these ancient mammals and geographically isolated populations, however, can prove useful also for resolving the phylogeny and molecular systematics of the Order.

In 2012 a novel genotype of *Theileria* was obtained from a boodie (*Bettongia lesueur*), sheltered in a wildlife rehabilitation centre near Perth, WA. When partial fragments of the 18S rRNA gene were analysed, the *Theileria* sp. appeared genetically close to known marsupial-derived protozoan species from the woylie (*Bettongia penicillata ogilbyi*), the quokka (*Setonix brachyurus*), and the long-nosed potoroo (*Potorous tridactylus*). The molecular classification was supported by microscopic observations of extracellular schizonts consistent with the distinguishing features of the Theilerias.

Interestingly, a new phylogenetic reconstruction conducted during the present study revealed that when a nearly complete fragment of the same gene was aligned, the boodie-derived *Theileria* sequence fell on a unique branch within the piroplasms, suggesting it may actually represent a new species. The novel analysis implemented in the present study represents the most comprehensive and robust phylogenetic reconstruction obtained for the piroplasms, up to now. The results also show that marsupials are hosts to multiple genera and highly divergent genetic variants of piroplasms, indicating lack of host-parasite co-speciation and suggesting that host switches, parasite extinction or other factors have shaped the host-parasite evolution and ecology.

F: Genes and genomics

Ref: P1F238/0618

Genomic scanning for drug resistance loci in *Haemonchus contortus*

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Understanding the evolutionary mechanisms of anthelmintic resistance is key to identifying putative mutations and mitigating the spread of resistant parasitic nematodes. We are employing a population genomic approach to elucidate genomic signatures of selection in the parasitic nematode *Haemonchus contortus*. We are using a ddRAD-seq approach (double-digest Restriction-site Associated DNA sequencing), in association with the *H. contortus* reference genome, to identify loci conferring resistance. Initially, we will investigate the region around the isotype-1 β -tubulin locus, which confers resistance against benzimidazoles, for a signature of selection. This will help us determine what the footprint of selection looks like around a locus known to be under selection due to drug treatment. Using that knowledge we will then conduct genome-wide scans to look for further regions associated with drug resistance. The regions and ultimately putative mutations elucidated through this approach will help our understanding of the origin and spread of resistance, and help control its prevalence.

F: Genes and genomics

Ref: P1F239/0626

Horizontally transferred genetic elements in the tsetse fly genome

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Tsetse flies (*Glossina* spp.) are the primary vectors of trypanosomes, which can cause Human and Animal African trypanosomiasis in sub-Saharan African countries. The flies also harbour a variety of bacterial microorganisms including three maternally transmitted endosymbionts, namely *Wigglesworthia*, *Sodalis*, and *Wolbachia*, which have a significant influence on the physiology of their host. In addition to the parasitism of bacterial organisms themselves, it is known that the genetic elements of microorganisms have been incorporated in the tsetse fly genome. The objective of this study was to explore the genetic elements horizontally transferred from microorganisms in the genome of *Glossina morsitans morsitans* using a composition-based bioinformatics program, so-called Batch Learning Self-Organizing Map (BLSOM). The method allows clustering of the sequence fragments based on the similarity of oligonucleotide frequencies independently of homologous sequences and thus has potential to discover horizontal gene transfer (HGT) events that could not be detected by conventional homology-based approaches such as BLAST. After initial scan of potential HGT events by BLSOM, nearly 5% of the tsetse fly genome was assigned as potential HGT candidates. The dominant phylum of HGT origin was Firmicutes, followed by phyla Actinobacteria, Bacteroidetes, and Thermoogae. Functional annotation using KEGG (Kyoto Encyclopedia of Genes and Genomes) database revealed that the detected HGT candidates encoded various functional pathways especially carbohydrate, lipid, and nucleotide metabolisms. We believe that the whole picture of HGT events in tsetse fly genome may lead to better understanding of co-evolutionary process of both host fly and microorganisms including trypanosomes.

F: Genes and genomics

Ref: P1F240/0729

***Sarcocystis tenella* natural infection in water buffaloes (*Bubalus bubalis*).**

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Large apicomplexan tissue cysts were identified in cardiac muscle samples collected from two adult female Murrah water buffaloes (*Bubalus bubalis*), from São Paulo – Brazil, using routine pathology techniques (haematoxylin and eosin). The samples were tested by immunohistochemistry for different protozoan parasites and positivity was found using anti-*Sarcocystis* spp. antibodies. DNA was extracted from these samples using a commercial kit and PCR reactions aimed on 18S gene were performed with generic primers. Resulting bands were purified and sequenced. Consensus sequences were identical and identified as *Sarcocystis tenella* using Blast search engine (BlastN 2.2.31+). This is the first identification of this *Sarcocystis* species in water buffaloes.

G: Co-infection

Ref: P1G076/0034

Studies on Blood Parasites of Goats in Mogadishu city of Somalia

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Blood smear of 100 goats from Mogadishu city goat-holders were examined for the presence of blood parasites using light microscope. Some ticks were also collected for the identification. The overall infection rate was 100% in investigated goats, 22 samples (22%) showed *Babesia* infection and 14 (14%) *Theileria* infection. Interestingly, the Remaining 64 infected goats (64%) showed co-infection of *Babesia* spp. with *Theileria* spp. No other blood parasites, Trypanosomes & Anaplasma, were found. Ticks observed on the investigated goats are: *Rhipicephalus evertsi* (72.84%), *Rhipicephalus pulchellus* (34.57%), *Amblyomma lepidum* (3.70%) and *Hyalomma rufipes* (1.23%). In conclusion, the results of this study revealed hyperparasitaemia as well as co-infection of *Babesia* and *Theileria* species. Although the animals are apparently healthy and this might be due to the unusual acaricidal practice among goat-holders or due to the host resistant to these parasites. In this issue, the need for further investigation using advanced molecular techniques is suggested.

G: Co-infection

Ref: P1G077/0076

Prevalence of equine theileriosis in and around Lahore, Pakistan

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Theileriosis is an infectious malady of equidae characterized by intermittent fever, loss of appetite, anemia, loss of weight, dyspnea, pale mucous membrane, jaundice and recumbency. Considering the significance and utilization of equines and the substantial losses rendered by Theileriosis. The present project was designed to study Prevalence and chemotherapy of Theileriosis in equines in and around Lahore. For this purpose 300 equines (Horse=100; Donkey=100; Mule=100) were examined for Theileria. The blood was collected from ear tip puncture and from Jugular venipuncture. Then thin blood smear slide was prepared and examined under microscope. *Theileria equi* were identified by their morphological characteristics. Data on prevalence of theileriosis was estimated by Pearson's chi-square test for significance using statistical software package STATA 9.1 (College Station TX 77845, USA).

Over all prevalence of *Theileria equi* in equines was estimated as 58%. There was also a mixed infection of *Theileria equi* and *Babesia caballi* in some cases. Species wise prevalence of *Theileria equi* was 54%, 64% and 56% in horses, mules and donkeys respectively. Prevalence of theileriosis was 50%, 75% and 60.75% in male horses, mule and donkey respectively while in female it was 57.1%, 33% and 38% in horses, mule and donkey respectively. Overall prevalence was significantly more in male as compared to female ($p < 0.05$). Prevalence of theileriosis was 50%, 100% and 67.7% in < 5 years age old horses, mule and donkey respectively while it was 52.3%, 60.4% and 75.4% in ≥ 5 years aged horses, mule and donkey respectively ($p < 0.05$). The overall therapeutic efficacy of Imidicarb Dipropionate in equines was found 91.7%. The efficacy of Buparvaquone was found as 66.7%, while of Aak (*Calotropis procera*) as 58.3% at 10th day after treatment. Among three drugs overall efficacy of Imidicarb Dipropionate was highest followed by Buparvaquone and Aak (*Calotropis procera*).

Key words: *Theileria equi*, Blood, Prevalence, Equine, Age

G: Co-infection

Ref: P1G078/0220

Endoparasitic infections of *Alectoris rufa* in an aviary in the Czech Republic

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For the first time, age-related dynamics of endoparasitic infections in the red-legged partridge *Alectoris rufa* were studied in an intensive breeding aviary in the Czech Republic, with special focus on *Cryptosporidium* spp. infections. **Materials and Methods:** Examinations were carried out during 2012 and 2013 (July-December). **a)** Cysts and oocysts of protozoans and helminth eggs were detected from fresh pooled (20-30 individual excrements) faecal samples. A total of 663 faecal samples were examined by the coprological flotation method (242 in 2012 and 421 in 2013) during bird rearing (at 7 day-intervals) for 1-22 weeks. **b)** Complete post-mortem examination of 89 birds (51 in 2012; 38 in 2013) of different ages were carried out by flotation, native preparations (digestive tract, bursa Fabricii, trachea) and mucosa on smears stained according to Giemsa, and histologically. **c)** Representative *Cryptosporidium* spp. oocyst isolates were also identified by PCR analysis of the HSP70, actin and GP60 genes. **Results:** Protozoans: during both years *Cryptosporidium meleagridis* occurred in 42 samples (prevalence 17%) and 99 samples (24%) mainly in 15-18, 19-21, and 22 week-old age classes, whereas 4 samples (1%) of the 5-7 week-old age class harboured *C. baileyi* in 2013. *Eimeria* spp. first occurred at age 2-3 weeks, 85 (35%) and 114 samples (27%) during whole rearing with a maximum in week 7-8 (2012) and 6, 20-21 (2013). Sequence analysis of isolates from both years confirmed the occurrence of *C. meleagridis* and *C. baileyi*. The GP60 subtype was identified as the IIIe family, named IIIeA16G2R1 variants b and c. Helminths: *Capillaria* spp. 113 (47%) and 241 (57%) positive samples during 11-22 weeks with a maximum in weeks 15-21. *Syngamus tracheae* 70 (29%) and 168 (40%) samples at 8 and 10-22 weeks with a maximum at 14-15, and 19-21 weeks. **Conclusions:** The main infections of *A. rufa* comprised representatives of the coccidian genera *Eimeria* and *Cryptosporidium* and of the nematode genera *Capillaria* and *Syngamus*. Further research will be focused on the potential to design control measures for these parasites using appropriate antiparasitic drugs.

This study was supported by the grants CIGA 20145011, and IGA FTA 20155014.

G: Co-infection

Ref: P1G079/0235

Anti- *Neospora caninum* and *Sarcocystis* spp. specific antibodies cross-react with *Besnoitia besnoiti*: influence of co-infections on serological diagnosis of bovine besnoitiosis

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Bovine besnoitiosis control remains to be a challenge since the disease continues spreading and control relies solely on accurate diagnosis coupled to management measures. However recent studies have reported that routinely used ELISAs may raise a high number of false positive results. ELISA cross-reactions between anti- *N. caninum* and/or *Sarcocystis* spp. specific antibodies and *Besnoitia besnoiti* antigens were investigated herein, since both infections are highly prevalent in cattle worldwide. The sera panel comprised the following categories: *B. besnoiti* seronegative (n=75) and ELISA false positive reactors (n=96), seropositive infected cattle sera (n=66) together with *N. caninum* (n=36) and *Sarcocystis* spp. (n=42) infection reference cattle sera. *B. besnoiti* tachyzoite based Western blot (WB) results classified animals as infected or non-infected. Sera were analyzed for the detection of specific anti-*N. caninum* by ELISA and WB and *Sarcocystis* spp. antibodies by IFAT and WB. Those samples recognizing *Sarcocystis* spp. 18-20 KDa antigenic area and *N. caninum* 17-18 KDa immunodominant antigen were considered as *Sarcocystis* spp. and *N. caninum* seropositives, respectively. The category of *B. besnoiti* false positive reactors showed the highest number of sera with specific anti-*Sarcocystis* spp. and *N. caninum* (74%) antibodies, followed by *N. caninum* infected cattle category (52.8%). The study revealed that *B. besnoiti* false positive ELISA results were associated not only to the presence of *N. caninum* and *Sarcocystis* spp. co-infections (χ^2 : 78.36; $p < 0.0001$; OR: 34.6; CI: 14-88), but also to high antibody levels against them ($p < 0.05$; *t*- Test). These results may explain why only some co-infected animals with *Sarcocystis* spp. and/or *N. caninum* are *Besnoitia* false positive reactors. Thus, sera comprising these requirements should be included in future validations of serological tests for bovine besnoitiosis.

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G: Co-infection

Ref: P1G080/0241

First case of a mixed infection by lungworms and heartworms in a domestic cat

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Aelurostrongylus abstrusus affects the lungs of domestic cats worldwide. Recently, other nematodes have been isolated from the respiratory system of cats either for the first time or after a long period since the first descriptions. *Troglostrongylus brevior* (that affects wild felids) has been found in cats from Southern and Central Italy and European Islands. The heartworm *Angiostrongylus chabaudi* has been recorded only in wildcats but an immature female nematode has been recently found in a domestic cat in Italy. The present report describes the first known mixed infection by *A. abstrusus*, *T. brevior* and *A. chabaudi* in a domestic cat. A ~6 month-old kitten dead for severe respiratory signs was necropsied and the cardio-respiratory tract was subjected to histological examinations. At necropsy lungs were edematous with scattered petechial areas, while histo-pathological findings revealed severe catarrhal bronchitis and a diffuse edema with emphysematous lobules. No evidence of adult or immature heartworms was recorded in histological sections. Thirty-two nematodes (30 *T. brevior* and 2 *A. abstrusus*) were recovered from the airways, while two parasites found in the proximal pulmonary arteries were microscopically and genetically identified as *A. chabaudi*. Nonetheless, morphological and morphometric differences were found in comparison with previous reports, thus calling for a re-description of this poorly known heartworm. These findings corroborate the existence of drivers currently fostering bridging infections from wild reservoirs to domestic hosts. Thus, a high level of vigilance is necessary to add new pieces to the intricate puzzle presently represented by extra-intestinal nematodes potentially infecting domestic cats.

G: Co-infection

Ref: P1G081/0307

Ecological analysis of structure helminth fauna of marals (*Cervus elaphus songarica*) in Kyrgyz Republic: biodiversity and parasite community

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The analysis helminth fauna domestic and wild animals shows that between them there can be an exchange of certain helminths. The helminthofauna of marals (*Cervus elaphus songarica*) under the conditions of highland ecological systems of in Kyrgyz Republic in terms of parasitology is insufficiently explored.

The focused helminthological examinations were launched in the natural conditions of Naryn State Nature Reserve. The objects of helminthological examination were marals (*Cervus elaphus songarica*) and domestic animals from households surrounding the nature reserve. Such examination method was applied to the liver and digestive system of marals and domestic animals (cattle, sheep).

The helminthological examinations held have shown that within the marals population the parasitizing helminths are of the families of *Dicrocoeliidae* (*Dicrocoelium lanceatum*) - 64,0%, *Trichostrongyloidea* (*Trichostrongylus capricola*, *Haemonchus contortus*, *Dictyocaulus filaria*), - 58,7%, *Anoplocephalidae* (*Moniezia expansa*) -23,1%, *Ascaridoidea* (*Neoascaris vutulorum*) - 17,8%, *Trichocephalidae* (*Trichocephalus ovis*) -16,5-%, *Paramphistomatidea* (*Paramphistomum cervi*) - 13,6%, *Fasciolidae* (*Fasciola hepatica*, *Parafasciolopsis fasciolaemorpha*) - 8,7% and parasitic protozoa – *Eimeriidae* (*Eimeria crandallis*, *Eimeria ovinoidealis*)- 38,9%. Nematodirus invasion prevails. Nematodiruses were found most frequently in young animals. The basis of parasite complex is made of *Trichocephalidae* and *Eimeriidae* oocysts that are dominant in terms of frequency of occurrence and that have epizootic importance.

Trematode prevalence in domestic cattle was 59.0%. The prevalence of *Fasciolidae* in cattle was 25-38% and intensity 62, *Dicrocoeliidae* in sheep 80% and 79-117.

The received research results show that the marals in are mainly affected by the helminthiasis, the causative agents of which are developed with the help of intermediary host. The spread of such helminthiasis is connected to the special features of ecology of terricole molluscum of *Bradybaena* genus and ants of *Formica* and *Proformica* genera. During our research *Bradybaena* species were encountered in various biotypes (the sections of mixed forest; the shores of basins covered with bushes; water meadows). They dwelled in leaf litter, in hollows, under stones, in grass, in floodplain bushes and trees. The population density per 1 m² reached 1.3 to 17.5 species.

G: Co-infection

Ref: P1G082/0395

Occurrence of cardio-pulmonary nematodes in European wildcats (*Felis silvestris silvestris*) from Italy

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Feral and wild animals have recently been incriminated as a source of infections of pets with cardio-pulmonary nematodes. Moreover, molecular studies have demonstrated that genetically identical populations of parasites (e.g. *Capillaria aerophila*, *Angiostrongylus vasorum*) can be shared between wildlife and pets. Data on the presence of lungworms in wildcats from central areas of Italy, where these parasites have high prevalences in cats and wildcat populations have one of their highest density, are lacking. In the present study, the carcasses of 16 animals were necropsied and examined. Adult nematodes, faeces, respiratory flushings were collected, and parasites were morphologically and genetically identified. Tissue samples were also processed for histology. Fourteen animals were positive for lungworms; *Aelurostrongylus abstrusus* was the most prevalent parasite (n. 10, 62.5%), followed by *Troglostrongylus brevior* (n. 8, 50%) and *C. aerophila* (n. 3, 18.7%). Nine animals had a mono-specific infection, whereas mixed-infections were observed in five cats, being *A. abstrusus* and *T. brevior* (n. 3, 18.7%) the most common association. Three nematodes belonging to the *Angiostrongylus* genus were found in the pulmonary arteries of a wildcat infected also by lungworms. The most common lesions were a mild-severe chronic catarrhal bronchitis and a chronic interstitial pneumonia with smooth muscle hypertrophy, fitting with findings in domestic cats infected by *T. brevior* and *A. abstrusus*, respectively. The present results demonstrate that *A. abstrusus* infects in wildcats in particular areas, thus confirming that different drivers may concur in changes of traditional epidemiological scenario of extra-intestinal nematodes both in wildlife and domestic hosts.

G: Co-infection

Ref: P1G083/0420

Molecular diversity of amoebas of *Entamoeba* genus in savannah chimpanzees

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Due to intensive human encroachment into the previously non-impacted forests, which increase the potential for disease transmission between human and non-human primates, we conducted a survey on the amoebas of the genus *Entamoeba* in a wild population of savanna chimpanzees living in the Issa Valley (Tanzania). We compared the results obtained by coproscopical and molecular diagnostics and conducted the molecular-phylogenetic analyses on *Entamoeba* isolates. Using a coprological technique we detected the cysts of complex *E. histolytica*/*dispar*/*moshkovskii*/*nuttalli* and *E. coli* only, while we recorded three *Entamoeba* species, namely *E. coli* (47%), *E. dispar* (16 %) and *E. hartmanni*-like (51%), using molecular species-specific PCR protocols. On the part of the sample dataset, we demonstrated that the molecular prevalence of entamoebas was higher than the prevalence based on the coproscopical analyses (9 % vs. 47 %). Our molecular phylogenies showed that our isolate of *E. dispar* and *E. coli* from Issa chimpanzees are closely related to isolates of primates and humans from GenBank. Our results showed that wild chimpanzees harbor some *Entamoeba* species similar to those occurring in humans, but further research is essential for determining if these infections are of natural or zoonotic origin. We argue that molecular methods are critical to improving diagnostics of entamoebas in wild primates.

G: Co-infection

Ref: P1G084/0452

Ticks and nematodes on hair sheep in the hot humid tropic of Mexico

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This study described the frequency of gastrointestinal nematodes (GIN) and ticks in hair sheep under hot humid tropical conditions. Ticks were also identified to report the genus. A prospective study was performed in a sheep farm during rainy season (August to November 2013). A total of seven visits were performed, once every 15 days. A variable number of hair sheep ewes were inspected in each visit (from 75 at 227 ewes). Ewes were inspected to find ticks present in the ears, inner part of the arms, the tail, and the perianal region. Additionally, all animals were faecal sampled directly from the rectum. Animals positive to GIN and or ticks were recorded on each visit. Twenty adult ticks were collected randomly and preserved in 70 % alcohol for identification. In total, 946 observations were recorded. The overall frequency of sheep positives to GIN was 22.8%, for ticks 20.8%, and for mixed infections 8.2%. The GIN frequencies were 26.4 %, 22.0%, 13.1 % and 85.3% in August, September, October and November. The frequencies of animals positive to ticks were 25%, 47.3%, 4.8% and 50.7%. Mixed parasite infection frequencies were 11.5, 13.2, 0.8 and 40% respectively. Ticks collected were all identified as *Amblyoma maculatum* (Acari: Ixodidae). In conclusion, continuous infestation with ticks, and mixed infections (GIN- ticks) is reported in hair sheep in the Mexican tropics. More studies need to determine the prevalence and other tick species, and the possible presence of haemoparasites that could transmit to sheep by these ectoparasites.

G: Co-infection

Ref: P1G085/0459

Protozoa infections in crab-eating fox (*Cerdocyon thous*) from the Northeast Region of Brazil

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Different parasites species may act in the control of population of wild hosts, preventing animal overpopulation and helping to maintain the genetic diversity of vertebrates and invertebrates. Wild carnivores may be infected by several pathogens, including protozoa such as *Toxoplasma gondii* and *Neospora caninum*. Both parasites have been poorly studied in wild animals and many biological aspects involving these animals remain unknown. In this study the occurrence of antibodies anti-*T. gondii* and *N. caninum* were investigated in crab-eating fox (*Cerdocyon thous*) from the Northeast region of Brazil. Sera samples of free-living animals (n = 20) were used in this study and Immunofluorescence Antibody Test (IFAT) was performed to detected antibodies anti-*T. gondii* and *N. caninum*. The presence of antibodies anti-*T. gondii* and anti-*N. caninum* was detected in 20 % (4/20) and 70 % (14/20) of the animals, respectively. These results demonstrate that *C. thous* have been exposed to these protozoa in the area of study. These findings indicate environmental contamination by the oocysts of *T. gondii* and *N. caninum*. However, it remains to be determined if these carnivores are able to eliminate *N. caninum* oocysts.

G: Co-infection

Ref: P1G086/0469

Is there an association between *Fasciola hepatica* and bovine TB?

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Bovine TB (bTB) is a mycobacterial disease affecting cattle in the UK. Despite an extensive test and cull programme aimed at eradicating bTB from England and Wales, the number of new bTB cases has continued to increase. *Fasciola hepatica* is a parasite of cattle, causing health and welfare issues as well as financial losses. *F. hepatica* has been shown to modify the immune response of cattle and is thought to affect the sensitivity of the bTB skin test. Field studies showed a significant negative association between herds diagnosed with *F. hepatica* and bTB. The aim of this study was to investigate this association in individual animals from commercial herds. Thirteen beef and dairy herds were recruited through their vets, and all or most of the herd (1843 cattle in total) were blood sampled at the time of their TB test. These samples were tested for antibodies to *F. hepatica* using an ELISA. IgG isotype ratios were also tested in a proportion of animals. There were no significant differences in *F. hepatica* exposure or IgG ratio between the TB reactors, Inconclusive Reactors and negative groups. However there were only a small number of TB cases (n=9). The study is ongoing.

H: Companion animal

Ref: P1H092/0006

Molecular detection and genotyping of *Enterocytozoon bieneusi* in pet shop puppies and breeding kennel dogs in Japan

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Enterocytozoon bieneusi is one of the most common Microsporidia and can cause diarrhoea in humans and dogs. Additionally, some *E. bieneusi* isolates from dogs have the potential for zoonotic transmission. Here, we determined the prevalence of *E. bieneusi* infection and the genotype of isolates from pet shop puppies and breeding kennel dogs in Japan. Fresh faecal samples were collected from 595 pet shop puppies (four pet shops) and 160 breeding kennel dogs (three breeding kennels). A nested polymerase chain reaction (PCR) assay targeting the internal transcribed spacer (ITS) region of ribosomal DNA was employed for detection of *E. bieneusi*, and 22 random samples of PCR amplicons (approximately 390 bp) were sequenced for genotyping. The overall prevalences of *E. bieneusi* in pet shop puppies and breeding kennel dogs were 7.7% and 16.3%, respectively. In pet shop puppies, the prevalence of *E. bieneusi* did not differ with faecal conditions; however, the prevalence significantly differed among the pet shops. In breeding kennel dogs, no significant correlation was observed between the prevalence of *E. bieneusi* and age or faecal condition; however, the prevalence significantly differed among the breeding kennels. All of the 22 sequenced samples were genotyped as PtEb IX, which belongs to the dog-specific genotype. In conclusion, *E. bieneusi* infections are common in pet shop puppies and breeding kennel dogs in Japan. However, the risk of zoonotic transmission of *E. bieneusi* from dogs to humans is likely low.

H: Companion animal

Ref: P1H093/0007

Molecular detection and characterization of *Cryptosporidium* species in private-household cats, and pet shop kittens in Japan

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Cryptosporidium species can cause diarrhoea in humans and animals, including cats. In addition, *Cryptosporidium* species harboured in cats have the potential for zoonotic transmission. Here, we determined the prevalence of *Cryptosporidium* species infection in private-household cats and pet shop kittens in Japan and performed a molecular characterization of isolates. Fresh faecal samples were collected from 297 private-household cats (from nine veterinary clinics located in six different regions) and 251 pet shop kittens (≤ 3 months old, from four pet shops located in two different regions). We used a semi-nested polymerase chain reaction (PCR) assay targeting the 18S rRNA gene of *Cryptosporidium* species for detection, and the nine samples showing PCR amplicons (approximately 820 bp) were sequenced. The prevalence of *Cryptosporidium* species from private-household cats and pet shop kittens was 2.7% and 0.4%, respectively. In private-household cats, the prevalence in <1 year cats (6.2%) was significantly higher than in ≥ 1 year cats (1.0%). No significant correlation was observed between prevalence and cat source (private owners vs. pet shops vs. breeding kennels), living conditions (indoor vs. outdoor), and faecal conditions (formed vs. unformed). In pet shop kittens, prevalence did not differ with faecal conditions and pet shops. All of the nine sequenced samples were identified as *C. felis*. The present study demonstrates a low prevalence of *Cryptosporidium* species infection in private-household cats and pet shop kittens in Japan. The risk of zoonotic transmission of *Cryptosporidium* species from cats to humans is likely very low.

H: Companion animal

Ref: P1H094/0008

Molecular detection and genotyping of *Enterocytozoon bieneusi* in private-household dogs in Japan

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Microsporidia are single-celled obligate intracellular protozoans, and widely infect animals. *Enterocytozoon bieneusi* is one of the most common Microsporidia and can cause diarrhoea in humans and dogs. Additionally, some *E. bieneusi* isolates from dogs have the potential for zoonotic transmission. We determined the prevalence of *E. bieneusi* infection and the genotype of isolates from private-household dogs in Japan. Fresh faecal samples were collected from 597 private-household dogs (from nine veterinary clinics located in six different regions). A nested polymerase chain reaction (PCR) assay targeting the internal transcribed spacer (ITS) region of ribosomal DNA was employed for detection of *E. bieneusi*, and 16 random samples of PCR amplicons (approximately 390 bp) were sequenced for genotyping. The overall prevalence of *E. bieneusi* in private-household dogs was 4.7%. Significantly different prevalences were found among dog source categories (private owners: 3.2% vs. pet shops: 4.3% vs. breeding kennels: 14.3%) and living regions (six regions: ranged 0-10.4%). However, no significant difference was observed between prevalence and age (<1 year: 8.3% vs. ≥1 year: 3.8%), living conditions (indoor: 4.9% vs. outdoor: 2.0%), and faecal conditions (normal: 4.7% vs. soft: 7.0% vs. diarrhoea: 0%). Of the 16 sequenced samples, all were genotyped as PtEb IX, which belongs to the dog-specific genotype. In conclusion, although *E. bieneusi* infections are relatively common among private-household dogs in Japan, the risk of zoonotic transmission of *E. bieneusi* from dogs to humans is likely low.

H: Companion animal

Ref: P1H095/0058

Parasitic infections of domestic dogs in western Hungary

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During the years 2012 and 2013, faeces from 296 domestic dogs from a rural area in western Hungary were examined for endoparasitism using standard techniques (modified McMaster, Baermann, Giardia coproantigen ELISA). The overall prevalence of dogs with evidence of endoparasitism was 47% (95%CI 41.2 – 52.8). The most frequently identified faecal forms of parasites were those of *Trichuris* (26.4%), ascarids (*Toxocara*, 10.1%; *Toxascaris*, 0.3%) and hookworms (10.1%), followed by those of *Capillaria aerophila* (4.1%), taeniid cestodes (1.0%), and *Crenosoma vulpis*, *Mesostephanus* trematodes and *Isospora canis* (0.3% each). *Giardia*-specific antigen was detected in 17.9% of the samples. Single and multiple infections with up to three parasites concurrently were recorded in 29.7% and 17.2% of the dogs, respectively. Young dogs (47, <1 year) tested more frequently positive for *Giardia* ($p=0.0004$) and overall gastrointestinal parasitism ($p=0.025$) than the older dogs.

In addition, ectoparasites collected from 260 dogs by body search and combing were identified. Fleas (2-60 per dog) and ticks (1-32 per dog) were isolated from 132 and 184 dogs, respectively; 56 dogs had concurrent flea and tick infestations. Regarding fleas, *Ctenocephalides canis*, *C. felis*, *Pulex irritans* and *Archaeopsylla erinacei* were identified on 127, 7, 4 and 2 dogs, respectively. Regarding ticks, *Dermacentor reticulatus*, *Ixodes hexagonus* and *I. ricinus* were identified on 143, 1 and 66 dogs, respectively.

By providing basic data on the epidemiology of parasitic infections, the results of this survey emphasize the need for continuous attention to the parasites of dogs from the veterinary and medical points of view.

H: Companion animal

Ref: P1H096/0072

Epidemiological Study of Canine Babesiosis in Gwagwalada Metropolis of the Federal Capital Territory Abuja, Nigeria

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Babesia parasites are the causative agent of Babesiosis, a tick-borne zoonosis which has been increasingly described throughout the world. In Nigeria, dogs are not only kept as pets, but are also used for hunting as well as a source of animal protein among some ethnic groups. There is limited information on the prevalence and distribution of canine Babesiosis in dogs and ticks in Nigeria. Epidemiological investigation was carried out to determine the prevalence of infection with *Babesia* parasite in dogs in Gwagwalada metropolis of the Federal Capital Territory, Abuja Nigeria, from November 2013 to January 2014. Blood samples were collected from 101 dogs and examined microscopically for the parasite. Data obtained were analyzed to determine the prevalence of *Babesia canis* and the correlation of infection with age, sex, breeds, types of management and presence or absence of tick infestation on the host animals. Dogs screened were those from randomly selected households in the study area. Overall results show an infection rate of 9/101 (8.9%). The prevalence was higher ($P < 0.05$) among adults than puppies and also higher ($P < 0.05$) among dogs with tick infestation than those without. This level of prevalence could be under reported and therefore of serious public health consideration due to close association of dogs and their owners.

H: Companion animal

Ref: P1H097/0098

Isolation and genetic characterization of *Toxoplasma gondii* in dogs in Brazil

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Dogs have an epidemiological importance because they act as human *Toxoplasma gondii* infection sentinels, being good indicators of environmental contamination. In these animals serological results for *T. gondii* were widely reported in Brazil, with percentages ranging from 4,9 to 91%. The aim of this study was to isolate and characterize genetically the *T. gondii* strains originating from dogs naturally infected tissues of the cities of Ilhéus and Itabuna, Bahia, Brazil. For this, 21 dogs blood samples were collected from the Zoonosis Control Centers of the respective cities. The sera were examined for *T. gondii* antibodies by the indirect hemagglutination test. Brains and hearts of seropositive dogs were bioassayed in mice for the isolation and characterization of the parasite by PCR-RFLP using 10 genetic markers (SAG1, newSAG2, SAG3, BTUB, c22-8, c29-2, GRA6, PK1, APICO and L358). The serology scored seven (33,3%) seropositive dogs. However, in the bioassay, *T. gondii* was isolated in only 04 (57, 1%), being designated TgDgBr6, 13, 17 and 21. All strains were virulent, causing clinical changes (rough hair coat, lethargy and abdomen distention) and death of all mice with intervals 08-20 days after inoculation. Genotyping of these four *T. gondii* isolates revealed four single genotypes, all with different clonal lineages combinations (types I, II and III) and two genotypes had atypical allele. Using PCR-RFLP, several markers showed be able to detect the genetic diversity of strains circulating in Brazil.

H: Companion animal

Ref: P1H098/0107

Molecular detection of tick-borne hemopathogens of stray dogs in East Malaysia (Borneo Island)

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This work is the first to report the prevalence of canine tick-borne hemopathogens in East Malaysia. Blood from 104 apparently healthy dogs were randomly collected from different locations within East Malaysia. Information regarding the age, sex and breeds of the dogs included in this study were also noted. DNA was extracted from the blood samples and polymerase chain reaction (PCR) technique was employed for the detection of various tick-borne hemopathogens, targeting the 16S rRNA gene for *Ehrlichia* and *Anaplasma* species and the 18S rRNA for the presence of *Babesia* and *Hepatozoon* species. 76% of the dogs were infected with at least one of the four hemopathogens; of which *Babesia* spp predominated with a prevalence of 65.4%, followed by *Ehrlichia canis*, *Anaplasma platys* and *Hepatozoon canis* with prevalence rates of 47.1%, 38.5% and 28.8% respectively. Male dogs showed a significantly ($p \leq 0.05$) higher infection rate for *E. canis* than their females counterparts, as did the adult compared to the young dogs; while there was no significant difference among sex, age and breed for the other hemopathogens. Co-infectivity status of the dogs examined showed that those infected with 2 hemopathogens at any one point in time predominated (44.2%), with *Babesia* spp and *E. canis* appearing together most commonly in the co-infections (35.6%). This study showed a high prevalence and co-infectivity status of tick-borne hemopathogens among dogs in East Malaysia. This necessitates the need to further investigate the epidemiology of these hemopathogens and their vectors in the area, and also other zoonotic species.

H: Companion animal

Ref: P1H099/0185

Efficacy and safety of monthly oral treatments with afoxolaner chewables (NexGard®, Merial) against flea infestations (*Ctenocephalides felis*) on dogs under field conditions in Australia

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A field study was conducted to assess the efficacy and safety of afoxolaner chewables (NexGard®) against fleas (*Ctenocephalides felis*) in Australia when administered to privately-owned dogs at monthly intervals for three administrations.

Thirty households from two geographical regions with high flea pressure were enrolled (15 in NSW and 15 in QLD). Informed owner consent was obtained and a total of 47 dogs of various breeds, ages, weights and sexes were included. The study was conducted from January to May 2012 (summer – autumn). Study personnel visited households at the start of the study, and then at monthly intervals for three months to perform flea counts and supervise treatment administration by dog owners.

All dogs were treated with NexGard® according to commercial dosing recommendations on Day 0, ~30 and ~60. Flea counts were performed on all dogs at enrollment. The dog with the highest flea count in each household was designated the sentinel dog for flea counts and therefore efficacy assessment at each subsequent monthly visit (Day ~30, ~60 and ~90). All dogs were assessed for tolerance or adverse events throughout the study.

Day 0 flea counts ranged from 12 to ~1100 (mean=145.7). Mean individual efficacy against fleas was 98.99%, 100% and 99.96%, at Day ~30, ~60 and ~90, respectively. Chewables were well accepted and there were no treatment related adverse events through the study.

This study demonstrated that NexGard® is a safe and highly efficacious monthly oral treatment against fleas (*C. felis*) on dogs under field conditions in Australia.

H: Companion animal

Ref: P1H100/0201

Parasitological survey of *Hepatozoon* spp. in ticks collected from rural dogs in São Paulo State, Brazil

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Hepatozoon canis is a common apicomplexan parasite of dogs, which is known to become infected by ingesting *Rhipicephalus sanguineus* ticks. However, some researchers have shown that *R. sanguineus* seems not to act as vector in Brazil. Although mature *H. canis* oocysts have been reported in *Rhipicephalus microplus* and *Amblyomma ovale* in this country, these tick species seem not to be highly efficient vectors. In order to determine the possible vectors for canine hepatozoonosis in an endemic rural area of Botucatu, São Paulo, Brazil, 158 dogs were examined for ticks. A total of 51 ticks were dissected in distilled water under a stereomicroscope. The tick fauna included five species: *R. sanguineus* (n=28), *Amblyomma cajennense* (n=12), *A. ovale* (n=8), *R. microplus* (n=2) and *Amblyomma brasiliense* (n=1). Out of 51 dissected ticks, in only one *R. microplus* partially engorged female (1.96%) we recovered sporulated oocysts similar to those of *H. canis*. This tick was collected from a *H. canis* naturally infected dog. *Rhipicephalus microplus* is a one-host tick, and although the detection of *H. canis* oocysts in this tick species has been reported before, its role as vector is questionable, because transovarial transmission does not occur in *H. canis*. Moreover, our findings, combined with results of previous studies, suggest that *R. sanguineus* may not act as *H. canis* vector in endemic areas of Brazil. This raises the question for future studies regarding the competent vectors of *H. canis*, since the invertebrate host range of this hemoparasite has not yet been fully elucidated.

H: Companion animal

Ref: P1H101/0203

Rural dogs of Southeastern Brazil as reservoirs of *Hepatozoon canis*

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Hepatozoonosis is a tick-borne disease caused by apicomplexan parasites. In dogs, the infection results in the development of schizonts within various tissues and gamonts in the peripheral blood. Molecular studies have shown that *Hepatozoon canis* is the etiological agent of canine hepatozoonosis in Brazil. In order to investigate the occurrence of *Hepatozoon* infection in dogs from rural areas of Botucatu, São Paulo, Brazil, blood samples from the marginal veins were taken from 158 apparently healthy dogs for light microscopy and for DNA isolation. Blood smears were prepared immediately after the collection, fixed with metanol and stained with Wright-Giemsa. A conventional PCR was used to amplify a 666 bp fragment of the *Hepatozoon* 18S rRNA gene. Blood parasitemia with *Hepatozoon* gamonts was found in 39 dogs (24.68%) and all tested positive by PCR. The parasitemia level was very low, ranging from 0.5 to 3% of the circulating neutrophils. The molecular investigation revealed 105 (66.45%) *Hepatozoon* naturally-infected dogs. *Hepatozoon* DNA sequences were obtained from 14 dogs. After sequencing, the found *Hepatozoon* sp. DNA showed 99-100% of identity with *H. canis* DNA isolates. The findings of the present study confirm the higher sensitivity of PCR when compared to blood smear examination and reinforce the presence of *H. canis* infecting dogs in rural areas of Southeast Brazil. Furthermore, canine infection by *H. canis* seems to be common among apparently healthy dogs, probably due to very low parasitemia, which causes a subclinical condition.

H: Companion animal

Ref: P1H102/0232

Parasites of the gastro-intestinal tract of dogs and cats in the megalopolis of Moscow

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About 1mln. of pet animals and 0.5 mln. of stray ones are inhabiting the territory of the Moscow megalopolis. The daily production of excrements by these animals results in massive insemination of soil in the city borders with eggs or invasive juveniles of helminthes and protozoan cysts. Stray dogs and cats are under especially heavy burden of parasites. In recent years important numbers of exotic pets and new breeds from other countries of Europe, Americas and Asia are imported. Such factors promote the rise in the frequency of known parasitic diseases and advent of new, previously unreported parasites. Scatoscopic analysis of excrements from dogs and cats was performed in Moscow in 2013-2014 godax. From total amount of samples 559 samples of dog excrements and 385 samples of cat excrements were randomly chosen. Eight species of helminthes were detected in dogs: *Toxocara canis* (2,32% of samples), *Toxascaris leonina* (0,35%), *Trichocephalus* sp. (0,53%), *Ancylostomatidae* gen. sp., (0,17%), *Spirocerca* sp. (0,17%), *Strongyloides* sp. (0,35%), *Capillaria* sp. (0,17%) *Dipylidium caninum* (0,17%), *Alaria alata* (0,17%) as also six species of Protozoa: *Sarcocystis* sp. (9,66%), *Cystoisospora canis* (0,71%), *Cystoisospora* sp. (2,5%), *Giardia* sp. (6,61%), *Cryptosporidium* sp. (1,96%), and oocysts morphologically similar to those of *Neosporum caninum* and *Hammondia hammondi* (0,71%). *Sarcocystis* was the most widespread parasite of adult dogs, when *Giardia* cysts and *Toxocara* nematodes were dominant in pups. During two last years the juveniles of *Strongyloides* were also reported in the dogs inside Moscow city borders. Five species of helminthes were reported from cats: *Toxocara cati* (5%), *Capillaria* sp. (0,27%), *Dipylidium caninum* (2,23%), *Hydatigera taeniaeformis* (0,27%), *Opisthorchis* sp. (0,27%), together with five species of Protozoa: *Giardia* sp. (3,63%), *Sarcocystis* sp. (1,67%), *Cystoisospora rivolta* (0,83%), *C. felis* (1,9%) and *Trichomonadidae* gen. sp.(2,51%). The most common infections of kittens were *Toxocara*, *Giardia*, *Cystoisospora* and *Trichomonadidae* gen. sp. (intestinal form).

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H: Companion animal

Ref: P1H103/0242

Misleading *Angiostrongylus vasorum* infections

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Although the geographic distribution of *Angiostrongylus vasorum* has recently expanded, information on its occurrence in certain regions is still fragmentary. This report describes three unexpected infections by *A. vasorum* in dogs from Italy. Two of these three cases (i.e. Dogs 1 and 2) were from Northern Italy, i.e. an area where the parasite is occasional. Clinical, radiographic, ultrasound and cytological examinations of lungs of Dog 1 were consistent with a neoplasia. A routine faecal examination showed the presence of *A. vasorum*. After a treatment with imidacloprid 10 %/ moxidectin 2.5% spot-on (Advocate®, Bayer) applied three times 15 days apart, clinical signs resolved, thoracic radiographs improved and lung lesions disappeared. Dog 2 was referred with dyspnoea and cough, and it scored positive for occult dirofilariosis by *Dirofilaria immitis*. After the detection of *A. vasorum* larvae at a routine copromicroscopy, the dog received a treatment with Advocate®. It clinically recovered and scored negative for *A. vasorum*. Dog 3 was diagnosed with *D. immitis* based on clinical and Rx findings, and positivity for the circulating antigen with a rapid test. Despite prompt parasiticide treatment the dog died suddenly for pulmonary haemorrhages. Post mortem results confirmed infection by *A. vasorum* only. This report indicates that canine angiostrongylosis may mimic and present clinical or diagnostic characteristics of more known canine diseases, thus impairing a prompt diagnosis. Therefore veterinarians should include canine angiostrongylosis in the differential of any cardio-respiratory distress of dogs, even when this parasitosis is unexpected in areas considered non endemic.

H: Companion animal

Ref: P1H104/0243

Successful treatment of microfilaraemia by *Dirofilaria immitis* and *Dirofilaria repens* in naturally infected dogs with a single administration of Advocate® (Bayer Animal Health)

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The current spread of *Dirofilaria immitis* and *Dirofilaria repens* in dogs and the rise of human cases have recently fostered new studies on the control of canine filariae. This study evaluated the field efficacy of a single application of a spot-on containing imidacloprid 10%/moxidectin 2.5% (Advocate®, Bayer Animal Health) in the elimination of *D. immitis* and *D. repens* microfilariae (mf). Fourteen dogs, i.e. six and eight infected with *D. immitis* and *D. repens* in endemic areas of north-eastern and central-southern Italy, respectively, were enrolled in the trial. *Dirofilaria immitis*-infected dogs received an adulticide treatment prior to the study. The animals were divided in blocks of two (1:1) animals each, where Day 0 (D0) had an interval of 15 days to compare treated vs. control dogs during the first fortnight of examination. At baseline (Days -60/-15) the animals had a range of microfilaraemia of 160-146,000 mf/ml (*D. immitis*) and 60-490 mf/ml (*D. repens*). All animals received a topical administration of Advocate® at D0 and were microscopically and genetically examined for mf at D15, D30, D60 and D90. All animals but one scored negative for mf at each post-treatment control. A *D. immitis*-infected animal (microfilaraemia baseline 13,550-30,050 mf/ml) had a 2 mf/ml count at Day 15, but it became negative from Day 30 onwards. The present study confirms the efficacy of moxidectin contained in Advocate® in suppressing microfilaraemia by *D. immitis* and *D. repens* in naturally infected dogs, previously shown under experimental and natural conditions.

H: Companion animal

Ref: P1H105/0254

Ticks in domestic dogs consulted at veterinary clinics in Culiacan, Sinaloa, Mexico

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Ticks are obligate hematophagous ectoparasites of wild, domestic animals, and humans; are important in both animal and human health because they can act in the maintenance and transmission of microorganisms pathogens such as bacteria, helminths, protozoa, and viruses that can cause a variety of zoonotic diseases such as anaplasmosis, babesiosis, borreliosis, rickettsioses and tick-borne encephalitis. The increasingly close coexistence between dogs and humans, as well as the high mobility of human populations and their pets, combined with changes in ecosystems, appropriate for the survival of ectoparasites, and the ease of these arthropods to adapt to the different climatic conditions and domestics, and considering wide distribution of dog ticks; are a cause for concern about human exposure to this vector with the consequent risk of acquiring zoonotic diseases; so the aim of this study was to characterize morphologically ticks present in dogs treated at veterinary clinics in the city of Culiacan Sinaloa, Mexico. for it, were collected 314 live ticks present on 157 canines, following the procedure by Needham 1985, and Gammons and Salam (2002), keeping them at -20 ° C., were identified with based on the Morphological keys Manual for the Taxonomic Identification of Ticks (DGSA, 2004), and with the Pictorial keys for identification ticks (Benavides and Lopez., 2005), each and every one of ticks were carefully observed, mainly the following the structures: capitulum (basis capituli, palps, hypostome), genital orifice, spiracular plate (shape), adanal plates, anal groove, 1st (internal and external spurs) and 4 coxa, dorsal scutum and festoons; resulting 314 (100%) ticks with the morphological characteristics of *Rhipicephalus sanguineus*; concluding that ticks present in dogs treated at veterinary clinics in the city of Culiacan, Sinaloa, Mexico, correspond morphologically to *Rhipicephalus sanguineus*, representing a risk to public health for possible involvement as a vector and / or reservoir host of causative pathogens of zoonotic diseases.

H: Companion animal

Ref: P1H106/0261

The efficacy of Milpro® tablets against adult *Toxascaris leonina* in experimentally infected dogs, randomized, parallel-group, blinded and negative controlled study

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Background: *Toxascaris leonina* is a common roundworm found in dogs (in cats, other species). It can infect humans representing a zoonotic risk and is a cause of visceral larva migrans in children. The study was designed in accordance with VICH GL19,[1] GL7[2] guidelines to evaluate the efficacy of a new formulation combining praziquantel 125mg and milbemycin oxime 12.5mg (Milpro®, Virbac, France) against this worm.

Material and Methods: Twenty dogs, 8-11 months of age, were included in the study at least 70 days after the inoculation of larvated eggs. On Day -1 the dogs were randomised according to the faecal eggs count and allocated to two study groups of 10 dogs each. Both sexes were represented in each group. On Day 0, all dogs assigned to group 2 were orally treated with Milpro®. The dogs in group 1 served as a negative control. Following the administration of Milpro® the dogs were observed for possible adverse events. At the end of the animal phase (Day +7) the gastrointestinal helminths were identified and counted during necropsy. Efficacy calculations were based on comparison between the treatment and the control groups.

Results and Discussion: All dogs in the control group were adequately infected by *T. leonina*. The difference between the two groups with regard to the numbers of *T. leonina* was statistically significant ($p < 0.0001$).

Conclusion: The efficacy of Milpro® tablets, based on the mean number of worm reduction, was 97.2 % against *Toxascaris leonina*, Milpro® was also well tolerated with no adverse effect reported.

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H: Companion animal

Ref: P1H107/0271

Irreversible pulmonary hypertension associated with *Troglostrongylus brevior* infection in a kitten

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A four month-old European male kitten was referred to the Veterinary Teaching Hospital of the University of Teramo, central Italy, for a bronchopneumonia unresponsive to antibiotics. The cat was severely dyspnoeic with open mouth breathing, respiratory rate of 50 breaths/min, abdominal breathing and pulse frequency of 150/min. The thoracic auscultation revealed an increased bilateral vesicular breath sound and a III/VI grade of right systolic heart murmur. Thoracic radiographs showed a significantly increased opacity of lung fields, an extensive bronchial thickening and a severe alveolar pattern. The two-dimensional and M-mode echocardiography showed a marked right-sided cardiac enlargement associated with Doppler evidence of pulmonary hypertension. Nematode larvae found at the Baermann examination of faecal samples were microscopically and molecularly identified as *Troglostrongylus brevior*. The kitten was treated with a single topical application of imidacloprid 10%/moxidectin 1% (Advocate[®], Bayer Animal Health), that was effective in stopping larval shedding up to three consecutive monthly follow-ups. Clinical, radiographic and echocardiographic signs of bronchopneumonia and pulmonary hypertension partially recovered but they still persisted at further follow-ups at seven, fourteen and ninety days post-treatment. In cats, pulmonary hypertension is a very rare condition described sporadically and, although cases are known in infections caused by other cardio-respiratory parasites, i.e. *Aelurostrongylus abstrusus* and *Dirofilaria immitis*, this is the first report of pulmonary hypertension in a kitten with troglostrongylosis. The severity of cardio-respiratory signs, despite a parasiticide treatment, further supports the high pathogenic potential of *T. brevior* in young animals.

H: Companion animal

Ref: P1H108/0278

The efficacy of Milpro® tablets against adult Echinococcus multilocularis in experimentally infected dogs, randomized, parallel-group, blinded and negative controlled study

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Background: Echinococcus multilocularis is a widespread tapeworm of serious zoonotic risk while infecting canids as definitive hosts. Therefore dogs in endemic areas of the E. multilocularis having access to intermediate hosts should be treated at four weekly intervals with an effective anthelmintic containing praziquantel to prevent this zoonosis.[1] The present study was designed in accordance with VICH GL19 guidelines[2] as a relevant model to evaluate the efficacy of new formulation combining praziquantel and milbemycin oxime (Milpro®, Virbac, France) against this worm.

Material and Methods: Twenty dogs of at least 8 weeks of age, were orally infected with approximately 15 000 E. multilocularis protoscoleces on Day 18 from a less than 10 year old European isolate. On Day -1 the dogs were randomised according to faecal eggs count into two study groups of 10 dogs of both sexes each. On Day 0 all dogs assigned to group 2 were orally treated with Milpro®. The dogs in group 1 remained untreated. At the end of the animal phase (Day +5) the gastrointestinal helminths were identified and counted during necropsy. Efficacy calculations were based on comparison between the treatment and the control groups.

Results and Discussion: Dogs in the control group were adequately infected with E. multilocularis scoleces. Seven of the dogs had more than 230 worms which supported the fact that adequacy of infection was obtained. In the treated group none of the animals were infected with E. multilocularis. The control and treatment groups differed significantly with regard to the number of worms recovered ($p < 0.0001$).

Conclusion: The treatment with Milpro® tablets was 100% effective against E. multilocularis. Milpro® was well tolerated with no adverse effects.

References:

1. ESCCAP: Worm Control in Dogs and Cats, ESCCAP Guideline 01 Second Edition. 2010:page 6.
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H: Companion animal

Ref: P1H109/0311

Spinal cord lesions in a cat infected with *Gurltia paralysans*

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Gurltia paralysans is a neurotropic metastrongyloid nematode that mainly resides in the leptomeningeal veins and parenchyma of the spinal cord of domestic and wild cats in Chile, Argentina, Brazil, Uruguay and Colombia. Here, the lesions of the lumbar spinal cord in a 3 year old male *G. paralysans*-infected domestic cat with chronic paraparesis (originating from Punucapa, Los Rios region, Chile) are characterized. Therefore, the nervous tissues of this cat were histologically processed and stained by hematoxylin-eosin, Luxol fast blue, van Gieson and Kongorot. Furthermore, the activation status of glial and endothelial cells as well as immune cell infiltration was visualized by specific immunohistological markers (GFAP, CNPase, Factor VIII, MAC 387, CD3, CD45R).

Histologically, intravascular nematode larvae and pre-adult stages being significantly associated with congestion, thrombosis and thickening of subarachnoidal vessels were detected in the meningeal veins of the spinal cord. The spinal cord parenchyma were affected by multiple hemorrhages and showed extensive foci of malacia with gitter cells and adjacent gliosis. The cellular infiltration of the meninges mainly consisted of lymphocytes intermingled with some macrophages forming a perivascular pattern.

In conclusion, the neurological signs driven by *G. paralysans* infections can be attributed to the severe chronic lesions in the spinal cord subsequent to ischemia due to parasitic vascular injury

H: Companion animal

Ref: P1H110/0327

Clinical evidence of nasal eucoleosis in dogs from Italy

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Eucoleus boehmi (syn. *Capillaria boehmi*) is a trichuroid nematode infecting the mucosa of the nasal cavities and paranasal sinuses of dogs, wolves and foxes. In the past few years, nasal eucoleosis has been increasingly reported in dogs from different areas, including Italy. Nonetheless, awareness on the clinical importance of this parasitosis is still poor. The present work describes twenty clinical cases of nasal eucoleosis diagnosed in dogs from Italy in 2012-2013, in order to provide new insights into clinical features of the infection and to promote awareness among veterinarians. Twenty dogs, scored positive for *E. boehmi* eggs at copromicroscopic examination, were selected and history details and clinical data were collected. The identity of the parasite was confirmed by a polymerase chain reaction based on amplification of *cox1* gene of the Subfamily Capillarinae. Most of the infected animals (70%) were hunting dogs which lived in rural or suburban areas of central Italy, often in crowded conditions. The most frequently observed clinical sign was nasal discharge (16/20), followed by repeated sneezing (11/20) and cough (7/20). Other respiratory signs, e.g. epistaxis, and impaired sense of smell were also found, although less frequently. No general clinical signs were present. All dogs were successfully treated with a single dose of a spot-on product containing imidacloprid 10%/moxidectin 2.5%. These results suggest that *E. boehmi* should be included in the differential diagnoses of upper respiratory tract disorders of the dog and show the promising activity of topical moxidectin in the treatment of the infection.

H: Companion animal

Ref: P1H111/0328

Occurrence of *Giardia duodenalis* and other intestinal parasites in three Dutch dog populations using four diagnostic tests

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Background: *Giardia duodenalis* is an intestinal parasite occurring in many animal species and also in humans. Symptoms may include diarrhoea, abdominal pain and weight loss, however, many infections occur asymptomatic. In dogs, it is one of the most prevalent intestinal parasites. Reported prevalences may vary depending on the tested population and diagnostic tests used, making comparisons difficult.

Aims: The aims of this study were: (1) to compare the performance of four different diagnostic tests -fecal flotation (CSF), immunofluorescence microscopy (DFA), a qPCR on rDNA and a rapid enzyme immunochromatographic assay (IDEXX SNAP *Giardia*®) - for detection of *G. duodenalis*; (2) to investigate how the presence of *G. duodenalis* is related to fecal consistency of the sample; and (3) similarly, whether *G. duodenalis* presence may be associated with the presence of other intestinal parasites.

Method: single individual fecal samples were collected from a large cohort of dogs (n=1291) belonging to three different groups: household dogs (n=551), kennelled dogs (n=343) and dogs with gastro-intestinal signs (n=397). Fecal consistency was scored. The four abovementioned tests were performed to detect *G. duodenalis* and other intestinal parasites (ascarids, whipworms, hookworms, cestodes and oocysts of coccidia) on the same samples.

Results: qPCR and DFA gave the highest number of positive samples. The prevalence of *G. duodenalis* as measured with DFA was highest among the group of kennelled dogs (25.1%), followed by clinically affected dogs (23.1%) and by household dogs (11.6%). Prevalence was especially high within hunting dogs, a subgroup of kennelled dogs (43.1%). Within different age groups, dogs below one year of age showed the highest prevalence (43.4%). Overall, a positive association was found between being *G. duodenalis* infected and having diarrhoea and also between being infected with *G. duodenalis* and having an infection with other intestinal parasites.

H: Companion animal

Ref: P1H112/0331

Parenteral infection of the land snail *Helix aspersa* with *Aelurostrongylus abstrusus* larvae.

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The maintenance of life cycles in a laboratory setting is crucial to support applied research and development activities in veterinary parasitology. The snail *Helix aspersa* is one of most used competent intermediate host of feline lungworms, *Aelurostrongylus abstrusus* and *Troglostrongylus brevior*. Although several methods of artificial infection of snails with larvae of feline lungworms have been described, these are time-consuming and do not ensure a satisfactory infection level of snails. The scope of this study was to compare different modes of infection to identify an optimized procedure to reduce the time of infection and to maximize the output of vital third-stage larvae (L3).

Four groups (A-D) of 15 *H. Aspersa* snails, each were formed and infected with 250 first-stage larvae (L1) of *A. abstrusus* following different methodologies. Snails of Group A were individually placed on a water-microfilm containing L1 for 48h while snails of Group B were individually placed with their foot on a potato slice that was covered with the infective dose of L1 suspension for 48h. Snails of Group C were inoculated by injection of 250 L1 in the posterior-ventral portion of the foot and snails of Group D served as negative controls. Eighteen days after infection all snails were sacrificed, separated from their shells, the whole tissues were artificially digested and the number of larvae retrieved were counted for each subject. There was no differences in mortality rates among groups. The mean number of retrieved L3 and the recovery rate of infective vital larvae (L3), was significantly larger in Group C (71.5 ±52.9; 28.6%) compared to Group B (38.2 ±44.9; 15.3%; $p=0.0161$) and Group A (19 ±23.3; 7.6%; $p<0.0001$). The parenteral infection method has been proven as a fast, easy to apply and effective system that is suitable to maximize the output of *A. abstrusus* L3 from infected snails.

H: Companion animal

Ref: P1H113/0391

Comparative efficacy of imidacloprid/permethrin/pyriproxyfen, afoxolaner, and fluralaner against tick (*Rhipicephalus sanguineus*, *Amblyomma americanum*) infestations on dogs

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This randomized controlled comparative laboratory study was designed to demonstrate the residual speed of efficacy of imidacloprid/permethrin/pyriproxyfen for the control of ticks (*Rhipicephalus sanguineus* and *Amblyomma americanum*) on dogs when compared to afoxolaner and fluralaner. Forty dogs were randomized into 4 groups: Group 1) imidacloprid/permethrin/pyriproxyfen, Group 2) afoxolaner, Group 3) fluralaner, and Group 4) non-treated controls. Dogs in Groups 1-3 were treated on day 0; Group 1 dogs were re-treated on days 28 and 56. Groups 1, 3, and 4 remained on study for 12 weeks, Group 2 dogs were removed after one month. All dogs were infested with 50 *R. sanguineus* and 50 *A. americanum* on days 3, 11, 21, and 28. Then, Groups 1, 3, and 4 were infested with 50 *R. sanguineus* and 50 *A. americanum* per dog on days 42 and 56 followed by 50 *R. sanguineus* per dog on days 70 and 84. Tick counts were performed on all groups at 3 and 12 hours post-infestation and statistical comparison performed between groups ($P < 0.05$). Efficacy against *R. sanguineus* 3 hours post-infestation ranged from 65-98% for Group 1, from -1-26% for Group 2, and from 13-53% for Group 3; efficacy at 12 hours ranged from 69-99%, 11.5-45%, and 52-98% for Groups 1, 2, and 3 respectively. Efficacy against *A. americanum* at 3 hours ranged from 69-99.5% for Group 1, from -6-60% for Group 2, and from -6-53% for Group 3; efficacy at 12 hours ranged from 80-100%, 6-49%, and 61-93% for Groups 1, 2 and 3 respectively. At 3 hours post-infestation, Group 1 dogs had significantly fewer ticks of both species compared to Group 2 and 3 dogs on most challenge days; tick counts in Groups 2 and 3 were never significantly different from control dogs 3 hours post-infestation. At 12 hours post-infestation, Group 1 dogs had significantly fewer ticks of both species compared to Group 2 dogs on most challenge days; tick counts in Group 2 were never significantly different from control dogs. The 12 hour kill efficacy in Group 3 decreased throughout the study period; during the last challenge month with both tick species the efficacy of Group 3 was inferior to Group 1 and comparable to control dogs. This study demonstrated that imidacloprid/permethrin/pyriproxyfen was highly efficacious at killing ticks on dogs just 3 hours post-infestation and was superior to afoxolaner and fluralaner at 3 hours on most challenge days.

H: Companion animal

Ref: P1H114/0393

Comparative efficacy of an imidacloprid/flumethrin collar, afoxolaner, and fluralaner against tick (*Dermacentor variabilis*, *Amblyomma americanum*) infestations on dogs

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This randomized controlled comparative laboratory study was designed to demonstrate the residual speed of efficacy of an imidacloprid/flumethrin collar for the control of ticks (*Dermacentor variabilis* and *Amblyomma americanum*) on dogs when compared to afoxolaner and fluralaner. Forty dogs were randomized into 4 groups: Group 1) imidacloprid/flumethrin collar, Group 2) afoxolaner, Group 3) fluralaner, and Group 4) non-treated controls. Dogs in Groups 1-3 were treated on study day 0. Groups 1, 3, and 4 remained on study for 12 weeks; Group 2 was removed after 1 month. All dogs were infested with 50 *D. variabilis* and 50 *A. americanum* on days 3, 14, 21, and 28. Then, Groups 1, 3, and 4 were infested with 50 *D. variabilis* and 50 *A. americanum* per dog on days 42 and 56 followed by 50 *D. variabilis* per dog on days 70 and 84. Tick counts were performed on all groups at 6 and 12 hours post-infestation. Both live and dead attached ticks were counted. Statistical comparison was performed between groups ($P < 0.05$). Efficacy against *D. variabilis* at 6 hours ranged from 95-100% for Group 1, 38.5-48% for Group 2, and 5-69% for Group 3; efficacy at 12 hours ranged from 97-100%, 27-59%, and 8.5-100% for Groups 1, 2, and 3 respectively. Efficacy against *A. americanum* at 6 hours ranged from 94-100% for Group 1, -2-38% for Group 2, and 4-57% for Group 3; efficacy at 12 hours ranged from 98-100%, 1-40%, and 34-97% for Groups 1, 2, and 3 respectively. At 6 hours post-infestation, there were significantly fewer live ticks of both species on Group 1 dogs compared to dogs in Groups 2-4. At 12 hours, there were significantly fewer live ticks of both species on Group 1 dogs compared to Group 2 dogs; for Group 3, the 12-hour efficacy declined throughout the study. Dogs in Group 1 also had significantly fewer total (live and dead attached) ticks of each species compared to Groups 2-4 at all time points. When the two species were combined (sum total of average of *D. variabilis* and *A. americanum*), total tick counts were up to 3.0 ticks on Group 1 dogs, 37.9 on Group 2 dogs, 28.3 on Group 3 dogs, and 52.4 on Group 4 dogs. This study demonstrated that an imidacloprid/flumethrin collar was highly efficacious at killing ticks on dogs just 6 hours post-infestation and was superior to afoxolaner and fluralaner at these counts and in the total number of ticks of each species on all challenge days at 6 and 12 hours.

H: Companion animal

Ref: P1H115/0404

Current surveys of the seroprevalence of *Borrelia burgdorferi*, *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Leishmania infantum*, *Babesia canis*, *Angiostrongylus vasorum* and *Dirofilaria immitis* in dogs in Bulgaria.

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Canine vector-borne diseases (CVBDs) have increasingly become a focus of interest in recent years. Some of the CVBDs are zoonotic and may therefore also represent a risk for the human population. Different factors are in discussion to explain the expansion of vectors and pathogens into formerly unaffected areas. Knowledge of the prevalence and distribution of CVBDs in Bulgaria is scant overall and most data rely on single case descriptions. The aim of the present study was to determine the seroprevalence of important CVBDs in 167 dogs from central-southern Bulgaria (Stara Zagora), with special emphasis on hitherto uninvestigated babesiosis and angiostrongylosis, on poorly investigated Lyme borreliosis and canine granulocytic anaplasmosis, and on the potentially zoonotic dirofilariosis and leishmaniosis. Relatively high prevalence rates were documented for anti-*Babesia canis* antibodies, *Dirofilaria immitis* antigen (16.2% each), anti-*Ehrlichia canis* (21%) and anti-*Anaplasma phagocytophilum* antibodies (30.5 - 46.1%), while *Borrelia burgdorferi* seroprevalence was low (2.4%). All samples were negative for *Leishmania infantum* antibodies and *Angiostrongylus vasorum* antigen and antibodies. In total, 64.7% (108/167) of the samples indicated infection or exposure to at least one agent and a high proportion of dual infections (43/108; 39.8%) was demonstrated. Multiple infections with up to four different organisms were also detected. Our data underline the importance of CVBDs and especially of co-infections which could influence the clinical outcome in dogs.

H: Companion animal

Ref: P1H116/0435

An epidemiological study of the impact of commercial insecticides in dogs in Canine Leishmaniosis seroprevalence in southeast Spain

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Sandfly transmitted *Leishmania infantum* causes canine Leishmaniosis (CanL) in Mediterranean countries and topical insecticide treatments are the most important means of preventing infection. This study investigates the relationship between CanL seroprevalence and preventive topical insecticide treatments in 800 dogs in Murcia, in southeast Spain. Dogs came from 17 veterinary practices, they were weighed and examined to select those with no CanL symptoms and blood samples were taken to analyze *L. infantum* antibodies using two commercial ELISAs. Owners were interviewed to gather data on the dog's date of birth, breed, home address and insecticide treatments used to prevent ectoparasite infections. Dog homes were georeferenced and the surrounding environment was characterized according to cartographic, climatic, land use and lithological variables using a geographical information system. CanL seroprevalence was 11% in dogs not using insecticide treatments (n=60) and 19% in those using them (n=740) (p>0.05). At least 16 different insecticide products were used and 76%, 23% and 1% of dogs received 1, 2 and 3 products simultaneously. The most frequent commercial brands were Scalibor collars (deltamethrin 40mg/g; MSD[®]), Advantix pipettes (permethrin 500 mg/ml and imidacloprid 100mg/ml; Bayer[®]) and ExSpot spot-on pipettes (permethrin 715 mg/ml; MSD[®]). Seroprevalence was 9%, 16%, 20%, 23% and 25% for dogs with Scalibor collars plus Advantix pipettes, Scalibor collars plus ExSpot pipettes, Advantix pipettes alone, Scalibor collars alone and ExSpot pipettes alone, respectively. A logistic regression model examining the relationship between the dogs serological status and insecticide treatment use, adjusted for dog's sex, age, weight and environmental variables of dogs residential area, confirmed a significant reduction in the risk of *Leishmania* seropositivity in dogs using the combination of Scalibor collars plus Advantix pipettes compared to those using other insecticide treatments. These results suggest that combining these two products could significantly reduce the incidence of CanL in *L. infantum* endemic Mediterranean areas.

H: Companion animal

Ref: P1H117/0438

First report of *Eucoleus boehmi* in a dog from Portugal

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Nasal eucoleosis is an uncommonly diagnosed parasitic infection, rarely observed in pets. Dogs with *Eucoleus boehmi* can have mild upper respiratory signs such as sneezing and nasal discharge, but may also not exhibit any clinical sign, depending on parasite load.

We describe a case of a 6 year-old mixed breed male dog, presented to FMV-ULisboa Hospital, with chronic sneezing and reverse sneezing of 2 months duration and mild bilateral nasal serous discharge. The patient was being treated with allopurinol for Leishmaniosis and a 3-year course of prednisolone for a carpal joint immune mediated arthritis. Physical examination, CBC, serum chemistry and thoracic radiography were unremarkable. A CT scan of the nasal cavity was compatible with bilateral chronic rhinitis of unknown etiology. For further investigation, a retrograde and anterograde rhinoscopy was performed, revealing erythematous mucosae with several white and serpentine-shaped worms on the turbinates surface. Multiple worms were retrieved, measuring between 15-30 mm. Histopathology analysis of the nasal mucosae revealed marked plasmocytic and eosinophilic inflammatory infiltration associated with multiple forms of nematodes, compatible with parasitic rhinitis. Morphological identification of the worms showed filiform nematodes, containing several bipolar plugged and barrel-shaped eggs in their medial segment. The eggs had a delicately pitted surface, containing a multicellular embryo that did not fill the entire egg and measured 54-60X30-35 µm. Morphological and morphometric characteristics were consistent with *E. boehmi* infection. Treatment with Fenbendazole (100 mg/kg) was prescribed, orally and daily for two weeks.

To the authors' knowledge, this is the first report of *E. boehmi* in a dog from Portugal. Respiratory capillariosis appears to be underestimated and should be considered as a differential diagnosis in dogs showing signs of upper respiratory disease.

H: Companion animal

Ref: P1H118/0446

***Tritrichomonas foetus* infection in cats with a history of chronic diarrhoea and multi-cat environments in Spain**

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Tritrichomonas foetus is a protozoan parasite which has been recently identified as a causative agent of feline chronic diarrhoea in domestic cats. Transmission of infection occurs by the faecal-oral route throughout direct contact among animals. Consequently, feline trichomonosis (FT) is more likely to be present in multi-cat environments. The objective of this work was to study the presence of *T. foetus* and some associated risk factors in cats with a reported history of chronic diarrhoea from a densely-housed origin. Animals enrolled in this study were family cats (n = 15) acquired from pet shops, shelters or breeding centres, and cattery cats belonging to one breeding centre (n = 28) and two cat shelters (A and B, n = 25 each). In the catteries, a follow-up analysis for a period up to 2 months was also performed to determine the parasite shedding pattern in faeces and the incidence. Faecal samples were analysed by means of *in vitro* culture and specific PCR. *T. foetus* was detected in a total of 38.7% (36/93) cats with chronic diarrhoea. Parasite infection was similarly detected in family cats and cattery animals (40% versus 38.4%). In the catteries, the parasite was detected in 50%, 44% and 20% of the animals from the breeding centre and the shelters A and B, respectively. Investigation of potential risk factors showed that cats ≤ 1 year old were more likely to be infected than older cats (57.1% versus 27.3%; $P < 0.05$). No significant differences were found when sex and breed factors were studied. The follow-up analysis showed that 58.3% infected cats shed trophozoites in their faeces intermittently and the incidence was 23.1%. These results confirm the importance of FT as cause of chronic diarrhoea in cats and highlight the relevance of close contact conditions for *T. foetus* transmission.

H: Companion animal

Ref: P1H119/0455

Evaluation of different parasitological techniques for diagnosis of intestinal parasites of dogs

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Dogs are animals infected by helminthes and protozoa, being some of them are threat for the public health. The diagnosis of helminthes is considered a challenge in the clinical routine of pet animals. Therefore, the aim of this study was to evaluate different techniques for detection of eggs/oocysts of intestinal parasites of dogs. Feces (n = 127) of animals living in shelters located in the Metropolitan region of Recife, Pernambuco, Brazil, were used in this study. Samples were divided in 30 pools (containing feces of four/five animals each) and were examined using the techniques of Willis-Mollay, Hoffman, Mini-FLOTAC and FLOTAC dual technique with Saturated sodium chloride (s.g., 1.20) and Zinc sulfate solution(s.g.1.20). Data were analyzed using the software InStar. Differences were considered statistically significant at $P \leq 0.05$. Out of all pools analyzed 93.3% (28/30) presented eggs and/or oocysts of intestinal parasites. In particular, eggs of Ancylostomidae, *Toxocara* sp., *Trichuris* sp., *Dipylidium caninum* and *Strongyloides stercoralis* were detected in 83.3%, 26.6%, 36.6%, 3.3% and 20%, respectively. In addition, oocysts of *Cystoisospora* sp. were observed in 20% of the pools. Considering the different diagnostic tests, 53.3% (16/30) of the positive samples were detected by the technique of Willis-Mollay, 20% (6/30) by the Hoffman technique, and finally 63.3% (19/30) and 90% (27/30) through the Mini-FLOTAC and FLOTAC techniques, respectively. In this study, the FLOTAC and Mini-FLOTAC techniques presented a higher sensitivity when compared with the others methods. Therefore, the use of these tools is suggested to diagnose intestinal parasites of dogs.

H: Companion animal

Ref: P1H120/0458

Gastrointestinal parasites in semi-domiciled cats from the Northeast region of Brazil

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Endoparasites have been recognised as important agents of infection in cats. Information on their distribution in some regions is still scant. Therefore, the present survey aimed to diagnose the main gastrointestinal endoparasites which affect cats in the Northeast region of Brazil. On January 2015 feces of semi-domiciled cats (n = 107) living in the Northeast region of Brazil were analyzed through the FLOTAC dual technique with Saturated sodium chloride (s.g., 1.20) and Zinc sulfate solution (s.g.1.20). The overall results showed that 75.7% (81/107) of the animals were positive for eggs and/or oocysts of intestinal parasites. In particular, eggs of Ancylostomatidae, *Toxocara cati*, *Strongyloides* sp., *Trichuris* sp. and *Dypilidium caninum* were detected in 72.8% (59/81), 44.4% (36/81), 23.4% (19/81), 2.4% (2/81) and 1.2% (1/81) of the positive animals, respectively. In addition, oocysts of *Cystoisospora* sp. were detected in 24.6% (20/81) of the positive samples. Out of all positive animals 13.6% (11/81) presented mixed protozoan and helminthic infections. Double and triple nematode infections were found in 27.1% (22/81) and 9.8% (8/81) of the cats, respectively. Curiously, in one animal 1.2% (1/81) four different helminth species were detected. The overall prevalence and variety of parasites found in this study are substantially high. Some of the parasites herein reported present zoonotic importance; therefore their control in the population of semi-domiciled cats living in urban areas is needed in order to promote the public health protection.

H: Companion animal

Ref: P1H121/0483

***Strongyloides stercoralis* found in imported dogs, kennel dogs and household dogs in Iceland**

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Introduction: *Strongyloides stercoralis* is a nematode primarily known in humans and dogs and has a zoonotic potential. It is endemic in the tropics but also found in several subtropical and temperate regions and can pose serious disease.

Material and methods: During 1989-2014 faecal samples from 2900 imported dogs in quarantine in Iceland have been examined for parasites. During 2012-2014 dogs in a big breeding kennel and also 88 household dogs have been subjects of a specific screening for *Strongyloides stercoralis*.

Results: *Strongyloides stercoralis* has been identified in 15 imported dogs in quarantine; two in 1994, 13 during 2008–2014. The first case outside quarantine was a diseased household puppy purchased from a kennel in 2012. Additional seven puppies from the kennel and two other dogs have been found to be infected. Specific anthelmintic treatments were performed and follow-up examinations indicated a successful removal. In 2012, examination of dogs in the above-mentioned kennel confirmed that dozens of dogs were infected with *Strongyloides stercoralis*. Follow-up studies indicate that repeated anthelmintic treatments, and other actions undertaken, have significantly reduced the prevalence of infection although some samples have been positive for *Strongyloides stercoralis* all through 2012-2014.

Conclusion: In recent years *Strongyloides stercoralis* is frequently found in imported dogs in Iceland, including dogs from Finland and Sweden. In spite of anthelmintic treatment of quarantined dogs the nematode is believed to have been brought to a breeding kennel with imported dog/dogs. So far, actions taken to eradicate the nematode in the kennel have not been successful.

H: Companion animal

Ref: P1H122/0512

Molecular identification of *Ehrlichia canis* and *Anaplasma platys* in dogs from La Comarca Lagunera, Mexico

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The tick-borne pathogens *Ehrlichia canis* and *Anaplasma platys* are the causative agents of canine monocytic ehrlichiosis (CME) and canine cyclic thrombocytopenia (CCT). Although serological studies have been done for *E. canis*, both pathogens have not been molecularly identified in Mexico, where the tick vector *Rhipicephalus sanguineus* is common and dogs are likely to be exposed to these pathogens. Therefore, the aim of this research was to identify *E. canis* and *A. platys* by PCR and phylogenetic analysis. 100 blood samples were collected from dogs (43 females and 57 males) that attended to veterinary clinics or lived in the dog shelter from La Comarca Lagunera, a region formed by three municipalities; Torreon, Gomez-Palacio and Lerdo, in the Northern states of Coahuila and Durango. All the sampled dogs were apparently healthy, but infested with *R. sanguineus* ticks. DNA was obtained from blood and PCR for 16S rDNA of *Anaplasma* and 16S rRNA of *Ehrlichia* were performed. PCR products were purified, cloned, sequenced and sequences aligned and compared with the sequence databases. 31 samples were positive to 16S rDNA of *Anaplasma*, while 10 were positive to 16S rRNA of *Ehrlichia*. The sequence alignment and phylogenetic analysis showed that 3 samples corresponded to *A. platys* and 3 to *E. canis*, concluding that these two pathogens are present in the study area. This is the first molecular identification of *A. platys* and *E. canis* in dogs from Mexico.

H: Companion animal

Ref: P1H123/0513

Efficacy of a new combination of fipronil and permethrin (Effitix® spot-on) against flea infestations in dogs

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Two single-center randomized, blinded, controlled clinical trials were performed to assess the efficacy of a spot-on treatment combining fipronil and permethrin (Effitix® Spot-on, Virbac, Carros, France) in the treatment and prevention of *Ctenocephalides felis* infestations in dogs. In each study, 16 Beagle dogs were randomly allocated to two groups: eight into an untreated control group, and eight into a group treated with Effitix® spot-on. Dogs were treated at the recommended dosage (at least 60 mg.kg⁻¹ for permethrin, and 6.7 mg.kg⁻¹ for fipronil). All dogs were infested with 100 *C. felis* on Days -2, 7, 14, 21 and 28 (Day 0 being treatment day). Flea counts were performed on Days 1, 8, 15, 22 and 29 in Study 1, and on Days 2, 9, 16, 23 and 30 in Study 2 (24 or 48 h post-product administration or flea infestation). In both studies, the treated group had statistically significantly ($p < 0.05$) fewer fleas compared to the control group on all assessment days. Based on geometric means, adulticidal efficacies (%) were 99.5, 100, 100, 100 and 100 on Days 1, 8, 15, 22 and 29 respectively in Study 1, and 99.7, 100, 100, 100 and 100 on Days 2, 9, 16, 23 and 30 respectively in Study 2. The results indicate that Effitix® spot-on has a rapid killing effect as more than 99% of fleas were killed within 24h. A persistent insecticidal effect is also demonstrated as it prevented further flea infestations for at least one month.

H: Companion animal

Ref: P1H124/0515

Speed of kill and adulticidal efficacy of a fipronil-permethrin spot-on (Effitix®) against ticks (*Demacentor variabilis* and *Rhipicephalus sanguineus*) on dogs

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A randomised, blinded, controlled study was carried out to assess the repellence and adulticidal efficacy of a spot-on treatment combining fipronil and permethrin (Effitix®, Virbac, Fort Worth, USA) against artificial challenges with *Demacentor variabilis* and *Rhipicephalus sanguineus* ticks on dogs. Twelve dogs were randomly assigned to two groups: six into an untreated control group, and six into a group treated with Effitix® on Day 0 at the label-recommended dosage (minimum dose of 50.0 mg.kg⁻¹ for permethrin and 6.7 mg.kg⁻¹ for fipronil). Each dog was infested with 30 ticks of each species (ten-minute exposure) on Days 1, 3, 7, 14, 21, 22 and 28. In situ tick counts were carried out three hours post-infestation, and a tick count plus removal was performed after 24 hours. Persistent acaricidal efficacies assessed 3 hours post-infestation were statistically significant (versus control group) at every interval, and were greater than 90% at 3, 7, 14 and 22 days post-treatment for both tick species. Acaricidal efficacies against *R. sanguineus* were 99% on Day 2, and 100% at every other interval throughout the study. Efficacies against *D. variabilis* were 97.6% on Day 2, and 100% at every other interval throughout the study except on D29 where it was 97.7%. The results indicate that Effitix® begins to repel ticks within ten minutes of exposure. The majority of the repelled ticks were dead within 24–48 hours, and ticks not repelled had died by Day 1. Most of these ticks were killed within the first three hours of exposure to the treated dogs.

H: Companion animal

Ref: P1H125/0535

First report of autochthonous canine *Angiostrongylus vasorum* infection in Belgium.

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Canine angiostrongylosis is considered as an emergent disease in Europe and Canada. A fatal case of *Angiostrongylus vasorum* infection is described in a four month old puppy born in Belgium. The dog was submitted for marked neurological disorders, body weight loss, a profound weakness and mild respiratory signs. The dog was given antibiotics and mucolytic compounds with very little improvement and consequently was referred to a specialist for additional examinations. As the general condition of the dog was rapidly declining, the animal was euthanized shortly after on its owners'request and a necropsy was carried out. Extensive gross pulmonary lesions were observed and histopathological examination revealed the presence of numerous larvae with morphology compatible with *A. vasorum*. Larvae were also found in the product of a bronchoalveolar lavage but faecal material was not examined. The presence of *A. vasorum* circulating antigen was demonstrated through ELISA; additionally an *A. vasorum* specific PCR was performed on brain material and yielded a positive result. This case confirms that the clinical diagnosis of canine angiostrongylosis can be very challenging especially when respiratory signs are absent or very mild such in the present case. This is the first reported case of canine angiostrongylosis in Belgium.

H: Companion animal

Ref: P1H126/0551

Infection rates of *Toxoplasma gondii*, *Encephalitozoon cuniculi*, feline coronavirus, feline leukemia virus, and feline immunodeficiency virus in cats in Japan

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Toxoplasma gondii (T. gondii), *Encephalitozoon cuniculi* (E. cuniculi), feline coronavirus (FCoV), feline leukemia virus (FeLV), and feline immunodeficiency virus (FIV) are infectious agents that can cause serious disease in cats. In addition, T. gondii and E. cuniculi are important infectious agents in both humans and cats. The seroprevalence of T. gondii, E. cuniculi, FCoV, FeLV, and FIV was investigated in 305 cats, both feral (134) and domesticated (171), from Nagano Prefecture in Japan. Of the cats investigated, 20.0% (61/305) were seropositive to T. gondii, 6.3% (19/300) to E. cuniculi, 23.4% (71/303) to FCoV, 2.4% (7/297) to FeLV, and 10.4% (31/297) to FIV. For T. gondii and FIV, feral cats showed significantly higher positive rates (32.8% and 16.7%, respectively) than domesticated cats (9.9% and 5.5%, respectively). No significant difference in seropositivities was observed in E. cuniculi, FCoV, and FeLV between feral and domesticated cats. For FIV, seropositivity was significantly higher in males (15.6%) than in females (7.9%). No significant difference in seropositivity between the sexes was observed for T. gondii, E. cuniculi, FCoV, and FeLV. Overall, 138 of 305 samples were positive for T. gondii, E. cuniculi, FCoV, FeLV, or FIV. Of the 138 samples that tested positive, 44 (31.9%) were positive to two or more of these agents. The seven samples testing positive for both T. gondii and E. cuniculi were all from feral cats.

H: Companion animal

Ref: P1H127/0558

Conjunctival swab in epidemiological surveys to detect canine visceral leishmaniasis by real time PCR

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Molecular diagnosis has been an important tool to detect canine visceral leishmaniasis (CVL). PCR shows high sensitivity and specificity in *Leishmania* spp. detection and is a useful tool to discriminate species. In this work, real time PCR (qPCR) was tested to amplify DNA from conjunctival swabs (CS), with the goal of providing a simpler, less invasive, and more owner-friendly method for biological sample collection from dogs, during epidemiological studies. Blood and CS samples were collected from 103 dogs during an epidemiological survey for CVL. Those samples were tested by real-time PCR (qPCR) and conventional PCR (cPCR) and their results compared. To do so, a qPCR and cPCR test targeting different regions of the kinetoplast minicircle DNA specific to *Leishmania* spp, previously described, were used. An endogenous control reaction was performed using a real-time PCR assay with beta-actin as a housekeeping gene. The obtained results showed that 22.3% (23/103) of tested dogs were positive by qPCR using CS samples, while 13.6% (14/103) were detected by cPCR. A 0.64 kappa value indicates a substantial concordance between tests, when compared. As for blood samples, 16.5% (17/103) and 18.4% (19/103) were positives in qPCR and cPCR respectively, resulting in a poor concordance between tests ($k=0.06$). A fair concordance was obtained ($k=0.30$), when compared concordance between qPCR of CS samples and qPCR of blood samples. Here, canine CS collected during an epidemiological survey in Brazil, were evaluated as a DNA source for qPCR diagnostics. The results showed that it proved to be a valuable tool, which can make easier the epidemiological studies, to monitor the presence of *L. infantum* infection in dogs.

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H: Companion animal

Ref: P1H128/0559

The investigation of *babesia* infection in companion dogs in Nanning, Guangxi, China

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Canine babesiosis is a hemoprotozoal tick-borne disease caused by canine babesia. This study aims to analysis the prevalence of babesiosis in companion dogs in Nanning, Guangxi, China. From November 2013 to November 2014, we collected 100 dog blood samples from Guangxi university animal hospital in Nanning. After DNA extracting, the sequence of the variable region of the 18S RNA gene was independently amplified from each sample by Babesia-specific PCR primers, the PCR products of positive samples were purified, cloned, sequenced and analysis. The results showed that, 3 were positive among the 100 blood samples, the infection rate of *Babesia spp.* in companion dogs was 3.0%. The three sequences showed a homology of 99% with *B.canis vogeli* in the GenBank. In the case records, two of the three babesiosis positive samples were infected with distemper and parvovirus. However, in field diagnosis, the babesiosis was missed diagnosis and no insecticide treatment, the dogs died at the end. Our results indicated that there are *Babesia* infection in companion dogs in Nanning, and these infection easily missed in the field diagnosis when mixed infected with bacterial and virus, and such mixed infection seem enhance the pathogenicity.

H: Companion animal

Ref: P1H129/0584

Retrospective review of centrifugal fecal flotation findings from 2 dog and 3 cat populations in central Oklahoma, U.S.A. for *Alaria* spp. infections with investigations into wildlife reservoirs.

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Alaria spp. are zoonotic trematodes of carnivores with a worldwide distribution. Alariosis in companion animals appears to have emerged in central Oklahoma within the past 16 years. Prior to 1990, the parasite was not considered endemic to this area. Retrospective review of centrifugal fecal flotation findings from 2 distinct dog and 3 distinct cat populations was performed to determine the prevalence of infection for *Alaria* spp. over the last 10 years. Flotation results for client-owned dogs and cats submitted between 2006 and 2014 to Oklahoma State University, Boren Veterinary Teaching Hospital (BVTH) and Oklahoma Animal Disease Diagnostic Laboratory (OADDL) were selected for the review. Flotation findings from shelter/rescue animals that were collected between 2013 and 2014 from BVTH and two feline veterinary practices were also included. Results for feral cats, collected through a neuter/release program operated by BVTH in 2015, were also evaluated. Additionally, 1 stray cat and 5 coyotes that had been hit by cars were submitted to OADDL for necropsy and gastrointestinal parasite recovery. Twenty-three feral hog tongues, provided through USDA APHIS in Oklahoma, were examined for *Alaria* mesocercariae. *Alaria* eggs were found from 9 of 5,417 (0.2%) client-owned dogs, 17 of 1,246 (1.4%) client-owned cats, 15 of 837 (1.8%) shelter/rescue dogs, 2 of 331 (0.6%) shelter/rescue cats, and 17 of 182 (9.3%) feral cats. Prevalence of infection in feral cats was significantly higher than that in shelter/rescue cats ($\chi^2 = 20.421$, df=1, $P < 0.001$) and in client-owned cats ($\chi^2 = 35.423$, df=1, $P < 0.001$). Prevalence of infection was also significantly higher in shelter/rescue dogs than in client-owned dogs ($\chi^2 = 45.975$, df=1, $P < 0.001$). Further confirmation of the occurrence of the parasite in reservoir hosts from the area came after recovery and identification of adult *Alaria* spp. from 1 stray cat and 5 coyotes. Five of 23 (21.7%) feral hog tongues were positive for mesocercariae. Expansion of wild carnivore and feral hog populations, changing climatic conditions, and suburban growth are factors associated with re-emergence of *Alaria* spp. in Europe; these factors may have impacted emergence of *Alaria* spp. in central Oklahoma. Since alariosis can be a fatal zoonotic disease of humans, it is important to keep veterinary practitioners and hunters warned about the potential human risk from handling and ingesting wild game.

H: Companion animal

Ref: P1H130/0591

Therapeutic efficacy of imidocarb dipropionate, atovaquone plus azithromycin or buparvaquone plus azithromycin in dogs with natural *t. Annae* infection in north-western Spain: preliminary results.

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Theileria annae (syn. *Babesia microti*-like) infection is considered endemic in northwestern Spain (García et al., 2006). Sick dogs feature an acute severe disease with pale mucous membranes, anorexia, apathy, severe regenerative macrocytic/hypochromic anaemia and thrombocytopenia (Miró et al., 2015). Piroplasmosis caused by *T. annae* is now recognized as a serious problem essentially because of its poor response to current piroplasmosis treatments. This study compares the efficacy of three treatment protocols and defines the most suitable protocol for treating this infection. Twenty dogs naturally infected with *T. annae* were enrolled in this multicenter randomized trial. All participating dogs were subjected to a physical exam and blood collection for full blood counts, biochemical profiles and parasite detection. Diagnosis was confirmed by light microscopy (LM) observation of Giemsa-stained blood smears and by genomic DNA isolation followed by PCR amplification and sequencing. Each dog was randomly assigned to a treatment group: 8 dogs were treated with imidocarb dipropionate (Imizol®) (5mg/kg SC as 2 doses 14 d apart); 5 with atovaquone (Malarone®) (13.3 mg/kg PO q 8 h, 10 d) plus azithromycin (10 mg/kg PO q 24 h, 10 d); and 7 with buparvacuone (Butalex®) (5mg/kg IM as 2 doses 2 d apart) plus azithromycin. After treatment, follow-up examinations were performed on days 15, 45 and 90 and included a physical exam, blood tests and parasite tests. The clinical efficacy of each protocol was assessed using categorized scoring systems from 0 to 3 and 0 to 2 (from low to high severity) for 19 clinical signs and 8 clinicopathological variables, respectively. On day 90, 80% of the dogs treated with atovaquone/azithromycin and 86% of those treated with buparvacuone/azithromycin tested negative for the parasite by LM and PCR, compared with only 37% of dogs treated with imidocarb dipropionate. In addition, clinical signs were mostly reduced (by 100%) in dogs treated with the buparvacuone-based combination while highest percentage reductions in laboratory abnormalities (78.8%) were detected in those treated with atovaquone/azithromycin. Among the treatments tested, buparvacuone/azithromycin showed the best therapeutic efficacy.

García AT. Piroplasma infection in dogs in northern Spain. Veterinary parasitology 2006, 138(1-2):97-102.

Miró, G., Checa, R., Paparini, A., et al., *Theileria annae* (syn. *Babesia microti*-like) infection in dogs in NW Spain detected using direct and indirect diagnostic techniques: clinical report of 75 cases. Parasit Vectors 2015.

H: Companion animal

Ref: P1H131/0592

First description of *Onchocerca lupi* infection in dogs in Spain

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Canine ocular onchocercosis has a worldwide distribution, including the south western United States, western Europe and northern Asia. *Onchocerca lupi* has been recognized as a zoonotic nematode that can cause various degrees of ocular lesions in dogs. Recently, it has been reported in dogs from Portugal and Greece (Otranto et al., 2013). An epidemiological study was performed to determinate the presence of *O. lupi* in dogs living in the south east of Spain (Huelva). A total of 104 dogs of different breeds, sex, and ages living in a shelter were included. Skin snip samples were collected using a disposable scalpel over an area of $\approx 0.2 \times 0.2 \times 0.2$ cm from the forehead and inter-scapular regions. Skin samples were stored in aliquots with saline solution (0.5 ml) before observation. Sediments (20 μ L) were individually observed under light microscopy. Microfilariae were identified according to morphologic keys and speciation for the most commonly subcutaneous filaroids retrieved in dogs from the Mediterranean region. Morphological features of *O. lupi* microfilariae include a short flattened unsheathed body (mean length 110.1 ± 7.5 μ m, width 6.8 ± 1.2 μ m) with a rounded head bearing a tiny tooth on the cephalic (Mutafchiev et al., 2013). Morphological identification was confirmed by molecular amplification and sequencing of the partial cytochrome oxidase subunit (*cox1*) gene, following procedures described previously (Otranto et al., 2013). Four out 104 dogs, (3.8%) were skin snip-positive for *O. lupi*, two skin samples were positive by microscopic observation and two more samples were positive by PCR and sequencing. One of the positive *O. lupi* infected dog showed neurological signs. This study reports for the first time *Onchocerca lupi* infection in dogs from South of Spain. We aimed to determine the clinical impact of the infection in those positive dogs and the epidemiological factors influencing the presence of this unknown parasite on the study area.

Mutafchiev Y, Dantas-Torres F, Giannelli A, et al. Redescription of *Onchocerca lupi* (Spirurida: Onchocercidae) with histopathological observations. *Parasit Vectors*. 2013 6(1):309.

Otranto D, Dantas-Torres F, Giannelli A, et al. Zoonotic *Onchocerca lupi* infection in dogs, Greece and Portugal, 2011-2012. *Emerg Infect Dis*. 2013;19(12):2000-3.

H: Companion animal

Ref: P1H132/0594

Canine babesiosis and hepatozoonosis in dogs from southeast of Bahia, Brazil

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Tick-borne diseases are common in tropical countries such as Brazil. *Babesia canis* and *Hepatozoon canis* are spread throughout the country with a high prevalence in urban and rural areas, respectively, causing subclinical or clinic disease in dogs, which can lead to death or enhance the concomitant diseases. The aim of this study was to determine the prevalence of these agents in the canine population in the state of Bahia, Brazil, and check for possible associated risk factors. Blood samples were collected from 380 dogs. The *Babesia* research was performed by capillary blood smears, Polymerase Chain Reaction (PCR) and Indirect Fluorescent Antibody Test Reaction (IFAT). *Hepatozoon canis* research was performed by capillary blood smears and PCR. Epidemiological data were collected through a structured questionnaire applied to dog owners. By cytology, only two animals were infected with *Babesia* spp. Anti-*B. canis* antibodies were detected in 140 (36.8%) dogs. By PCR, 147 (38.7%) and 163 (42.9%) dogs were positive for *Babesia* spp. and *Hepatozoon canis*, respectively. Co-infection by the two agents was observed in 102 (26.8%) dogs. The condition of the semi-restricted dogs not sleeping at home ($p = 0.02$) was considered a risk factor for babesiosis. The observed prevalence for both agents can be related to peri-urban feature of this municipality. In conclusion, local dogs are exposed to infection and co-infection by these pathogens. Moreover, this study is reporting the first case of *H. canis* infection in the state of Bahia.

H: Companion animal

Ref: P1H133/0681

Two cases of cysticercosis in dogs in the Czech Republic

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Taenia crassiceps tapeworms are intestinal parasites of carnivores (definitive hosts), mostly foxes and dogs, in North America, Europe, and Russia. Rodents are natural intermediate hosts with cysticerci localized in body cavities or subcutaneously. Cysticerci proliferate by asexual budding.

Dogs, cats and humans can occasionally also serve as intermediate hosts. We documented two cases of larval taeniosis crassiceps in dogs during the years 2012 and 2014 in the Czech Republic.

Case 1 - only 1.5 years old yorkshire terrier with more than an year prolonged respiratory problems; it was unsuccessfully treated with different antibiotics. At the end the disease was diagnosed as cysticercosis by thoracocentesis and later autopsy. It was a unique case of the occurrence of cysticerci in pleural cavity.

Case 2 - geriatric patient 10 years old dog shi-tzu with many chronic diseases; it had oedema in the hip area. After incision and surgical intervention subcutaneous cysticercosis was diagnosed. On the basis of microscopic examination and sequencing of a part of the cytochrome oxidase 1 gene we confirmed in both cases the development stages of *Taenia crassiceps*.

These two cases also point to the risk of zoonotic transmission of *T. crassiceps*.

H: Companion animal

Ref: P1H134/0727

Prevalence and genetic diversity of the intestinal parasites *Blastocystis* sp. and *Cryptosporidium* spp. in household dogs in France and assessment of zoonotic transmission

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Several parasites including the protozoa *Blastocystis* sp. and *Cryptosporidium* spp. may represent causative agents of gastrointestinal symptoms in household dogs and a potential risk of transmission to the owners. Even if France is one of the largest European countries in terms of canine population, only few data are available about the molecular epidemiology of these two parasites. The purpose of this study was to determine the prevalence of intestinal parasites in household dogs in France and evaluate the zoonotic risk of *Blastocystis* sp. and *Cryptosporidium* spp. through the genotyping of the corresponding isolates.

Hundred and sixteen faecal samples were collected from household dogs regardless of race, age or gender, living in the Lyon area, France, and presenting or not diarrhea. Various intestinal protozoa, helminthes, and fungi were identified by light microscopy. Screening for *Blastocystis* sp. and *Cryptosporidium* spp. was performed by PCR followed by direct sequencing of the PCR products and analysis of the obtained sequences for genotyping.

The overall prevalence of dogs infected with at least one gastrointestinal parasite was 47.4% (55/116). By light microscopy examination of stools, the most common parasites were the protozoa *Giardia* spp. (25.0%) and *Cytoisospora* spp. (19.8%). Using molecular methods, four dogs (3.4%) were shown to be infected by *Blastocystis* sp. and carried either ST2 commonly identified in various animal groups or ST10 frequently found in bovid. Three dogs (2.6%) were positive for *C. canis*, infecting episodically humans. No association between either *Blastocystis* sp. or *C. canis* infection and gastrointestinal symptoms might be clearly established from our data.

The present study reports the first molecular epidemiological data regarding the zoonotic parasites *Blastocystis* sp. and *Cryptosporidium* spp. in household dogs in France. The low prevalence of both parasites coupled to the identification of *C. canis* as well as of *Blastocystis* sp. ST2 and ST10 in the canine population strongly suggested that dogs are not natural hosts of *Blastocystis* sp. and play a negligible role as zoonotic reservoirs for both parasites.

I: Novel diagnostics

Ref: P2I331/0038

Reliability of PCR as a diagnostic method of *Encephalitozoon cuniculi*

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The detection of nucleic acid sequences from many parasites, including *E. cuniculi* in urine samples for routine diagnostics is currently performed by PCR in most laboratories. The success and reliability of nucleic acid sequences amplification require efficient procedures of extraction and purification from other microorganism components, biological substances, and contaminating materials which could function as PCR inhibitors. Primers are used to amplify portions of the small subunit ribosomal RNA (SSU-rRNA) gene, which allows increased sensitivity and specificity compared to non-molecular methods of detection. In the present study, a total number of 274 urine samples were collected from animals of different host species including cattle, buffaloes, sheep, goat, rabbit, rat and dogs from different provinces of Egypt (Alexandria, Behera and Assuit). Samples were collected from veterinary medical convoys, veterinary clinics, faculty hospital and farms and examined using PCR. DNA was extracted from urine samples of different animals using phenol chloroform extraction method. The PCR was performed using *E. cuniculi*-specific primers that amplified a 549 bp fragment of the small subunit ribosomal RNA (ssrRNA) gene. The forward and reverse primers were: (Ence F: 5'-ATGAGAAGTGATGTGTGTGCG-3') and ;(Ence R: 5' TGCCATGCACTCACAGGCATC-3'). The present study revealed that there were only five positive samples with a percentage of 1.82% including two samples from cattle with a percentage of 5.7%, two from buffaloes with a percentage of 4.44% and only one from rabbits with a percentage of 2.85%. It could be suggested that negative PCR results may be due to intermittent shedding or low numbers of spores being shed that are below the detection threshold of the PCR assay. This data could also indicate that urine has been found to be particularly a difficult specimen for PCR based amplification, due to the presence of many possible inhibitors. It could be concluded that PCR is an unreliable method for diagnosis of *E.cuniculi* infection, since the presence of DNA is not correlated with disease, and even in clinically ill animals, DNA detection can be negative.

I: Novel diagnostics

Ref: P2I332/0049

Acute trypanosomiasis due to *Trypanosoma theileri* in a dairy cattle in Kurdistan Province, Iran (first report)

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In July 2013 a seven year-old dairy Holstein cattle was referred to the Veterinary Clinic in Baneh town; Kurdistan Province. The cow had a history of pyrexia, anemia, weakness and lymphadenopathy. Laboratory findings revealed evidence of anemia and a lymphocytosis. Examination of Giemsa - stained blood smears revealed the presence of a large number of *Trypanosoma theileri*. Also, the animal was negative for other blood protozoal and anaplasma infections. After administration of Diminazene (5 mg/kg/IM two doses) along with Dexamethazone for 48 hours clinical cure was observed. Our report indicates that *Trypanosoma theileri* should be considered as a pathogen for cattle unlike present believes.

I: Novel diagnostics

Ref: P2I333/0093

***Sarcocystis* sp. parasites in the Mexican Great-tailed Grackle (*Quiscalus mexicanus*), Bronzed Cowbird (*Molothrus aeneus*), and Stripe-headed Sparrow (*Aimophila ruficauda*)**

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The objective of this study was to describe the morphological and ultrastructural characteristics of *Sarcocystis* sp. Parasites were identified in the muscles of seven wild great-tailed grackles (*Quiscalus mexicanus*), six bronzed cowbirds (*Molothrus aeneus*), and two stripe-headed sparrows (*Aimophila ruficauda*) in Mexico. Histopathological analysis revealed thin-walled mature parasite cysts. Ultrastructurally, the cyst wall consisted of a granular layer with villar protrusions and numerous microtubules. Bradyzoites measured $4.1 \times 1.6 \mu\text{m}$, and micronemes appeared in the anterior third of the conoid. The sequences and phylogenetic analysis of a specific fragment of internal transcribed spacer 1 (ITS-1) was amplified using the 25/396 primers by polymerase chain reaction and restriction fragment length polymorphism (PR-RFLP) results. *Hinf* I and *Hind* III did not cut the fragment. The sequencing results indicated a 100% similarity among the *Sarcocystis* parasites from the three bird species, and a BLAST search revealed 96% sequence similarity with *S. neurona*. Phylogenetic analysis showed that studied sequences are topologically distant to those reported for *S. neurona* in the USA and in South America and are also unrelated to any previously reported group. Although our morphological and molecular analysis data provide strong evidence that *S. neurona* uses these bird species as intermediate hosts, future molecular studies with additional DNA fragments, combined with biological studies, will ultimately allow us to convincingly identify these parasites. This is the first report of a *Sarcocystis* sp. parasite in wild birds in Mexico that may be *S. neurona*.

I: Novel diagnostics

Ref: P2I334/0103

Species specific pcr primer pairs to identify *Haemonchus contortus* and *Haemonchus placei*

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Haemonchus contortus (Hc) and *Haemonchus placei* (Hp) are two closely related parasitic nematode species that predominantly infect small ruminants and cattle, respectively. They are blood-sucking nematodes that cause significant losses of productivity in ruminants. These species are capable of hybridisation in experimental and natural conditions when they co-infect the same animal. Although morphological criteria can be used to identify most of *Haemonchus* adults to the species level, the values of the characters often overlap and mistakes can occur. For differentiation between Hc and Hp we present a combined approach, using morphological analysis and PCR primer pairs which will also allow the identification of hybrids. Genomic DNA was extracted from a single specimen of each of the species. PCR primers 75 and 86 (Zarlenga et al., 1994) were used to amplify Hp rDNA and products were purified and sequenced. All sequences were compared against the National Center for Biotechnology Information database in order to identify the rDNA regions. Then, two species specific PCR primer pairs were designed. The *BotuHpF/R* primer pair amplified a 459 bp band with Hp DNA samples and the *BotuHcF1/R2*, resulted in a 260 bp band using Hc DNA samples. Six different lineages of Hc and three of Hp were evaluated to validate the results and confirmed the primers specificity. Three of the Hp and four of Hc lineages are from São Paulo state. One of the Hc lineages is from Ceará state, Brazil. Another Hc lineage employed is from the Moredum Research Institute, UK.

I: Novel diagnostics

Ref: P2I335/0104

Diagnosis of species of goats haemonchus on production system in the Valley of the Cauto in Granma.

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The parasite *Haemonchus* is causing heavy losses in livestock production goats, but not an accurate diagnosis has been made. This work was performed with the aim of identifying and characterizing this genus present in the Valley Cautious in Granma. Were collected worms present in the abomasum for determination. Genre is identified based on the general morphology (size and shape), depending on the species the morphometric characteristics of the spicules of males, the structural identification and crown gall cervical cephalic. We measured the total length of the spicules, distance from the right end and left to its respective hook. Morphology was further characterized vulvar fin adult females. The results determined that goats Cauto Valley are affected by *H.* and *H. contortus placei*, with a predominance of the first 89%, which showed spicules of 398 microns and 429 microns respectively. In the characterization of females identified three types of vulvar flaps (buttons, lingüiformes smooth) with predominance of the first. We conclude that in the province of Granma identified two *Haemonchus* species in goats: *placei contortus* and the latter with hosts pecificity.

Key words: Gastrointestinal nematodes / goats / *Haemonchus contortus* / *Haemonchus placei*.

I: Novel diagnostics

Ref: P2I336/0126

Epidemiological status of theileriosis in small ruminants in Kurdistan Province, western Iran: over a five year period

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Due to the lack of any information about the prevalence of ovine theileriosis in Kurdistan Province, west of Iran; the present study was aimed to determine, the prevalence of the *Theileria* infection and identification of their species in small livestock in Kurdistan Province of Iran. In this survey, 9180 small ruminants, including sheep and goat, were randomly sampled and examined from 600 flocks in Kurdistan Province from June 2008 to August 2013. Thin and thick peripheral blood smears were taken and then stained by Giemsa staining method. A total of 9180 collected samples, 5460 were sheep and 3720 were goat. *Theileria spp.* is detected in 1115 (20.4%) out of sheep samples and 1393 (37.4%) out of goat samples by direct examination of blood smear. Overall, the prevalence rate of *Theileria* infection was 27.3% (n=2508) in both small ruminants. Moreover, *Th. lestoquardi* was the most common (65.6%) and (52 %) species found in these animals using microscopic examination and also among 100 randomly selected positive smears by semi-nested PCR respectively. The high prevalence of *Theileria* infection in small ruminants indicates the enzootic status of malignant theileriosis in the western region of Iran.

Key words: Ovine theileriosis, *Th. Lestoquardi*, *Th. Ovis*, *Prevalence*, Semi-nested PCR

I: Novel diagnostics

Ref: P2I337/0202

Sexual transmission of *Toxoplasma gondii* in sheep after insemination with experimentally contaminated semen: diagnosis by PCR, MAT and bioassay

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The objective of this study was to evaluate the sexual transmission of *Toxoplasma gondii* in sheep inseminated with semen experimentally contaminated with tachyzoites. Part of the ram semen collected was experimentally contaminated with 4×10^7 *T. gondii* tachyzoites of the RH strain. Another part of the semen was free of tachyzoites and used to inseminate the control group. Straws filled with semen were submitted to cryopreservation and afterwards assessed by PCR and bioassay to identify the parasite and its viability and subsequently be used for artificial insemination via intrauterine laparoscopy. Five ewes were inseminated with contaminated semen and four were inseminated with semen of the control group. Serum samples and buffy coat of all sheep were collected on days -14, -7, and 0 before insemination and on days 7, 14 and weekly up to two months after insemination and submitted to MAT for antibody detection and PCR of the 529bp repetitive element of *T. gondii* DNA. The parasitic agent was isolated in experimentally infected semen and all mice inoculated with infected semen became ill and died of acute toxoplasmosis. Seven days after the insemination, all females submitted to the procedure with contaminated semen exhibited anti-*T. gondii* antibodies. While the DNA of *T. gondii* was isolated from buffy coat of only one sheep 35 days after insemination with contaminated semen. All sheep in the control group continued negative. These results demonstrate the possibility of sexual transmission of *T. gondii* via insemination when using semen containing tachyzoites. Moreover, it is concluded that the cryopreservation of the semen do not inhibit the infectious potential of the parasite.

I: Novel diagnostics

Ref: P2I338/0304

Hematologic parameters of Thoroughbred horses naturally infected with Cyathostomins

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Cyathostomins (small strongyles) are recognized as the principal parasitic pathogen of horses and may be associated with several clinical and sub-clinical effects in the host. The aim of this study was to evaluate the hematological parameters of horses naturally infected with >98% of cyathostomins. The research was carried out from July/2014 to January/2015 with 31 naturally infected with cyathostomins young Thoroughbred horses (22 females, 9 males) from eight to 12 months of age. The animals belonged to Sao Jose da Serra Stud, in Sao Jose dos Pinhais, Brazil. Individual blood and faecal samples were collected from all animals every two months (July, September, November and January). The egg count per gram of feces (EPG) was performed with the mini-FLOTAC technique with the sensitivity of x10, whereas blood samples was performed for leukocyte count and the percentage of packed cell volume. All animals were dewormed with ivermectin and pyrantel in September as a routine spring-preventive treatment. The mean of EPG was 848.4; 0.0 (IVM+PYR treatment); 1228.4; and 2066.5 for July, September, November and January respectively. Despite the high EPG, all animals were clinically healthy and the relative leukocyte count was within the reference standards for Thoroughbred horses. We found no significant ($p>0,05$) correlation between EPG and the eosinophil count. Packed cell volume was within the reference standard for all months. In the present study, the evaluated hematologic parameters did not change according to EPG, preventing their use as diagnostic markers for animals with different cyathostomin EPG counts.

I: Novel diagnostics

Ref: P2I339/0324

A 'specific' issue: The use of universal primers to detect *Coxiella* sp. in the brown dog tick (*Rhipicephalus sanguineus*)

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The zoonotic bacterium *Coxiella burnetii* (*C. burnetii*), the causative agent of 'Q fever' in humans, has been detected worldwide in a large number of tick species. Although ticks may play a role in the natural transmission cycle of *C. burnetii*, the role of Australian brown dog ticks, *Rhipicephalus sanguineus* (*R. sanguineus*), harbouring this pathogen is currently unknown. The primary aim of this study was to detect *C. burnetii* in 107 brown dog ticks from urban Western Australia and the Northern Territories. The *C. burnetii*-specific primer targeting the IS1111a transposase element gene was employed using qPCR. All tick samples failed to amplify the IS1111a gene, while the two positive controls amplified the 498bp region. Following the negative detection of *C. burnetii* in *R. sanguineus* ticks, a secondary aim was introduced; a Next-Generation Sequencing (NGS) approach using the Ion Torrent™ semiconductor platform, in conjunction with universal bacterial primers targeting the 16S rRNA gene (V1-2), were used to evaluate the bacterial microbiome and to determine the presence of *Coxiella* sp. in *R. sanguineus* ticks. NGS results revealed *Coxiella* sp. was present in 53/59 (90%) tick pools. Further analyses showed these sequences had a 100% sequence homology to a *Coxiella*-like sp. previously identified from *R. sanguineus* in the Philippines (Accession No. JX185722). This study illustrates the value of using universal primers rather than species-specific primers, to detect novel bacterial genera. The identification of this *Coxiella*-like sp. in *R. sanguineus* ticks in Australia, requires further investigation into whether this bacterium causes cross-reactivity in immunologic assays used for the diagnosis of coxiellosis.

I: Novel diagnostics

Ref: P2I340/0361

Fluke infection in large ruminants: a preliminary result

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A study was conducted in six cattle farms and one abattoir to determine the occurrence of fluke infection in large ruminants in Kuala Terengganu, Malaysia. Faecal samples were collected from each animal and were subjected to McMaster method, larval culture and sedimentation method. Results obtained as in March 2015 revealed that for the field study, out of 60 animals diagnosed, 66.7% was found to be positive with only *Fasciola* eggs, 5% was found with only *Paramphistomum* eggs and 10% and was positive for both *Fasciola* and *Paramphistomum* eggs. The mean worm egg counts for fluke ranged from 2.22 ± 0.77 to 32.08 ± 18.47 e.p.g. All the animals which were found positive for *Fasciola* was found to be negative for strongyle eggs. As for the abattoir study, result from December to March 2015 revealed that 231 cattle and 110 buffalo were slaughtered and out of this numbers 11 livers (3.2%) were condemned. *Fasciola* (2.3%) and *Paramphistomum* (0.6%) were found in the livers, while 0.3% was condemned due to abscess. The number of adult *Fasciola* found from each liver ranged from 27 to 551 while for *Paramphistomum* the number ranged from 33 to 1188 individuals. Infection of both liver fluke and stomach fluke were found to be more in buffalo than in cattle. Results obtained from this study are still insufficient to make a clear conclusion on the current status of fluke infection in large ruminants in Terengganu. An ongoing project is now conducted which involve detection of IgG and molecular identification of *Fasciola*. Intermediate host morphological identification is also included, and the study will also include small ruminants and will involve the other 6 districts in Terengganu. This study will benefit farmers as the current status of fluke infection will be known and further control strategies could be planned based on future results.

I: Novel diagnostics

Ref: P2I341/0366

Evaluation and application of a semi-automated multiplexed-tandem PCR for the specific diagnosis of gastrointestinal nematode infections in sheep in the UK and Europe

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Gastrointestinal nematodes (GINs) are a major constraint on small ruminant production worldwide. However, sustainable parasite control is threatened by climatic changes in some parts of the world and the emergence and spread of anthelmintic resistance globally. Rapid detection and species identification are key prerequisites for improving our understanding of GIN epidemiology, for identifying pathogenic species and for monitoring the response of parasite populations to drug treatment. Current diagnostic methods are time-consuming, labour-intensive and require input from experienced personnel. DNA-based methods have the potential to address some of these issues and offer rapid, sensitive and specific testing options that can be offered by veterinary diagnostic laboratories. Multi-species helminth infections continue to pose a challenge for diagnosis, but this has been circumvented through the development of a semi-automated multiplex-tandem PCR (MT-PCR) platform, which targets the key GIN species affecting sheep (*Teladorsagia circumcincta*, *Trichostrongylus* species, *Haemonchus contortus*, *Cooperia* and *Oesophagostomum*). MT-PCR has been successfully evaluated under Australian conditions and is now available commercially. The aim of the present study was to evaluate the MT-PCR platform for detecting the key GIN genera infecting sheep in temperate countries, using ovine samples from the UK and Europe. Preliminary results indicate high analytical specificity and sensitivity, using mono-specific isolates of known provenance. MT-PCR also showed good agreement with archived field samples of known species composition, as determined by previous coproculture and multiplex PCR analyses. The test is currently being evaluated on field-derived faecal material collected throughout the UK sheep grazing season.

I: Novel diagnostics

Ref: P2I342/0397

Morphometric study of infective larvae of Cyathostome (Nematoda: Cyathostominae) of horses from Parana State, Brazil

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Small strongyles or cyathostomins account for more than 50 internal parasites of horses. As the free-living infective larvae (L3) are alike, it becomes a challenge to identify these nematodes. The available morphological keys consider the number, shape and arrangement of the intestinal cells (ic) of L3 that are classified in: Type A (2 + 6 ic); B (4 + 4 ic); C (2 + 2 + 4 ic); D (8 and 6 ic in a single line); M (7 ic); G (8 ic in varying arrangements); M (9 ic), *Gyalocephalus capitatus* (12 ic); and *Posterostomum* sp. (16 ic). Another way to identify L3s is using biometric measurements as the total length of the larvae, considering the length from the mouth to the tail end of the sheath. In this sense, only type A, C and D are of any value because type A is considered the most prevalent L3 throughout the world with 6 out of 10 of the most common species of equine gastrointestinal parasites. This research was conducted to identify and to measure L3 in order to monitor their distribution in Paraná, Brazil. Horse faeces were collected from the rectum of naturally infected animals and made into a pool of fecal culture of three studs. After L3s were collected, the larvae (n=300) were identified by their type using optical microscopy and measured with microscopes equipped with a macro imaging station. The average size was 731,5µm (±47) for Type A; 739,1µm (±48) for Type C; and 749,3µm (±37,8) for Type D. L3s were identified as Type A, D, C, H, B, F, and E at 56.7; 22.1; 8.9; 6.4; 4.4; 3.9; and 1.0%, respectively. Type A was the most frequent L3 type in Parana, followed by Type D. However, the average of their size was smaller than those already described in the literature. These results emphasize the importance of studying in detail the biology and the prevalence of cyathostomins to complement epidemiological and drug efficacy-based studies.

I: Novel diagnostics

Ref: P2I343/0421

Separation and purification of FhESPs making antibody against it and detection with developing an Elisa method

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Fascioliasis is caused by trematodes belonging to the genus *Fasciola*. In the past, infection was limited to specific geographical areas, but is now widespread throughout the world. It was previously believed that humans became infected only occasionally from livestock, evidence now suggests that there are endemic foci in which human-to-human transmission may occur. As a consequence, human fascioliasis should be considered a disease of major global public health importance.

Excretory-Secretory products have been recognized as playing an important role in inducing antibody formation so developing a method for antibody detection specific for ESPs will be a good way for Fasciolosis diagnostic and further investigations.

In this study the adult *Fasciola hepatica* worms were collected from the livers of infected bovines obtained from slaughterhouse. The flukes were washed with PBS and incubated in RPMI1640, the medium was centrifuged and the supernatant collected and put in refrigerator over night then lyophilized and kept at freezer. The protein concentration was tested according to Bradford method.

The antigen was examined by Fast Protein Peptide Liquid Chromatography (FPLC) and Poly acrylamide Gel Electrophoresis (PAGE).

Anti FhESPs were collected immunizing mice with it. An Elisa method was developed using FPLC fractions as antigens against polyclonal antibodies in mice blood sample and human positive serum sample.

According to the Bradford calibration curve the protein concentration in sample was 54.69 µg/ml. Running the sample on Poly acrylamide gel and dyeing it with coomassie brilliant blue only one band observed. FPLC calibration curve obtained by injection of five samples to the column and the molecular weight of ESP samples were: 17 and 44 KD. An ELISA method was developed and so 8ng/ml of ESP as coated antigen against the polyclonal antibody was detected. Using the human samples of people infected with *fasciola hepatica* the produced antibody had specific binding to ESP fractions.

I: Novel diagnostics

Ref: P2I344/0440

Validation of an *In Vitro* Culture Method for Cestodes

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Diagnosing *Dipylidium caninum* and *Taenia taeniaeformis* can be difficult due to the inconsistent shedding of proglottids and eggs in the faeces. Frequently, false negative results are obtained with the current diagnostic methods. While neither *Dipylidium caninum* nor *Taenia taeniaeformis* normally pose an important health issue to cats, they are both zoonotic. Diagnostic methods using excretion/secretion (E/S) products in the faeces could decrease the number of false negatives. However, E/S products frequently are limited in quantity, making development of these types of new diagnostic methods challenging. The purpose of this study was to determine how long these cestodes could be maintained in culture for the collection of E/S products and if length of culture impacted E/S product secretion.

Dipylidium caninum and *Taenia taeniaeformis* were harvested from the small intestine of cats that were euthanized for health issues. The cestodes were transferred to 150 cm² culture flasks within 2 h of euthanasia and maintained in a growth media at 36°C and 5% CO₂. The media (Eagle's Minimal Essential Medium with 6 mg/ml D-glucose; gentamicin 200 µg/ml; and Fungizone® (250 ng/ml)) was changed 6-8 h and 12 h after the cestodes were first placed in the flasks and then every 24 h, until the worms were sacrificed. For media changes, the media was poured out and new media gently added to the flask. Collected growth media was frozen until analysis (-20°C).

After 14 d, both *Dipylidium caninum* and *Taenia taeniaeformis* were alive, growing and releasing segments. A decision was made to sacrifice them on day 14. Preliminary E/S product data indicate that E/S was successfully collected throughout the 14 d. Based on this study, these cestodes can be maintained for an extensive period of time in culture, providing an opportunity to collect larger quantities of E/S products for development of diagnostic methods. The length of time that the cestodes lived also provides an opportunity to study the impact of stress (e.g., crowding, inter-species competition, nutrition) on E/S products.

I: Novel diagnostics

Ref: P2I345/0465

Detection of circulating cathodic antigen in the urine of raccoons (*Procyon lotor*) infected with *Heterobilharzia americana*

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Heterobilharzia americana is a trematode parasite (family: Schistosomatidae) that lives in the mesenteric veins of mammals in North America. The raccoon (*Procyon lotor*) is a main wildlife host, and dogs are occasionally infected resulting in gastrointestinal and hepatic disease. Diagnosing this parasite can be challenging due to potentially low and intermittent fecal egg shedding. A point of care immunodiagnostic assay (POC-CCA; Rapid Medical Diagnostics, Pretoria, RSA) has been developed to detect a gut-associated circulating cathodic antigen (CCA) in the urine of humans infected with *Schistosoma mansoni*, a related parasite. To determine whether this test will detect *H. americana*, the assay was compared with a fecal saline sedimentation test using raccoon samples. Urine, fecal, and tissue samples were collected from a total of 95 raccoon carcasses at an organized group predator hunt in central Texas, USA. Both urine and feces were available from 49 raccoons. Thirty-six of 49 (73.5%) were positive by POC-CCA and 9 of 49 (18.4%) samples were positive by fecal saline sedimentation. Two of the 9 positive fecal saline sedimentation tests were negative by POC-CCA. Of the 36 positive POC-CCA tests, 29 were negative by fecal sedimentation. Agreement between these two tests was poor (<0) and the proportion of test results differed significantly ($p < 0.000001$) using McNemar's test. The significantly higher proportion of positive test results by POC-CCA could be due to higher test sensitivity or to lower specificity. Raccoons were also parasitized with at least seven other respiratory and gastrointestinal helminths based on fecal sedimentation testing, so the possibility of cross reactivity should be explored further. Detection of trapped *H. americana* eggs in liver, small intestine, and pancreas tissues from these animals by histopathologic examination will help to estimate the sensitivity and specificity of the POC-CCA test.

I: Novel diagnostics

Ref: P2I346/0485

Development of a pen side diagnostic test for fasciolosis in sheep and cattle

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Fasciola hepatica, the common liver fluke is a parasite that raises severe health and welfare concerns for infected cattle and sheep. Over recent years, it has been shown that prevalence of *F.hepatica* is increasing significantly, especially within the UK, creating considerable costs to the UK farming economy of more than £300 million annually. Diagnostics for liver fluke are primarily based on faecal egg counts (FEC), involving a long and time-consuming method and collection of samples is not always practical, particularly on extensively managed cattle. Other diagnostic tests include ELISA based assays for antibody detection in bulk milk for dairy herds or for individuals, using serum or milk samples. These tests however are only able to indicate exposure to infection and are not reliable for identifying current infection. None of the tests available are able to identify acute fasciolosis, which mainly affects sheep and can cause losses of up to 10% of a flock in a matter of days. Neither are they ideal for use in cattle as treatment can only be administered at drying off or at housing. The aim of this project is to produce a novel lateral flow pen-side diagnostic test to allow farmers to quickly identify infected individuals. Serum samples from experimentally and naturally infected cattle and sheep identified immunodominant antigens recognised early in infection using western blotting. The major antigens detected were the fluke cysteine peptidases, Cathepsin L1 and L2, which were detected by 4 weeks post infection in experimentally infected sheep and calves following natural, primary infection. Recombinant Cathepsin L1 is being evaluated as the basis for a lateral flow test and these results will be presented. This technique will be evaluated for its use as a novel diagnostic test to aid in treatment of infected animals.

I: Novel diagnostics

Ref: P2I347/0486

Evaluation of species-specific peptides for early detection of antibodies to canine monocytic ehrlichiosis and anaplasmosis.

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Canine monocytic ehrlichiosis (CME) is caused by *Ehrlichia canis* (Ec) and causes severe clinical symptoms in infected dogs. *Anaplasma phagocytophilum* (Aph) causes canine granulocytic anaplasmosis (CGE) whereas *Anaplasma platys* (Apl) causes canine cyclic thrombocytopenia (CCT) in tropical and warm regions of the world, like the Mediterranean, Asia, Middle East, Africa, Australia, and the USA. In this study, we evaluated new peptide-based assays¹ for species-specific detection of CME, CGE and CCT using temporal samples from experimentally infected dogs. All peptide-based microtiter assays were optimized before evaluation. The Ec assay was able to detect antibodies in infected dogs (n=11) between days 7-14 post infection coinciding with the acute phase of infection (elevated body temperature) and approximately, 14 days prior to conventional markers. The Aph and Apl peptide assays detected antibodies in samples from infected dogs (n=12 for Aph, n=6 for Apl) 7-14 days post infection when all dogs were positive by PCR. All 3 peptide-based assays were species-specific and detected antibodies only from homologous experimental infection temporal samples. In conclusion, the results suggest that new species-specific peptide immunoassays may be useful for early antibody detection in dogs infected with *E. canis*, *A. phagocytophilum* and *A. platys*.

¹Qurollo BA, Chandrashekar R, Hegarty BC, Beall MJ, Stillman BA, Liu J, Thatcher B, Pultorak E, Cerrito B, Walsh M, Breitschwerdt EB. A serological survey of tick-borne pathogens in dogs in North America and the Caribbean as assessed by *Anaplasma phagocytophilum*, *A. platys*, *Ehrlichia canis*, *E. chaffeensis*, *E. ewingii*, and *Borrelia burgdorferi* species-specific peptides. Infect Ecol Epidemiol. 2014, 4: 24699

I: Novel diagnostics

Ref: P2I348/0571

Small ruminant parasite management on St. Kitts: FAMACHA and the Five Point $\sqrt{\text{®}}$

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On St. Kitts, an Island in the West Indies, anthelmintic resistance is of large concern in small ruminants due to the high level of *Haemonchus contortus* and the limited classes of anthelmintics available. While many of the sheep and goats are free ranging, to improve production and increase food security, the government is promoting enclosed pastures with more intensive management and breed improvements. This raises concerns about parasite load and infections in the grazing stock.

As part of a health management program for local sheep and goat producers, Ross University School of Veterinary Medicine (RUSVM) is assessing the ability to introduce the use of the FAMACHA selective treatment technique. Preliminary work on resistance and FAMACHA validation with the sheep herd owned by RUSVM in 2013-2014 demonstrated multi-drug resistance and some challenges in interpreting FAMACHA scores due to hydration levels, nutritional status (based on body condition scores (BCS)), and potentially another cause of anaemia (e.g., *Babesia* spp.). Further validation is now being conducted with goats and non-RUSVM sheep. Preliminary data indicated that the challenges seen with the RUSVM herd also were on local farms. Sheep with FAMACHA scores of 1 and 2 (no anaemia) had PCV values as low as 15% while sheep with FAMACHA scores of 4 (anaemic) had normal PCV values (>30%).

Due to the lack of correlation, the Five Point $\sqrt{\text{®}}$, nasal discharge and bots (sheep), coat condition (goats), FAMACHA, submandibular anaemia, BCS, and dag score, also is being evaluated to determine if it may be a better tool to assist farmers in making parasite and health management decisions. Preliminary data indicate that the Five Point $\sqrt{\text{®}}$, particularly the BCS combined with FAMACHA, might be a better indicator for treatment in this situation of heavy *H. contortus* infections and low production inputs.

I: Novel diagnostics

Ref: P2I349/0579

Detection of *Leishmania* spp. in conjunctival swab samples from dogs, cats and horses by real-time PCR

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Domestic dogs are considered the main reservoir of Visceral Leishmaniasis (VL) in Brazil. However, some authors suggest that other mammals species can be reservoirs of the parasite that causes VL. In this context, the present study aims to investigate *Leishmania* spp infection by real-time PCR (qPCR) and conventional PCR (cPCR) tests and compare both techniques, using conjunctival swab (CS) samples from 204 dogs, 108 cats and 54 horses collected in São Paulo state, Brazil. To do so, a qPCR and cPCR targeting different regions of the *Leishmania* spp kinetoplast minicircle DNA, previously described, were used. The qPCR assay was based on Taqman probe. The results showed that 1.96% (4/204) of the dogs were positives by cPCR and 0.98% (2/204) by qPCR, with a 0.32 kappa value that indicates a fair concordance between the tests. Regarding the cats, 1.8% (2/108) of tested cats were detected by cPCR and qPCR, which leads an almost perfect concordance between the tests (kappa =1). Nonetheless, less than chance agreement (kappa<0), was observed using samples from horses. Thirty six samples 66.7% (36/54) were detected by cPCR, while 12.9% (7/54) of the tested horses were detected by qPCR. In conclusion, non-invasive sampling of CS tested by qPCR proved to be effective for *Leishmania* spp detection in dogs, cats and horses samples. CS is an easy and practical alternative to obtain biological samples, especially in cats and can represent a great contribution for diagnosis of leishmaniasis.

Financial Support: FAPESP n: 2013/19821-4

I: Novel diagnostics

Ref: P2I350/0586

Use of a specific IgG-avidity ELISA to characterize recent and chronic *Toxoplasma gondii* infections in piglets and sows

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In order to diagnose recent or long-term *Toxoplasma gondii* infections in piglets and sows a commercial ELISA kit (PrioCHECK Toxoplasma Ab porcine ELISA, Prionics, Switzerland) has been adapted to measure specific IgG avidity (functional affinity). The avidity assay is based on the fact that antibodies produced early after an infection have lower binding affinities than those produced later on and can be eluted by including an additional incubation step with urea. Six 4.5 week-old piglets and 8 pregnant sows were orally inoculated with 5,000 and 10,000 *T. gondii* (European type II isolate) oocysts, respectively. Serial serum samples were obtained from the inoculated animals and from 14 piglets delivered by three of the sows and tested by ELISA. All absorbance values were normalized by calculating a percentage of positivity (PP) value relative to the O.D. of the positive control. For the assessment of IgG avidity, positive sera were titrated in duplicate with and without an additional incubation step with 6M urea in washing buffer (10 min, RT), and two end point titres were determined using PP 15 as cut-off. The avidity index (AI) was calculated as endpoint titre with urea/ endpoint titre without urea x 100. All animals seroconverted by 2-3 weeks post inoculation (wpi). The specific IgG avidity increased during the course of infection in all inoculated animals. The piglets showed lower AI, with values <12% (mean $8.9 \pm 1.8\%$) at 4 wpi and $\geq 40\%$ only at 8 wpi or later. Moreover, one piglet had low ($\leq 28\%$) AI values for at least 11 wpi. At 4 wpi the sows showed AI values ≥ 12 (mean $30.3 \pm 10.8\%$) and at 8 wpi all animals had values $\geq 40\%$ (mean $58.5 \pm 14.4\%$). Piglets from sows inoculated at mid-pregnancy were seronegative at birth and showed positive ELISA values after colostrum ingestion. Although the ELISA values decreased over time and were undetectable at 3 months of age, the AI remained constantly high (mean $58.3 \pm 10.2\%$) in seropositive piglets when tested at 7, 28 and 56 days of age and were comparable to those of the dams at the time of farrowing. A high AI ($\geq 40\%$) could be used to rule out a recent infection (<8 weeks) in most animals. Low AI values might be suggestive of recent infections; however, they can persist in some animals for longer periods. The IgG avidity-ELISA might represent a valuable diagnostic tool for epidemiological studies and can aid in the diagnosis of clinical *T. gondii* infections in pigs.

J: Genetics of host resistance

Ref: P2J244/0043

T helper cell transcription factor polymorphisms in nematode-infected sheep

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Teladorsagia circumcincta is a common parasitic nematode of the sheep abomasum. Immunity is acquired through repeated exposure to the parasite. However, the immune response, pathology and clinical outcome vary greatly between animals. The aims of this project are: 1) understand how sheep respond to worm infection; 2) understand and identify genes associated with the response; 3) identify variation within those genes which may contribute to resistance. T helper cell (Th)1 and Th17 activation is associated with susceptibility to the parasite (low antibody, high worm numbers) while a Th2 response is associated with resistance (high antibody levels and clearance of infection), which was illustrated by Affymetrix Microarray, validated by RT-qPCR and DGE analysis. The transcription factors that define the T helper cell lineages (GATA3, TBX21, RORC and RORA) have been sequenced and splice variants and SNPs identified. Initial results suggest up-regulation of GATA3 in resistant animals and up-regulation of TBX21 (T-bet) in susceptible animals. Interestingly RORC (ROR γ t) appears to be up-regulated, while RORA is down-regulated, in resistant animals, suggesting differential function of these Th17 transcription factors in disease outcome. The RORA variant transcripts also show differential expression. Absolute quantitation analysis of all transcripts has correlated gene expression with phenotypes of helminth resistance.

J: Genetics of host resistance

Ref: P2J245/0305

Body growth and development of Thoroughbred horses naturally infected with Cyathostomins

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Parasite infections are responsible for important economic losses in horse farming, either directly in animals that develop clinical signs or indirectly by body condition loss and performance. The objective of this study was to evaluate the body growth and development of horses naturally infected with cyathostomins. The survey was conducted from August 2014 to January 2015 with 30 Thoroughbred horses (21 females and 9 males), eight to twelve months of age and naturally infected with >98% of cyathostomins. The animals belonged to the Sao Jose da Serra Stud, in Sao Jose dos Pinhais, Brazil. Weight and height data and stool samples were collected monthly. The faecal egg count (EPG) exam was performed with the mini-FLOTAC technique with the sensitivity of 10x. All animals were dewormed with ivermectin plus pyrantel in September as a routine spring-preventive treatment. From August to January, the weight gain and the monthly growth of the animals was on average 16.6; 28.2; 20.9; 14.0; and 17.3 kg and of 1.9; 1.6; 1.0; 1.6; and 0.3 cm, respectively. The five-month EPG average was 291.0; 0.0 (IVM+PYR treatment); 635.7; 1230.7; 986.0; and 2064.7. Although the highest monthly weight gain was observed in September ($p < 0.00$), the month in which the animals had zero EPG, it did not improve the growth of the foals. There was no correlation ($p > 0.05$) between EPG and body growth during the period. The EPG did not differ between males and females and did not affect their development when evaluated separately.

K: Lifelong education (UG and PG)

Ref: P2K248/0284

Teaching collections: importance in learning and parasitological diagnosis

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The teaching of parasitology is gradually adapting to new technologies including the availability of content on-line. Undergraduate and post-graduate disciplines taught in the Department of Animal Parasitology (DPA), use digital learning booklets for theory and practice, in the form of texts and images. Moreover, the DPA has established individual collections in boxes, containing 50 important ectoparasite specimens mounted on slides. The modernization of the Didactic Parasitological Collection aims to provide practical material, through the preparation and mounting of specimens on slides or as photographs and is intended for teaching, demonstrations, training, and basic research in many disciplines, focusing on diagnosis, classification, the medical and veterinary importance and the evolutionary history of the parasites. The parasite collections are constantly updated to ensure optimal maintenance of the specimens and to organize and increase the longevity of the material. Specimens, including some rarities, have been digitally photographed to establish a virtual collection. Access of students to the collections facilitates the study of the biology and morphology of the parasites, so that actions related to diagnosis and control, to be implemented by future professionals are based on solid foundations. The collections serve to stimulating interest in research and to improve the performance of students in the disciplines of the DPA. These effects have been observed over time, and represent a significant development of human resources in teaching and research in Animal Parasitology.

Late breaking posters

L: Late breaking abstracts

L001

***Trypanosoma lewisi* – a rare parasite of wild rats in Malaysia**

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A study on the parasites of 105 wild rats caught in the urban areas around Kuala Lumpur indicated the presence of *Trypanosoma lewisi* in blood smears of 13 rats. *T. lewisi* is a lesser known parasite among other common parasites such as *Taenia taeniaformis*, helminths, Blastocystis, Sarcocystis and ectoparasites such as lice and mites. It was clearly identified on blood smears and confirmed by PCR by authorized laboratory at Universiti Putra Malaysia. Rats as pests transmit several zoonotic diseases and this study was carried out with the cooperation of the Kuala Lumpur Municipal Council which is empowered to control and eradicate these vermins. Due to the rise of zoonotic diseases such as leptospirosis, melioidosis and toxoplasmosis, which are purportedly transmitted by rodents, there is a need to carry out in depth studies on emerging and re-emerging diseases which may be potentially carried by rats. *T. lewisi* is a known zoonotic parasite and considering almost 15% are infected with it can cause disease conditions in individuals with low immunity and in high risk patients.

L: Late breaking abstracts

L002

Efficacy between *Azadirachta indica* (Neem) and *Jacaranda filicifolia* (Jambul Merak) leaves water extracts against gastrointestinal nematodes in local goats

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The situation of severe drug resistance to gastrointestinal helminthes in small ruminants has led to further research on medicinal plants with anthelmintic properties. An *in vivo* study was carried out involving 24 Katjang goats which were randomly selected from a private farm located in Sungai Siput, Perak from January to February 2014. Two (2) groups (n=8) of goats were treated orally with two different herbal remedies, namely; *Azadirachta indica* (Neem) leaf water extract and *Jacaranda filicifolia* (Jambul Merak) leaf water extract for 6 consecutive weeks. Faecal egg count (FEC), FAMACHA and Packed Cell Volume (PCV) data were taken within that period. Results indicated that Neem leaf water extract was efficacious for helminth control as compared to *Jacaranda filicifolia* leaf water extract, which had no significant difference with the untreated Control group at $P > 0.05$. Therefore, herbal remedies can be a potential method for use in smallholder goat farms which cannot use anthelmintics, as it is a eco green method as well as safer in terms of food safety, with less drug residues in livestock.

L: Late breaking abstracts

L003

The history of *Theileria orientalis* infections of cattle in Japan, Australia and New Zealand- similarities and contrasts in disease syndromes

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In the past decade, epidemics associated with *Theileria orientalis* in cattle herds in Australia and New Zealand have resulted in thousands of mortalities. However a similar disease has been recognised in Japan for many decades. This paper examines the features of disease in the three countries by studying the epidemiologic features, genotypes, transmission and diagnostic methods.

In Japan, *T. orientalis* is a common pathogen, with distribution in cattle across the entire archipelago but favouring areas of higher tick concentration. *Haemaphysalis longicornis* was long thought to be the main biological vector, but research has shown other arthropods may play a role in transmission. Outbreaks are seen most frequently in herds of cattle de-pastured for the summer where ticks are present and disease, characterised by severe anaemia, inability to walk, inappetance, abortion and death, is seen in stressed cattle such as those approaching parturition. Studies using Polymerase Chain Reaction (PCR) to identify the Major Piroplasm Surface Proteins (MPSPs) have shown that both benign and virulent genotypes exist, often in the same herd.

Prior to 2006, organisms resembling *T. orientalis* were frequently seen in blood smears of Australian cattle, but clinical cases associated with this infection were rare. The genotype seen in Australia ('Buffeli') was transmitted by ticks of native animals (*H. bancrofti*) and considered benign. From 2006, veterinarians in New South Wales and later Victoria, Western Australia, Queensland and South Australia reported dozens of cases of clinical disease associated with *T. orientalis* infection. PCR analysis revealed the presence of the Ikeda genotype, long suspected to be the major virulent strain in Japan.

In New Zealand 60 cases of mild infection with *T. orientalis* were recorded in 1984-5 but serious clinical cases were rare until late in 2012, when an epidemic occurred, starting in Northland and subsequently moving south across the North Island and into the South Island. The occurrence of cases followed the distribution of *H. longicornis*. Clinical disease was confirmed to be associated with the presence of the Ikeda genotype. PCR testing of historically collected blood samples (2008-2012) showed this genotype had been a recent introduction to New Zealand; the first detection occurring in blood collected in late 2011.

L: Late breaking abstracts

L004

Variant antigen profiling: a novel approach to population genomics of Variant Surface Glycoproteins in *Trypanosoma congolense*

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Animal African Trypanosomiasis (AAT) is endemic in East Africa, where it is primarily caused by *Trypanosoma congolense*. AAT causes considerable animal morbidity and losses of livestock, having a detrimental effect on economic development. Effective control of AAT, which would improve animal welfare while increasing productivity and economic prosperity across Africa, requires a precise assessment of disease risk, for the effective application of vector control, drug therapy and animal stocking. However, while the epidemiology of human trypanosomiasis is relatively advanced, our knowledge of the distribution and diversity of animal trypanosomes is poor, and this limits our ability to assess risk or prioritize resource use. We introduce the 'variant antigen profile' (VAP) as a novel means of dissecting pathogen variation and understanding its impact on disease. AAT is a particular challenge due to antigenic variation of the variable surface glycoprotein (VSG) coat surrounding the parasite. At any time, a single VSG gene is expressed from hundreds of alternatives and sequential expression of different VSG enables the parasite to evade the immune response. Previously, we have shown that *T. congolense* VSGs segregate into defined phylotypes according to genetic proximity, between which recombination does not occur. We wish to exploit this predictable repertoire to produce a VAP for any *T. congolense* strain. In this project, we are estimating VAPs for an historical collection of 35 strains to quantify population variation in VSG repertoire and confirm the universality of VSG phylotypes, as well as understand their link to population structure and geography.

L: Late breaking abstracts

L005

The isolation and identification of intestinal parasites of *Apodemus agrarius* in Korea

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In the isolation and identification of the intestinal parasites from *Apodemus agrarius* in Korea, ninety three of *Apodemus agrarius* were captured during 2012. It was detected several nematodes in the intestinal contents of 20 (21.5%) mice and in the feces of 1 (1.1%) mice. Ninety five of *Apodemus agrarius* were captured during 2013. It was detected many nematodes in the intestinal contents of 84 (88.4%) mice. It was detected some cestodes in the intestinal contents of 2 (2.1%) mice. And also, it was detected some trematodes in the intestinal contents of 3 (3.2%) mice. Thirty seven of *Apodemus agrarius* were captured during 2014. It was detected many nematodes in the intestinal contents of 30 (81.1%) mice, such as *Ascaris* sp (5.4%). It was detected some cestodes in the intestinal contents of 12 (32.4%) mice, they were all of *Hymenolepis* spp. And also, it was detected some trematodes in the intestinal contents of 3 (8.1%) mice, they were all of *Neodiplostomum seoulense*.

All kind of parasites will be identified by PCR and sequencing.

L: Late breaking abstracts

L006

***Anaplasma* spp. in wild ungulates and carnivores in northern Spain and their associated ticks**

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Wild species are essential hosts for tick-borne diseases but data on the prevalence and diversity of *Anaplasma* spp. in wildlife are scarce. In this study, we used real-time PCR to investigate the distribution of *Anaplasma* species in spleen samples collected from 627 wild animals (137 cervids, 227 wild boar, 263 wild carnivores) in two regions in northern Spain. A first generic real-time PCR assay was used to screen for the presence of *Anaplasma* spp. followed by a second species-specific multiplex real-time PCR or partial sequencing of the 16S rRNA gene for species identification. *Anaplasma phagocytophilum* was highly prevalent in wild ruminants (61.3%), but it was absent from wild boar and carnivores. Interestingly, *Anaplasma marginale* and *Anaplasma ovis* were not detected in wild ruminants, but *Anaplasma centrale* was identified in 1 roe deer and 1 red deer, *A. bovis* in 4 roe deer, 2 red deer and 1 fallow deer, and a novel *Ehrlichia* sp. in one badger. A total of 298 of the 627 animals could be examined for ticks. *Ixodes ricinus*, the recognised vector of *A. phagocytophilum* in Europe, was the main tick species parasitizing cervids (93.5%, 1674/1791), *Dermacentor marginatus* (76.1%, 35/46) in wild boar, and *Ixodes hexagonus* in carnivores (58.4%, 265/454). *A. phagocytophilum* infection rates were highly associated with the tick burden identified in the different hosts. More investigations are needed to assess the impact of the different *Anaplasma* species in wildlife and the risk of transmission to domestic animals.

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L: Late breaking abstracts

L007

Preliminary data of frequency of mutations A286S / L and V317I in the GABA-gated chloride channel gene of fipronil-resistant *Rhipicephalus microplus* populations from Uruguay and Brazil

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The southern cattle tick, (Canestrini), is one of the most damaging pests that parasites cattle in tropical and subtropical regions of the world. Recently, cross-resistance between fipronil and lindane has been reported on *Rhipicephalus microplus* populations from Uruguay and Brazil. Polymorphisms in the GABA-gated chloride channel gene of fipronil-resistant ticks was described and some of them were associated with resistance to fipronil. Five aminoacid substitutions were present in the resistant strains. Two of them may be linked to resistance (A286S / L, V317I), and the alanine to serine or leucine mutations at residue 286 are located in the same alanine residue mutated in fipronil-resistant individuals of other species. The objective of the present study was to determine the frequency of these mutations on fipronil-resistant populations from Uruguay and Brazil (n=11) confirmed with toxicological bioassays. DNA was obtained from engorged females (n= 5-18). The frequency of A286S / L mutation on 5 field populations and 3 fipronil-resistant strains varied between 11 and 100% but on 6 fipronil-resistant populations no mutations were registered. The frequency of mutation V317I was lesser and it was observed only in 3 populations. We hypothesize that other mechanisms of resistance are present.

Funding: ANII, INIA, FAPESP, FAPERGS, CNPq, FDRH-RS

L: Late breaking abstracts

L008

Genetic acclimation and ecological impacts

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Free-swimming *Argulus* specimens were collected from polluted freshwater in China, at low pH and low oxygen. The 18S and 28S rDNA were partly amplified using TD-PCR. We attribute this phenomenon to “genetic acclimation”. This hypothesis is strongly supported by the high transition/transversion ratio in our investigation, where the transitions are more frequent than transversions. Environmental genetic acclimation comes as response to the increasing influence of changeable ecosystem, population dynamics, demography, genetic structure and geographic resources. In addition, the gene flow between two populations can result in those populations becoming differentiated, principally at farm sites. The exposure of fish lice to chemotherapeutants, might also lead to genetic divergence. Furthermore, genetic structures of parasites are believed to be associated with high levels of inbreeding, low intra-population genetic variability and large genetic differentiation among populations from different geographical regions and/or hosts. Since hosts represent the parasite's primary environment, the selection of free-swimming individuals, in our study, were because we believe that, the host-parasite interaction plays an important role in the genetic content. A host-parasite association can closely co-evolve, likewise the absence of congruence may not always signify lack of historical association between the two components. In addition, in many systems, host–parasite evolutionary dynamics have led to the emergence and maintenance of diverse parasite and host genotypes within the same population. Thus, the host–parasite interactions influence evolutionary and ecological dynamics in important ways. However, the role of surrounded conditions in maintaining genetic variation in parasites is still generally poorly understood.

L: Late breaking abstracts

L009

Comparison between 18S and 28S rDNA genes in *Argulus* species

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18S and 28S rDNA of eight free-swimming *Argulus* spp. were partly amplified using TD-PCR. Obtained sequences were compared with others available in GenBank data. The phylogenetic trees of nineteen 18S and ten 28S rDNAs sequences showed different phylogenetic relationships. 28S gene was highly conserved comparing with 18S gene. The 18S phylogenetic tree revealed that the geographic could play a role in the genetic variation. The Chinese specimens obtained in our study are placed in one "major clade", as well as the Indian specimens. The geographic integrity of the majority of the clades identified thus far provides support for the phylogenetic history inferred from our study. Furthermore, the *Argulus* specimens were placed in different levels and some beyond outgroup. In all probability, this phenomenon could only be explained by the environmental impacts on the 18S gene, as well as the parasitic phase. Despite the difference in the phylogenetic relationships based on 18S and 28S analysis, it is possible to propose that environmental conditions may not have an impact on the genetic content of 28S. This proposal is supported by the results obtained from 28S phylogenetic tree, where two *Argulus* species, were placed in same subclade with high bootstrap support (ML 88, ME 97). In addition, contrary to the results obtained from the 18S tree, *A. foliaceus* C38 clustered with other *A. foliaceus* specimens, as well as specimens were clustered by parasite species. This finding indicates the possibility of identifying the *Argulus* species by using 28S as markers.

L: Late breaking abstracts

L010

Phenotypic expression of Pelibuey sheep experimentally challenged with *Haemonchus contortus*

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The objective was to determine whether a single experimental infection with *Haemonchus contortus* L₃ was sufficient to classify Pelibuey hair sheep, into responders and non-responders, using the eggs per gram of feces (EPG) as a phenotypic marker. Twenty Pelibuey male sheep, raised free of gastrointestinal nematodes, 6-7 months old were included in the study. They were challenged with *H. contortus* L₃ larvae (7500 ± 1412) orally on day 0 of the study. Faecal samples of each lamb were obtained daily from day 21 to 41 post-infection and their mean FEC, determined with the McMaster technique. The mean EPG value of each lamb during that period was used to classify lambs into responders and non-responders using the general EPG mean of all the lambs ± 2 SD. A second artificial infection was superimposed to all the lambs on day 42 (8420± 1545 L₃ larvae) and a new period of daily faecal samples was performed from day 65 to 78 of the study to determine the EPG of the super-imposed challenge. After the first infection, mean EPG of the responder lambs was 2449 ± 194, while the non-responder lambs had 14461 ± 1044 EPG (P<0.05). After the second challenge, responder lambs had lower FEC than non-responder lambs (650±220 vs. 5933±1189; P<0.05). Thus, a single artificial infection was enough to segregate Pelibuey lambs into responder and non-responders. This methodology could be used to develop Pelibuey selection protocols to resist *H. contortus*.

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L011

Infectivity of *Hyalomma* Ticks for *Theileria annulata* using 18S rRNA PCR

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Among the ixodid ticks, species of genus *Hyalomma* are of prime importance as they can survive in harsh conditions better than those of other species. Similarly, among various tick-borne pathogens, *Theileria* (*T.*) *annulata*, the causative agent of tropical theileriosis in large ruminants, is responsible for reduced productivity and ultimately substantial economic losses due to morbidity and mortality. The present study was planned to screening of vector ticks through molecular techniques for determination of tick-borne theileriosis in district Toba Tek Singh (T.T. Singh), Punjab, Pakistan. For this purpose, among the collected ticks (n = 2252) from livestock and their microclimate, *Hyalomma* spp. were subjected to dissection for procurement of salivary glands (SGs) and formation of pool (averaged 8 acini in each pool). Each pool of acini was used for DNA extraction, quantification and primer-specific amplification of 18S rRNA of *Theileria* (*T.*) *annulata*. The amplicons were electrophoresed using 1.8% agarose gel following by imaging to identify the band specific for *T. annulata*. For confirmation, the positive amplicons were subjected to sequencing, BLAST analysis and homology search using NCBI software. The number of *Theileria*-infected acini was significantly higher (P<0.05) in female ticks vs male ticks, infesting ticks vs questing ticks and riverine-collected vs non-riverine collected. The data provides first attempt to quantify the vectoral capacity of ixodid ticks in Pakistan for *T. annulata* which can be helpful in estimation of risk analysis of theileriosis to the domestic livestock population of the country.

L: Late breaking abstracts

L012

Preliminary data of frequency of mutations A286S / L and V317I in the GABA-gated chloride channel gene of fipronil-resistant *Rhipicephalus microplus* populations from Uruguay and Brazil.

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The southern cattle tick, (*Canestrini*), is one of the most damaging pests that parasites cattle in tropical and subtropical regions of the world. Recently, cross-resistance between fipronil and lindane has been reported on *Rhipicephalus microplus* populations from Uruguay and Brazil. Polymorphisms in the GABA-gated chloride channel gene of fipronil-resistant ticks was described and some of them were associated with resistance to fipronil. Five aminoacid substitutions were present in the resistant strains. Two of them may be linked to resistance (A286S / L, V317I), and the alanine to serine or leucine mutations at residue 286 are located in the same alanine residue mutated in fipronil-resistant individuals of other species. The objective of the present study was to determine the frequency of these mutations on fipronil-resistant populations from Uruguay and Brazil (n=11) confirmed with toxicological bioassays. DNA was obtained from engorged females (n= 5-18). The frequency of A286S / L mutation on 5 field populations and 3 fipronil-resistant strains varied between 11 and 100% but on 6 fipronil-resistant populations no mutations were registered. The frequency of mutation V317I was lesser and it was observed only in 3 populations. We hypothesize that other mechanisms of resistance are present. Funding: ANII, INIA, FAPESP, FAPERGS, CNPq, FDRH-RS

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L013

Laboratory evaluation of the efficacy of 10% imidacloprid + 2.5% moxidectin topical solution (Advantage[®] Multi, Advocate[®]) for the treatment of *Dirofilaria immitis* circulating microfilariae in dogs

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This study examined the efficacy of 10% imidacloprid + 2.5% moxidectin topical solution (Advantage[®] Multi, Advocate[®], Bayer) for the treatment of circulating microfilariae from dogs naturally infected with *Dirofilaria immitis*. The study included two groups of 11 dogs each that consisted of two replicates. Replicate 1 contained 12 dogs (6 treated and 6 controls) and replicate 2 contained 10 dogs (5 treated and 5 controls). Six of the 10 dogs in replicate 2 were the controls from replicate 1. All dogs entering the study completed a physical examination including chest radiographs, blood collections for examination of *Dirofilaria immitis* circulating microfilariae, serum chemistry, complete blood counts and urinalysis. To qualify for the study each dog was required to have a geometric mean ≥ 300 microfilariae per ml of blood from 3 consecutive samples collected during the acclimation period and a heartworm disease classification of 1 or 2. Dogs were treated on study days 0 and 28. Microfilarial counts were performed on post-treatment. Percent microfilarial reduction was determined by comparing the geometric mean number of circulating microfilaria between the two groups post-treatment. Seven days after the first treatment, the geometric mean microfilarial counts in treated dogs were reduced by >99% compared to the controls and remained at >99% through the end of the study. The results of this study demonstrated that Advantage[®] Multi for dogs is efficacious for treatment of circulating *D. immitis* microfilariae in naturally infected heartworm-positive dogs with no treatment-related adverse events observed.

L: Late breaking abstracts

L014

Intergrated control of cowdriosis and the vector *amblyomma variegatum*

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Cowdriosis is a fatal septicaemic disease of cattle, sheep and goats. The disease is caused by a rickettsial organism *Cowdria ruminantium* located in the brain capillaries of infected animals. It is transmitted by the colourful bont tick *Amblyomma variegatum*.

This same tick transmits a debilitating skin disease of ruminants called Streptothricosis [krichi]. Control of this tick has been difficult over the years. The use of acaricides alone has proved inadequate. Scientists have advocated an integration of one or more strategies to control the tick. For example, the use of a combination of Acaricides and the DECOY Technology by my colleagues in Florida has been largely successful when applied in the Carribean Islands.

In our poster presentation, we present alternate strategies to reduce the numbers of this tick drastically on animals thereby advancing the control of both Cowdriosis and Streptothricosis.

L: Late breaking abstracts

L015

Monitoring Field Susceptibility to Imidacloprid in the Cat Flea: An Update on Progress from a Fourteen Year Initiative

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2015 marks fourteen years since the establishment of an international Flea Susceptibility Monitoring (FSM) initiative by Bayer Animal Health, the first of its kind in the companion animal parasiticide arena. The program incorporates collaborating laboratories in the USA, Europe and Australia, utilising a validated larval development inhibition assay to track the susceptibility of cat flea isolates, field collected from veterinary practices, to imidacloprid. Since 2002, an established diagnostic dose threshold of 3ppm has been employed to identify isolates that require further investigation. In the thirteen year period to the end of 2014, a total of 2838 cat flea isolates were received across all participating sites; 2042 of these submissions were suitable for placement into the bioassay, resulting in a total of 1683 valid assays over this period. Adult flea emergence 5% or greater at 3ppm imidacloprid was observed in 56 of these valid assays (3.3%). For these isolates that reached the threshold for further investigation, re-conduct of the assay using either a repeat challenge dose of 3ppm of imidacloprid or a dose response probit analysis confirmed their susceptibility to imidacloprid. These findings affirm retained susceptibility of cat flea isolates to imidacloprid after almost twenty years of its worldwide use as a companion animal pulicide.

L: Late breaking abstracts

L016

Isolation of *Hc-fau* and its role in regulating *Haemonchus contortus* diapausing L4

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The gastrointestinal nematode *Haemonchus contortus* is a major blood feeding pathogen of sheep and goats. Diapause is a strategy to adapt this nematode to hostile environmental conditions. Therefore, the research on diapause is essential for preventing and controlling the disease. In this research, diapausing larvae of *H. contortus* ZJ strain were obtained and characterized. The complete cDNA and gene of *Hc-fau* were isolated through RACE and Genome walking. Microinjection and RNAi were conducted in *C. elegans*. Research on functions and relationship between domains of *Hc-FAU* was performed through transfection in HEK293 cell line. Research showed the larvae had a length of $1067 \pm 97 \mu\text{m}$ ($n=18$) and rod-like shape of crystalline inclusion in the intestinal cells. *Hc-fau* encodes two proteins, *Hc-FAUA* and *Hc-FAUB* consisting of 130 and 107 amino acids, respectively. *Hc-FAU* possesses a diverged ubiquitin-like (UBiL) protein domain and a conserved ribosome protein S30 domain. However abundance of *Hc-fau* transcript decreases significantly in diapausing L4. In *C. elegans*, knockdown of *Ce-rps30* confers extended lifespan, increased lipid storage in the intestine and shortened body length. Shortened lifespan is observed in *C. elegans* overexpressing *Hc-fau*, especially *Hc-faub*, where hatching failure is detected. The genes of insulin/IGF-1 signaling, TGF- β , cGMP, dafachronic acid, apoptosis and fatty acids metabolism are all down-regulated in *Ce-rps30*RNAi worms. The S30 domain stays in the nucleus, while UBiL accumulates in the cytoplasm. Results of UBiL domain and S30 domain overexpressions indicate synergism in regulating lifespan and reproduction. In all, *Hc-FAU* may play an important role in L4 diapause.

L: Late breaking abstracts

L017

Macrocyclic Lactone Resistance – or what?

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Treatments with anthelmintic drugs remain the principal means of control of parasitic gastrointestinal nematodes in conventional cattle farming. A new challenge however, is emerging anthelmintic resistance (AR), and mechanisms behind its development have not been fully investigated. The aim of these studies was therefore to investigate the effect of one of the most commonly used anthelmintic groups (macrocyclic lactones, ML) against *Cooperia oncophora* and *Ostertagia ostertagi* in Swedish cattle, and to seek an explanation for AR by further characterising worm isolates *in vitro* before and after treatment.

Two field trials carried out in consecutive years in Northern Europe and in Sweden showed reduced efficacy of ML in first season grazing cattle, with *C. oncophora* being the predominant survivor post anthelmintic treatment. However, in a controlled pen trial, using isolates collected from the field trials, results were ambiguous when animals were successfully dewormed according to the standard. Nevertheless several adult worms of *C. oncophora* survived the treatment.

Gene expression regarding P-glycoproteins, was investigated in the same worm isolates before and after ML treatment. Surviving male worms showed a tendency for increased gene expression of putative *Con-pgp-9*, but AFLP testing showed no changes in gene diversity. Pharmacokinetic behaviour of ML was also studied in interaction with concomitant use of dexamethasone in another controlled pen trial, showing a significant difference in plasma levels of ML in combination with dexamethasone.

The differing results of these studies illustrate the complexity of the AR problem, which poses a great challenge for future research in this area.

L: Late breaking abstracts

L018

The role of Eid Celebration on transmission of *Taenia spp.* and *Echinococcus granulosus* in dogs

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A total of 504 dogs were tested in three different time periods, between February 2012 and March 2013, to determine whether the Eid Celebration-Feast of Sacrifice has a significant impact on the transmission of *Echinococcus granulosus* (G1), *Taenia hydatigena* and *Taenia ovis* in Kosovo. Using a modified flotation-ovassay and flotation-sieving techniques, taeniid eggs were isolated from dogs for DNA extraction and PCR species identification. In Phase I (February to April, 2012), taeniid eggs were detected in 31 samples (6.2%, CI 4.2-8.6), six being positive for *E. granulosus* (sheep strain G1), and 31 containing DNA of *Taenia spp.* (29 *T. hydatigena* and one *T. ovis*). Around 4-6 months later, just before the Eid Celebration-Feast of Sacrifice, the same dogs were sampled (phase II) and six contained taeniid eggs (1.2%, CI 0.4-2.6; five were positive for *T. hydatigena*, one for *T. ovis* and none for *E. granulosus*). The dogs were re-treated with praziquantel before the Eid Celebration-Feast of Sacrifice, which was celebrated on 25-27 October 2012. In phase III, around 2-3 month after the religious event, the dogs were tested again and taeniid eggs were found in 21 samples (4.3%, CI 2.6-6.3; three were positive for *E. granulosus*, 18 for *T. hydatigena* and one for *T. ovis*). The study indicates that transmission of taeniids, including *E. granulosus*, increased after the Eid Celebration-Feast of Sacrifice. The results of this study are important for the design of control measures against cystic echinococcosis in large parts of the Balkan area where home slaughtering is practised and associated to this Muslim celebration.

L: Late breaking abstracts

L019

Frequency of vector-borne infections in pet-owned and stray dogs residing in the Turks and Caicos Islands

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Vector-transmitted diseases are common in dogs in the Caribbean. A 2004 serosurvey in the Turks and Caicos Islands reported 58% and 71% of feral dogs positive for *Dirofilaria immitis* antigen and *Ehrlichia canis* antibodies, respectively. None had antibodies to *Babesia canis*. The aim of this study was to evaluate the current seroprevalence of vector-transmitted diseases in pet-owned and stray dogs.

Anti-coagulated whole blood and serum were collected as part of a routine check-up for 101 dogs. A complete blood count (ProCyte Dx®) and SNAP® 4Dx® Plus test were performed on site.

The average age was 2.45 years (6 wks-13 yrs). Fifty-seven dogs were over 6 months of age. Of these 56% were infected with *D. immitis*; 25 were positive by antigen and microfilaria, 4 by antigen only and 3 by microfilaria only. On North Caicos, 85% (23/27) of pet-owned dogs were infected with *D. immitis*. *Ehrlichia* antibodies were the next most common with 28/97 (29%) testing positive including 7 puppies 6-8 wks of age. *B. canis* IFA identified 15/100 dogs with antibody titers of 1:64 or greater. Anemia (79/100 dogs) and thrombocytopenia (31/100 dogs) were the most common hematologic abnormalities. Vector-borne panel PCR was positive for *E. canis* in 8 dogs, for *A. platys* in 3 dogs and *Babesia* spp. in 2 dogs. Two dogs had *E. canis*/*A. platys* co-infections by PCR.

In conclusion, relative to the 2004 serosurvey, there was an increased frequency of dogs with *B. canis* antibodies despite a lower overall seroprevalence of *E. canis*.

L: Late breaking abstracts

L020

Characterisation of cellular and humoral immune responses during the periparturient period in *Teladorsagia circumcincta* infected ewes

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A major aspect of *Teladorsagia circumcincta* epidemiology is the increased ewe egg output around lambing which is the principal source of infection for newborn lambs. This is thought to be due to a relaxation of immunity during the periparturient period; however information on the exact nature of anti-parasite immune responses during this period is limited. We determined parasite-specific cellular and antibody responses in ewes experimentally infected with *T. circumcincta* over the periparturient period, and whether these responses were influenced by dietary protein levels. Twin-bearing Texel-cross ewes were trickle-infected with *T. circumcincta* L3 from day -40 relative to lambing then fed from day -25 until the end of the study at day 32 post-lambing with either 0.8× or 1.2× metabolisable protein (MP). Parasite-specific cytokine-release from peripheral blood mononuclear cells (PBMC) and serum levels of parasite-specific antibodies were determined. Diet group had no effect on any immune measure nor faecal egg output. However, an increase in IL-10 production by PBMC stimulated with L4 or adult antigen was seen from 2 weeks prior to lambing until the end of the study ($P<0.001$). In contrast, interferon- γ production was reduced post-lambing in PBMC stimulated with adult antigen ($P=0.036$). L3- and L4-specific IgG declined around the time of lambing ($P<0.001$), whereas adult-specific IgG levels were unchanged. This suggests that cellular immunity is skewed to a regulatory-type response during the periparturient period, and this effect is independent of MP levels. The drop in serum L3 and L4-specific IgG may further contribute to the periparturient relaxation of immunity.

L: Late breaking abstracts

L021

First finding of antibodies to *Encephalitozoon cuniculi* in chickens in Egypt

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In the present study, the presence of antibodies against *Encephalitozoon cuniculi* in chickens of different breeds was investigated by the method of enzyme linked immunosorbent assay (ELISA). A total number of 88 serum samples were collected randomly from chickens raised in houses and commercial farms in different locations of Behera province, Egypt. The breeds sampled included Egyptian native breed (balai) chickens (n=35), commercial egg-laying (Hy-line) breed (n=40) and commercial broiler Sasso breed (n=13). The age of the tested birds ranged from one month up to 20 months. Antibodies against *E. cuniculi* were detected in 13/88 (14.77%) of sera examined. *E. cuniculi* antibodies were detected only in the sera of egg-laying chickens. Results of this study indicate that chickens are exposed to *E. cuniculi* infection in Egypt. These results are of epidemiological relevance and public health importance; as the presence of *E. cuniculi* in raised chickens indicates a risk of infection to humans, mainly chicken breeders. Therefore, routine screening examinations of large-scale breeding of chickens are advised considering the zoonotic potential of these parasites.

L: Late breaking abstracts

L022

Geographic divergence of *Calicophoron microbothrioides* by analysis of the COI mtDNA

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Paramphistomosis is a frequent parasitosis in South American cattle. So far, *Calicophoron microbothrioides* was the only species identified in Peru, identified by the ITS2 rDNA. In order to search for additional molecular markers, 15 flukes were obtained from cattle of Cajamarca (7°09'S 78°30'W) and another 10 of Amazonas (6°13'S 77°51'W), both neighbor departaments from Northern Perú. The ITS2 (391 bp) plus a partial fragment (373 bp) of the COI subunit from the mtDNA were PCR amplified, sequenced and compared by analysis of the haplotypes number and diversity (Hd), Tajima's D and Fu's Fs neutrality tests, fixation index (Fst) and gene flow (Nm). The ITS2 elicited *C. microbothrioides* as the only species, without variation, but two haplotypes (H1 and H2) were identified for COI, which diverged in sites 82 (A x G), 298 (C x T) and 301 (T x G). Cajamarca's flukes shared H1 and H2, but the Amazonian were almost dominated just by H1 (p=0.01). Neutrality tests (Tajima's D=2. 3, p<0.05; Fu's Fs=4. 65), indicate that a selection process can take place, and the Fst value (0,46) confirms the population structure. In conclusion COI could be useful for the geographically differentiation of *C. microbothrioides*. Further studies are needed to compare more distant isolates and to know about the epidemiological implications of these findings.

L: Late breaking abstracts

L023

***In vitro* and *ex vivo* evaluation of the anti-*Giardia duodenalis* activity of the supernatant of SLAB51 (Sivoy™)**

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The effects on *Giardia duodenalis* growth (G), adherence (A), and viability (V) of Sivoy™ probiotic supernatant were evaluated *in vitro* and *ex vivo*. Sivoy™ (10¹ UFC) was *in vitro* cultured and the obtained supernatant was filtered, adjusted at pH 7, and added (100 µl/ml) as such (FS) or after heat-treatment to *G. duodenalis* (5×10⁴) cultures in TYI-S-33 medium. Negative and metronidazole 20 µg/ml (M) treated controls were used. *Ex vivo*, five- 1 cm long mice duodenal portions were cultivated in standard conditions with 5×10⁵ *G. duodenalis* trophozoites/ml, while to further five duodenal portions similarly cultured and infected, FS 200µl was added. After 12 and 18h, samples were fixed in 10% buffered formalin and histologically processed to score *Giardia* infection and cell damage. Cells proliferation/apoptosis were scored by TUNEL, Caspase-3, and Ki67 tests. All data, in triplicate, were statistically evaluated (P< 0.05). Results showed that FS significantly reduced *Giardia* G and V respect to negative controls but its efficacy was lower than that of M, while the inhibition of A was similar to that of M. Moreover, the effects of FS were significantly lowered by heat-treatment and the reduction was statistically higher at 90°C than at 56°C, indicating a heat-sensitive nature of active FS compounds. In *ex vivo* trials, in intestinal sections treated with FS respect to untreated controls viable *G. duodenalis* trophozoites and enterocyte TUNEL+ and Caspase-3 expression were significantly reduced, while enterocyte Ki67 expression was significantly increased, confirming the anti-*G. duodenalis* activity of FS observed *in vitro*.

L: Late breaking abstracts

L024

Performance of the rapid immunochromatographic test (Dual Path Platform – DPP®) officially adopted by the Brazilian Ministry of Health for the diagnosis of canine visceral leishmaniasis

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Despite the fact that systematic culling of seropositive dogs in Brazil was not able to significantly reduce the number of human cases of visceral leishmaniasis (VL), the official program of Ministry of Health based on identification of reservoirs by serodiagnosis operates continuously for 35 years. In order to improve this control program, it was incorporated recently a new immunochromatographic rapid test based on dual-path platform (DPP®), which was assessed in this study for the diagnosis of canine visceral leishmaniasis (CVL) and compared with an immunoenzymatic assay (ELISA) and an indirect immunofluorescence antibody test (IFAT). The specificity was evaluated using 74 serum samples from unexposed non-endemic dogs and 45 serum samples from dogs infected with other pathogens (*Babesia canis*, *Ehrlichia canis*, *Hepatozoon canis*, *Mycoplasma haemocanis* and *Anaplasma platys*). Serum samples from 52 naturally infected dogs with parasitological diagnosis confirmed (cytological examinations and cultures of bone marrow aspirates, qPCR and xenodiagnosis) were used to evaluate the sensitivity. The DPP® showed higher specificity [DPP®: 98.32% (02/119); IFAT: 84.03% (19/119); ELISA: 86.55% (16/119)] and lower sensitivity [(DPP®: 92.31% (48/52); IFAT: 100% (52/52); ELISA: 100% (52/52)] than the traditional serological techniques. When compared to IFAT and ELISA, DPP® shows good diagnostic performance, what qualifies it as a reliable screening tool for VL control programs.

L: Late breaking abstracts

L025

Fasciola hepatica: spread of albendazole resistance in Central Eastern Argentina

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Recently, a *Fasciola hepatica* field isolate resistant to albendazole (ABZ) and susceptible to triclabendazole (TCBZ) was recovered from sheep of Entre Ríos, Argentina (32°02'S, 60°16'W). In order to know about the regional status of resistance to drugs, a FECRT test was carried out on cattle. Four farms located at approximately 150 km around of the former description were selected. They belonged to the departament of Gualaguay (Gy), Gualaguaychu (Gch), Rosario del Tala (Rt) and Concepcion del Uruguay (Cu). Sixty cows per farm were assigned to four groups according to the treatments they received, as follows: ABZ group, 10 mg/Kg (n=15); TCBZ group, 10 mg/Kg (n=15); G3, Closantel group (CSTL) 15 mg/kg (n=15); G4, untreated controls (n=15). On day 0 cattle were drenched and sampled for feces. Fecal samples were taken again on days 7 and 21 post treatment.

ABZ resistance was detected in all the farms, giving by the following efficacy percentages (upper and lower limits of 95% confidence interval): Gy farm, ABZ 47% (70-25), TCBZ 100%, CSTL 93%(100-81); Gch farm, ABZ 25% (47-3), TCBZ 100% (100-100), CSTL 90% (100-76); Rt farm, ABZ 0%, TCBZ 100%, CSTL 100%; Cu, ABZ 0%, TCBZ 100%, CSTL 96% (100-86).

ABZ resistance seems to be widely spread in the studied region, although ABZ drenching not quite frequent. Other possibilities might take place, like the transport of snails infected with resistant *F.hepatica* across the basins.

L: Late breaking abstracts

L026

Serological survey of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* infections in domestic cats

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Anaplasma phagocytophilum (Aph), vectored by *Ixodes* spp., displays positive tropism to granulocytes, and is associated with fever and polyarthritis in cats, dogs, and humans. *Borrelia burgdorferi* (Bb) is also vectored by *Ixodes* spp., and while it is known to infect cats, syndromes associated with Bb infections in cats are unclear. Bb is associated with several abnormalities in dogs and humans. In dogs, lameness, anorexia, lethargy, and nephritis can develop. Cardiac or nervous system diseases also occur in some humans and rarely in dogs. The purpose of the study was to evaluate the presence of antibodies to Aph and Bb using peptide immunoassays.¹ Samples obtained from IDEXX Reference Laboratories, Inc. (n=4,172) located in *Ixodes* spp. endemic areas were screened by Aph ELISA and Bb (VlsEC6) ELISA. The data are shown in Table 1.

Species	No. Pos/Total tested	% Positive
Aph	347/4172	8.3
Bb	138/4172	3.3
Aph+Bb	66/4172	1.6
Aph-A. <i>phagocytophilum</i>		
Bb-B. <i>burgdorferi</i>		

The results indicate that in this sample set, antibody positive rates for Aph infection were higher than that for Bb. In addition, 1.6% of the feline sera tested contained antibodies to both Aph and Bb. These results support those of other studies showing cats can be infected with Aph and Bb.² This is the first report of a large scale serosurvey of cats naturally exposed to Aph and Bb.

¹Qurollo BA et al. Infection Ecology and Epidemiology 2014, 4: 24699.

²Magnarelli LA et al. Am J Vet Res. 2005; 66(11):1895-9.

L: Late breaking abstracts

L027

Detection of *Angiostrongylus vasorum* in Red Foxes (*Vulpes vulpes*) from Brandenburg, Germany

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Angiostrongylus (A.) *vasorum* is a nematode that causes angiostrongylosis in domestic and wild canids. Red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) are suspected of providing a wildlife reservoir for A. *vasorum* infections in pet dogs. To obtain data on the occurrence of A. *vasorum* in wildlife, red fox and raccoon dog carcasses (hunted or found dead) were collected from January to September 2009 in the Federal State of Brandenburg, Germany. Lung tissue samples were subjected to DNA extraction and examined for the presence of A. *vasorum* DNA by means of real-time PCR. A. *vasorum* DNA was detected in 11 out of 122 (9.0 %) lungs of red foxes and in none of the lung samples of raccoon dogs. These data suggest that red foxes are a reservoir of A. *vasorum* infections for pet dogs in this area.

L: Late breaking abstracts

L028

Protozoan Parasites in Faeces of Small Felidae Kept at the Czech Zoos

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This study was focused on prevalence of zoonoses in different species of small cats kept at zoos in the Czech Republic. The main aim of the research was the verification of presence of various protozoal parasites in cat faeces with special attention to small felidae. Sample collection was done from July 2013 to January 2014 in three regions in Moravian districts. In total, 700 samples from nine species of small felidae were investigated: Arabian wildcat (*Felis silvestris gordonii*), Jungle cat (*Felis chaus*), European wildcat (*Felis silvestris silvestris*), Amur leopard cat (*Prionailurus bengalensis euptilura*), Geoffroy's cat (*Leopardus geoffroyi*), Leopard cat (*Prionailurus bengalensis*), Fishing cat (*Prionailurus viverrinus*), Sri Lankan rusty-spotted cat (*Prionailurus rubiginosus phillipsi*) and Serval (*Leptailurus serval*) were examined. Parasite prevalence was determined by flotation method, using sucrose solution and subsequently by microscope examination of samples. Overall, there were found and positive findings of *Toxoplasma gondii* (1.43%), *Toxascaris* spp. (3.14%), *Toxocara* spp. (4.57%), *Giardia* spp. (8.57%), *Isospora* spp. (1.14%) and *Cryptosporidium* spp. (0.14%) among the tested samples. European wildcat and Geoffroy's cat were the species mostly affected by intestinal parasites. The occurrence of parasites positively correlates with seasons, cats are more susceptible to infection in summer and autumn months. However, the prevalence of these protozoal parasites depends on many factors, including: age, type of environment, geographical region, breeding and hygienic conditions, diet and many others. These zoonoses may be an important source of infection not only to bred felids and other animals at zoos but even for zoo keepers and visitors. Therefore, it is necessary not to underestimate the health risks connected with these parasites and monitor their epidemiology.

L: Late breaking abstracts

L029

Weight as a basis for targeted selective anthelmintic treatment of lambs at weaning

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The existence of gastrointestinal nematodes resistant to broad spectrum anthelmintics threatens the anthelmintic-dominant approach to the control of gastrointestinal nematodes in sheep in Ireland and the adoption of practises that can slow the development of resistance is required. The maintenance of refugia, the proportion of the parasite population that escapes exposure to anthelmintic, is considered important in delaying the build-up of anthelmintic resistance in worm populations. Conventionally, in Ireland, anthelmintics are delivered on a whole group basis. One proposal for maintaining refugia is part-flock treatment. There is a need for information on which individuals in the flock should be left untreated and an assessment of any negative effects of this strategy on animal performance. A study to examine the effect of anthelmintic treatment at weaning on lamb performance on a number of farms over 2 years was undertaken. Weaned lambs were weighed and assigned to one of 3 weight classes light, medium or heavy. The light and heavy lambs were used in the study and were randomly assigned to one of 2 anthelmintic regimes ($\pm$ anthelmintic; STARTECT, Zoetis, administered in accordance with manufacturer's instructions) and co-grazed on 'contaminated' pastures. The results for faecal egg count clearly show that lambs were exposed to GIN infection. Mean faecal egg count (FEC_{OT}) for 'other' trichostrongyles (excluding *Nematodirus battus* and *Strongyloides papillosus*) was 464 eggs per gram of faeces at Day 0. FEC measurements at 14 days post treatment indicated 100% efficacy of the anthelmintic used. Treatment was significant for FEC_{OT} and dag score (at Day 28; dosed lambs had lower values than undosed lambs. Treatment also had a significant effect on final live weight; undosed lambs were 1.2 (s.e. 0.20) kg lighter than lambs that had been dosed. There was no evidence for an interaction between treatment and weight class. As weight class did not affect FEC and since heavy lambs are likely to be sold earlier and taking account of the required withdrawal period prior to slaughter, it would be appropriate to selectively choose lighter lambs for treatment at the post-weaning period.

L: Late breaking abstracts

L030

Serological survey of *Leptospira interrogans* *Toxoplasma gondii* and *Trypanosoma cruzi* in free roaming domestic dogs and cats in a marginated rural area of Yucatan Mexico

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Zoonotic diseases in the poorest rural communities from Mexico are usually endemic in domestic and peridomestic animals. Leptospirosis, American Tripanosomiasis and Toxoplasmosis are relevant infectious diseases that may impact over public health, making their control and prevention imperative in order to avoid emerging outbreaks in the human population in close contact with pets.

A cross sectional study were performed in order to evaluate the serological status of *Trypanosoma cruzi*, *Toxoplasma gondii* and *Leptospira interrogans* in free roaming dogs and cats from a marginated rural community from Yucatan Mexico (Mayapan), 100 households were visited and animals sampled.

From the 106 samples, 93 were from dogs and 13 were cats. Frequency of positive cases for *T. gondii*, *T. cruzi* and *Leptospira* ssp was 97.8%, 9.7% and 45.2% for dogs and 92.3%, 0.0% and 15.2% for cats respectively. No associations with age, sex and body condition was found for *T. gondii* and *Leptospira* ssp neither for the place where pets sleep, fumigation or presence of the triatome in the household in the case of *T. cruzi*. For Leptospirosis, the most common serovars found were Canicola, Australis and Bratislava in dogs and cats with titers from 1:100 to 1:200 only one case of 1:400 was found in a dog.

There is an important circulation of the infectious agents in the studied population of free roaming owned dogs and cats, probably due to the lack of preventive medicine, vectorial control and consume of not well cooked food. Multifactorial lacks allow an increased exposition of dogs and cat populations with infectious agents. Domestic animals in those rural communities can be considered as important sentinels where show the risk of human infection in that rural community, because it is demonstrate that animals living in those area are able to become infected even if they are very young.

L: Late breaking abstracts

L031

Parasite load of *Trypanosoma cruzi* in dogs and owners from a rural community Yucatan, Mexico

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American trypanosomiasis is a zoonotic infectious disease caused by the hemo flagelated protozoa *Trypanosoma cruzi*. It is endemic in urban as in rural areas from Southern Mexico, and prevalence rates around 1-10 % has been reported. The prevalence of *T. cruzi* infection persists in the area due to the lack of vector control and infection both domestic and peridomestic animal species. Particularly in dogs from Yucatan, prevalence of infection can range from 10 to 17.5 %. Although several serological studies has been done in dogs, there is not information about parasite load as well in people as domestic reservoirs. The objective of this study was to determine the parasite load of *T. cruzi* in inhabitants and owned dogs in a rural region of Mexico. A convenience sampling was performed in owners (n=89) and their dogs (n=89) from the rural community of Molas, Yucatan. The parasite load was determined with an absolute quantification with a sybergreen real time PCR beside the assessment of DNA samples obtained from blood. In a total of 10.1 % of sampled inhabitants from Molas were determined the presence of *T. cruzi* DNA, finding low parasite loads (<10 parasites/mL) in 3.37%, medium parasite load (10-20 parasites/mL) in 5.6% and high parasite load in 5.12%. By other hand, in a total of 29.21% of sampled dogs were recorded the amplification for *T. cruzi* DNA, showing low parasite loads (<10 parasites/mL) in 15.7%, medium parasite load (10-20 parasites/mL) in 2.24% and high parasite load in 11.23%. These findings are clearly indicating the circulation of the parasite in persons and their dogs, highlighting that 11% of positive animals show high levels of parasitemia in peripheral blood, which may involve a significant risk during coexistence with their owners in household due to lack of vector control in the community.

L: Late breaking abstracts

L032

Parasitic zoonoses in rural village inhabitants of tropical Mexico

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A longitudinal study was made in 89 inhabitants from a rural community of Yucatan Mexico to determine the serological prevalence of the zoonotic agents (*Toxocara canis*, *Toxoplasma gondii* and *Trichinella spiralis*). Associations of seroprevalences with age, gender, presence of eye lesions and history of deworming were evaluated. Dogs owned to the participants were also serologically evaluated to determine IgG antibodies against *T. gondii* and fecal samples were taken to monitor the presence and intensity of infection with gastrointestinal parasites. From the 89 blood samples taken from dogs, 87 (97.6%) were seropositive to *T. gondii*; only 52 viable fecal samples were collected from dogs and 46.2% had the presence of parasites with low to moderate intensity; from those 12% had the presence of *T. canis*. In humans, the serological prevalence of *T. canis*, *T. gondii* and *T. spiralis* was 29.2%, 91.0% and 6.7% respectively. No associations were found between the positive cases with studied variables. This study demonstrate the high circulation of the studied zoonotic agents in the area particularly *T. gondii* which suggest a common source of infection in dogs and humans. Preventive measures must be designed towards good prophylactic practices in domestic and backyard animals (*T. canis* and *T. spiralis*). Contaminated sources with *T. gondii* (food and water) should be further determinate for control measures.

L: Late breaking abstracts

L033

Concordance between taqman B1 Real time-PCR and SAG1 Nested-PCR for the *Toxoplasma gondii* detection in women with recent abortion and its potential use in animals

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The infection caused by *Toxoplasma gondii* is worldwide distributed and the clinical implications are mainly associated with abortions in seronegative infected-pregnant women and in immunosuppressed individuals. However the opportune detection of the parasite is not easy and the molecular tools for the detection has been improved and implemented in order to establish an accurate diagnosis. Real time PCR is a variant of conventional PCR that allows detecting the presence of parasite DNA and estimating a hypothetical amount of parasite load in a clinical sample. On the other hand, nested PCR is another variant of PCR where is possible to detect a minimal amount of DNA from the protozoa due two different continuous amplification reactions.

In order to stablish the concordance between both molecular detection tools, 161 DNA samples purified besides blood samples from women with recent abortion were assessed. Two molecular methods were performed: a taqman real time PCR and a nested-PCR. Kappa value, sensibility and specificity were calculated with the software Epidat 3.0. With a taqman real time-PCR from B1 gene there were identified 3 cases, with the nested-PCR of SAG1 marker were detected 7 cases, and just 2 samples were detected with both methods. Due the obtained results, the kappa value between both tests was of 0.25 considered as acceptable (according Landis and Koch, 1977) or weak (according to Altman et al., 1991). Sensibility of real time PCR in comparison with nested-PCR was of 22.22% and sensibility of 98.03 %.

Although nested-PCR does not allow the accurate determination of the amount of parasite DNA present in clinical specimens, allows the detection of the agent even under conditions of low parasite loads. As based on these results, the nested-PCR appears to be a more reliable for the opportune detection of *T. gondii* for patient evaluation tool. This model may also be applied to animals suffering from abortions to improve the diagnosis.

L: Late breaking abstracts

L034

Analysis of P-glycoprotein gene transcripts in *Fasciola hepatica* and its relation with resistance to triclabendazole

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Fasciolosis is a parasitic disease caused by the trematode *Fasciola hepatica*. Its control is mainly based on the use of triclabendazole (TCBZ), a halogenated benzimidazole with excellent efficacy against juvenile and adult stages. Resistance to TCBZ in *F. hepatica* has been reported in many parts of the world. Benzimidazole resistance in nematodes is mainly caused by specific amino acid substitutions in β -tubulin resulting from a nucleotide substitution in the gene. However, the resistance to TCBZ in *F. hepatica* is not associated with changes in this molecule. The increased cellular efflux of TCBZ in *F. hepatica* is related to P-glycoproteins (PGP) and a T687G single nucleotide polymorphism (SNP) was initially suggested as responsible for the resistant phenotype. In the present work, a complete PGP sequence has been assembled based on sequences available in databases. We analyzed transcripts from this gene region in TCBZ resistant strains from Peru and Argentina and in susceptible strains from Mexico and Ireland. We observed that the T687G SNP is not associated with resistance to TCBZ. Instead, we identified other SNPs, including variants in the coding region and in introns. Two SNPs A617T and T267G, which fall in a PGP intron, are shared by all resistant strains but also are present in some susceptible ones. RT-PCR from adult RNA showed no evidence of alternative splicing. Our results highlight the lack of clear association of SNPs in this PGP gene with resistance to TCBZ. The recent release of two assemblies of the *F. hepatica* genome revealing the presence of multiple ABC transporters and multidrug resistance genes opens new avenues for investigating variants associated with the resistance of *F. hepatica* to TCBZ.

L: Late breaking abstracts

L035

The Function of Metacaspase in *Toxoplasma gondii* in the Apoptotic Cell Death

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Metacaspases, are caspase-like Cysteine protease found in plants, fungi, and protozoa which caspases are missing. Previous studies demonstrated that metacaspases are multifunctional protease involved in apoptosis-like cell death, insoluble protein aggregates clearance and cell proliferation. To investigate the function of metacaspase of *Toxoplasma gondii* (TgMCA), we studied the characterization of TgMCA knockout and overexpressed strains. Bioinformatics analysis that TgMCA contain a Ca^{2+} dependent C2 domain at N-terminal and a catalytic His-Cys dyad dependent p20 domain at C-terminal. An anti-TgMCA antibody was prepared and an 80kDa band was identified in the cell lysates of tachyzoites by western blot. Immunofluorescence studies showed TgMCA was well-distributed in the cytoplasm of the intracellular parasites, and localized to nuclear when treated in extracellular buffer. The lack of TgMCA did not affect proliferation, but the apoptotic-like response was diminished when detected by FACS analysis and TUNEL assay, indicating that TgMCA contributed to the cell death. Proliferation of TgMCA overexpressed strain decreased significantly compared to the wild type and were less virulent in mice. We suggest that TgMCA play roles in *Toxoplasma gondii* apoptotic cell death and cell cycle.

L: Late breaking abstracts

L036

Overexpression of TgSCD induces endoplasmic reticulum stress in tachyzoites

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Stearoyl-CoA desaturase (SCD) is a key enzyme in the synthesis of unsaturated fatty acid. A ~110 KDa SCD protein was demonstrated to exist in *Toxoplasma gondii* by C-terminal endogenous tagging (3×HA) in $\Delta ku80$ strain. The HA tagged protein colocalized with ER Tracker Red and TgDer1-GFP, two endoplasmic reticulum (ER) markers, confirming the ER localization of TgSCD, which is consistent with the ER localization of SCDs previously described in other eukaryotic cells. Under TgGRA1 promotor, a TgSCD-GFP overexpression plasmid was constructed. GFP fluorescence could be observed at the ER in the tachyzoites after electroporation. The TgSCD overexpressed tachyzoites formed smaller plaques in 7 days in HFF cells, compared to the parental RH strain. BALB/c mice infected with 100 TgSCD overexpressed tachyzoites survived for at least 15 days while all mice infected with equal RH tachyzoites died in 8 days post infection. The 40-fold upregulation of TgSCD transcripts determined by qPCR implied increased protein accumulated at the ER, which may induce ER stress. In the response to ER stress, transcription of many genes playing an integral role in protein processing, lipid biosynthesis and oxidative stress enhanced, such as glycosyl transferase, Derlin-1, caltuticulin and PUF genes. Transcripts of these genes all increased several folds in TgSCD overexpressed tachyzoites, indicating ER stress was induced indeed. Induction of ER stress in *T. gondii* may provide a new direction to control toxoplasmosis.

L: Late breaking abstracts

L037

Anti-*Toxoplasma gondii* effect of sterculic acid and its analogues in vitro

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Lipid metabolism is very important for the intracellular stage of *Toxoplasma gondii*. Many lipids need unsaturated fatty acids in the side chains. The key $\Delta 9$ -desaturase was predicted to exist in *T. gondii* by blastp with *Plasmodium falciparum* stearoyl-CoA desaturase (SCD). Sterculic acid has been shown to specifically inhibit $\Delta 9$ -desaturation and its analogues (methyl sterculate) exhibited antimalarial activity against asexual blood stages. Here, we examined whether these compounds also exhibit anti-*T. gondii* effect in vitro. In MTS assay of Vero cells infected with *T. gondii* tachyzoites, sterculic acid showed an EC_{50} value of 16 μM , and the EC_{50} values of the four analogues (INT13, INT14, INT15 and INT21) were 357 μM , 428 μM , 306 μM and 248 μM , respectively. The IC_{50} value for sterculic acid to Vero cells was 215 μM while the analogues showed no effect to Vero cells at the concentration up to 1 mM. In the proliferation assay, replication of invaded parasites slowed in HFF cells under 0.1 mM sterculic acid or 1 mM analogues. Only 3% vacuoles contained eight parasites in the INT21 group in 18 hours, in contrast with over 40% in the mock group. In the observation of anti-*T. gondii* effect under the microscope, most Vero cells disintegrated at 36h because of the propagation and egression of tachyzoites in the mock group. Only a small part of cells ruptured in the sterculic acid and analogues groups. Our study demonstrated that sterculic acid and its analogues may be an anti-*T. gondii* drug candidate.

L: Late breaking abstracts

L038

Progress on World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines on faecal egg count reduction test

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Guidelines for performing a faecal egg count reduction test (FECRT) were incorporated in the 1992 World Association for the Advancement of Veterinary Parasitology (WAAVP) publication on how to detect anthelmintic resistance in nematodes of veterinary importance. However, since the publication of these guidelines some limitations have been noted, highlighting the need for an update. We are therefore drafting a WAAVP guidelines on FECRT in order to improve the standardization and performance of the FECRT across a variety of both animal and parasite species.

Compared to the previous guidelines we suggest five important differences. First, the current guidelines will be structured into three parts. In the first part, general recommendations on experimental design, choice of FEC method, statistical analysis and interpretation of FECRT results will be given. In the second part, animal specific guidelines will be provided to perform a FECRT on small ruminants (sheep and goats), cattle, horses and pigs. In the final part, confounding factors affecting anthelmintic drug efficacy are highlighted. Second, we recommend performing the FECRT based on pre- and post treatment FECs of the same animals. Third, it is recommended to count at least a predefined number of eggs under the microscope across a group of animals regardless of the sample size and the FEC method applied. Fourth, 95% confidence intervals are based on a hierarchical Bayesian instead of a frequentist framework. Finally, additional thresholds for defining reduced efficacy are provided for horses.

L: Late breaking abstracts

L039

Genetic variation within *Taenia multiceps* cysts (coenurus) from ruminants in Greece

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This study was designed to genetically characterise the larval stage (coenurus) of *Taenia multiceps* from ruminants in Greece utilizing DNA regions (designated *pcox1* and *pnad1*) within the cytochrome c oxidase subunit 1 (*cox1*) and NADH dehydrogenase 1 (*nad1*) mitochondrial genes, respectively. A molecular-phylogenetic approach was used to analyse the *pcox1* and *pnad1* amplicons derived from genomic DNA samples from individual cysts (*n* = 105) from cattle (*n* = 3), goat (*n* = 5) and sheep (*n* = 97). Results revealed five and six distinct electrophoretic profiles for *pcox1* and *pnad1*, respectively, using single-strand conformation polymorphism. Direct sequencing of selected amplicons representing each of these profiles defined five and six different sequence types for *pcox1* and *pnad1*, respectively, amongst all 105 isolates. Phylogenetic analysis of individual sequence data for each locus, including a range of well-defined reference sequences, inferred that all isolates of *T. multiceps* cyst from ruminants in Greece clustered with previously published sequences from different continents. The present study provides a foundation for future large-scale studies of the epidemiology and ecology of *T. multiceps* in ruminants and dogs (the final host of the parasite) in Greece.

L: Late breaking abstracts

L040

Effect of the saprophytic *Mucor circinelloides* on the survival of eggs of *Calicophoron daubneyi*

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The aim of the study was to ascertain if the soil fungus *Mucor circinelloides* could be useful as an agent to develop biological control of trematode.

A total of 40 tubes containing 1 mL solution with 500 eggs of *C. daubneyi* / each were prepared. One batch of 20 tubes were added 0.5 mL with $5 \cdot 10^6$ spores of *Mucor circinelloides*, while other batch remained without spores as control. The tubes were maintained at 15-18°C or at 25°C during 29 days.

In the control tubes, the viability of the eggs of the gastric fluke decreased significantly from the 17th day, in coincidence with the observation of miracidia. Percentages of 32-41% miracidia were obtained at the end of the study. A significant reduction on the viability of the *Calicophoron* eggs by adding the spores of *Mucor* from the 4th day was noted. Miracidia were detected from the 17th day also, but 6-15% values were reached. No differences regarding the temperature were recorded. It is concluded that the utilization of *Mucor* spores could reduce the risk of infection by one half.

This work was partially supported by the Research Project AGL2012-34355 (Ministerio de Economía y Competitividad, Spain; FEDER).

L: Late breaking abstracts

L041

Industrial pelleting of feed with fungal spores to prevent equine strongylosis

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The possibility to add chlamydospores of a predatory fungus (*Duddingtonia flagrans*) during the industrial manufacturing of pellet feed has been analyzed. Firstly, chlamydospores were exposed to 72°C during different time intervals to assess their resistance under pellet manufacturing conditions (70-75°C for 90s). Secondly, one batch of concentrate feed was elaborated with fungal spores. In both experiences, the biological activity of chlamydospores was determined by measuring the mycelial growth and the sporogenesis, while their parasiticide activity was assayed on third-stage strongyle larvae (L3).

By heating the spores for ≤5 min in the first assay, hyphal growth was observed after 5 days, sporogenesis occurred after 17 days, and a significant reduction (94-97%) in the L3 counts was obtained ($\chi^2 = 26.187$, $P = 0.001$). No differences were observed between the spores in liquid solution or in pellet feed regarding the mycelial growth rates or the counts of chlamydospores, but lower percentages of reduction of L3 were achieved in the pellets (84-89%) ($\chi^2 = 9.396$, $P = 0.002$).

It is concluded that *D. flagrans* chlamydospores can survive the industrial manufacturing of pelleted feed, which enhances the possibility of the biological control of strongyles affecting horses. Because this formulation does not imply an additional task for horse owners or keepers, its practical utilization would be guaranteed.

This work was partially supported by the Research Project AGL2012-34355 (Ministerio de Economía y Competitividad, Spain; FEDER).

Molecular detection of *Ehrlichia canis* in dogs and their respective ticks in two regions endemic

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Canine ehrlichiosis has a high occurrence in routine medical clinic of small animals. This bacterium multiplies in epithelial cells of the intestine, hemocytes and cells of the salivary glands of the tick *Rhipicephalus sanguineus*, which occurs transtadial transmission and probably no transovarial transmission. The aim of our work was molecularly detect the occurrence of *Ehrlichia* sp. in dogs and their respective ticks in two endemic regions. Thus 720 ticks (ten of each animal) were removed from 72 dogs selected randomly in Zoonoses Control Center of Municipalities of Araçatuba, São Paulo and Campo Grande, Mato Grosso do Sul. After that, were made aspirations with needle of lymph nodes and bone marrow. After dissection of ticks, organs (intestine, ovary and salivary gland) and the punched sample of bone marrow and lymph nodes of the dogs were tested by polymerase chain reaction (PCR) for amplification of the 16S ribosomal acid ribonucleic (rRNA). Through the McNemar test, we observed that in PCR of lymph node 80.5% (58/72) were positives, while the PCR of bone marrow found 44.4% (32/72) of positivity, there was a significant difference between these lymphoid organs ($p < 0.05$). In relation to the PCRs of the organs of ticks observed positivity of 22% (16/72) in the intestines, 11% (8/72) in the ovaries and 7% (5/72) in the salivary glands. Thus, we concluded that the detection of *E. canis* through PCR was more sensitive to the use of deoxyribonucleic acid (DNA) extracted from lymph nodes and the presence of the bacterium in the ovaries of ticks leads us to consider a possible transovarial transmission.

L: Late breaking abstracts

L043

Spontaneous shedding of metastrongyloid third-stage larvae by experimentally infected *Limax maximus*

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Felids and canids acquire infection of metastrongyloid nematode parasites (*Aelurostrongylus abstrusus*, *Angiostrongylus vasorum*, *Crenosoma vulpis*, *Troglostrongylus* spp.) by the ingestion of infective third-stage larvae (L3) in the tissues of gastropod intermediate hosts (IMH) or paratenic hosts. Speculation on potential exposure due to L3 shed into the environment has arisen due to reports of L3 released by laboratory aquatic snails experimentally infected with *A. abstrusus*, *A. vasorum* and *Troglostrongylus brevior*. Spontaneous shedding has not been reported for these species in the terrestrial gastropod natural IMH (slugs, land snails). First-stage larvae of *C. vulpis*, *A. vasorum*, and *Troglostrongylus wilsoni* were each placed on lettuce (400 – 1600 L1/slug) and fed to laboratory raised *Limax maximus* in separate exposure groups of 22-37 slugs. A fourth exposure consisted of a mixture of *A. abstrusus* and *T. wilsoni* L1 (recovered from the lungs of a co-infected bobcat) were used to infect 30 *L. maximus* (1400 L1/slug). Slug feces was examined for L3 (2x/week) using a modified Baermann method. Shedding of L3 was detected in all groups beginning 20-30 days PI and continued as long as 198 days PI. No L3 were detected from unexposed control group slugs. Weekly shedding levels for the various infection groups ranged from 0 – 4.77 L3/slug. Based on identification using morphology, L3 of both *T. wilsoni* and *A. abstrusus* were shed by the mixture-exposed slugs. Detection of spontaneous shedding in all 4 parasite species indicates that spontaneous shedding of L3 into the environment may be a characteristic of the metastrongyloids.

L: Late breaking abstracts

L044

***Sarcocystis* sp. and *Toxoplasma gondii*: seroprevalence, in vitro isolation and PCR identification in indigenous sheep from northern Italy**

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The farming of indigenous sheep breeds is an important economic and cultural activity in many parts of the Mediterranean and genetic diversity offered by these breeds plays an important role in production and resistance to disease. The Cornigliese sheep breed is indigenous to northwestern Italy. It is transhumant and is known for the excellent dye uptake of its wool, for the quality of its milk for cheese production and for its meat. It is currently considered at risk of extinction and counts 1,600 head. The aim of the study was to determine seroprevalence for *T. gondii* by meat juice ELISA and evaluate the presence of *T. gondii* and *Sarcocystis* spp. within host tissue by histology, PCR and in vitro isolation, in indigenous Cornigliese sheep. Seventeen out of 24 (70.8%) sheep were positive for *T. gondii* by meat juice ELISA. Twenty sheep (83.3%) were positive by PCR for *T. gondii*, while 24/24 sheep (100%) were positive by PCR for *Sarcocystis* spp. Tissues cysts compatible with *Sarcocystis* spp. were visible in all animals on histology. PCR confirmed the presence of *T. gondii* after three weeks of in vitro culture on Vero cells in only one sample. *T. gondii* and *Sarcocystis* spp are present in a high percentage of Cornigliese sheep in northern Italy. Future studies should concentrate upon genotyping of *T. gondii* and species identification of *Sarcocystis* in order to better evaluate the reproductive and economic effects of these parasitic infections, in light of the necessary conservation of this local, indigenous sheep breed.

L: Late breaking abstracts

L045

In vitro expression of ATP Binding-Cassette (ABC) proteins in an *Ixodes ricinus* cell line following treatment with ivermectin

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Ticks are among the most important vectors of a wide range of human and animal diseases. Acaricides are widely used to control infestations by ticks, however, there are increasing reports of resistance. Ixodid tick cell lines represent a potentially interesting model for the study of drug resistance mechanisms, including expression of the ABC Cassette Transporter proteins. The cell line IRE/CTVM19 was used to determine the expression of PGP's following treatment with ivermectin. Cells were treated with different concentrations of IVM in DMSO. Negative controls were either left untreated or treated with DMSO only (0,1%). Cell cultures were maintained for approximately 20 days and media was changed weekly. Cell viability in flow cytometry (LIVE/DEAD Fixable Near-IR stain kit; Life Technologies) and Real-Time PCR for ABC protein subfamilies B1, B6, B8, B10 were carried out at days 10 and 20. was measured either through Trypan Blue exclusion assay and flow-cytometer. No significant differences in cell viability between controls and treated cells were observed. For all conditions, live cells were approximately 84-92%. Real-Time PCR showed that ABC pump expression was not significantly modulated. Expression of B1 subfamily was not detectable at any time point in any condition. B6, B8 and B10 were detected, but no significant differences were visible among untreated and treated tubes, although a positive trend in B8 expression in particular was observed with increasing concentrations of ivermectin. It would be of interest to develop this in vitro model and evaluate other cell lines and in the presence of different acaricides.

L: Late breaking abstracts

L046

Diagnosis of anthelmintic resistance in gastrointestinal nematodes of bovines

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Livestock farming is playing an important role in the economy of Pakistan by contributing about 11.5% to the GDP of Country. The total population of cattle is 35.6 million and population of buffalo 31.7 million, which yields about 44,827,000 tons of milk and 1,711,000 tons of meat per annum and this profitable farming is at risk due to infection with anthelmintic resistant populations of nematodes. Timely diagnosis of resistance and use of most effective drug is a key to profitable farming. That is why a study was conducted in District Toba Tek Singh of Punjab, Pakistan for the first time to evaluate the prevalence of anthelmintic resistance in the buffalo and cattle calves by using a Faecal Egg Count Reduction Test. Three commonly used anthelmintics, ivermectin and benzimidazole, were used to detect the development of resistance. A total of 30 randomly selected properties were surveyed for this purpose. Only one showed resistance to ivermectin, whereas 25 showed suspected resistance in the study area. In case of levamisole resistance, 2 farms showed slight resistance and the 24 farms showed suspected resistance against levamisole in district Toba Tek Singh. While with oxfendazole no property was found to resistant population of nematodes. This result indicates that resistance against commonly marketed anthelmintics is present in Pakistan and survey in other districts should also be conducted.

L: Late breaking abstracts

L047

Validation of FAMACHA[®] system in different sheep breeds of Pakistan

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Sheep and goats are of utmost importance in Pakistan and they are reared all over the country mostly by poor and landless farmers. Unfortunately gastrointestinal nematodes particularly *Haemonchus contortus* (*H. contortus*) and development of anthelmintic resistance are the major issues for sheep industry. Use of FAMACHA[®] system can help to slow down the development of anthelmintic resistance. The objective of present study was to check the sensitivity and specificity of FAMACHA[®] system in Lohi and Kajli sheep breeds located in different geographical regions of Pakistan. The eye color scores of 60 and 80 Lohi and Kajli sheep were checked by the same trained person and experiment was performed in triplicates. The packed cell volume (PCV) served as the gold standard for clinical evaluation of FAMACHA[®] system. For calculation of sensitivity and specificity, two different criteria were adopted: animals classified as FAMACHA[®] (F[®]) 4 and 5 or 3, 4 and 5 were considered anaemic (positive test) and animals classified as F[®] 1, 2 and 3 or 1 and 2 were considered to be non-anaemic (negative test). The highest sensitivity in Kajli breed was found at PCV value < 17% in both evaluation criteria. But in Lohi breed, the highest sensitivity was found at PCV values < 22% and ≤ 12% in first and second evaluation criteria respectively. This study suggests that animals in the FAMACHA[®] category 3 should be drenched in order to increase the sensitivity of the method. Therefore, FAMACHA[®] system is a very valuable additional tool of TST.

L: Late breaking abstracts

L048

The societal cost of *Taenia solium* cysticercosis in Tanzania

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Taenia solium is a zoonotic parasite prevalent in many low income countries and recognised as a public health threat, however the burden it poses on human and pig populations in Tanzania is unknown.

The aim of this study was to estimate the societal cost of *T. solium* cysticercosis in Tanzania, by assessing both the health and economic burden.

The health burden was assessed in terms of annual number of neurocysticercosis (NCC) associated epilepsy incident cases, deaths and Disability-Adjusted Life Years (DALYs), while the economic burden was assessed in terms of direct and indirect costs imposed by NCC-associated epilepsy and losses due to porcine cysticercosis.

Taenia solium cysticercosis was present in 10 regions of the country and contributed to reduced societal and economic wellbeing. The annual number of NCC-associated epilepsy incident cases and deaths were 22,410 (95% Uncertainty Interval (UI), 5,860 - 49,272) and 324 (95% UI, 54 - 929), respectively. More than 11% of the pig population was infected with the parasite disproportionately affecting small-scale farmers. The number of DALYs per thousand person-years for NCC-associated epilepsy was 0.98 (95% UI, 0.23 - 2.35). Around 6 million USD were spent due to NCC-associated epilepsy and 6.5 million USD were lost due to porcine cysticercosis.

Our results show that *T. solium* imposes a serious public health, agricultural and economic threat. We urge that a One Health approach is taken involving all relevant governmental agencies to find sustainable solutions for prevention, control and elimination of *T. solium*.

L: Late breaking abstracts

L049

Comparative study of four different diagnostic methods for detection of *haemonchus contortus* eggs

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Haemonchus contortus is a blood-feeding nematode of small ruminants. Recent studies indicate the presence of emerging multi-drug resistant isolates, which makes correct diagnosis of this parasite more critical than ever. In this study we compared four diagnostic methods based on different principles. Faeces were collected at a commercial laboratory receiving diagnostic samples from sheep flocks in Sweden. Samples (n=37) were analysed individually with all four diagnostic methods, namely: microscopy and McMaster egg counting (1) before and (2) after staining with peanut agglutinin (PNA), and by detection of DNA following amplification by (3) quantitative PCR and (4) Loop-Mediated Isothermal Amplification (LAMP). Differences between tests $\pm$ SD were analysed with Bland-Altman plots. Strongylid faecal eggs counts ranged from 200–12,100 epg (mean $\pm$ SD = 1,278 $\pm$ 2,049). Microscopy showed that *H. contortus* was present in 27 unstained samples and in 28 stained with PNA, whereas 29 LAMP and 34 qPCR results were positive. Ct-values with LAMP ranged between 13–38 (mean $\pm$ SD = 21 $\pm$ 7), and in qPCR between 25–49 (mean $\pm$ SD = 33 $\pm$ 6). There was agreement between the different methods, however, bias between the microscopy methods was less than for the molecular tests, -4.2 $\pm$ 11 and -9.8 $\pm$ 10, respectively. Interpretation in terms of test sensitivity was: microscopy<PNA<LAMP<qPCR. For high-throughput diagnostic laboratories, it seems possible to identify *H. contortus* directly with the McMaster method, without making mistakes with false positives. However, both molecular methods were more sensitive compared to microscopy. The molecular methods provide an opportunity to diagnose with increased accuracy.

L: Late breaking abstracts

L050

Detection of *Dirofilaria immitis* DNA in host serum by nested PCR

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The heartworm *Dirofilaria immitis* is a parasitic filarial nematode and the causative agent of cardiopulmonary dirofilariasis in dogs. Studies have shown that parasite-derived cell-free DNA (cfDNA) can be detected in host body fluids (e.g., blood and urine) and may be a promising diagnostic marker for parasitic infections. Thus, our aim was to detect *D. immitis*-derived cfDNA in host serum by nested PCR. Sera were collected from 12 dogs with natural *D. immitis* infections; eight were microfilariae (mf)-positive and the remaining four were mf-negative. Culture fluids derived from single sex adult *D. immitis* worms were also tested for cfDNA. All mf-positive sera were positive by nested PCR, whereas no amplification products were detected in mf-negative sera. Parasite DNA was detected only in the culture fluids of mf-producing females. These results suggest that *D. immitis* DNA circulate freely in the host serum. Additionally, *D. immitis* cfDNA may be primarily derived from the mf, and adult worms appeared to be minor contributors of cfDNA concentrations in serum; however, the derivation of *D. immitis* DNA from other developmental stages (i.e., third, fourth, and fifth stage larvae) is unclear. An evaluation of the kinetics of *D. immitis* cfDNA in host serum throughout the parasite life cycle could facilitate the development of early molecular diagnostic techniques.

L: Late breaking abstracts

L051

Cohort study of CVL to evaluate the dynamics of the disease in dogs, geographical distribution and the use of deltamethrin-impregnated collar as a prevention method in an endemic city, using serological and qPCR tests

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Canine visceral leishmaniasis (CVL) is an important zoonose and its epidemiological pattern is been changing constantly, with rapid geographical spread. Understanding the dynamics of the disease is one of the basis to achieve better methods of control and prevention. In Panorama city, SP, Brazil the seroprevalence of CVL was 41% in 2010. Based on this data we analyzed the dynamics of CVL and their geographical distribution on site, as well as evaluating the use of one of the existing control measures. A longitudinal study was conducted in 125 dogs in Panorama city, distributed in two groups geographically homogeneous. 69 dogs were placed in the control group and 56 dogs received deltamethrin-impregnated collars. All dogs were submitted to serological tests (DPP and ELISA) and qPCR of whole blood samples and lymph node aspirates, collected in three periods with an interval of six months each. CVL geographic distribution was considered homogenous in qPCR results, but heterogeneous in serology for both groups. 24% died after positive qPCR diagnostic, and 9% after positive serology. 29% and 17% presented positive qPCR and positive serology, respectively in at least one of the tested samples. Among the animals that were not infected in the first sampling, 23%(7/34) and 32%(16/32) animals became infected during the study period in the group with collar and in the control group, respectively, with statistical significantly difference ($P < 0.1$). The use of collars showed some protection against LVC. The prevalence of the disease continues to grow and is present throughout the city.

L: Late breaking abstracts

L052

Secretion of rna-containing extracellular vesicles by the porcine whipworm, *trichuris suis*

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Trichuris suis is a parasitic helminth of pigs and ensures its own survival by evading host immune responses, but little is known about how this is achieved. Micro RNAs (miRNA) has been shown to be involved in post-transcriptional regulation of genes associated with immunological processes. Recent findings show, that some adult parasites secrete miRNA-containing extracellular vesicles (EV). Fusion of these structures with host cells indicates a role in host gene regulation. The aim of this study was to demonstrate the secretion of miRNA-containing EV by *T. suis* first stage larvae (L1). Infective *T. suis* eggs were mechanically stimulated to hatch and obtained L1 larvae were cultivated in culture media (37°C). Larvae were separated from the media where after EVs were purified from the media by ultracentrifugation. The obtained the pellet, as well as larval surfaces, were then examined for EVs by electron microscopy. Another EV pellet was analysed for RNA using a Bioanalyzer. We show here, that EVs are secreted by *T. suis* L1 larvae and demonstrate the presence of RNA, of miRNA-size, inside them. In addition, an electron-dense layer covering the surface of the larvae was observed. A role of the RNA molecules in host-parasite interactions is suggested, and the surface coat may play a function in host immune evasion. Future studies should focus on the characterization of the surface coat as well as the *T. suis* EV-derived RNA and the potential, of using these molecules as biomarkers of infection or targets in new anti-parasitic drugs, should be explored.

L: Late breaking abstracts

L053

Quantitative PCR to assess *in vitro* efficacy of different anticoccidials on *Eimeria tenella*

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Emergence of resistant *E. tenella* isolates toward different anticoccidial agents is considered a challenge in controlling this disease. The aim of this study is to establish an *in vitro* assay using real-time quantitative PCR (qPCR) to assess *E. tenella* sensitivity profile toward the corresponding anticoccidials.

In vitro models were designed to determine minimum inhibitory concentrations (MIC) of monensin (Mon), maduramicin (Mad), salinomycin (Sal), lasalocid (Las), and toltrazuril (Tol) using a laboratory reference **Houghton** strain. In the first study, minimum inhibitory concentrations (MIC) of Mon, Mad, Sal, and Las were determined that are able to inhibit more than 95 % of Houghton sporozoites development to mature merozoites (treatment duration 24 hours). The defined MIC for Houghton strain were 0.5, 2.5, 1, and 0.5 µg/ml for Mon, Mad, Sal and Las, respectively. A second study was conducted to determine reproduction inhibition exerted by Tol. Tol was added to infected MDBK cells with sporozoites after 48 hours of establishing the infection (treatment duration 48 hours. Tol at 5 µg/ml reduced reproduction of Houghton strain by 95 %. Different *E. tenella* field isolates were evaluated *in vitro* for their sensitivity for all named anticoccidials using the previously defined anticoccidial MIC. All isolates show similar general ability to invade and grow in MDBK cells with different sensitivity profiles toward different anticoccidials.

The described models may be suitable and provide elaboration of reliable *in vitro* *E. tenella* sensitivity profiles toward the tested anticoccidials. However, whether this applies to *in vivo* conditions; remains to be demonstrated.

L: Late breaking abstracts

L054

The transcriptome of *Parascaris* spp. – assembly, annotation and gene expression induced by ivermectin exposure

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Parascaris are amongst the most pathogenic equine endoparasites in foals and yearlings. Macrocytic lactone (MLs) resistance is widespread in *Parascaris* populations but its mechanisms are poorly understood. Next-generation-sequencing was used to assemble a comprehensive *Parascaris* transcriptome, annotate it with an emphasis on genes potentially involved in ML action or resistance and the response of adult worms to ivermectin (IVM) exposure.

A draft transcriptome has been assembled following the sequencing of a normalised library from a single male and female worm using 454 pyrosequencing. This assembly was substantially improved using data produced by HiSeq technology. The combined assembly contains approximately 54,000 contigs (51% exceeding 1kb in length; N50 = 1862 bp) and covers 80% of the amino acid sequences encoded in the *Ascaris suum* transcriptome. Twenty contigs are predicted to encode P-glycoproteins. The Pgp-11 sequence in *Parascaris* corresponds to the allele recently reported to be associated with ML susceptibility; additionally, a Pgp-11 paralog was found. The transcriptome also encodes 5 GluClIs, representing *glc-2*, *glc-3/avr-15*, *glc-4*, *glc-5* and *avr-14* orthologs of *C. elegans*. We also explore synonymous/non-synonymous polymorphisms and reveal positive selection for particular genes. Following 12 h *in vitro* culture with or without a sublethal concentration of IVM, individual male adults (n = 5 per treatment) were subjected to Illumina sequencing and their transcription profiles compared. IVM significantly regulated transcription more than 2-fold in less than 100 genes. None of these genes represents a gene family previously implicated in ML resistance, and there was also no over-representation of stress-response genes.

L: Late breaking abstracts

L055

Prevalence and molecular characterization of *Toxocara* spp. from dogs in the Southeastern part of Brazil

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The aim of this study was to estimate the prevalence and infection intensities of *Toxocara* spp. in dogs from the Southeastern part of Brazil. In addition, sequence analysis of the *cox1* gene will be used to confirm the morphological findings and evaluate the genetic variability among worms. A total of 37 dogs up to six months old, of both sexes and different breeds, from public kennels, in Uberlandia and Belo Horizonte cities were treated with a single dose of piperazine sulphate 46% (180 mg/kg bodyweight) and expelled worms collected. Worms were enumerated, morphologically identified and stored at -20°C for further molecular analysis. All worms were identified as *T. canis* by morphology. The prevalence was found to be 100% with a mean worm intensity of 5.2 To confirm the morphological identification and to genetically compare the worms to *T. canis* from other regions in the world 609 bp of the *cox1* gene will be PCR amplified and sequenced. Population genetic tools and cluster based methods will be used to analyze the sequence data

L: Late breaking abstracts

L056

BKI 1294 as an effective treatment of cryptosporidiosis in calves?

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Background: *Cryptosporidium parvum* is a protozoan agent that infects humans and animals. In calves *C. parvum* is one of the most common causes of heavy neonatal diarrhoea. So far medical treatment options are not satisfactory. Here we present data on the novel CDPK1 inhibitor - BKI 1294 - that was able to substantially reduce the shedding of *C. parvum* oocyst by calves.

Methods: In this double blinded study 24-48 hour old calves were infected with *C. parvum* and treated five times with BKI 1294 (400 mg/animal), vehicle or were left untreated. Treatment was applied five times every second day and started one hour after infection. Oocyst output, health status, dehydration and blood chemistry was assessed.

Results: Oocyst shedding was most prominent between day 4 and 11 post infection and significantly ($P < 0.05$) reduced by 79% and 73% in this period compared to the mock control group and the untreated group respectively. Faecal consistency was similar in all groups but the number of days animals experienced dehydration was slightly lower in the treated group. Treated animals experienced on average 0.8 days of dehydration with a score above two whereas mock treated and untreated animals displayed 2.3 or 4.5 days of dehydration, respectively. Analysis of the blood chemistry and histological sections of various organs revealed no major differences between the groups indicating the low toxic potential of BKI 1294.

Conclusions: Altogether BKI 1294 appears to be an attractive drug candidate for the control of cryptosporidiosis in calves.

L: Late breaking abstracts

L057

Diagnosis of malaria infection by fluorescence in situ hybridization (FISH) with group of rRNA-targeted oligonucleotide probes

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Plasmodium species are not only the cause of the most important parasitic disease of humans, but are much more widespread and prevalent in animals than commonly realized. Malaria in animals is not necessarily a tropical disease; for example, around 30 % of bird species in Britain are infected with malaria. More than one hundred species of *Plasmodium* have been described from non-human primates, birds, rodents, reptiles (lizards, snakes), and bats. Most of these species have only been defined morphologically. Unlike in humans, most animals show simultaneous infections with more than one species of *Plasmodium* and most species descriptions originated from such multiple infections. This prevents the molecular characterization of most *Plasmodium* species, because sequence data cannot be assigned to a particular morphospecies. Surprisingly, even for the five human species of *Plasmodium*, no species-specific FISH probes have been developed. Here I report the successful development of species- and genus-specific FISH probes based on the 18S small and 28S large ribosomal units for *P. falciparum* as a proof of concept for the interrogation of animal morphospecies of *Plasmodium*. This will open up future molecular screening for animal malaria, not only in blood samples but likely also in fecal samples, and might lead to a better understanding of the epidemiology and ecology of malaria parasites in many animal species in temperate and in tropical regions. It will also contribute to a better phylogenetic understanding of this important parasite group.

L: Late breaking abstracts

L058

Molecular Characterization of *Cryptosporidium* spp. in Rabbits in Brazil

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The number of farms aiming the raising of rabbits as pets or for meat production is increasing in Brazil. *Cryptosporidium cuniculus* infection are related to clinical disease in rabbits or to zoonotic infection in humans. The objective of this study was to determine the occurrence of *Cryptosporidium* spp. in fecal samples from rabbits farms in Brazil according to the age group in pet rabbits and meat rabbits. 514 fecal samples were collected from 22 farms and stored at 5% potassium dichromate solution. Fecal samples were purified by centrifugal flotation in Sheather's solution and *Cryptosporidium* oocysts were screened using the negative malachite green technique. Molecular characterization of *Cryptosporidium* spp. was accomplished in microscopy positive samples using nested PCR for amplification of a fragment of the 18S rRNA gene and GP60 gene, following the sequencing of the amplified fragments. Eight (1.56%) and seven (1.36%) samples were positive for *Cryptosporidium* spp., respectively by microscopy and nested PCR. Molecular analysis revealed *C. cuniculus* subtype VbA21. This is the first report of *C. cuniculus* in rabbits in Brazil.

L: Late breaking abstracts

L059

Molecular Characterization of *Eimeria* spp. in Rabbits in Brazil

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The rabbit's production is increasing in Brazil as pets or for meat production. *Eimeria* infection is common in rabbit's farms in Brazil and has been related to mortality outbreaks in rabbits of different ages. The objective of this study was to determine the occurrence of *Eimeria* spp. in fecal samples from rabbits, according to the age including pet rabbits and meat rabbits. A total of 514 fecal samples from 22 rabbit farms were collected and stored at 5% potassium dichromate solution. Oocysts were purified by centrifugal flotation in Sheather's solution and *Eimeria* oocysts were screened using microscopy. In samples positive by microscopy, the detection of the eleven *Eimeria* species of rabbits was accomplished by PCR using species-specific primers for amplification of a fragment of the ITS1 ribosomal DNA. One hundred samples were positive by microscopy. Species-specific PCR allowed for the identification of *E. exigua* (6.74%), *E. flavescens* (22.47%), *E. intestinalis* (8.99%), *E. irresidua* (5.62), *E. magna* (21.35%), *E. media* (2.25%), *E. perforans* (11.24%), *E. piriformis* (7.86%), *E. stiedai* (6.74%) and *E. vej dovskyi* (6.74%). This is the first report of molecular classification of rabbit's *Eimeria* species in Brazil.

L: Late breaking abstracts

L060

Seroprevalence, risk factors and genetic characterization of *Toxoplasma gondii* in free-range white yaks (*Bos grunniens*) in China

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The objective of this study was to determine the seroprevalence, risk factors and genotypes of *Toxoplasma gondii* in white yaks (*Bos grunniens*) in China. A total of 974 yak serum samples were collected from Tianzhu Tibetan Autonomous County (TTAC), Gansu province, northwest China for detecting *T. gondii* antibodies by the modified agglutination test (MAT), and 414 tissues belonging to 138 white yaks were collected for detecting *T. gondii* DNA by amplification of B1 gene with a semi-nested PCR. 155 serum samples (15.91%, 95% CI=13.62-18.21) were seropositive for *T. gondii* antibodies at a 1:100 cut-off, and 10 DNA samples (7.25%, 95% CI=2.92-11.57) were positive for the *T. gondii* B1 gene, which were genetically characterized using multilocus PCR-RFLP. Only two showed complete genotyping results (ToxoDB#9). Statistically significant differences were not observed between *T. gondii* seroprevalence and gender, season or pregnancy in the logistic regression analysis ($P > 0.05$). Ages of white yaks was considered as a main risk factor associated with *T. gondii* infection. Our results indicated a widespread exposure to *T. gondii* among white yaks, and revealed the genotype ToxoDB#9 of *T. gondii* in white yaks for the first time in China.

L: Late breaking abstracts

L061

Intensity of infestation of chewing lice (Phthiraptera) and fleas (Siphonaptera) on poultry (*Gallus gallus domesticus*) in a region of Southern Mexico

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Chewing lice (Phthiraptera, Ischnocera) are economically-important poultry ectoparasites that live mainly on the skin. Amblyceran lice may cause irritation of the skin, restlessness, overall weakening, cessation of feeding, loss of weight, reduced laying capacity and skin lesions that may become sites of secondary infection. The fleas (Siphonaptera) are obligate parasites that live, feed and shelter beneath the surface of their host's epidermis, hair or feathers. The aim of this study was to determine the frequency of ectoparasites in poultry Macuilxóchitl, Oaxaca, Mexico.

A total of 45 adult hens, reared in family farms, with makeshift facilities and floor were analyzed. Ectoparasites were collected after slaughter, using a swab with alcohol on the feathers, body, head and feet. The number of ectoparasites was estimated, as not all observed parasites were collected. Specimens were transferred to the laboratory of the Department of Parasitology of the Faculty of Veterinary Medicine of the National Autonomous University of México.

All hens tested positive for ectoparasites. In chickens, four species of chewing lice were identified: *Menopon gallinae* (86%), *Menacanthus stramineus* (88%), *Chelopistes meleagridis* (64%), *Lipeurus caponis* (33 %) and three species of fleas, *Echidnophaga gallinacea* (75 %), *Ctenocephalides felis* (66 %), *Ctenocephalides canis* (60 %). This is the first study on the frequency and intensity of infestation of lice in chickens of backyard farms.

L: Late breaking abstracts

L062

First description of subtypes iiaa18g3r1 and llaa15g2r1 of *cryptosporidium parvum* in female foals in Brazil

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The aim of this study was to detect the infection by *Cryptosporidium* spp. in three female foals in Brazil. Fecal samples were collected from Quarter Horse with 10, 35 and 75 days of age, with normal stool consistency and asymptomatics. We evaluated the samples by nested polymerase chain reaction targeting the 18S rRNA gene and the GP60 gene and subsequent sequencing of the amplified fragments. The sequencing of the amplified fragments revealed the presence of *C. parvum* with 100% genetic similarity with two subtypes different: in the sample the filly 10 days, we found 1 llaA18G3R1 (sample 1), llaA15G2R1 (sample 2); these two subtypes are of common occurrence in calves. Based on these results, we conclude that equine can be hosts of *C. parvum* subtype llaA18G3R1 and llaA15G2R1, first described in Brazil.

First molecular characterization of *cryptosporidium* spp. infecting buffalo calves in Brazil

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With the aim of determining the occurrence of *Cryptosporidium* spp., 222 fecal samples were collected from Murrah buffaloes calves aged up to six months and genotyped with a nested polymerase chain reaction targeting the 18S rRNA gene and sequencing of the amplified fragment. Nested 18S PCR was positive for 48.2% of the samples. Sequence analysis showed that the most frequent species in these animals was *Cryptosporidium ryanae*, which was present in buffalo calves as young as five days of age. The zoonotic species *Cryptosporidium parvum* was detected in one animal. An uncommon *Cryptosporidium* 18S genotype was found in buffaloes.

L: Late breaking abstracts

L064

Risk factors associated with pesticides resistance in horn flies populations

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The parasitic pests are considered one of the main limiting factors in animal production worldwide and, although rarely associated with high mortality, determine significant economic impacts due to reduce the productivity and increases the costs. Ultimately, the application of higher doses and more frequents treatments in addition to raising the production costs, increase the level of contaminations so much in the environment as in the livestock products. Adding to the complexity and difficulty of reversal, the development of resistance involves not only the pesticide base that the populations were exposed, but all chemical group to which it belong. In order to identify and study the probabilistic scenarios that determine the setting of resistance to pesticides in Brazilian horn fly populations were interviewed 33 cattle breeders in an important cattle production region. Samples of horn flies were collected In the herds visited for carrying out of the bioassay to verify the presence of the pyrethroid resistance in these populations. Aspects concerning the use of pesticides, frequency of the treatments for the control of ectoparasites in the herds, flock fitness and perception of the farmer in relation to the highest incidence of flies and ticks were addressed in the questionnaire. With the answers and the resistance factors of the horn fly populations evaluated was built a database using the Epi-Info version 3.5.2 software, the same software was used in the database analysis, which showed that the largest parasitic problem for the herds analyzed is the infestation of cattle tick, which is the determinant factor for the ectoparasites control treatments. It is observed that about 50% of the herds are made up to six annual treatments targeted to the control of the cattle tick infestations, but in some herds observed the realization of up to 26 annual treatments. It is observed by the risk analysis that the development of pesticide resistance in the horn fly populations is unleashed by the misuse of pesticides, mainly for the control of ticks population, which determine a selection pressure of resistant individuals in the horn fly populations.

L: Late breaking abstracts

L065

About a case of invasion of *Echidnophaga gallinacea* Insecta Siphonaptera on *Canis familiaris* in Mexico

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The presence of fleas *Echidnophaga gallinacea* on Dalmatian dog communicates Mexico City. Fleas are insects belonging to the order Siphonaptera are a wide range of genres families and species, these insects have a great importance in both animal health and public health is why if in some species animals mentioned as *Ctenocephalides canis* or *C. felis*, does not mean that these species are found only on those animals, in the course of an investigation of ectoparasites isolated *Canis familiaris*, breed Dalmatian male, was isolated to 17 fleas, fleas were separated from the body animal, underwent clearance and mounted between slide and cover slip for identification, after observing them under the microscope, it was concluded that it was *Echidnophaga gallinacea* of which there are some mentions of dog but this is the first time their presence is reported in dogs Mexico

L: Late breaking abstracts

L066

Evaluation and impact assessment of *post mortem* detection techniques for bovine cysticercosis in Belgium

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Bovine cysticercosis (bcc), caused by the tapeworm *Taenia saginata*, is responsible for important economic losses. Control is based on routine meat inspection (MI), which has a low sensitivity. In Belgium, the observed prevalence of bcc is 0.28%; yearly taeniasis incidence is $\approx$ 11000 cases. We compared routine MI, MI with additional cuts in the heart, and detection of circulating antigen (Ag-ELISA). Serum samples and predilection sites (PS) of 101 MI positive carcasses (G1) and of 614 randomly selected MI negative carcasses (G2) were collected. Complete dissection of PS was the reference test. In G2 only four new cases (0.6%) of bcc were found by making additional cuts in the heart. However, by dissecting PS of the G2 animals, 144 (23.5%) carcasses were found to contain cysticerci. Ag-ELISA identified another 40 positives in MI and PS negative carcasses (8.5%). These results were used to model the current situation in Belgium. Of 500,000 cattle slaughtered yearly, 191,946 (38.4%) contain cysticerci, and only 1429 infected carcasses are detected at MI. An average of 125,223 viable cysticerci can pass the MI undetected yearly. The sensitivity of MI is estimated at 0.76%. Models calculating the infection risks for the consumer according to the used detection test and their impact in short and long term were developed. Implementation of Ag-ELISA may lead to a 43.6% decrease in the number of viable cysticerci passing the slaughterhouse undetected. On a longer term, implementing Ag-ELISA would lead to an overall decrease of infections in bovines and humans.

L: Late breaking abstracts

L067

Specific alleles of the Major Histocompatibility Complex class II and age influence prevalence or abundance of intestinal parasites in Alpine marmots (*Marmota marmota*)

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The Major Histocompatibility Complex (MHC) transcript genes, coding for antigen presenting molecules, represent an important functional complex of genes of vertebrates due to its key role in host-parasite interactions. Individuals with a high MHC diversity and/or carrying specific MHC alleles are expected to be more resistant to parasite infection. Hosts characteristics (age with acquired immunity and gender) are obviously associated with MHC to explain the variations in parasite burden usually observed in wild animals. Here, we investigated the influence of MHC characteristics, age and gender on the abundance or prevalence of three intestinal parasites (*Eimeria monacis*, *Eimeria sp.* and *Ctenotaenia marmotae*) in a wild population of Alpine marmots, an hibernating mammal. Our results revealed that (1) MHC diversity did not affect the abundance or prevalence of the three intestinal parasites, (2) the presence of specific alleles of MHC-DRBI genes played not the same role on oocyst excretion for the 2 eimerian species, (3) oocyst excretion decreased more drastically with age for *Eimeria sp.* than for *E. monacis* and (4) prevalence of the cestode *C. marmotae* increased as the capture period progressed but was not affected by age or MHC. These results suggest that MHC diversity may play a weak role on the associations between the Alpine marmots and their intestinal parasites and that their defensive strategies may use not the same way for the 2 coccidian species. We emphasize the need for empirical studies of host-parasite interactions in the wild to understand how hosts may resist and/or tolerate its parasites.

L: Late breaking abstracts

L068

Genome-wide expression patterns of calcium-dependent protein kinases in *Toxoplasma gondii*

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Background: Calcium-dependent protein kinases (CDPKs) were found in plants and some Apicomplexan parasites but not in animals or fungi. CDPKs have been shown to play important roles in various calcium-signaling pathways such as host cell invasion, egress and protein secretion in *Toxoplasma gondii*. The objectives of the present study were to examine the *T. gondii* CDPK genes expression patterns during different development stages and stress responses.

Methods: We carried out a comprehensive expression analysis of CDPK genes based on previously published microarray datasets, and we also used real time quantitative RT-PCR to study ten *T. gondii* CDPK genes expression patterns under acid, alkali, high temperature and low temperature conditions.

Results: Microarrays analysis indicated that some *Tg*CDPK genes exhibited different expression levels in IFN- γ stimuli conditions or at different developmental stages, suggesting that CDPK genes may play different roles in these processes. Expression profiles under low temperature, high temperature, acid and alkaline indicated that most CDPK may be involved in regulating high temperature, acid and alkaline signaling pathways.

Conclusions: We present a genome-wide expression analysis of CDPK genes in *T. gondii* for the first time, and the mRNA levels change with abiotic and biotic stresses, suggesting functional roles in these processes. These results will provide a solid basis for future functional studies of the CDPK gene family in *T. gondii*.

