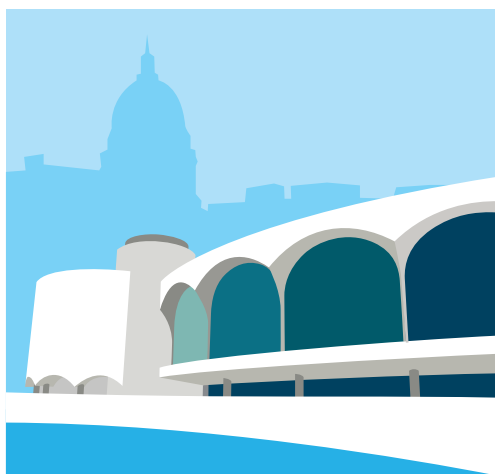




WAAVP

27th Conference of the World Association for
the Advancement of Veterinary Parasitology

JULY 7 – 11, 2019 | MADISON, WI, USA

Dedicated to the legacy of Professor Arlie C. Todd

Sifting and Winnowing the Evidence in Veterinary Parasitology



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Abstract Book

*Joint meeting with the 64th American Association of Veterinary Parasitologists
Annual Meeting & the 63rd Annual Livestock Insect Workers Conference*

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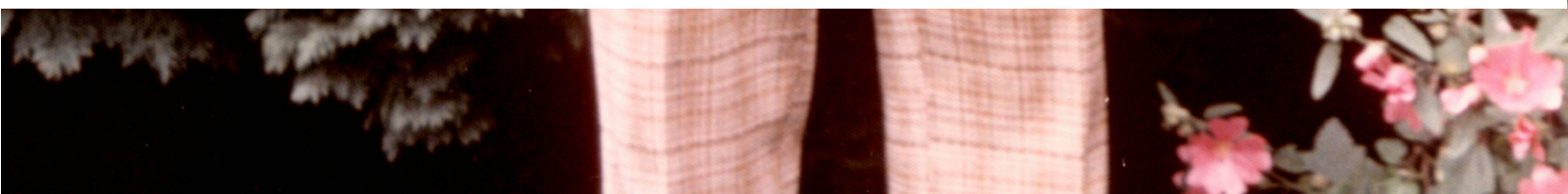
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KEYNOTE PRESENTATION

With an overall theme of Sifting and Winnowing the Evidence in Veterinary Parasitology, the Conference reminds us that we cannot for a moment believe that knowledge has reached its final goal. We honor the tremendous contributions to veterinary parasitology by University of Wisconsin Emeritus Professor [Arlie C. Todd](#), mentor of 59 graduate students, 44 of which earned a Ph.D.



Keynote Presentation

Keynote Address and Dedication Demystifying One Health: Sifting and Winnowing the Role of Veterinary Parasitology

July 7, 2019, 13:30 - 15:30

Plenary Hall, Madison Ballroom (ABCD), Level 4

Demystifying and Demonstrating the Value of a One Health Approach to Parasitological Challenges

**Professor Rosina C. Krecek¹, Professor
Patricia A. Conrad², Professor Peter M.
Rabinowitz³**

¹*Global One Health, College of Veterinary
Medicine and Biomedical Sciences, Texas A&M
University and University of Johannesburg,
South Africa, College Station, United States,*

²*Department of Pathology, Microbiology &
Immunology, School of Veterinary Medicine,
University of California, Davis, United*

*States, ³Departments of Environmental and
Occupational Health Sciences, Global Health,
Family Medicine, University of Washington
Center for One Health Research, Seattle,
United States*

A panel of three One Health advocates will illustrate with zoonotic parasite cases, key principles, skills and outcomes what they have learned from applying a One Health approach to medical and veterinary clinical practice.

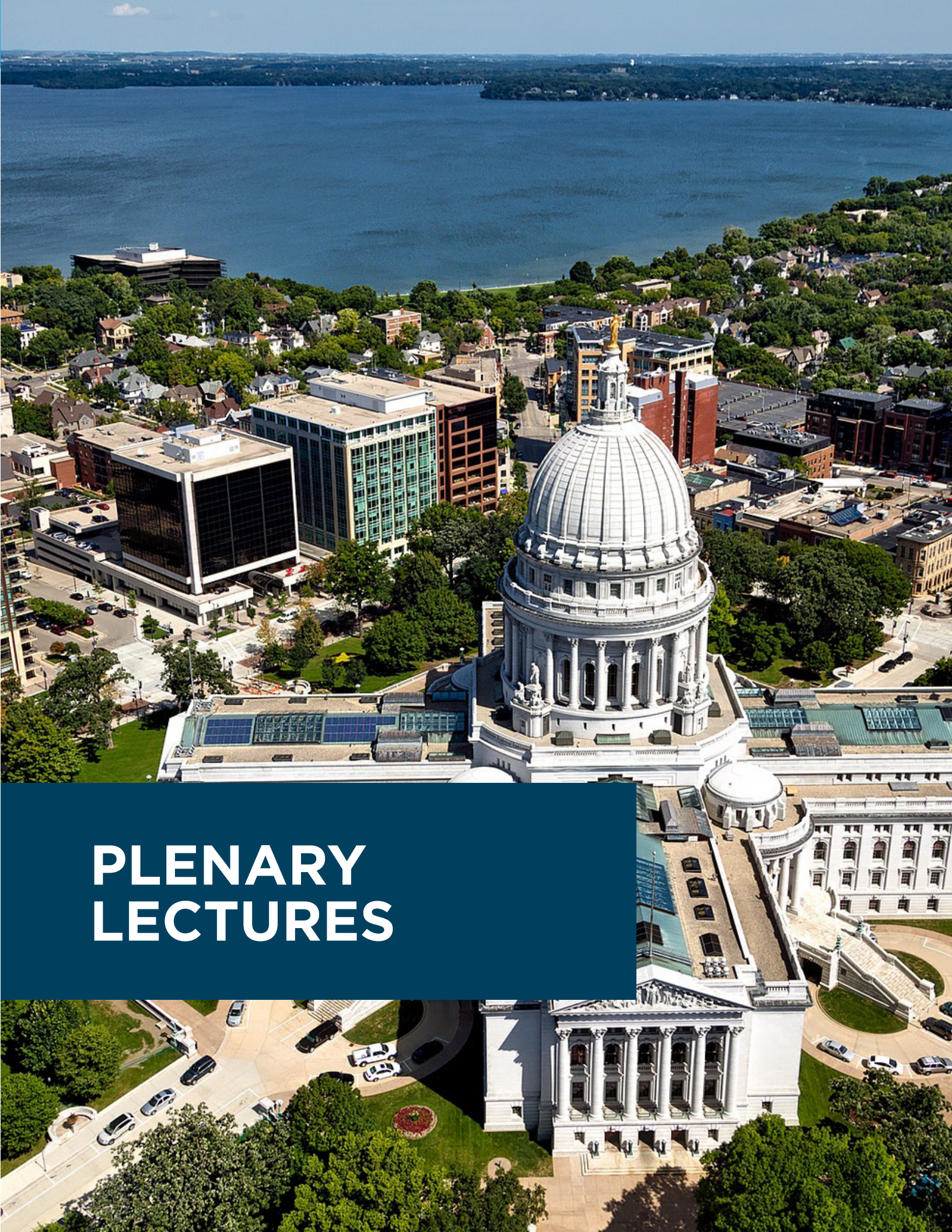
a. Rosina C. Krecek: Title: "Pork Tapeworm: A One Health Model". This veterinary scientist educator and researcher will illustrate how *Taenia solium*, a neglected tropical helminth parasite, infects pigs and 50 million humans across developing countries, and is preventable with tools available today. This disease limits pig production, causes human epilepsy and threatens food security necessitating a new One Health approach that incorporates vaccination, antiparasitic therapies and educational materials.

b. Peter M. Rabinowitz: Title: "A Clinical Perspective on One Health Challenges for

Parasitology". This medical doctor and scientist will explore how parasites move between animals and people and current status of clinical information sharing between human and animal clinicians. He will compare clinical diagnostics for parasitic diseases in humans, animals, and barriers to information sharing using *Giardia* as example. This discussion will describe the microbiome as a crossing point between humans, animals and environments.

c. Patricia A. Conrad: "Babesia and Toxoplasma Discoveries at the Human-Animal-Environment Interface". As a veterinary protozoologist and scientist she will describe how a One Health approach transformed her research and resulted in surprising discoveries of new parasite species and the impact of environmental factors on protozoal pathogen transmission. Lessons learned in challenging the dogma in protozoology, working with productive teams, and making parasitological research fun will be shared.

This session will illustrate an interdisciplinary One Health approach in veterinary parasitology to encourage research, education, and a scientific basis for local and global health policy decisions. Further, the need to improve quality of evidence-based metrics, development of useful tools for tracking One Health outcomes and better ways to assess impact will be explained.



PLENARY LECTURES

Plenary Lectures

Plenary Lecture 1.0 Evolving Approaches to Drug Discovery

July 7, 2019, 16:00 – 18:00

Plenary Hall, Madison Ballroom (ABCD), Level 4

PL01.01 Data-Driven Revolutions in Antiparasitic Drug Discovery

Prof. Timothy Geary¹

¹*Institute of Parasitology, McGill University, Ste-Anne-de-Bellevue, Canada, 2Queen's University of Belfast, Belfast, Northern Ireland, UK, 3CryBioMed, Philadelphia, United States*

Parasite infections remain major and global impediments to human and animal health. Control measures involving environmental interventions have led to significant improvements in the prevention of some of these infections, but are necessarily supplemented with chemotherapeutic options. For many parasites, available drugs are less than optimal and drug resistance poses real and potential threats. New developments in bioinformatics and functional genomics – addressed to various extents in 'big data' science – are poised to revolutionize our understanding of parasite biology and our ability to manipulate the expression of specific parasite genes in ways that facilitate the discovery of new antiparasitic drugs. These drugs are likely to improve the treatment of parasitic infections of both human and veterinary importance and will include agents that influence the interface to favor the host. Improvements in diagnosis will enable us to move from very broad-spectrum agents to more targeted treatments in the future, potentially changing the rate of resistance selection. Research that enables better insights into fundamental aspects of parasitism can be anticipated to pay off in the discovery and development of better ways to control these pathogens, and we must take full advantage of new science and new technologies to make this a reality.

PL01.02 Target-Based Drug Development for Cryptosporidiosis and Other Apicomplexa Diseases

W.C. Van Voorhis¹, M.A. Hulverson¹, R. Choi¹, W. Huang³, S. L. M. Arnold¹, D. A. Schaefer², D. P. Betzer², R. S. R. Vidadala⁴, G. R. Whitman¹, L. K. Barrett¹, D. J. Maly^{4,3}, M. W. Riggs², E. Fan³, and K. K. Ojo¹.

¹*Department of Medicine, Division of Allergy and Infectious Disease, Center for Emerging and Reemerging Infectious Disease (CERID), University of Washington, United States*

²*School of Animal and Comparative Biomedical Sciences, College of Agriculture and Life Sciences, University of Arizona, United States*

³*Department of Biochemistry, University of Washington, United States*

⁴*Department of Chemistry, University of Washington, United States*

Bumped kinase inhibitors (BKIs) have shown promise in animal models of cryptosporidiosis, toxoplasmosis, neosporiasis, and sarcocystosis. They were designed to target calcium-dependent protein kinase 1 (CDPK1), an enzyme for these organisms that has descended from the plant kingdom and appears to control cell entry and replication of these obligate intracellular parasites. Using the knowledge from the co-structures of CDPK1 and BKIs, improvements have been made to the safety and efficacy of BKIs, and they are advancing toward human and animal use for treatment of cryptosporidiosis. As the understanding of BKI pharmacodynamics for cryptosporidiosis therapy has increased, it has become clear that better compounds for efficacy do not necessarily require substantial systemic exposure. We now have BKIs with reduced systemic exposure, acceptable safety parameters, and efficacy in both the mouse, newborn calf, and piglet models of cryptosporidiosis. These BKIs are promising pre-clinical lead for cryptosporidiosis therapy in animals and humans. Since toxoplasmosis, neosporosis, and sarcocystosis require more systemic distribution, we have alternative BKIs that display superior systemic distribution and

safety properties, and have efficacy in mouse models of these diseases. These are coming under later stage investigations in farm animal models.

Supported by US NIH/NIAID R01AI089441, R01AI111341 and R01HD080670 and USDA 2014-06183.

PL01.03 Excretory/Secretory Products of Nematode Parasites: Significance and Therapeutic Implications

Dr. Lucienne Tritten¹

¹University of Zurich, Zurich, Switzerland

Parasitic nematodes are highly successful pathogens, inflicting diseases on humans, animals and plants. Despite great differences in their life cycles, preferential hosts and transmission modes, these parasites share a common capacity to manipulate their host's immune system. This is known to be achieved through the release of excretory/secretory proteins, the most well-characterized component of nematode secretomes, which are comprised of functionally diverse molecules. Recently, among others, non-coding RNA has been proposed as potential new player in host manipulation.

This lecture will summarize the current status of research on excretory/secretory products of nematode parasites and their expected biological significance, with a focus on proteins and microRNA. Furthermore, how molecular mediators of host-parasite interactions may be translated into new targets for therapeutic interventions will be discussed.

PL01.04 Next Generation Parasiticides: A Mission We Can Accomplish Together

Dr. Daniel Kulke¹

¹Bayer Animal Health, Germany

Following more than 100 years of antiparasitic drug discovery, Bayer currently celebrates its centenary of animal health dedicated antiparasitic products with the launch of Neguvon for the treatment of scabies in 1919. Since then a series of chemical drug

classes repetitively revolutionized our understanding of state-of-the-art antiparasitic chemoprophylaxis/chemotherapy and thereby also re-defined the expectations towards future generations of effective, safe and convenient antiparasitic products.

In simplified terms, antiparasitic drug discovery was historically performed by testing compounds, sometimes compound libraries, against a pathogen of interest in vitro, followed by an in vivo validation in infected animals. Once proof of concept against a particular pathogen was demonstrated, both parasite and host species were expanded to evaluate the spectrum of efficacy and safety. This approach resulted e.g. in the discovery of the endecto-parasiticide ivermectin, one of the top-selling veterinary medicines in the world.

However, the evaluation of approaches to tackle parasites became and still becomes more diverse and creative. Novel approaches include high-throughput phenotypic or target-based screenings of large compound libraries, identification of cellular and molecular pathways and potential targets via -omics approaches, re-evaluation of traditional medicines, repurposing of old drugs alone or in combination, vaccination strategies, but also consider breeding, microRNA or gene therapy as potential antiparasiticide innovations of the future.

However, once an efficacious drug candidate has been identified, complexity is added: the future drug needs to be safe for target animals and humans, needs to be suitable for formulation, and should have favorable pharmacokinetic properties and reasonable production costs, to mention just a few aspects.

The purpose of this presentation is to share not only the hurdles and challenges but also the enormous potential and great opportunities towards broadening the antiparasitic treatment options of the future for both veterinary and human medicine by all the great achievements contributed by academia and pharmaceutical industry as well as numerous governmental and non-governmental organizations and initiatives as part of our worldwide community for the advancement of veterinary parasitology.

Plenary Lecture 2.0 Genes and Genomics in Parasite Control

July 8, 2019, 8:30 - 10:30

Plenary Hall, Madison Ballroom (ABCD), Level 4

PL02.01 Enlisting Spatiotemporal Transcriptomics to Identify Nematode Receptor Drug Targets**Dr. Mostafa Zamanian¹**¹*University of Wisconsin-Madison, Madison, United States*

Filarial nematode parasites continue to pose a major challenge to animal and human health, and new treatment options are needed for both heartworm and human filariasis. Transcriptomic tools are essential for the discovery of potential therapeutic targets that facilitate the development and survival of parasitic nematodes within their vertebrate hosts. Transcriptome studies in filarial nematode parasites have thus proved useful in detecting bulk changes in gene expression throughout the parasite life cycle and as a function of chemical or environmental perturbation. However, localization of gene transcripts to specific cells or tissues has yet to be realized at an appreciable scale. Established *in situ* methods of gene transcript localization are low in throughput and complicated by the lack of fully defined cell and neuronal architecture in filarial parasites. Further, single-cell methods to associate gene expression with individual cells can often be prohibitive in terms of costs and tissue requirements. In light of these limitations, we show how spatial RNA sequencing and single-cell approaches can be optimized and enlisted to generate high-resolution transcriptional maps in filarial nematode parasites. We discuss how these methods can be used to prioritize putative receptor drug targets associated with parasite sensory behaviors and secretory function. Together, these data help provide the first steps towards a spatially resolved cell and transcriptome map in a filarial nematode parasite and present a roadmap that may be extensible to other clinically relevant parasitic nematodes.

PL02.02 Extracellular vesicles: A Conserved Nematode Strategy for Host Manipulation?

Dr. Wang Yuan¹, Ms Hannah Loghry¹, Dr. Mostafa Zamanian², Dr. Yoko Ambrosini¹, Dr. Karin Allenspach¹, Dr. Jonathan Mochel¹, Dr. **Michael Kimber**¹

¹*Iowa State University, Ames, United States,*²*University of Wisconsin-Madison, Madison, United States*

Parasitic nematodes have evolved diverse evasion mechanisms to manipulate host biology and create conditions that promote parasitism. Although the importance of parasite excretory/secretory products in host modulation is well-recognized, we currently have a less developed understanding of the specific molecules parasitic nematodes use to manipulate the host, how those molecules are trafficked to host cells, and how they manipulate host cells at the genetic and cellular level. We, and others, have identified that parasitic nematodes actively secrete modulatory proteins and small, regulatory RNAs (miRNAs), and deliver them to host tissues via extracellular vesicles (EVs). These EVs are a mechanism by which discrete populations of parasite-derived effector molecules can be protected from the host environment and trafficked to host cells where they exert their effects. Here we describe how filarial nematodes (*Brugia*) prodigiously release EVs throughout their life cycle that contain a stage- and sex-specific cargo enriched in proteins with known and putative modulatory functions. RNA-seq analysis reveals an EV miRNA complement that includes parasite miRNAs identical to host immunomodulatory miRNAs. The EVs are internalized by vector and definitive host cells and we will discuss potential host modulation at the transcriptional level. Gastrointestinal nematodes (*Ascaris*) also release EVs that, in comparison to filarial nematodes, contain a more complex cargo of proteins that also includes putative host modulators. The miRNA cargo of *Ascaris* EVs shares similarities to that of *Brugia*, suggesting conserved mechanisms of host manipulation. We will describe how *Ascaris* EVs are internalized by host mucosal epithelium to drive the earliest determinants of the mutually beneficial Type 2 response using a canine enteroid model. We conclude that investigating

the structure and function of nematode EVs will generate new mechanistic understanding of how parasites modulate the host at the genetic and cellular level.

PL02.03 A Multimodal Approach to Understanding Macrocyclic Lactone Resistance in *Dirofilaria Immitis*

Dr. Cassan Pulaski¹

¹UGA, United States

PL02.04 Deep Amplicon Sequencing as a Powerful New Tool to Screen for Sequence Polymorphisms Associated With Anthelmintic Resistance in Parasitic Nematode Populations

Dr. Russell Avramenko¹, Dr. Elizabeth Redman¹, Lynsey Melville², Dr. Janneke Wit¹, Camila Queiroz¹, Dr. Dave Bartley², Dr. John Gilleard¹

¹University of Calgary, Calgary, Canada,

²Moredun Research Institute, Penicuik, United Kingdom

Parasitic gastrointestinal nematodes contribute to significant human morbidity and cause billions of dollars per year in lost agricultural production. Control is largely dependent on the use of anthelmintic drugs, which in the case of livestock parasites, are severely compromised by the widespread development of drug resistance. Consequently, there is an urgent need for scalable and accurate diagnostic tools to detect the emergence of anthelmintic resistance particularly in its early stages. These diagnostics are often hindered, by the need to discriminate between multiple co-infecting parasite species, each with varying levels of anthelmintic resistance. Detecting and measuring the frequency of resistance-associated mutations in mixed parasite populations has the potential to provide sensitive and quantitative assessment of resistance emergence from an early stage. We describe the development and validation of deep amplicon next-generation sequencing as a powerful new approach to detect and quantify the frequency of single nucleotide polymorphisms (SNPs) associated with benzimidazole resistance. We have used parasite communities in sheep, which provide an excellent system in which to undertake the proof-of-concept of this approach, as there are multiple co-infecting trichostrongylid nematode species, each with varying levels of benzimidazole resistance. We demonstrate that this approach provides an accurate measure of resistance allele frequencies, and can reliably detect resistance alleles down to a frequency of 0.1%, making it particularly valuable for screening mutations at the early stages of resistance. We cover several examples to illustrate the use of this technique to screen for benzimidazole resistance, both

when resistance is already well established, but also as resistance is emerging in parasite populations. This approach provides a powerful new tool to screen for the emergence of anthelmintic resistance mutations in parasitic nematode populations of both animals and humans.

Plenary Lecture 3.0 Leishmaniasis, Leishvet and One Health

July 9, 2019, 8:30 - 10:30

Plenary Hall, Madison Ballroom (ABCD), Level 4

PL03.01 Dog Vaccination to Decrease Transmission of Parasitic Zoonoses: Notes from Leishmaniasis

Dr. Gaetano Oliva², Dr. Laia Solano-Gallego¹

¹Dep. Medicina I Cirurgia Animals, Universitat Autònoma de Barcelona, Spain, ²Department of Veterinary Medicine and Food Production, University of Naples Federico II, Italy

Dogs are the main reservoir for *Leishmania infantum* infection and are an important target for public health intervention to prevent transmission to people. Vaccination is an important preventative tool against this infection in humans and in dogs. A protective vaccine should provide robust memory T cells able to mount a recall response at the time of (re)exposure to parasites and reactivate polyfunctional T cell effector functions. The development of safe, effective, durable and low-cost prophylactic vaccines against leishmaniasis is still a major challenge. Despite the efforts, there is still no effective vaccine licenced for human use. However, four commercial vaccines have been licensed for the control of canine leishmaniasis (CanL) in Brazil and Europe. These vaccines provide at least 12 months of protection with the indication to reduce the risk of developing an active infection and clinical disease. The main controversy regarding CanL vaccines is that they do not block the establishment of infection. This controversy has been poorly explored due to difficulty of xenodiagnosis in vaccinated dogs to evaluate transmission. Easy biomarkers to determine dogs who are “super-spreaders” of infection

need to be investigated. Moreover, limited information is available regarding the impact of canine vaccination in reducing human leishmaniasis both in Europe and in Brazil. Another important use of vaccination is as immunotherapy to reduce the progression of disease in infected dogs. However, limited studies are available with current anti-*Leishmania* commercial vaccines. A further limitation of some commercial vaccines includes interference with serological diagnostic methods as well as side effects. Vaccination may be considered as a part of a comprehensive control program for CanL, in which repellents against sand flies are used concurrently with dog vaccination. Finally, leishmaniasis is one of the best examples of a disease whose successful control depend on the use of a One Health strategy.

PL03.02 Treatment of Canine Leishmaniasis and Drug Resistance – Implications on the Transmission of Infection and One Health

Prof. Guadalupe Miro², Prof. Gad Baneth¹

¹The Hebrew University of Jerusalem, Rehovot, Israel, ²Universidad Complutense de Madrid, Madrid, Spain

Leishmania infantum causes a zoonotic infection endemic in the Mediterranean Basin, the Middle East, central Asia, South and Central America. It is also a cause of disease in dogs in the USA, and countries where there is no known sand-fly transmission due to vertical transmission, travel and importation of infected dogs. Infected dogs may develop severe clinical disease or remain sub-clinically infected but infectious to sandflies. Treatment of canine leishmaniasis (CanL) is warranted when clinical disease is present, however, there are very few available effective drugs and most of them are used for treatment of human disease with the possibility of drug resistance development. This situation calls for coordination between veterinary and human health under the umbrella of One Health to decrease the risk of drug resistance transmission.

The main drugs employed for the treatment of CanL caused by *L. infantum* are the pentavalent antimony meglumine antimoniate,

miltefosine, and allopurinol. Additional immune potentiating compounds such as domperidone, dietary nucleotides and active hexose have been licensed for dogs in some countries as ancillary treatment to enhance immune responses against *L. infantum* infection. Anti-leishmanial treatment often achieves clinical cure in dogs with leishmaniosis but it is frequently not associated with a parasitological cure. Treated dogs may remain carriers of the disease, experience clinical relapses and can be infectious to sand flies.

Drug resistance has been widely described in human cutaneous and visceral leishmaniosis but reports on drug resistance in CanL are relatively scarce. Resistance to allopurinol may develop in dogs experiencing disease relapse which may transmit resistant parasites to other dogs and also enhance the danger of the parasite's transmission to humans. In conclusion, new treatment and disease control strategies should be coordinated between human and veterinary officials in a One Health manner to improve the prevention of disease transmission.

PL03.03 Treatment of Canine Leishmaniosis and Drug Resistance – Implications on the Transmission of Infection and One Health

Patrick Bourdeau², Christine Petersen^{2,3,4}

¹Laboratoire de Dermatologie, Parasitologie et Mycologie, ONIRIS, Ecole Nationale Vétérinaire, France, ²Department of Epidemiology, College of Public Health United States, ³Center for Emerging Infections Diseases, United States, ⁴Immunology Program, Department of Internal Medicine and Microbiology, Carver College of Medicine, University of Iowa, United States

Leishmania has biologically adapted to specific Phlebotomine sand flies through long co-evolution. The ability of *Leishmania* spp. to bind to sand fly midgut allows each *Leishmania* species to propagate and differentiate into infectious promastigotes and be transmitted. Sand fly feeding upon a mammalian host is the first step towards being infected and a host of *Leishmania*. Once deposited into the skin, host susceptibility to infection vs. ability to

mount a sterilizing immune response predicts which hosts could be reservoirs of different *Leishmania* spp. Materials in addition to parasites are expelled during sand fly feeding, including salivary antigens and other factors that promote local inflammatory responses and visceralization of infection increasing the likelihood that systemic infection is established. Any environmental factor that increases sand fly biting of a particular host increases that host's role in *Leishmania* transmission.

First descriptions of reservoir species were based on association with local human disease and ability to observe infected leukocytes on cytology. This approach was one pathogen for one reservoir host. Advances in sensitive molecular tools greatly increased the breadth of mammals found to host *Leishmania* infection. Visceralizing forms of *Leishmania*, particularly *L. infantum*, are now known to have multiple mammalian hosts. *L. donovani*, long been described as an anthroponotic parasite, was recently identified through molecular and serologic surveys to have additional mammalian hosts. The epidemiological role of these animals as a source of parasites to additional hosts via vector transmission is not known.

Current evidence may suggest that dogs and other domestic animals may either control infection or do not have sufficient skin parasitemia to be a source of *L. donovani* to *P. argentipes*. Further xenodiagnosis and characterization of skin parasitemia in these different hosts is required to more broadly understand which *Leishmania* hosts can be a source of parasites to sand flies and which ones are dead-end hosts.

Plenary Lecture 4.0 Veterinary Entomology: Outbreak and Advancements

July 10, 2019, 8:30 - 10:30

Plenary Hall, Madison Ballroom (ABCD), Level 4

PL04.01 Introduction to Veterinary Entomology: Outbreak and Advancements**Prof. Mason Reichard¹**¹*Oklahoma State University, United States***PL04.02 Discovery, Surveillance, and Control of *Haemaphysalis longicornis*, the Asian Longhorned tick, in New Jersey****Dr. Nicole Lewis¹**¹*NJ Department of Agriculture, Ewing, United States*

On November 7, 2017, *Haemaphysalis longicornis*, also known as the Asian Longhorned tick, was confirmed by the National Veterinary Services Laboratory (NVSL) on a sheep in New Jersey. This was the first finding of the tick infesting livestock in the country. Known as a serious pest of livestock in the Australasian and Western Pacific Regions, this species has never previously established a population in the United States, that we knew of. In a multi-agency effort, this finding led to the identification of *H. longicornis* in several counties in New Jersey as well as eight other states. The investigation found the tick on several species including dogs, cats, cows, goats, sheep, white-tailed deer, opossum, grey foxes, coyotes, groundhogs, raccoons, horses, and humans. Testing of both the tick and the index case animal, a sheep, were negative for various human and animal pathogens. Currently, state, federal and academic organizations are performing surveillance to better understand the spread of this tick and to help determine the best methods for control. The involved agencies developed educational and outreach material that is available to the public at the NJ Department of Agriculture website. The public is encouraged to submit ticks for identification and a county drop of location has been established for each of the 21 counties. Research is ongoing to better understand disease transmission, pesticide resistance, and spread of this pest throughout the country.

PL04.03 One Health Approach to Manage the Threat of Ticks and Tick-borne Diseases in North America**Dr. Adalberto Pérez de León¹**¹*USDA-ARS Knippling-Bushland U.S. Livestock Insects Research Laboratory & Veterinary Pest Genomics Center, Kerrville, United States*

Ticks are more important than mosquitoes as vectors of pathogens causing morbidity and mortality in domestic animals and wildlife. Around 80% of the cattle in tropical and subtropical regions of the world are affected by economically important ticks and tick-borne pathogens. Most tick-borne diseases of public health importance are zoonotic. Estimates indicate that Lyme disease and other diseases caused by tick-borne pathogens could burden over 30% of the global human population by 2050. Some tick species are invasive and transmit pathogens causing transboundary diseases of high consequence for domestic animals, humans, and wildlife. Intricacies of the livestock-wildlife-human interface represent another aspect of global change adding complexity to efforts trying to solve the problem with ticks and tick-borne diseases (TTBD). "One Health" is a term used to describe approaches to optimize health outcomes for humans, other animals, and the environment. Strategies based on the One Health concept facilitate research on the interplay between climate, habitat, and hosts driving tick population dynamics. This enhances our epidemiological understanding of tick-borne diseases. Applying the One Health concept provides a holistic framework to address the global importance of TTBD. Effective and safe TTBD management requires rational tactics involving multiple technologies to avoid the intense use of chemical treatments, which can harm the environment and public health. Examples of One Health research to manage the threat of TTBD emphasizing integrated tick management through the optimal use of compatible methods in a way that is safe, economically viable, and environmentally sustainable will be presented. Our experience indicates that grasping the expectations of end-users of technology is fundamental to realize a shared vision of improving the outcomes of tick control interventions. Scientific evidence can be used to generate the support to establish the capacities required for the effective management of ticks to mitigate the health burden of TTBD.

PL04.04 Ecology and Behavior of Triatomines and *Trypanosoma Cruzi* at the Human-Wildlife-Dog Interface in the Southern United States

Dr. Sarah Hamer¹

¹*Texas A&M University, College Station, United States*

Despite over 100 years of research on *Trypanosoma cruzi*, this parasite continues to cause significant morbidity and mortality in human and animal populations across the Americas. Parasite transmission cycles are vastly different across geographic regions, ranging from domestic infestations with high human contact to sylvatic cycles among wildlife with limited spillover to humans. Using a one health approach, we developed a multidisciplinary program to study Chagas disease ecology in the southern US, where there is increasing recognition for locally-acquired disease in both humans and dogs. We use citizen science to empower the public with disease prevention information while accepting citizen-collected triatomines. Analysis of over 5,000 triatomines submitted to this program from 27 states since 2013 revealed >55% *T. cruzi* infection prevalence with two genetic variants of the parasite. Peak vector encounters with humans occurred in mid-summer months, and vector bloodmeals were acquired from diverse wildlife, domestic animals, and humans. We developed triatomine colonies of locally-relevant species for use in behavioral and transmission studies. Using time lapse photography in our colony as well as in infested dog kennels, we found that triatomine activity, and therefore risk of host contact, is not limited to the nocturnal hours. Further, using an indirect xenodiagnostic approach, we found variation in the infectivity of chronically-infected dogs to triatomines, in which some dogs consistently infected all naïve bugs to which their blood was fed, whereas others infected fewer insects. In all cases, xenodiagnoses was more sensitive than PCR of blood to detect host infection. Given the limited antiparasitic treatment options and lack of a Chagas disease vaccine, disease risk reduction must focus on controlling the vectors in the environment. Citizen science is enabling new insights in vector infection, behavior, and host encounters that can ultimately be useful in developing interventions to protect human and animal health.

A nighttime photograph of a city skyline. The central focus is a large, white, classical-style building with a prominent green copper dome, illuminated from within. It is surrounded by various modern and older city buildings, some with lit windows. In the foreground, a body of water reflects the city lights and the capitol building. A dark teal rectangular box is overlaid on the bottom left of the image.

ORAL SESSIONS

Oral Sessions

OA01 Canine Heartworm I

July 8, 2019, 11:00 - 12:30

Plenary Hall, Madison Ballroom (ABCD), Level 4

OA01.01 Regional and Local Trends in the Prevalence of Canine Heartworm: United States 2012–2018

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Aims

Canine heartworm disease is a potentially fatal disease for which treatment is financially burdensome for many pet owners. Prevention is strongly advocated by the veterinary community along with routine testing for infection during annual wellness examinations. Despite the availability of efficacious chemoprophylaxis, recent reports have suggested that the incidence of heartworm disease in domestic dogs is increasing. Identification of areas of increasing prevalence is imperative for effective client education, intervention efforts, and focusing research.

Results

Using heartworm testing data for the United States from January 2012 through September 2018, a Bayesian spatio-temporal binomial regression model was used to estimate the regional and local temporal trends of heartworm prevalence. The region with the greatest increase in prevalence was found in the Lower Mississippi River Valley. Statistically significant increases in prevalence occurred throughout the southeastern states and extended northward into Illinois and Indiana. Local trends varied across the United States, with positive trends along most of the Atlantic coast, central United States, and western states, while clusters of negative trends were present along the Mississippi Alluvial Plain

(a historically endemic area), Oklahoma and Kansas, and Florida.

Conclusions

Canine heartworm prevalence is increasing in much of the United States both regionally and locally despite veterinarian recommendations on prevention and testing. Additional steps should be taken to protect dogs, cats, and ferrets. Further work is needed to identify the driving factors of the local negative trends present along the Mississippi Alluvial plain, Florida, and other areas.

OA01.02 A Moxidectin Extended-Release Injectable Suspension (PROHEART® 12) for the Prevention of Heartworm (DIROFILARIA IMMITIS) Disease in Dogs in the USA for 12 Months

Dr. Tom McTier¹, Ms. Martha Wachowski¹, Dr. Kristina Kryda¹, Mr. Sean Mahabir¹, Dr. Doug Rugg¹, Dr. Mark Mazaleski¹, Dr. Dwight Bowman²

¹Zoetis, Kalamazoo, United States, ²Cornell University, Ithaca, United States

ProHeart® 6 (PH 6) (0.17 mg/kg moxidectin) and ProHeart® SR-12 (PH 12) (0.5 mg/kg moxidectin) are extended-release microsphere injectable products providing continuous heartworm prevention for 6 and 12 months, respectively, in dogs. Over 12.4 million PH 12 doses have been sold globally outside the United States (USA) since 2008. The current studies were conducted to confirm the efficacy of PH 12 for preventing the development of *Dirofilaria immitis* in dogs in the USA. Two separate laboratory studies were conducted, each with 20 Beagles, ≥12 months of age, of mixed sex, weighing between 6.7 and 12.5 kg and determined to be free of existing heartworm infection prior to study initiation. Dogs were then randomly allocated to receive a single subcutaneous injection of either saline (control) or PH 12 on Day 0. PH 12 was reconstituted to deliver moxidectin at 0.5 mg/kg. Dogs were inoculated with 50 *D. immitis* third stage

larvae on Day 365. Two different heartworm isolates (GCFL and ZoeAL) collected from naturally infected dogs in the USA were used. All dogs were necropsied ~5 months post-inoculation, and examined for adult heartworms. In both studies, none of the PH 12-treated dogs had any adult heartworms, whereas all control dogs in both studies had heartworms, with a geometric mean of 30.2 (range, 22-37) for Study 1 and 32.6 (range, 22-44) for Study 2. Mean counts for PH 12 for both studies were significantly different from mean counts for comparable controls ($p < 0.0112$). One PH 12-treated dog and two saline-treated controls had mild injection site reactions within 24 hours post-treatment that resolved within one month. In conclusion, a single dose of PH 12 (0.5 mg/kg moxidectin) was 100% effective in preventing heartworm (*D. immitis*) disease in dogs for 12 months in two laboratory studies using recently collected heartworm isolates from the USA.

OA01.03 A Comparison of Dog Owner Satisfaction and Preference for Flea and Tick Medications in the US, UK and Australia

Dr. Robert Lavan¹, Dr. Rob Armstrong¹, Dr. Dorothy Normile¹, Hannah Newbury¹, Karen Lipworth¹
¹Merck Animal Health, Perkasie, United States

A dog owner survey was administered to over 1500 dog owners in the US, UK and Australia to assess their opinions on an extended duration flea and tick medication (Bravecto® [fluralaner], Merck Animal Health) and compare this experience to using monthly flea and tick products. Additionally, at least one veterinarian in each clinic provided their annual flea and tick recommendation.

While veterinarians, on average, recommended 12 months protection for fleas and 10-12 months for ticks, dog owners usually believed that they needed less protection for their dogs. All dog owners were current users of the extended duration fluralaner and most dog owners (75%) had used other monthly flea and tick products. Satisfaction was very high among respondents (over 90%) and most (over 90%) preferred the extended duration product. The chief benefit

of fluralaner was identified as the longer dosing period and the next most frequent benefit was convenience. The proportional owner responses were surprisingly consistent across the three countries. Several questions compared 12-week dosing with monthly dosing to assess whether the longer dosing was a burden or more difficult to accomplish. Most responders indicated that they did not have difficulty providing follow-up doses and that they were more likely to dose fluralaner on time compared to monthly products.

OA01.04 Macrocytic Lactone Resistance in *Dirofilaria immitis*: Extent of Resistance and Investigation of the Genomic Changes Underlying Resistance

Prof. Roger Prichard¹, Dr Cristina Ballesteros¹, Prof Timothy Geary¹, Ms Kathy Keller¹, Dr Catherine Bourguinat¹, Dr Cassan Pulaski²
¹McGill University, Sainte Anne-de-Bellevue, Canada, ²Louisiana State University, Baton Rouge, United States

Prophylaxis with macrocyclic lactone (ML) endectocides is the primary strategy for heartworm control. Recent evidence has confirmed that ML-resistant *Dirofilaria immitis* isolates have evolved. In this study, participating veterinary clinics helped recruit heartworm positive dogs. Blood was taken prior to treatment and then positive dogs were treated with a single standard dose of Advantage Multi® and another blood sample taken 2-4 weeks post-treatment. A modified Knott's Test was undertaken on each blood sample, followed by isolation of microfilariae, genomic DNA extraction and MiSeq sequencing of regions encompassing 10 SNP sites correlated with ML resistance. We observed significant correlation of SNP loci frequencies with the ML microfilaricidal response phenotype. A 2-SNP model was superior to other models tested. Subsequently, we have undertaken whole genome sequencing on a number of the isolates that are phenotypically ML-sensitive (S) or ML-resistant (R). *Fst* values (an index of identity for which an *Fst* = 0 means identity, and an *Fst* = 1 means complete difference) of the S and R isolates have been determined and single nucleotide polymorphisms (SNPs)

in which the S versus R $F_{st} \geq 0.5$ have been identified. Sequences with high F_{st} values were blasted against the *Brugia malayi* (a filarial nematode phylogenetically very close to *D. immitis*) and the *Caenorhabditis elegans* genomes to identify orthologous genes in *D. immitis* and to map them to the *B. malayi* genome. These comparisons revealed that a high proportion of the genetic differences between S and R isolates map to a region of one of the five chromosomes in *D. immitis*; genes in that region may contribute to ML resistance. Work is continuing to define more precisely the genetic change(s) responsible for ML resistance and to understand the underlying mechanism of resistance in heartworm.

OA01.05 Compliance With Veterinary Recommendations for Canine Flea and Tick Medications in Spain and the United States

Dr. Robert Lavan¹, Dr. Federica Burgio², Dr. Kaan Tunceli³, Dr. Dorothy Normile¹
¹Merck Animal Health, Perkasie, United States,
²MSD Animal Health, Zaragosa, Spain, ³Merck, Inc., Kenilworth, United States

Improvements in patient health outcomes are associated with development of longer duration medications for chronic diseases and chronic administration. In 2014, a longer duration isoxazoline product was launched to treat or prevent ectoparasite infestation on dogs. This product (Bravecto® [fluralaner]; Merck Animal Health) is an oral chew that is effective for up to 12 weeks. Most other available topical or oral flea/tick products are re-dosed monthly.

Studies were completed on large databases in Spain and the United States to look at dog owner adherence to veterinary recommendations around fleas and ticks. The studies demonstrated that, regardless of flea/tick medication brand, 50-60% of dog owners purchased only 1-2 doses per year. Also, owners who purchased the extended duration flea and tick product obtained significantly more months of coverage per year than owners who purchased monthly products. In both countries, owners who purchased a topical product purchased the least number of annual doses, on average, compared to oral products. Dog owners who

purchased monthly topical or oral products were significantly more likely to purchase 1-6 months of protection relative to the extended duration flea/tick product. Owners who purchased the longer acting product were significantly more likely to purchase 7-12 months of protection and thereby come closest to adhering with veterinary flea/tick control recommendations. Regardless of flea/tick product prescribed or dispensed, veterinarians have a lot of room to improve owner adherence for flea and tick protection.

OA01.06 Functional Expression of Glutamate-Gated Chloride Channels from the Parasitic Nematode *Brugia malayi*

Melanie Abongwa¹, Bukunmi Akanji¹, Sudhanva Kashyap¹, Aaron Hoisington¹, Shivani Choudhary¹, Saurabh Verma¹, Gunnar Mair¹, Richard Martin¹, **Alan Robertson**¹
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Filarial nematodes cause significant disease in humans and animals. Control and treatment are severely hampered by the lack of drugs available to kill adult worms. Current therapies are only effective against larval microfilariae, with limited activity against adult worms. Therefore, there is a need to develop safe and effective adulticides. The elucidation of the pharmacology of potential drug targets is a key element in reaching this goal. Glutamate-gated chloride channels (GluCl_s) are targets for the macrocyclic lactone class of anthelmintics which include the 'wonder drug', ivermectin. Here, we report on the cloning and functional characterization in *Xenopus* oocytes of two *B. malayi* GluCl_s; Bma-GLC-3 and Bma-AVR-14a. Functional, homomeric channels were formed when oocytes were injected with either glc-3 or avr-14a capped RNA (cRNA). Two-electrode voltage-clamp recordings revealed significant pharmacological differences between the channels formed by GLC-3 and AVR-14a. GLC-3 was more sensitive to L-glutamate compared to AVR-14a; EC₅₀ values were 64.8 μ M and 1.6 mM, respectively. Current-voltage relationships demonstrated that the channels were selectively permeable to chloride ions. No responses were detected following the application of 1 mM L-aspartate, glycine,

γ -aminobutyric acid (GABA), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), or N-methyl-D-aspartate (NMDA). AVR-14a was also more sensitive to the chloride channel antagonists picrotoxin and fipronil. Ivermectin exhibited an unusual and complex mode of action on GLC-3 involving direct activation of the channel ($EC_{50} = 4.1$ nM) and inhibition of the responses to L-glutamate ($IC_{50} = 95.1$ pM). Further investigation of these subunits and the in vivo subunit composition and functions of the different GluCl α s present in *B. malayi* will provide insights to ivermectin's limited activity on adult filaria.

OA02 Diagnosis and Decision Support for GI Nematodes in Ruminants I

July 8, 2019, 11:00 - 12:30

Breakout Room 2, Hall of Ideas E&H, Level 4

OA02.01 Further Progress on Developing New World Association for the Advancement of Veterinary Parasitology (WAAVP) Guidelines for the Fecal Egg Count Reduction Test

Ray Kaplan¹, Bruno Levecke², Paul Torgerson³, Robert Dobson⁴, Stig Thamsborg⁵, Matt Denwood⁵, Martin Nielsen⁶, Jozef Vercruyse²
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In recent years there have been many new insights into the fecal egg count reduction test (FECRT) regarding optimal experimental design and the analysis and interpretation of data. Additionally, there are important host-specific and parasite-specific differences that require protocol modifications to

address these distinctions. These issues highlight the necessity for developing new World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines that improve both the general methodology and standardization of the FECRT. Extensive data simulations and analyses identified many factors that can affect the outcome and interpretation of a FECRT. One important factor is the level of over-dispersion in the pre-treatment FEC on a given farm (k). While k is unknown before FEC are measured, k is known to differ among hosts and age groups, and this heavily impacts the amount of data necessary to have sufficient power to make a correct interpretation of the observed results. Proper consideration of this and other factors made it obvious that a simple protocol would be unlikely to yield consistently accurate results. However, there is a strong demand among the veterinary parasitology community for a simplified guideline that will facilitate the performance of FECRT at the individual farm level. To address these conflicting demands, we have developed a guideline that has several components. In the first part, we address the major issues relevant to experimental design and make a series of general recommendations. We then provide two separate guidelines for the performance of the FECRT, (1) a more rigorous version that is intended for use in scientifically-based studies (e.g. in studies intended for publication or registration of new drugs), and (2) a simpler version that has fewer experimental and analytical demands, and is intended for use by veterinarians and livestock owners.

OA02.02 Calf Behaviour as a Targeted Selective Treatment (TST) Indicator

Dr. Andrew Greer¹, Dr. Rachael Bryant¹, Mr. Robin McAnulty¹
¹*Lincoln University, Christchurch, New Zealand*

Targeted selective anthelmintic treatments (TSTs) rely on being able to accurately identify individuals within a herd or mob that are likely to benefit from receiving an anthelmintic treatment. Given that one of the ubiquitous signs of sub-clinical parasitism in grazing livestock is a reduction in voluntary feed

intake, the objective of this pilot study was to evaluate whether grazing and/or activity behaviour could be used as a TST indicator in grazing calves. Twenty-five 10-month-old Friesian x Jersey calves naturally infected with gastro-intestinal nematodes, and with faecal egg counts ranging between 0 and 250 eggs per g, were fitted with Sensor™ activity and behaviour ear tags which record grazing time, ruminating time and activity. On one of four treatment days each animal was administered anthelmintic and their grazing and activity pre and post treatment were compared. Grazing time initially decreased from a mean of 322 ± 23.9 minutes per day pre-treatment to 223 ± 14.6 minutes per day in the 24h immediately following treatment ($P=0.04$) and then increased to 422 ± 27.1 minutes per day post treatment ($P=0.003$, compared with pre-treatment). Rumination time also initially decreased from a mean of 288 ± 21.0 minutes per day pre-treatment to 95 ± 4.6 minutes per day in the 24h immediately following treatment ($P=0.001$) and then increased to 362 ± 18.8 minutes per day post treatment ($P=0.01$, compared with pre-treatment). There were no differences in the time spent being active ($P=0.71$), not active ($P=0.41$) or highly active ($P=0.14$). Overall, these results indicate that both grazing time and rumination time, but not activity time, respond to anthelmintic treatment and may provide a useful indicator for treatment as part of a TST regime.

OA02.03 Effects of Nematode Parasitism on Activity Patterns and Rumination Behaviour in First-Season Grazing Cattle

Niclas Högberg¹, Professor Lena Lidfors², Associate professor Anna Hessel², Professor Johan Höglund¹

¹Swedish University of Agricultural Sciences, Department of Biomedical Sciences and Veterinary Public Health; Parasitology Unit, Uppsala, Sweden, ²Swedish University of Agricultural Sciences, Department of Animal Environment and Health, Skara, Sweden

We investigated the effects of gastrointestinal nematode (GIN) challenge on activity and rumination patterns in first season grazing steers exposed to two different levels of *Ostertagia ostertagi* and *Cooperia oncophora*

during ~140 days. At turnout, experimental animals were allocated to one of two replicate treatment groups (H1; H2; L1; L2) grazing in different enclosures. Infected groups (H) received 10.000 third stage (L3) *O. ostertagi* (50%) and *C. oncophora* (50%) larvae; whereas control groups (L) were dewormed monthly with 0.5 mg ivermectin (Ivomec®, Pour-on) per kg bodyweight. Activity and rumination patterns were monitored by fitting animals in each group (H1, n=15; H2, n=17; L1, n=17; L2, n=11) with leg mounted (IceQube, IceRobotics) and neck mounted (Heatime LD tag, SCR) loggers. Body weight gain (BWG) was recorded every fortnight, whereas faecal and blood samples were collected every four weeks for nematode faecal egg count (EPG) and serum pepsinogen concentrations. EPG levels were affected by treatment ($P < 0.0001$). However, BWG was not affected ($P = 0.997$) by treatment, probably due to extreme drought. Preliminary results shows an increase in lying time ($P = 0.0369$) recorded by IceQube loggers and a trend of increase in individual behaviour variation for rumination ($P = 0.0556$) and decrease in individual behaviour variation for activity ($P = 0.0654$) in infected groups, recorded by Heatime loggers during the 30 first days. In addition, a trend of an increase in total step count ($P = 0.0565$) and activity ($P = 0.0509$) in infected groups, recorded by IceQube loggers over ~140 days, was seen. In conclusion, our preliminary data supports that changes and variation in activity and rumination patterns monitored with loggers could contribute to the identification of animals challenged with GIN, even when no differences can be observed with traditional measures, such as BWG.

OA02.04 Construction of a Decision Tree for Targeted-Selective Treatment of Dairy Cows Against Gastrointestinal Nematodes

Nadine Ravinet¹, Anne Lehébel¹, Nadine Brisseau¹, Yann Quenet¹, Nathalie Menudier², Aurélien Madouasse¹, Christophe Chartier¹, Alain Chauvin¹

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In dairy cows, the milk production (MP) response to anthelmintic treatment (AT) against gastrointestinal nematodes is highly

variable. This study aimed to develop a decision tree based on nested criteria to increase the probability of MP response.

A randomized controlled trial was conducted using injectable eprinomectin at housing in 123 French grazing dairy herds. Monthly individual milk yields were obtained. Linear mixed models were used to assess the effect of AT on MP. Starting with all herds (Step 0), the herd level criterion associated with the highest MP response to AT was identified (step 1). Then, among farms meeting this first criteria, a second criteria associated with the highest MP response was identified (step 2). This was repeated a third time on herds meeting the first 2 criteria (step 3). Furthermore, at each step, within the selected herds, the cow characteristics associated with the highest MP gain were identified.

When considering all the herds (step 0), the effect of AT was small but significant (+0.3 kg/cow/day). The first criterion (step 1) was the % of grazed grass in the diet: no MP gain in low-pasturing herds versus +1 kg/cow/day in moderate/high-pasturing herds. In this subset (n=50), the second criterion (step 2) was the TEC (Time of Effective Contact with GIN infective larvae before the first calving): +0.6 kg/cow/day in high-TEC herds versus +1.4 kg/cow/day in low-TEC herds. In this new subset (n=17), the third criteria (step 3) was the bulk tank milk Ostertagia ODR: +0.2 versus +1.8 kg/cow/day when ODR < 0.9 and ≥ 0.9, respectively. In steps 0, 1 and 2, best responding cows had calved during the grazing season and/or were low-producing cows within their herd.

Those herd and cow-level criteria, used sequentially, can be useful to build a decision tree that increases the probability of MP gains.

OA02.05 Ostertagia Ostertagi Antibodies in Bulk Tank Milk in Autumn: Variability of the ELISA Results According to the Date of Sampling

Nadine Ravinet¹, Nadine Brisseau¹, Anne Lehébel¹, Yann Quenet¹, Nathalie Menudier², Aurélien Madouasse¹, Christophe Chartier¹, Alain Chauvin¹

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In dairy cows, the bulk tank milk (BTM) Ostertagia antibody level (ELISA, results as optical density ratio, ODR) is often used as a criterion for anthelmintic treatment at housing, one single BTM sample being collected between October and December. However, this autumnal BTM ODR could vary depending on the date of sampling. The objective of the study was to quantify the autumnal variability in BTM Ostertagia ODRs, taking into account the variability related to the measurement method.

Ostertagia antibody levels were measured 16 times in 6 BTM samples (O. ostertagi-Ab ELISA kit, SVANOVIR®) (4 days, 2 experiments per day, samples tested twice per experiment, same operator). The coefficient of variation (CV), calculated for each series of 16 ODRs, ranged from 0.08 to 0.10.

In 125 French grazing dairy herds, BTM samples were collected fortnightly from the 1st of October until housing and analysed with the same ELISA kit: 3 to 7 autumnal ODRs per herd were obtained. The effect of the sampling date on the ODR was assessed (linear mixed model, herd as random effect) and the CV of each within-herd series was calculated.

November and December ODRs were slightly lower than October ODRs : -0.03 and -0.07, respectively (p = 0.0002). The maximal ODRs were obtained during October in 99/125 herds (79%). Within-herd CVs ranged from 0.02 to 0.38 (mean 0.10) and 44 herds (35%) showed a CV > 0.10, indicating that the autumnal variation in BTM ODR values could be higher than the variability related to the method of measurement.

Those results confirm that the BTM *Ostertagia* antibody level can vary during the autumn and this variability can have consequences on the decision to treat. To minimize the risk to under-estimate the exposure to *Ostertagia*, the optimal month for sampling BTM should be October.

OA02.06 A Bulk Milk Tank Survey of *Ostertagia* and *Fasciola Hepatica* Antibodies in Australian Dairy Enterprises.

Dr Gareth Kelly¹, Grant Richards², David Homer³

¹Boehringer Ingelheim, Sydney, Australia,

²Parasite Diagnostic Services, Hastings, Australia, ³Landmark, Melbourne, Australia

Between 2010 and 2016, 2493 bulk milk samples were analysed to determine the prevalence of *Ostertagia* and Liver Fluke antibodies (measured as Optical Density Ratios (ODR)) to help inform farmers on the health status of their milking herds. Samples from herds with no exposure to anthelmintic treatment within the previous four months were submitted to a single laboratory by sales managers. Results were collated according to the region covered by the sales manager, closely approximating biogeographical zones of Australia and enabling samples to be categorized according to the major dairy producing regions within Australia. Overall, the average ODR for *Ostertagia* was 0.788, indicative of a moderate infection (higher than published surveys where year-round grazing is not routine). Based on previous published validation work in other countries, this level of immune response to *Ostertagia* challenge in dairy cows equates to an estimated milk loss of 0.9 kg/day/cow. Furthermore, 92% of herds indicated that productivity may have been reduced with an ODR greater than 0.501 when tested. No seasonal variation was evident, but regional variation was evident with lower ODR values in the sub-tropical region of NSW compared to temperate dairy production regions. For fluke, 38% of all farms tested positive with moderate to high ODRs. Herds with high fluke ODR levels also had significantly higher ODR levels for *Ostertagia* than herds with low fluke ODRs (0.851 vs

0.755, $P < 0.01$). These results together suggest that the productivity of Australian dairy farms has the potential to improve with better animal health management.

OA03 North American Ticks

July 8, 2019, 11:00 - 12:30

Breakout Room 3, Hall of Ideas F&I, Level 4

OA03.01 Show Us Your Ticks: A Survey of Ticks Infesting Dogs and Cats Across the United States

Dr. Meriam Saleh¹, Kellee Sundstrom¹, Dr. Kathryn Duncan¹, Michelle Ientile¹, Julia Jordy¹, Parna Ghosh¹, Dr. Susan Little¹

¹Oklahoma State University, Stillwater, United States

A variety of tick species infest dogs and cats in the United States. To identify the species and stages of ticks on pets and characterize host attachment site preferences, tick submissions were invited from veterinary clinics in all 50 states. Upon receipt, ticks were identified to species and stage using morphologic keys; if damage precluded identification by morphology, species was confirmed molecularly. From February 2018 to January 2019, 10,885 ticks were submitted from 1,487 dogs and 341 cats in 49 states; ticks were collected in every month. Dog and cat infestation intensities ranged from 1-4,765 and 1-52 (median = 1, mean = 6.7 and 2.8), respectively. Dogs were primarily infested with *Dermacentor variabilis* (527/1487; 35.4%), *Ixodes scapularis* (400/1487; 26.9%), *Amblyomma americanum* (348/1487; 23.5%), and *Rhipicephalus sanguineus* (172/1509; 11.6%). Cats were primarily infested with *I. scapularis* (156/341; 45.7%), *A. americanum* (100/341; 29.3%), and *D. variabilis* (62/341; 18.2%). Other submitted ticks included *A. maculatum*, *Haemaphysalis longicornis*, *Otobius megnini*, and less common *Dermacentor* spp. and *Ixodes* spp. Co-infestations were documented in 93 dogs and 14 cats. Reported attachment site by tick species differed significantly for both dogs ($\chi^2 = 221.213$, $df = 15$, $p < 0.0001$) and cats ($\chi^2 = 244.689$, $df = 10$, $p < 0.0001$). In dogs, *A.*

americanum was most commonly attached to the abdomen, axillary, and inguinal regions, *D. variabilis* and *I. scapularis* the head and ears, and *R. sanguineus* head, ears, legs, and feet. In cats, *D. variabilis* and *I. scapularis* were most commonly attached to the head and ears, but *A. americanum* was most commonly attached to the tail and perianal region. These data confirm that dogs and cats in the United States are at risk of tick infestation every month of the year, and that tick species differ in their reported attachment sites.

OA03.02 Dermacentor spp. from Dogs and Cats in North America: Diversity and Geographic Distribution

Kathryn Duncan¹, Meriam Saleh¹, Kellee Sundstrom¹, Susan Little¹

¹Oklahoma State University, Stillwater, United States

Dermacentor spp. ticks are known to commonly feed on dogs and cats throughout North America and transmit important tick-borne pathogens, including spotted fever group Rickettsia spp. However, the geographic distribution of the different species of Dermacentor in this region is not completely understood. To better define the identity and distribution of Dermacentor spp. removed from dogs and cats in the United States, 495 Dermacentor spp. ticks submitted from 260 dogs (n=449 ticks) and 39 cats (n=46 ticks) from veterinary practices in 40/50 states were identified morphologically and molecularly. Amplification and sequencing of the ITS2 region and a 16S rRNA gene fragment confirmed that 97.8% (484/495) were *D. variabilis* and 1.6% (8/495) were *D. albipictus*. In total, only three (0.6%) *D. andersoni* were identified, one each from one cat and one dog from Montana and one cat from Idaho. While translocation of pets prior to tick removal cannot be discounted, the majority (36/45; 80%) of Dermacentor spp. ticks removed from dogs and cats in the Rocky Mountain states, where *D. andersoni* has been thought to predominate, were actually *D. variabilis*, suggesting this species may be more widespread in the western United States than is currently recognized, or that *D. andersoni*, if also present in the region, is preferentially feeding on hosts other than

dogs and cats. Together, these data support the interpretation that *D. variabilis* is the predominant Dermacentor species found on dogs and cats throughout the United States, a finding that may reflect somewhat recent shifts in tick distribution.

OA03.03 Ixodes spp. from Pet Dogs and Cats in the United States: Geographic Distribution and Prevalence of Borrelia burgdorferi

Ms. Parna Ghosh¹, Dr. Meriam Saleh¹, Ms. Kellee Sundstrom¹, Ms. Michelle Lentile¹, Dr. Susan Little¹

¹Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, United States

Ixodes spp. are commonly found on dogs and cats in many areas of the world. In the eastern United States, *I. scapularis*, the predominant species and primary vector of *Borrelia burgdorferi*, can be divided into two genotypes – American and Southern – on the basis of 16S rRNA gene sequence. To confirm the species and characterize the genotype of *Ixodes* ticks submitted from dogs and cats, we examined ticks morphologically and, when indicated, evaluated a 16S rDNA sequence from, 342 ticks submitted from 185 dogs, 63 cats, and one rabbit from 34 states; to estimate prevalence of infection, *flaB* of *B. burgdorferi* sensu stricto was amplified from a subset of ticks. Almost all *Ixodes* spp. submitted from the Northeast (n=93/94; 98.9%) and Midwest (n=55/57; 98.2%) were *I. scapularis*, American genotype (primary haplotype F), and 26/140 (18.6%) were positive for *B. burgdorferi*. One *I. affinis* was identified from a shelter cat in New York, one *I. cookei* from a dog in Ohio, and one *I. scapularis* Southern genotype (O) from a dog in Kansas. In contrast, 77/144 (53.5%) *Ixodes* spp. from the South were *I. scapularis*, American genotype (F and K); 50/144 (34.7%) were *I. scapularis*, Southern genotype (M, N, and O); 7/144 (4.9%) were *I. affinis*; and 10/144 (6.9%) were *I. cookei*. None of the *Ixodes* spp. from the South tested positive for *B. burgdorferi*. In the West, most (40/47; 85.1%) *Ixodes* spp. were *I. pacificus*, with *I. angustus* (n=3) submitted from dogs in Alaska and Oregon and *I. haerlei* (n=4), preliminarily

identified from a dog in Montana. Although *I. scapularis*, American genotype predominated in the Northeast, a diverse array of *Ixodes* spp. were found on dogs and cats in the South and West; the significance of these less common *Ixodes* spp. as disease vectors, if any, warrants further investigation.

OA03.04 Characterization Of Voltage Sensitive Sodium Channel Gene Of *Haemaphysalis longicornis* (Acari: Ixodidae), A New Invasive Tick Species In The United States

Dr. Guilherme Klafke^{1,2}, Jason Tidwell¹, Dr. Robert Miller¹, Prof. Dina Fonseca³, Dr. Denise Bonilla⁴, Dr. Adalberto Pérez de León⁵
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Haemaphysalis longicornis (Neumann), the Asian longhorned tick, is a three-host tick species that originates from temperate and subtropical areas of East Asia where it infests and transmits zoonotic pathogens to livestock and humans. *H. longicornis* was detected in the United States outside of quarantine for the first time on August 2017 infesting sheep in New Jersey (NJ). Since then, this invasive tick has been detected in Arkansas, Connecticut, Maryland, New York, North Carolina, Pennsylvania, Virginia, and West Virginia. Treatment of livestock with acaricidal drugs has been the main strategy to control ticks. However, the development of acaricide resistance needs to be anticipated. Acaricide resistance is an inherited phenotypic trait frequently conferred by mutations in the pesticide's target sites. Pyrethroid resistance in several arthropod species can be caused by conserved mutations in the voltage sensitive sodium channel gene (*vssc*). In order to detect future development of pyrethroid resistance in

H. longicornis using molecular techniques, our objective was to characterize the *vssc* gene. RNA was obtained from a pool of *H. longicornis* collected in NJ. Degenerate primers were used to amplify two conserved segments of the *vssc* gene and a 3'/5' rapid amplification of cDNA ends approach was used to get the full-length transcript. The translated amino acid sequences and the putative protein secondary structure was compared to the homologs *vssc* of the tropical cattle tick, *Rhipicephalus microplus* (Canestrini), the brown dog tick, *Rhipicephalus sanguineus* (Latreille) and other parasitic arthropods. No mutations previously associated to pyrethroid resistance were detected in the NJ *H. longicornis* samples analyzed. To the best of our knowledge, this is the first characterization of a gene in *H. longicornis* associated with acaricide resistance.

OA03.05 Dynamic Expression Analysis of microRNA Profiles During *Haemaphysalis longicornis* Different Stages by Deep Sequencing of Small RNA Libraries

Wenge Liu¹, Junhui Guo¹, Jin Luo¹, Hui Shen¹, Qiaoyun Ren¹, Ze Chen¹, Zhiqiang Qu¹, Zegong Wu¹, Jun Ni¹, Xiaofeng Xu¹, Jianxun Luo¹, Hong Yin¹, **Guangyuan Liu**¹
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Haemaphysalis longicornis, a three-host life cycle tick, is an obligate specialized blood-sucking parasite. MicroRNAs (miRNAs) show an abundance of expression changes during the different developmental stages of ticks. Here, deep sequencing technology was used to profile the conserved and potential novel miRNAs expressed during different developmental stages of *H. longicornis*. In total, 111,192,069 reads were obtained and four small RNA libraries (egg, unfed larvae, unfed nymph and unfed adults) of *H. longicornis* were built after deep sequencing on a HiSeq 4000. Among these, 78 conserved miRNAs and 55 potential novel miRNAs were identified, including stage-specific miRNAs and differentially-expressed miRNAs. GO analysis indicated that significantly-enriched GO terms related to cell proliferation and differentiation, comprising the specific terms

developmental process, growth, metabolic process, regulation of biological process, reproduction and membrane enzyme regular activity. KEGG analysis revealed significantly-enriched signaling pathways related to growth and development including the insulin signaling pathway, the notch signaling pathway, the Hippo signaling pathway and the Wnt signaling pathway. Our data suggested that the abundance of miRNA changes (both conserved and potential novel) in the different developmental stages of *H. longicornis*, especially for stage-specific miRNAs, may indicate the importance of miRNAs as essential regulators during the development of *H. longicornis*.

OA03.06 The *Haemaphysalis longicornis* Larvae Needs Glutathione S-Transferase in the Metabolism of Flumethrin

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[Introduction] *Haemaphysalis longicornis* is a tick of health importance as it serves as vector of several disease-causing pathogens. Presently, the major method to control *H. longicornis* is by using chemical acaricides. Flumethrin is a common pyrethroid pesticide that has specificity towards acarids. Glutathione S-transferases (GST) system on ticks is one of the mechanisms for metabolizing these acaricides. Two GSTs

from *H. longicornis* (HIGST and HIGST2) were identified until now. However, the role of GSTs in pyrethroid metabolism of ticks has still been unknown.

[Objective] In this study, we tried to elucidate the role of GST in flumethrin metabolism by determining their possible interaction, its ability to induce GST gene and protein expression, and effect of GST knockdown on tick survival upon exposure to flumethrin.

[Methods] Enzyme kinetic studies were conducted with recombinant *H. longicornis* GSTs. Using the Vmax and Km values of recombinant GSTs with and without acaricides, the type of interaction between recombinant GSTs and flumethrin was determined. The sublethal doses of flumethrin were determined using a modified larval packet test. The gene and protein expression of adult female ticks exposed to sublethal doses of flumethrin were also determined using real-time RT-PCR and Western blot analysis. RNA interference of GST genes was conducted on different stages of development to determine the effect of GST knockdown on tick survival upon exposure to flumethrin.

[Results] Flumethrin showed uncompetitive inhibition on the activity of recombinant HIGST, while recombinant HIGST2 activity was not affected. Both GST genes and proteins were upregulated upon exposure to sublethal doses of flumethrin. Knockdown of GST genes did not affect the survival of the nymphs and adults, while HIGST knockdown resulted to the increased susceptibility of larvae to sublethal doses of flumethrin.

[Conclusion] These findings suggest that HIGST is vital for the metabolism of flumethrin in the larvae.

OA04 Coccidia

July 8, 2019, 11:00 - 12:30

Breakout Room 4, Hall of Ideas G&J, Level 4

OA04.01 Bumped Kinase Inhibitor 1369 Is Effective Against Porcine Cystoisosporosis

Aruna Shrestha¹, Florian Koston¹, Bärbel Ruttkowski¹, Ryan Choi², Grant R. Whitman², Samantha A. Michaels², Edelmar Navaluna², Kayleigh F. Barret², Lynn K. Barret², Carlie S. Dorr², Kayode K. Ojo², Matthew A. Hulverson², Andrew Hemphill³, Wesley C. Van Voorhis², Anja Joachim¹

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The chemo-prophylactic control of cystoisosporosis in the European Union currently relies on the use of toltrazuril. Recently, resistance against toltrazuril in a Dutch field isolate of *Cystoisospora suis* has been described, and therefore, the need of new therapeutics to overcome the limited treatment options is of immediate importance. Bumped kinase inhibitors (BKIs) specific for parasite calcium-dependent protein kinases (CDPKs) have already been shown to reduce infection in several apicomplexan parasites of human and veterinary importance, including *Toxoplasma gondii*, *Neospora caninum*, *Cryptosporidium parvum*, and *Sarcocystis neurona*. In the present study, the efficacy, safety and pharmacokinetics of BKI 1369 in piglets experimentally infected with *Cystoisospora suis* was determined using an established animal infection model, and in cell culture. Five-day treatment with BKI 1369 effectively suppressed oocyst excretion and diarrhea and improved body weight gains in treated piglets without obvious side effects for both toltrazuril-sensitive (Wien-I) and resistant (Holland-I) *C. suis* strains. In vitro, BKI 1369 inhibited merozoite proliferation in intestinal porcine epithelial 1 (IPEC-1) cells by at least 50% at a concentration of 40 nM and

proliferation was almost completely inhibited (>95%) at 200 nM. Nonetheless, exposure of infected cultures to 200 nM BKI 1369 for five days did not induce phenotypic morphological alterations in surviving merozoites as confirmed by transmission electron microscopy. During treatment in piglets, plasma concentration of BKI 1369 increased to 11.7 μ M, suggesting constant exposure of parasites to the drug. Oral application of BKI 1369 could be considered as a new therapeutic alternative against porcine cystoisosporosis. For the use in pigs, future studies on BKI 1369 should be directed towards ease of drug handling and minimizing treatment frequencies.

OA04.02 In Vivo Anti-Cryptosporidial Efficacy of Novel Lactate Dehydrogenase Inhibitors

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¹*University Of Illinois At Urbana-champaign, College of Veterinary Medicine, Champaign, United States*

Cryptosporidium parvum is a highly prevalent zoonotic and anthroponotic protozoan parasite that causes a diarrheal syndrome in children and neonatal livestock, culminating in growth retardation and mortalities. Despite the high prevalence of *C. parvum*, there are no fully effective and safe drugs for treating infections, and there is no vaccine. We have previously reported that the bacterial-like *C. parvum* lactate dehydrogenase (CpLDH) enzyme is essential for survival, virulence and growth of *C. parvum* in vitro and in vivo. In the present study, we screened compound libraries and identified inhibitors against the enzymatic activity of recombinant CpLDH protein in vitro. We tested the inhibitors for anti-*Cryptosporidium* efficacy using in vitro infection assays of HCT-8 cell monolayers and identified compounds A1.0 and C2.0 that inhibited the proliferation of intracellular *C. parvum* in vitro, with IC₅₀ values of 14.88 and 72.65 μ M, respectively. At doses tolerable in mice, we found that both A1.0 and C2.0 consistently reduced the shedding of *C. parvum* oocysts to undetectable levels in infected immunocompromised mice's feces, and prevented intestinal villous atrophy as

well as mucosal erosion due to *C. parvum*. Together, our findings have unveiled promising anti-Cryptosporidium drug candidates that can be explored further for the development of the much needed novel therapeutic agents against *C. parvum* infections.

OA04.03 A *Cystoisospora Suis* In Vitro Assay for Compound Screening: The Example of Bumped Kinase Inhibitor BKI 1369

Mrs. Baerbel Ruttkowski¹, PhD Aruna Shrestha¹, Prof Anja Joachim¹

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Currently the only drug registered for treatment of piglet coccidiosis caused by *Cystoisospora suis* is toltrazuril. However, in the light of the first recorded toltrazuril-resistant field isolates alternatives must urgently be sought. Screening for compounds in vitro can guide the selection of compounds with assumed efficacy in vivo and give hints at mechanisms of efficacy and stage-specific activity. A previously developed in vitro cultivation system for *C. suis*, using sporozoites obtained from oocysts ex vivo to infect semi-permanent IPEC-J1 cells to sustain the development of all endogenous life-cycle stages, was adapted to quantify merozoites developed from initial sporozoite infection. A serial dilution of sporozoites was used to adapt the culture conditions and to evaluate the growth curve of merozoites during 5-9 days of cultivation. Along with in vivo efficacy testing, the bumped kinase inhibitor BKI-1369 was evaluated in vitro for efficacy against *C. suis*. The standard treatment for five days from the day of infection could be reduced in vitro to three days, starting two days post infection in vitro. Compound testing includes cell toxicity of the compound and its solvent, titration of effective drugs with determination of IC 50/IC90 and the evaluation of treatment duration. Test readout was either microscopical evaluation by counting of free merozoites harvested 9 days after infection from culture supernatants or by quantitative real-time PCR with determination of the total amount of merozoites in supernatant and cells followed by DNA preparation

and amplification of the *C. suis* gene CSUI_005805. Three different standards – DNA from merozoite dilutions, direct DNA dilution standards and a cloned plasmid standard – were compared for efficacy and sensitivity. On the basis of this assay, in vivo efficacy testing can be tailored to reduce the number of animals used and provide information on the possible mechanisms of action of tested compounds.

OA04.04 New Treatment Options for Piglet Coccidiosis: Injectable Toltrazuril in Combination With Iron (As Glettoferon) Is Effective Against Experimental *Cystoisospora Suis* Infections on the 1st or 3rd Day of Life

Prof. Anja Joachim¹, Dr. Nicolas Guerra², Dr. Barbara Hinney¹, Dr. Adnan Hodžić¹, Dr. Hamadi Karembe², Dr. Aruna Shrestha¹, Dr. Daniel Sperling²

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Piglet coccidiosis is one of the most common diarrheal diseases of suckling piglets. Infection causes poor growth and seriously compromises animal health. Where available, toltrazuril is applied orally in a metaphylactic oral single-use individual application to prevent disease. Recently, an injectable toltrazuril has been developed for parenteral application on the 1st - 3rd day of life (dol) together with iron for anemia prevention in a single application (Forceris®, 45 mg toltrazuril + 200 mg iron/piglet). In two experimental trials with infection of piglets on the 1st resp. 3rd dol (1000 *C. suis* oocysts/piglet) the efficacy of this product (application on the 2nd dol) was evaluated in comparison to a control group (iron treatment only). Oocyst excretion, diarrhea and weight gain were compared between groups. The presence of bacterial enteropathogens was determined in selected samples. For each group, 10-13 piglets finished the study and were included in the evaluation. In the control groups, all piglets excreted oocysts and had diarrhea for at least one day. In the treated groups oocyst excretion was completely suppressed and diarrhea was reduced to a one resp. two

piglets and five resp. three diarrheic samples. Differences between treated and control groups were significant for all parameters. Weight gain was suppressed during the acute phase of infection (8th – 15th dol), resulting in reduced body weight in the controls compared to the treated groups by the end of the study (29th dol). Infection on the 1st dol induced more intense oocyst excretion while diarrhea was less severe, probably due to insignificant levels of *Cl. perfringens* type A and *E. coli* compared to the trial with infection on the 3rd dol. Forceris® treatment on the 2nd dol was highly effective against experimental *C. suis* infections on the 1st – 3rd dol in the presence of bacterial enteropathogens.

OA04.05 Molecular Mechanisms Involved in Interaction of SRSs of *Toxoplasma Gondii* Exosomes with Host Proteins

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Knowledge of the exosomes secreted by *T. gondii* parasites or *T. gondii*-infected host cells is still very limited. Apart from their role in cell-to-cell communication, exosomes secreted by *T. gondii* are able to stimulate host immune response directly or indirectly, via dendritic cells and other APCs, which could also lead to specific pathological changes in the host. In a previous study, we successfully identified some surface antigen 1-related sequences (SRSs) proteins in *T. gondii* exosomes, beside other *T. gondii* specific proteins. *T. gondii* SRS proteins are involved in the process of attachment to host cells, along with stimulating immune reactions and regulating parasite virulence, which is thought to promote the formation of tissue cysts in intermediate hosts in order to establish persistent, latent infections that facilitate transmission of infection to the definitive host. In our current study, three SRSs were identified and characterized from *T. gondii* (designated TgSRSs). Recombinant TgSRSs (rTgSRSs) were expressed in *Escherichia coli* and purified. Three SRSs native proteins of *T. gondii* or

T. gondii exosomes were recognized by the antisera against rTgSRSs protein. Indirect immunofluorescence assay results indicated that three TgSRSs were located either on cell surface or cytoplasm of *T. gondii* tachyzoites. Applying CRISPR/CAS9 technology, three gene deletion and overexpression plasmids targeting TgSRSs were constructed. To understand the complex mechanisms behind the pathogenicity of *T. gondii*, and thereby facilitate the development of proper preventive measures against Toxoplasmosis, we analyzed the structural characteristics and functions of these proteins in toxoplasma invasion, reproduction and interaction with host cell proteins, in addition to other possible roles of these three rTgSRSs.

OA04.06 TKL1-Deficient *Toxoplasma Gondii* Is a Potential Live-Attenuated Vaccine Against Acute, Chronic and Oocysts-Caused Congenital Toxoplasmosis in a Murine Model

Dr. Jin-lei Wang¹, Mr. Qin-Li Liang¹, Miss Ting-Ting Li¹, Dr. Jun-Jun He¹, Miss Meng-Jie Bai¹, Prof. Hany M. Elsheikha², Prof. Xing-Quan Zhu¹
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Toxoplasma gondii is an important pathogen for which a vaccine would address a significant unmet public health and medical need. We tested whether deletion of tyrosine kinase-like 1 (tkl1) can be an effective strategy for developing a live-attenuated vaccine, with controllable attenuation and potent immunogenicity. The protective efficacies of vaccination using RHΔtkl1 tachyzoite against acute, chronic and congenital toxoplasmosis in Kunming mice were evaluated. Mice vaccinated with RHΔtkl1 developed a strong humoral and cellular response as indicated by a high level of *T. gondii*-specific IgG, IL-2, IL-12, IFN-γ and IL-10. All vaccinated mice survived a lethal challenge with 103 tachyzoites of RH or ToxoDB#9 (PYS or TgC7) strains, as well

as 100 tissue cysts or oocysts of Pru strain, whereas all non-vaccinated mice infected with these parasites died. Vaccination also protected against cysts or oocysts caused chronic infection, reduced the vertical transmission caused by oocysts, increased the litter size, and maintained pups' body weight in dams challenged with 10 oocysts on day 5 of gestation. In contrast, all non-vaccinated + oocysts-infected dams had abortions and no fetus survived. Vaccinated dams remained healthy after infection and their brain cyst burden was significantly reduced compared with non-vaccinated + oocysts-infected dams. A single vaccination of mice with RHΔtkl1 tachyzoites induced a balanced and strong immunity against *T. gondii* with different stages, and protected mice against congenital oocysts infections. This is, to the best of our knowledge, the first to demonstrate the efficacy of live-attenuated vaccine against oocysts-caused congenital toxoplasmosis.

OA05 Worldwide Vector-Borne Infections in Companion Animals

July 8, 2019, 11:00 - 12:30

Breakout Room 5, Meeting Rooms KLOP, Level 4

OA05.01 Highly Specific Screening Tests Maintain Actionable Positive Predictive Values in Areas with Low Heartworm Prevalence

Prof. Dwight D. Bowman¹, Jennifer Braff², Jesse Buch², Andrei Rakitin², Ramaswamy Chandrashekar², Melissa Beall²
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²IDEXX Laboratories, Inc, Westbrook, United States

At low prevalence, the positive predictive value of a diagnostic screening test is directly proportional with disease prevalence within a given region. Consequently, where the prevalence of heartworm infection has dropped below the claimed false positive rate (1 – specificity) for a screening test, rare positive test results may be suspected as false positives. This study re-evaluated the field-observed specificity of the SNAP® 4Dx® Plus test for heartworm (HW) antigen and estimated the local positive predictive value (PPV) for specific

California counties. To better understand the diagnostic value of HW screening results in low-prevalence areas, in-clinic SNAP® 4Dx® Plus-HW results from 2013 to 2016 were examined for California and for Scandinavia where virtually no autochthonous HW transmission is believed to occur. For this analysis, all positive HW tests from Scandinavia were conservatively treated as false positives. The observed specificity was defined as the proportion of Scandinavia HW tests that returned a negative result. Using the observed Scandinavian specificity, SNAP® 4Dx® Plus-HW results from California with the stated test sensitivity, we calculated the positive predictive values in California counties. Of the 14,778 Scandinavian tests results, 18 were positive giving a positive rate of 0.12% and an observed specificity of 99.88%. For the same period, there were 301,997 test results from 476 California veterinary clinics with 1,742 being HW positive with a positive rate of 0.58%. The positive rate for the SNAP® 4Dx® Plus-HW tests in California was significantly higher than that observed in Scandinavia by chi-square test ($P < 0.001$). Therefore, the higher than expected rate of SNAP® 4Dx® Plus-HW test positives cannot be solely attributed to a lack of specificity (false positives) but strongly suggests the actual presence of *Dirofilaria immitis* in California. The results support that any HW-positive test results on the SNAP® 4Dx® Plus-HW, due to its high specificity, are highly probable to be true positives.

OA05.02 Trends in Global Clinic-Based ELISA Seropositive Rates for Canine Vector-Borne Disease

Dr. Jesse Buch¹
¹IDEXX Laboratories, Inc., Westbrook, United States

Current trends in animal relocation and vector range expansion have increased the importance of studying the global epidemiology for canine vector-borne disease (CVBD). In some regions, epidemiological data on vector-borne zoonotic pathogens is available to a far greater extent for canine populations than humans. Thus, CVBD data is becoming an important component of public health as an increasing emphasis is being placed on studying canine populations as

sentinels for human vector-borne disease. This talk will review recent global trends in CVBD clinic-based ELISA results for *Dirofilaria immitis* antigen and antibodies to *Borrelia burgdorferi*, *Ehrlichia* spp., *Anaplasma* spp. and *Leishmania*. The trends are based on more than 60 million in-clinic serological CVBD test results reported from more than 11,000 veterinary clinics located in 79 countries from 7 regions. The strengths and limitations of these data will also be discussed.

OA05.03 Comprehensive Survey of Parasitic and Infectious Diseases in Feral & Domesticated Cats in Grenada, West Indies

Dr. Tara Paterson¹, Tamara Hockley¹, Dr. Dave Elsemore², Dr. Jancy Hanscom², Dr. Phyllis Tyrrell², Dr. Christian Leutenegger², Dr. Ramaswamy Chandrashekar²

¹St. George's University, School Of Veterinary Medicine, St. George's, Grenada, ²IDEXX Laboratories, Inc., Westbrook, United States

Little data exists on feline infectious diseases in the Caribbean even though some are zoonotic and are of significant public health concern. The objective of this study was to survey domesticated and feral cats for parasitic and infectious diseases using a comprehensive diagnostic approach. Seventy-one cats (46 feral, 25 domestic) presenting to the St. George's University (SGU) Small Animal Clinic for care or sterilization through the SGU Feral Cat Project were sampled. Sixty-five blood samples were tested using the IDEXX SNAP® Feline Triple® test – 13.8%, 13.8% and 9.2% were positive for *Dirofilaria immitis*, feline leukemia virus (FeLV) and feline immunodeficiency virus, respectively. Fifty-seven fecal samples were evaluated at SGU using centrifuged fecal floatation – 82.5% of cats were infected with ≥ 1 parasite. The most common parasite observed was hookworm (*Ancylostoma* sp., 78.9%), followed by *Mammomonogamus* sp. (40.4%), whipworm (*Trichuris* sp., 33.3%), roundworms (*Toxocara cati* 8.8%, *Toxascaris leonina* 1.8%), *Isospora felis* (8.8%), and *Dipylidium caninum* (3.5%). Fecal Dx® antigen testing was performed on 44 fecal samples at IDEXX Laboratories with the following results: hookworm 56.8%, whipworm 29.5%,

and roundworm 9.1%. Larva were observed in 3 of 49 fecal samples assessed by Baermann technique for *Aerulostrongylus abstrusus* but none were identified as *A. abstrusus*. Results from IDEXX RealPCRTM analysis of 69 blood samples yielded: *Mycoplasma haemominutum* 20.3%, *Bartonella* sp. 17.4%, *Mycoplasma haemofelis* 7.2%, feline panleukopenia 4.4%, *Mycoplasma turicensis* 4.3%, FeLV 1.4% and *Ehrlichia ewingii* 1.4%. Forty-two (60.9%) cats were positive for ≥ 1 infectious organism. Of these, 18 were co-infected with up to 4 organisms. Feral cats were infected with parasites and infectious organisms more frequently than domesticated cats. This is the most comprehensive assessment of infectious diseases in Grenadian felids. The results of this investigation will aid in the diagnosis and treatment of cats in Grenada and the broader Caribbean region.

OA05.04 Prevalence of Babesia Spp. And Clinical Characteristics of Babesia Microti-Like Infections in North American Dogs

Dr. Nanelle R. Barash¹, **Dr. Barbara Qurollo**¹, Ms. Brittany Thomas¹, Dr. Adam J. Birkenheuer¹, Dr. Edward B. Breitschwerdt¹, Ms. Erica Lemler¹
¹North Carolina State University, College of Veterinary Medicine, Department of Clinical Sciences, Raleigh, United States

Babesia microti-like infection, reported in European dogs and North American foxes, has rarely been reported in domestic North American dogs. We determine the prevalence of *Babesia* spp., including *B. microti*-like, in a large convenience set of North American dog blood samples submitted to a reference laboratory for vector-borne disease testing between June 2015 and June 2018. In addition to *Babesia* spp. testing, comprehensive canine vector-borne disease diagnostics were performed to identify co-infecting pathogens. *Babesia* spp. DNA was PCR amplified from 269/9376 (2.9%) dogs. The most prevalent *Babesia* species were *B. gibsoni* (187; 2.0%) and *B. microti*-like (48; 0.51%), of which 30 (0.32%) dogs were co-infected both *Babesia* spp. Limited clinicopathologic data in *B. microti*-like infected dogs revealed proteinuria (75%), hyperglobulinemia (67%), thrombocytopenia (60%), anemia (58%), and

hypoalbuminemia (56%). Co-infections with *Mycoplasma*, *Dirofilaria immitis*, or *Wolbachia* and co-seroreactivity to *Bartonella*, *Ehrlichia*, and *Rickettsia* spp. were documented in *B. microti*-like infected dogs. In North America, dogs can be infected with *B. microti*-like and develop clinicopathological abnormalities consistent with babesiosis caused by other *Babesia* spp.

OA05.05 Hepatozoon Canis in Hunting Dogs From Southern Italy: Distribution and Risk Factors

Dr. Laura Pacifico¹, Dr. Melissa Beall², Dr. Francesco Buono¹, Dr. Jennifer Braff², Giovanni Sgroi¹, Dr. Jesse Buch², Dr. Diego Piantedosi¹, Dr. Benedetto Neola³, Dr. Christian Leutenegger², Dr. Ramaswamy Chandrashekar², **Dr. Vincenzo Veneziano¹**
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Hepatozoon canis infects domestic and wild carnivores in Europe. Hepatozoon spp. are mainly transmitted through ingestion of infected ticks containing mature oocysts. In Europe, the principal vector of *H. canis* is *Rhipicephalus sanguineus*, and the distribution of the disease correlates with the range of the vector. However, *H. canis* has been reported outside of these areas, often related to infection in wild carnivores. Because hunting dogs can act as sentinels for monitoring wildlife diseases, this study was performed to determine the prevalence and risk factors associated with *H. canis* infection in hunting dogs from Southern Italy. Blood was collected from 1,433 dogs in Napoli, Avellino and Salerno provinces as part of a Campania region hunting dog health assistance program. Samples were tested by real-time polymerase chain reaction (PCR) to amplify *H. canis* DNA. PCR identified 200 positives, indicating a prevalence of 14.0%. Multiple logistic regression on *H. canis* PCR results determined that province ($p < .0001$), coat length ($p = .026$), *E. canis* serological status ($p < .0001$), and breed ($p < .0001$) were significant predictors of *H. canis* infection. Risk factors for positive *H. canis* PCR results included living in Salerno

province (OR 4.93 vs. Avellino, 95% CI 3.18-7.64), long coat length (OR 1.79 vs. short, 95% CI 1.16-2.77), seropositivity for *E. canis* (OR 2.84, 95% CI 1.72-4.69), and hound breed (OR 2.97 vs. pointing, 95% CI 1.88-4.70). The significant association with *E. canis* seropositivity and lack of association with the observed presence of ticks could be an indication that the duration of exposure to the vector is important. Although hunting dogs in Southern Italy are frequently exposed to *H. canis*, infections could be underestimated because clinical signs overlap with other tick-borne diseases. Further studies are necessary to determine the epidemiological relationships between hunting dogs and wild animal populations sharing the same environments in Southern Italy.

OA06 Canine Heartworm II

July 8, 2019, 13:30 - 15:30

Plenary Hall, Madison Ballroom (ABCD), Level 4

OA06.01 Colorado Dog Importation and Increases in Heartworm Prevalence

Jason Drake¹, Rudolph Parrish¹
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Animal welfare groups, attempting to increase adoptions and reduce euthanasia, transport thousands of rescued dogs state-to-state annually. A recent survey reported that approximately only 1/3 of animal welfare groups test, treat or provide prevention for heartworm (*Dirofilaria immitis*) infection prior to shipping dogs. Studies have reported prevalence of heartworms in dogs in shelters or rescued following natural disasters ranging from 14.6 – 48.8%. For this study, we obtained data from Companion Animal Parasite Council (CAPC) heartworm prevalence maps and from the Colorado Department of Agriculture Pet Animal Care Facilities Program (PACFA) and investigated changes in heartworm prevalence and the number of dogs shipped into Colorado. The CAPC heartworm maps reveal that the prevalence of heartworms in Colorado dogs has increased 67.5% from 2013-2017. The PACFA data from

2014-2017 show that over 130 animal welfare organizations in Colorado received more than 114,000 dogs from out-of-state sources, representing approximately 9.5% of the total estimated 2017 Colorado population of nearly 1.2 million dogs. Three large Colorado-based organizations responded to requests for details regarding the originating states from which they received dogs. The majority of these dogs were apparently shipped to Colorado from states with higher heartworm prevalence. New Mexico represented the source of the greatest number of relocated dogs, accounting for just over 30%. Nearly half (49%) of the dogs relocated by these three organizations came from either Texas or Oklahoma. Animal welfare organizations and veterinarians should increase testing and prevention of heartworm infections in dogs prior to, and following, shipment from areas with endemic heartworm. Repeated testing is recommended due to the 6-month pre-patent-period associated with *D. immitis*. Veterinarians and pet owners should increase vigilance with heartworm testing and prevention, even in areas with historically low heartworm prevalence. Movement of dogs from highly endemic areas may increase the risks of local transmission.

OA06.02 Improved Real-Time PCR Diagnostics for *Dirofilaria immitis* Utilizing a Next-Generation Sequencing-Based Approach to Target Selection and Assay Design

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Dirofilaria immitis is a mosquito-borne pathogen with a wide range of hosts in both the Canidae and Felidae. Current diagnostic practices involve either direct microscopic observation of microfilaria or the use of ELISA assays targeting antigens released by adult female worms. While commonly employed, the diagnostic use of microscopy can lead to misdiagnosis or under diagnosis, and significant concerns regarding the sensitivity and specificity of available ELISA tests exist. Attempting to

overcome these diagnostic shortcomings, multiple real-time PCR-based diagnostic assays have been developed in recent years. Capable of detecting *D. immitis* in both vector mosquitoes and definitive hosts, these assays provide a powerful new diagnostic option. However, to date, all such diagnostics have utilized sub-optimal mitochondrial or ribosomal targets. Maximizing the diagnostic sensitivity and specificity of real-time PCR requires the targeting of highly divergent, high copy-number repetitive DNA sequences. Accordingly, we now describe the development of a novel real-time PCR diagnostic, targeting a highly repetitive genomic DNA element. This element was identified using a next-generation sequencing-based approach that we have previously employed to improve diagnostic options for human-infecting soil-transmitted helminthes. Our target represents the most highly repetitive DNA element within the *D. immitis* genome. Accordingly, assay sensitivity and specificity has been maximized targeting this element. Comparative testing against genomic DNA isolated from various filarial nematodes confirmed assay specificity and a panel of field-collected samples was employed to demonstrate improved sensitivity of detection vs. established molecular diagnostic options. Furthermore, due to the evolutionarily divergent nature of such DNA targets, the adaptability of this assay to conventional PCR-based approaches was also evaluated. Such adaptability could expand the utility of this assay to settings where real-time PCR is not available.

OA06.03 Do Canids Infected with *Dirofilaria immitis* Release Unique Volatile Organic Compounds in Their Breath?

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Dirofilaria immitis, causative agent of canine heartworm disease, is a mosquito transmitted parasite. Mosquito vectors use host semiochemical signals such as CO₂ and odorant compounds to locate viable hosts. Here, we aim to examine the role of canine heartworm infection on the chemical fingerprints of breath volatiles which may facilitate mosquito attraction and therefore parasite transmission dynamics. Specifically, we hypothesize that infected dogs will release unique volatile organic compounds (VOCs) in their breath which are mosquito attractants and may be used to non-invasively detect heartworm infection status. We developed a rapid (under 2 minutes) method to capture and store 1 liter of exhalant from dogs for downstream thermal desorption and processing by gas chromatography mass spectrometry (GC-MS). Using this method we sampled 25 dogs with known heartworm infection status, and identified the compounds using automated and manual extraction methods (AMDIS). Over 500 unique VOCs were identified and classified into chemical subclass. GC arrays (heat maps) of the volatile breathomes were generated and show significantly more unique VOCs in infected dog breath than in non-infected dogs, and more phenols, which are known attractants. However, contrary to our hypothesis, alkane hydrocarbons such as decanes and nonanes, known mosquito repellent compounds, were found in non-infected dogs and these compounds were largely absent from infected dogs. The emerging field of breathomics has been used to examine VOCs as biomarkers of diseases in humans; however, to our knowledge no one has used breath biomarkers as signals of pathogen detection in animals. Here, we find that breath VOC composition differs significantly between *D. immitis* infected and non-infected dogs as does the presence of mosquito attractant and repellent compounds. These results suggest that mosquito-host interactions may be manipulated by the metabolic by-products of parasitic infection driving parasite transmission dynamics.

OA06.04 Collection and Molecular Detection of Dog Heartworm, *Dirofilaria immitis*, DNA in Wild Caught Mosquitoes

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Dirofilaria immitis, the causative agent of dog heartworm disease, is responsible for a disproportionate amount of canine morbidity and mortality; in addition to being expensive to treat, severe infections are often fatal. Much is known about the pathogen in the canine host, yet little is known on the basic biology of the nematode in the mosquito vector. Thus, to evaluate the effectiveness of collection techniques on ability to capture dog heartworm infected mosquitoes, we conducted a field study spanning 111 weeks. Four methods were used to collect mosquitoes; two aspirators types, sweep netting, and a CDC trap. Sites with canines present in either residential yards (n=4) or dog kennel facilities (n=3) were utilized. Collected mosquitoes were sorted by site, trap, species, and date, then pooled into groups of up to 25 individuals. Mosquito head and thorax pools were extracted for DNA, that was screened using currently available protocols. These protocols were found unreliable, thus, we developed a novel qPCR primer and probe set.

Using this method, the original samples were re-assayed and provided 644 positive pools. Approximately 10% of positive samples were confirmed by Sanger sequencing. Twenty-three species tested positive for dog heartworm DNA, including a new association with *Wyeomyia mitchellii*. *Aedes atlanticus*, *Anopheles crucians*, and *Culiseta melanura* composed nearly 28% of the total collection, yet were responsible for 66% of the qPCR positive pools. Infection rates for commonly collected mosquitoes ranged up to 2.4% with more rarely collected species ranging up to 20%.

We present a new qPCR primer and probe set for detecting dog heartworm DNA. The CDC trap appears to be the most effective

collection method, yet other techniques should be utilized when targeting mosquito species that this trap is biased against.

OA06.05 Evaluation of Heat Treatment of Serum Prior to Testing for Detection of Antigen of *Dirofilaria immitis* in Necropsy Positive and Negative Dogs from Florida, USA.

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Multiple studies have used heat treatment to determine changes in prevalence of *Dirofilaria immitis* antigen in serum of naturally infected dogs in the absence of necropsy data. In order to determine changes in test sensitivity and specificity following heat treatment, serum from 547 dogs (259 necropsy positive and 288 necropsy negative samples) were evaluated using the DiroCHEK® (Zoetis) antigen assay. Positive results were determined visually and by spectrophotometry at 650nm at 5 minutes. All samples were prepared and subjected to heat treatment by an operator that was unaware of necropsy results using 300µl of serum. Positive antigen results were interpreted based on necropsy results for collection years 2011-2015 (n=223) and 2017-2019 (n=36). Necropsy negative samples from 2011-2015 (n=224) were randomly selected from 367 available samples determined as antigen negative. Of the necropsy positive samples, 225/259 (86.9%) and 245/259 (94.6%) samples tested positive before and after heat treatment, respectively. For the necropsy negative samples, 288/288 (100%) and 277/288 (96.2%) tested negative before and after heat treatment, respectively. Using necropsy status as the reference, sensitivity was improved by 7.7% and specificity decreased by 3.8%. Heartworm intensities ranged from 1-30 for post-heat antigen positive samples and 1-62 for those remaining antigen negative. Results will be discussed in context of parasite life cycle, necropsy results, and application of optical density as an indicator of positive or negative infection.

OA06.06 Canine Heartworm Infection and Prophylaxis Use among Pet Caretakers from the Cumberland Gap Region of Tennessee, USA

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The incidence of canine heartworm (CHW), *Dirofilaria immitis*, infection continues to increase in the United States, despite the availability of effective prophylaxis. Current reports of CHW prevalence based on use of veterinary services likely underrepresent the true magnitude of the issue because pet dogs not receiving regular veterinary care, and dogs in residing in animal shelters are often excluded from distribution maps. This study estimated the prevalence of CHW in a pet dog population and prophylactic use by pet caretakers, irrespective of their use of veterinary services. Pet dogs from the Cumberland Gap region in Tennessee, USA were serologically tested for CHW and their caretakers interviewed about monthly CHW prophylaxis use and perceptions regarding knowledge and prevention of CHW. This was accomplished by pedestrian neighborhood survey. CHW prevalence was 2% (3/125) in the tested population. Non-use of monthly prophylaxis was present in 42% of surveyed pet caretakers, implying that 54 of the 125 dogs tested are potentially at risk for infection with CHW. Significant ($p < 0.05$) predictors of prophylaxis use identified by a logistic regression model include household income, use of veterinary services, altered reproductive status (spay/neuter), knowledge of CHW, and confinement outdoors. Pet caretakers utilizing regular veterinary services were more likely to perceive CHW as an important health factor, and administer monthly prophylaxis to their pets. These results underscore the importance of veterinary service mediated interaction with pet caretakers to enhance awareness of CHW and facilitate adoption of effective prophylactic programs.

OA06.07 First Case of a Natural Non Patent Infection in a Domestic Cat (*Felis catus*) With the Canid Heart Worm *Angiostrongylus Vasorum*

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Cardiopulmonary nematodes in cats include different parasite species affecting feline lungs and the heart, with the metastrongyloid *Aelurostrongylus abstrusus* being the most frequent feline lungworm worldwide. The present case report describes a 11 month old male neutered European short hair cat which presented with generalized subcutaneous oedema and pleural and peritoneal effusions. According to clinical examination, abdominal imaging and laboratory analyses, a tentative diagnosis of severe glomerulopathy with massive proteinuria was made. Due to worsening of the clinical signs despite therapeutic interventions and a poor prognosis, the cat was euthanized. Necropsy and histological examinations revealed severe bilateral collagenofibrotic glomerulopathy, generalized oedema and a focal verminous pneumonia with thrombosis in arterial lung vessels containing nematode cross sections. A serum sample was tested for the presence of antibodies against the cat lungworm *A. abstrusus*, resulting negative. Genetic analyses confirmed the presence of nematode DNA; and, after exclusion of common lung and heart parasites occurring in cats, DNA of the canid heart worm nematode *A. vasorum* was identified. This is the first description of a naturally occurring infection with *A. vasorum* in a cat.

Previous experimental studies demonstrated the development of adult male and female *A. vasorum* worms containing eggs in cats, but no larval excretion in the faeces. Although cats did not become patent, *A. vasorum*

infections were clinically relevant. As *A. abstrusus* and *A. vasorum* are both gastropod transmitted nematodes, they may share the same intermediate hosts within overlapping areas. In addition, especially chronic *A. abstrusus* infected cats become non-patent and do not excrete L1. Considering that patent *A. vasorum* infections are widespread in the dog and fox population in Switzerland but are apparently not patent in cats, we cannot exclude that infections with *A. vasorum* may be misinterpreted and occur more frequently than expected, particularly in highly endemic areas.

OA06.08 Heartworm Control in Grenada, West Indies: Results of a Field Study using Imidacloprid 10% + Moxidectin 2.5% (Advantage Multi®, Advocate®) Spot-on and Doxycycline for Naturally Acquired *Dirofilaria immitis* Infections

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Melarsomine dihydrochloride (Immiticide®) is an effective adulticidal therapy in dogs with canine heartworm disease (CHD), however the risk of post-therapy pulmonary thromboembolism and expense of treatment warrants exploration of alternative treatment options for CHD. This randomized, controlled field study compares the adulticidal efficacy of 10% imidacloprid + 2.5% moxidectin (Advantage Multi®, Advocate®) and oral doxycycline with that of Immiticide®. Thirty naturally-infected dogs with class 1, 2 or early class 3 CHD were evaluated for up to 18 months. Dogs were randomly assigned to a control (CP, n=15) or investigational treatment (IVP, n=15) group. CP dogs received two injections of Immiticide® (2.5mg/kg) 24-hours apart -according to US label- and monthly ivermectin/pyrantel pamoate (Heartgard® Plus). IVP dogs were treated with oral doxycycline (10mg/kg q12h for 28 days) and topical Advantage Multi® / Advocate® once monthly for 9 months. Dogs were evaluated up to 18 months - monthly for the first 9 months, then every 3 months. IVP

data was analyzed for non-inferiority defined as $\leq 15\%$ margin of difference. Parasitocidal efficacy was based on antigen status using the IDEXX PetChek® 34 Heartworm-PF Antigen test. One dog in each treatment group remained antigenemic by month 15. Based on antigen status, non-inferiority was confirmed at months 12, 15 and 18. Non-inferiority was also demonstrated at months 12 & 18 based on results of heat-treatment of antigen-negative samples. Non-inferiority of the ultrasonography data at month 12 was demonstrated as no parasites were observed in 50% and 60% of IVP and CP dogs, respectively. Monthly application of Advantage Multi®/Advocate® combined with oral doxycycline has proven to be an effective, safe and affordable therapeutic alternative for the treatment of CHD and should be considered particularly in cases where finances or debilitated health preclude treatment of an infected individual. This study was supported by Bayer Animal Health.

OA07 Host Responses Against Helminths in Ruminants

July 8, 2019, 13:30 - 15:30
Breakout Room 2, Hall of Ideas E&H, Level 4

OA07.01 IL-5 Dependent Modulation of Eosinophil Responses Augments Haemonchus Contortus Infection in Sheep

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Eosinophils have been associated with protection against *Haemonchus contortus* in sheep both in vitro and in vivo. IL-5 is a key cytokine in eosinophil induction and activation in vivo. In this study, impairment of the eosinophil response was attempted by the administration of three subcutaneous immunizations of Canaria Hair Breed (CHB) lambs with recombinant ovine IL-5 (rIL-5ov) prior to infection with *H. contortus*. Sheep vaccinated with rIL-5ov in Quil-A adjuvant,

had lower blood eosinophil counts and higher mean worm burdens than control sheep vaccinated with Quil-A adjuvant alone. In addition, adult worms in IL-5 vaccinated sheep were significantly longer with higher eggs in utero in female worms. These results suggest that eosinophil plays a direct role in effective control in *H. contortus* infection in CBH sheep.

OA07.02 Fasciola Hepatica Infection in Cattle: Analysing Responses of Peripheral Blood Mononuclear Cells (PBMC) Using a Transcriptomics Approach

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The parasitic helminth *Fasciola hepatica* (liver fluke) causes economic loss to the livestock industry globally and also represents a zoonotic disease. New control strategies such as vaccines are urgently needed, due to the rise of drug resistance in parasite populations. Vaccine development requires a comprehensive understanding of the immunological events during infection. Previous in vivo studies by our group have investigated global differentially expressed genes (DEGs) in ovine peripheral blood mononuclear cells (PBMC) in response to both acute and chronic *F. hepatica* infection. This work demonstrated that pathways involved in the pathogenesis of ovine fasciolosis included inhibition of macrophage nitric oxide production, fibrosis, and antibody isotype switching, among others. Transcriptomic changes in PBMC populations following *F. hepatica* infection in cattle, in which the disease phenotype is quite different, have not yet been examined. Using RNA sequencing we investigated gene expression changes in PBMC isolated from 9 non-infected and 11 *F.*

hepatica-experimentally-infected calves at the day of infection, at 1 week and at 14 weeks post-infection. Longitudinal time-course comparisons among groups revealed 21 and 551 DEGs driven exclusively by *F. hepatica* infection in cattle at acute and chronic stages, respectively. In addition, significant numbers of DEGs related to growth and maturation were detected in both groups at week 14. These results show that fewer DEGs at the acute stage of infection can be identified in cattle, as compared with sheep. Our results reflect the major differences in the disease phenotype between cattle and sheep and may reveal pathways to target in vaccine development. It also highlights the importance of including an uninfected group in the experimental design for transcriptomics-based infection time-course studies to control for physiological changes due to growth and development. The significant differences in DEGs, immunological pathways, and their relevance for the development of anti-liver fluke vaccines are discussed.

OA07.03 Host Effects on *Haemonchus contortus* Larval Traits

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Nematode parasite control is currently based largely around the use of anthelmintics however, alternative approaches including the breeding of animals for resistance to nematode infection is based on the capacity of grazing ruminants to mount a strong immune response against nematode infection. It has been shown that resistant animals are able to influence the percentage establishment of ingested larvae, the survival rate of adult worms and the fecundity of adult females. In this work we investigate the influence of host animal on a range of life-history variables associated with the free-living stages of *H. contortus*.

A group of 5 lambs was infected with the same batch of *Haemonchus contortus* and after patency individual faecal samples were collected, separately cultured and a series of experiments assessing different larval traits

were undertaken: the length of the larvae, larval survival, response to ivermectin in a migration assay, the ability of the larvae to exsheath in vitro, and establish and develop to the adult stage in young lambs.

The results for all traits indicate a significant difference between the host animals, with larvae from specific hosts following a consistent pattern with the highest or lowest trait results. Compared to larvae from Host 1 the larvae from Host 5 were shorter (741 to 692 μm , $p < 0.05$), had a longer median survival (3.6 to 6.4 days), were less susceptible to ivermectin as indicated by their EC_{50} (1.2 to 4.5 μM), exsheathed to a lesser degree (83.6 to 58%), but showed a higher establishment rate in the consecutive host (15.2 to 31.4%). Regarding the survival time, anthelmintic susceptibility and establishment rate as indicators for fitness, the parasites populating Host 5 produced progeny of greater fitness. The findings indicate that the host animal of the parental parasite generation has a significant effect on the parasite progeny.

OA07.04 Performance of Barbervax® Vaccination in Lactating Merino Ewes

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Barbervax® vaccination against *Haemonchus contortus* has been commercially available in Australia since 2014. The optimum use of booster vaccination in lambing ewes has not been identified. The aim of this experiment was to determine the performance of Barbervax® in lactating ewes when administered one week prior to lambing or at lamb marking (seven weeks after lambing began) to reduce worm burden. The experiment used 400 single bearing Merino ewes, previously primed with Barbervax®, on a commercial sheep property in the New England region of New South Wales. The experiment had a complete 2 x 2 factorial design with two treatments administered

pre-lambing [booster vaccination (V) or anthelmintic treatment with Startect® (T) and the same two treatments administered at lamb marking time (7 weeks after the commencement of lambing) providing 4 treatment combinations of 100 ewes each (VV, VT, TV, TT). Taking the start of lambing as week 0 (W0), body weight and blood samples for serological and haematological analysis were conducted at W-1 (prelambing), W7 (lamb marking) and W18 (weaning). Additional bloods were sampled at W9 for antibody test. Worm egg counts (WEC) were taken at lamb marking, six weeks later and at weaning. The results revealed that pre-lambing Barbervax® booster vaccination significantly reduced worm egg output at lamb marking compared to anthelmintic treatment. Booster vaccination given at lamb marking was not effective at reducing WEC compared to anthelmintic treatment and there was no interaction between the effects of pre-lambing and marking treatments. Body weight and packed cell volume did not differ between the treatment groups throughout the trial. It can be concluded that Barbervax® booster vaccination at pre-lambing, but not lamb marking time conferred additional protection for reproducing ewes treated with an effective short acting anthelmintic at these times.

OA07.05 Early Host Immune Responses to the Cattle Gastrointestinal Nematode *Ostertagia Ostertagi*

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Ostertagia ostertagi is an abomasal mucosa-dwelling parasite of cattle that imposes significant economic impact on the US cattle industry. With the rise in drug resistance among nematodes, vaccination is advanced as the most cost-effective control alternative; however, nematode vaccines have met with lackluster results thus far. Hence, elucidating cellular and molecular mechanisms underlying *O. ostertagi* infection is important for developing efficient vaccines. Reports show that a primary

O. ostertagi infection in cattle does not elicit the classic Th2 (anti-inflammatory) paradigm suggesting that an overall mixed response (Th1, Th2, and other types of Th) may dominate. Following infection, infective third stage larvae (L)3s develop into L4s and then to early adults in the abomasal glands and actively produce excretory-secretory (ES) antigens while causing significant tissue damage in abomasum. Little is known about the regulation of immune responses at the host-parasite interface during these early and most important phases of the infection. Our research investigated the early onset of the host immunity. We first determined the kinetic responses of genes involved in innate immunity, such as Toll like receptors (TLRs), cytokines and co-stimulatory markers in peripheral blood mononuclear cells using quantitative real time PCR (qRT-PCR). We then examined the influence of *O. ostertagi* ES antigens on host innate immune cells, i.e., macrophages, by in-vitro cell activation assays, qRT-PCR and immunocytochemistry. Results show that upon progression of the disease, gene expression of a subset of TLR increased indicating active involvement of cellular immune response. Additionally, changes in cell morphology coupled with transcriptional changes in TNF α and TGF β suggest a regulatory phenotype that is consistent with classical signs of macrophage activation. These results demonstrate that the TLR and macrophage mediated responses are initiated early during infection and may be key to elucidating the complex and intimate relationship between *O. ostertagi* and its bovine host.

OA07.06 Abomasum Histomorphometric of Lambs Infected by *Haemonchus Contortus* and Fed with Different Protein Levels

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The aim of this study was to evaluate the effects of dietary protein levels (high: 17% and low: 10%) and *Haemonchus contortus*

infection in sheep. Twenty four Santa Inês lambs, six months old, randomly distributed in a factorial arrangement 2x2: high protein control (HPc n = 5), high protein infected (HPI n = 7), low protein control (LPc n = 5) and low protein infected (LPI n = 7). Lambs were infected in a single oral dose of 10,000 *H. contortus* L3 larvae. After 42 days of the infection, the animals were slaughtered for sample collection of abomasum, that was preserved on formalin 10%, embedded in paraffin for cutting which were stained by hematoxylin and eosin. Ten images from each abomasum were evaluated in 100x lens light microscopy to measure: length of the epithelial and glandular layer (CEG), mucosal muscular thickness (MM), total length of mucosal layer (CM); stereological analysis to calculate density (SD) and surface volume (SV). For the variables CEG, CM and SD, there were effects of diet and infection, but there was no interaction between them, with lower values for HP compared to LP and higher for infected compared to controls ($P < 0.05$). In MM there was only effect of the infection with greater muscle layer for the infected compared to controls ($P < 0.05$). In SV there were no differences between treatments and no interaction ($P > 0.05$). The HP group showed thinner abomasal mucosa. The LP and infected groups had thicker mucosa. In the first case to allow a better digestibility of the lower amount of protein and in the second, the inflammatory process induced by parasitism led to the thickening of the mucosa, the same occurring with muscular layer with increased contractions to eliminate the worms.

OA07.07 Assessment of an Enhanced Sampling Protocol and Use of a ddPCR Assay for Detection of *Haemonchus Contortus* on Commercial Sheep Farms

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A novel sampling strategy based on faeces sampling covering approximately 10% of the animals in the flock and with focus on *Haemonchus contortus* was evaluated. During 76 different sampling occasions 810 individual

samples were collected from 20 conventional and 19 organic sheep farms with between 70 and 250 lactating ewes. The samples were pooled into three to five triplets per flock on each sampling occasion (in total 270) and the fresh samples were examined by microscopy. Droplet digital (dd)PCR assay was also carried through using DNA extracted from frozen faeces. Most farms (95%) were investigated on two occasions, first samples were collected from ewes prior to turn-out and then from lambs after they had been on grass for at least 5 weeks. By microscopy, extra information about the *Haemonchus* status was provided on 48% of the sampling occasions by including more than two triplets which was the standard method irrespective of flock size applied before 2015 in Sweden. At the farm level, detection was further enhanced by 13% by replacing microscopy with the ddPCR assay. There was moderate agreement between the two diagnostic tests (Cohen's kappa 0.49 ± 0.11). On five farms *Haemonchus* was diagnosed by ddPCR only, whereas on one farm the parasite was only found with microscopy. Ewes were more often *Haemonchus* positive than lambs irrespective of the diagnostic method used indicating that the level of parasite control was acceptable on most farms. Although more ewes were infected no difference in FEC was observed between age groups or production systems (conventional or organic). Combined our results show that *Haemonchus* infection is widespread but it can be controlled using information obtained with this sampling strategy. In conclusion, we have developed a practical tool for sheep producers to assess *Haemonchus* infection which can be further enhanced by replacing microscopy with ddPCR.

OA08 Tick Disease Transmission

July 8, 2019, 13:30 - 15:30
Breakout Room 3, Hall of Ideas F&I, Level 4

OA08.01 Immunogens Expressed by Tick Transmitted *Borrelia burgdorferi*

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Majority of human vector borne diseases in the US is primarily transmitted by hard ticks. Lyme disease (LD) caused by spirochetes, *Borrelia burgdorferi* (Bb) and the recently described *B. mayonii*, is the most common human vector borne disease in the US. These LD spirochetes are transmitted by the deer tick, *Ixodes scapularis* during feeding. Nearly 30,000 LD cases are reported to the US CDC each year with 300,000 cases estimated. In order to develop effective methods to prevent LD, understanding the molecular basis of how LD spirochetes interact with their tick vectors and vertebrate hosts is needed. The goal of this study was to identify Bb immunogens that are expressed in both its arthropod (tick) and vertebrate stages. Antibodies to tick transmitted Bb from rabbits that were infested by Bb-infected *I. scapularis* nymphs was used to probe western blots of cultured Bb protein extracts to identify Bb immunogens. More than 20 immunodominant protein bands were excised and processed for LC-MS/MS sequencing and identification. Further, immune sera to tick transmitted Bb will be used to immuno-screen a Bb-genomic DNA phage display library. We discuss our findings with reference to understanding the molecular basis of Bb transmission and progress toward identification of vaccine antigens against LD.

OA08.02 *Borrelia burgdorferi* Infection Modifies Protein Content in *Ixodes scapularis* Nymph Tick Saliva

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Majority of human vector borne diseases in the US is primarily tick borne. Lyme disease (LD) caused by spirochetes, *Borrelia burgdorferi* (Bb) and *B. mayonii* is the most common human tick-borne disease (TBD) in the US. LD is primarily associated with *Ixodes scapularis* nymph tick bites. There is evidence that proteins in saliva of *I. scapularis* nymphs play significant roles in transmission of LD spirochetes. However molecular identities of *I. scapularis* nymph ticks have not been determined. The goal of this study was to identify proteins that Bb-infected *I. scapularis* nymph inject into the host during feeding. We successfully used LC-MS/MS sequencing to identify proteins that uninfected and Bb infected *I. scapularis* nymphs sequentially inject into the host every 12 h during feeding. Overall, we have identified an additional more than 100 proteins in saliva of Bb-infected *I. scapularis* nymphs that were not found in uninfected ticks. These data for the first time reveals molecular identities of tick saliva proteins that might play important roles in transmission of Bb.

OA08.03 Prevention of *Babesia rossi* Transmission by *Haemaphysalis elliptica* on Dogs Treated With Nexgard®

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The objective of this study was to determine the efficacy of afoxolaner (NexGard®) to prevent *Babesia rossi* transmission by *Haemaphysalis elliptica* ticks on dogs. The study was conducted with 3 groups of 7 dogs: G1 and G2 were negative control groups,

infested on Day 2 and Day 28, respectively. G3 dogs were treated group on day 0 and infested by 50 *Haemaphysalis elliptica* ticks on Day 2 and on Day 28. These ticks were originated from nymphs having fed on *B. rossi* infected dogs. Infection rate of the ticks was PCR assessed and it was 12.8% at Day 2 (from a batch of 40 ticks) and 6% at Day 28 (from a batch of 50 ticks). All these dogs had a weekly veterinary examination and a daily visual observation. On Days 0, 7, 14, 21, 28, 35, 42, 49 and 56, and in case of suspicion of fever (i.e. body temperatures > 39.4 °C) or any other babesiosis sign, blood samples were collected for blood smears and PCR detection of *Babesia rossi* DNA. The *B. rossi* infection rate in the untreated control group challenged on Day 2 (group 1) was 100% (6/6). It was 57.1% (4/7) in the control group challenged on Day 28 (group 2). The rate of *B. rossi* infection in the afoxolaner treated groups was 0% (0/7) after the two tick challenges and until Day 56. The ticks were thumb counted at 48 hours and counted and removed at 144 hours after each infestation. The untreated control groups had an arithmetic mean of ticks ranging from 23.8 to 26.8 on these assessment days. Based on arithmetic mean values of live ticks, NexGard® provided 100% acaricidal efficacy against *H. elliptica* ticks on all assessments. This study demonstrated that NexGard® protected dogs against infection by *B. rossi* for a month.

OA08.04 *Haemaphysalis Spinigera* a Vector for Outbreak of Kyasanur Forest Disease in Shimoga: A Malnad Region of Karnataka

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Ticks are next to mosquitoes which are involved in the transmission of variety of disease causing pathogens of public health and veterinary importance. After the first report of fatal Kyasanur Forest disease

(KFD) in the year 1957 from a small pocket of Karnataka state in India, the prevalence of virus in human subject has been reported from adjoining states till 2019. Different species of ticks have been incriminated as possible vector of the pathogen. After the recent reports of monkey's death from local region of Shimoga district, Karnataka, on recommendation of local health care professionals, the ticks were collected from the local cattle breed, malnad gidda, and forest vegetation. The people living adjacent to the forest region of Shimoga are maintaining this breed and leaving the animals in the forest for grazing. A detail history was collected from the people who are living in that area and surrounding forest regions revealed several cases of skin rashes due to tick bites. The ticks were processed and morphologically identified as nymphal and adult stages of *Haemaphysalis spinigera*. The animal owners reported tick bite which causes itching, erythematous, popular lesions on the hands, neck and leg region. The suspected samples from human, dead monkeys and ticks were submitted to National Institute of Virology (NIV), Pune, the nodal Indian agency for case confirmation of KFD. The report confirms the presence of KFD virus in the submitted samples. The health care professionals were suggested to carry out immediate vaccination in order to prevent the outbreak of KFD. The peoples living adjacent to the forest were advised to apply tick repellent oil before entering in to the forest. The spraying of acaricide (Malathion) in the outbreak areas was carried out to manage the tick population.

OA08.05 No Evidence of *Bartonella Henselae* Transmission From Larva to Nymph Stages of *Rhipicephalus Sanguineus* by Using Artificial Membrane Feeding System

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Bartonella spp. are fastidious gram-negative bacteria which confirmed to be or as potential to be human and animal pathogens. *B. henselae* are the cause of cat scratch disease (CSD) in humans, transmitted by cat scratch with claws contaminated with *Bartonella* positive flea feces. There has been considerable interest in ticks as potential vectors for *Bartonella* spp. The aim of the present study was to clarify the vector competency of *R. sanguineus* ticks for *B. henselae* transmission. An artificial membrane feeding system was used to infect ticks with *B. henselae* and to evaluate bacteria transstadial transmission within tick developmental stages. 400 larvae were engorged with *Bartonella*-infected blood, and, after molting, 60 nymphs were engorged on non-infected blood. Blood culture from a feeder was then performed at day 4 of nymph feeding. 200 larvae and 40 nymphs fed with non-infected blood were considered as the control groups. In both experimental and control groups, PCR were applied on various samples, including blood from a feeder, pooled engorged larvae, pooled semi-engorged larvae, pooled larva feces, pooled nymphs and pooled nymph feces. The PCR results showed that all samples were negative for *Bartonella* spp. except blood sample from the experimental feeder. In addition, no bacterial colonies were found after 14 days of incubation from blood culture after feeding of nymphs infected as larvae. These results indicated no evidence of *B. henselae* infection of larvae after tick feeding on infected blood through membrane feeding and no transstadial transmission from larva to nymph stage of *R. sanguineus*. In future, transstadial transmission of *B. henselae* from nymph to adult and transovarial transmission will be evaluated.

OA08.06 Potential Involvement of Salivary Cholinesterase Activity in Arthropod Vector-Borne Disease Transmission

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Arthropod vectors transmit pathogens responsible for 17% of all infectious diseases

globally. Most of these pathogens are transmitted through the vector's saliva during blood feeding. Ticks transmit bacteria, viruses, and protozoans, and in some parts of the world are more important than mosquitoes as vectors of zoonotic diseases affecting humans. The southern cattle fever tick, *Rhipicephalus microplus*, is the most economically important ectoparasite of cattle worldwide. Our previous research documenting the presence of acetylcholinesterase (AChE) in the saliva of *R. microplus* proposed this enzymatic activity could: 1) decrease the toxicity of acetylcholine in the blood meal, 2) modulate host immune responses to facilitate tick parasitism, and 3) facilitate salivary assisted transmission (SAT). We hypothesized further that if tick salivary acetylcholinesterase participates in pathogen SAT then similar enzymatic activity would be present in the saliva of other arthropod vectors. This reports includes evidence of AChE-like activity in the saliva of several ticks, mosquitoes, sand flies, and apparently biting midges. Salivary AChE-like activity was not detected in the horn fly, stable fly, or house fly. Salivary cholinesterase (ChE) activities detected in arthropod vectors exhibited Michaelis-Menten KM values lower than the KM value for bovine serum AChE. A lower KM value is indicative of higher affinity for substrate, and is consistent with a hypothesized role in localized depletion of host tissue acetylcholine and modulation of host immune responses at the vector bite site that might favor hematophagy, which could enable infection by vector-borne pathogens.

OA08.07 Trends in Canine Infection with *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in the Eastern United States: 2010–2017

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Borrelia burgdorferi and *Anaplasma phagocytophilum* are tick-borne infections transmitted by *Ixodes scapularis* in the eastern United States; both agents cause disease in dogs and people. Cochran Armitage trend test ($P < 0.0001$) was used to evaluate changes in annual percent positive results for antibodies to *B. burgdorferi* and *Anaplasma* spp. in approximately 20 million canine tests in 25 states in the eastern United States. From 2010 to 2017, a decreasing trend in percent positive test results for *B. burgdorferi* that ranged from 16.7% to 48.6% was evident in 8 states along the mid-Atlantic coast from Virginia to New Hampshire, and in Wisconsin. In contrast, a continued increasing trend was evident in 7 northeastern and midwestern states where Lyme borreliosis is endemic or emerging, as well as in 3 southern states where endemicity has not yet been established, while no overall trend was evident in Vermont or 5 other midwestern states. Similarly, percent positive test results for *A. phagocytophilum* showed a significant, although smaller, decreasing trend in 6 states along the mid-Atlantic coast from Virginia to Connecticut and Rhode Island, as well as in most states in the Midwest. However, a strong increasing trend was evident in Massachusetts and northern New England as well as Appalachian regions in West Virginia and Pennsylvania. Although percent positive test results continued to increase in regions where Lyme disease and anaplasmosis are more newly endemic, the significant decrease evident in other areas over the 8-year period considered was unexpected. This change may reflect the combined positive influence of canine vaccination, tick control, and routine testing of dogs in regions where these infections have long been endemic. Analysis of trends in canine test results for tick-borne infections continues to be a valuable tool to understand relative geographic and temporal risk for these zoonotic agents.

OA08.08 Species Diversity and Seasonal Distribution of Hard Ticks (Acari: Ixodidae) Infesting Mammalian Hosts in Various Districts of Riyadh Province, Saudi Arabia

Dr Abdullah Alanazi¹, Prof Hamdan Al-Mohammed², Prof Mohamed Alyousif³, Prof Asraf Said⁴, Dr Bashir Salim⁵, Dr Sobhy Abdel-

Shafy⁶, Dr Raafat Shaapan⁷

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Hard ticks are among the most important blood sucking arthropods that transmit pathogens to humans and animals. This study was designed to determine the prevalence, map the geographical distribution and examine the seasonal activity of hard tick species infesting the most common domestic and wild mammals in various districts of Riyadh Province, Saudi Arabia during the period of January to December 2017. A total of 11,587 hard ticks were collected from the bodies of 8,435 animals belonging to 18 different mammalian species. The ticks were preserved in 70% alcohol, and microscopy was used to identify species. Two genera, *Hyalomma* and *Rhipicephalus* were identified, comprising ten species of hard ticks, with *Hyalomma* comprising 68.3% and *Rhipicephalus* comprising 31.7% of species. The most common species found on domestic mammalian hosts was *Hyalomma dromedarii* (Koch, 1844) (39.9%) followed by *Rhipicephalus turanicus* (Pomerantsev, Matikashvili & Lotosky, 1936) (34.9%); while on wild mammalian hosts *Rhipicephalus sanguineus* (Latreille 1806) was by far the most prevalent species (83.0%). These ticks were most abundant from May through July (36.0%) in the studied areas, and tick abundance differed significantly among seasons ($t = 7.854$, $df = 11$, $P = 0.000$). Regression analysis for tick intensity was highly significant on domestic animals ($F = 5.365$, $P = 0.006$), while it was not significant on wild animals ($F = 2.494$, $P = 0.088$). However, this study provides new data on the current status of ticks for human and animal health service managers, as well as for governmental authorities, to gain a better understanding of the occurrence of hard ticks infesting mammalian hosts in Riyadh Province, Saudi Arabia, which can help improve prevention and control of tick-borne diseases, especially during outbreaks.

OA09 Wildlife Parasites

July 8, 2019, 13:30 - 15:30
Breakout Room 4, Hall of Ideas G&J, Level 4

OA09.01 Ticks, Keds, and Citizen Scientists: Working With Hunters to Better Understand Wildlife Parasites

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Ticks are frequently encountered by hunters, outdoor enthusiasts, and rural residents and can carry pathogens that cause diseases such as Lyme disease, Powassan virus Disease, and Anaplasmosis. Deer keds are less well-known ectoparasites of Cervidae that occasionally bite humans. Recent small-scale studies have sequenced pathogens traditionally considered to be tick-vectored from keds, but pathogen distribution, diversity, and occurrence of co-infections in this group are unknown. In addition, despite their actual and potential medical importance, data on the distribution of ticks, keds, and tick-borne diseases in Pennsylvania is lacking. This information, along with basic vector education, is critical to mitigate potential disease risks faced by recreational hunters and their animals. To address this, in 2018 we developed the Pennsylvania Parasite Hunters (www.paparasitehunters.com) citizen science project to engage hunters in surveillance of ticks and keds in the State. Participating hunters ordered postage-paid kits to collect specimens from harvested deer. We had a return rate of 28.4% representing 30 counties in Pennsylvania. In addition, we sampled 85 deer from 24 counties at participating deer processing locations for a combined coverage of 43 of 67 Pennsylvania counties and a total of 753 ked and 1,048 tick specimens. Overall, the pilot was a successful and efficient way to survey wildlife parasites from a wide geographic area and participants were engaged in the study. Pathogen status and multi-state tick and ked behavior observations will be discussed.

OA09.02 Effects of Sarcoptic Mange on American Black Bear Activity and Movement Patterns in Pennsylvania

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Since 1991, sarcoptic mange has increased in American black bears (*Ursus americanus*) in Pennsylvania, both in incidence and geographic distribution and has recently expanded into surrounding states. This skin disease is caused by a parasitic mite, *Sarcoptes scabiei* that burrows under the host's skin as part of its life-cycle. This causes a hypersensitivity reaction in the host that can lead to significant skin disease, as well as atypical behavior, altered activity patterns causing health declines, substantial weight loss, as well as secondary infections. In some cases, these effects can be severe enough to cause mortality. This study aims to characterize changes in movement, resource utilization, and health parameters in bears with sarcoptic mange. Over a two-year period, 36 bears will be fitted with GPS-collars and followed for five years. Between April – October 2018, 28 bears were collared. Bears were collared in triplicates according to gender and similar habitats, consisting of one bear without mange (i.e. healthy control), one bear with confirmed sarcoptic mange treated with a single injection of ivermectin, and one bear with confirmed sarcoptic mange that was untreated. Data were analyzed in R Studio to study differences in movement and activity patterns between these three groups. These data will be used to inform management decisions on whether bears with sarcoptic mange should be treated, will define the efficacy of treatment with a single dose of ivermectin, and will contribute to the present understanding of the effects of sarcoptic mange in black bear populations.

OA09.03 First Report of *Cryptosporidium Parvum* Subtype IIaA16G3R1 in Cervids

Dr. Weslen Teixeira¹, Dr. Márcio de Oliveira³, Dr. Pedro Peres³, Ms/ Walter Bertequini Nagata², Bruna Nicoleti Santana², Ms. Bruno Oliveira², Dr. José Duarte³, Dr. Marcelo Meireles², Dr. Welber Lopes¹, **Unesp Katia Bresciani²**

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We characterized genetically the infections by *Cryptosporidium* in *Mazama gouazoubira*. By a non-invasive harvest methodology using trained sniffer dogs to locate fecal samples of cervids, 642 fecal samples were obtained from six Brazilian localities. The cervid species responsible for the excretion of each faecal sample were identified by the polymerase chain reaction (PCR) performed from the genomic DNA previously extracted. From this identification, 437 fecal samples of *M. gouazoubira* were selected for research of *Cryptosporidium* spp. performed through negative staining with malachite green and polymerase chain reaction (nPCR) followed by sequencing the amplified products. In the samples that were diagnosed the presence of parasite species with zoonotic potential, genotyping was also performed using nPCR with the subunit of GP60 gene. Statistical analysis consisted of the Fisher exact test to verify the association of the presence of the enteroparasite in relation to the presence of cattle in each locality, and the McNemar tests and Kappa correlation coefficient used to compare the results obtained between the two diagnostic techniques. In the fecal samples of *M. gouazoubira* the occurrences of *Cryptosporidium* were diagnosed in 1.6% (7/437) and 1.1% (5/437), respectively, through nPCR and microscopy. *C. parvum* was diagnosed in 100% (7/7) of the samples submitted to sequencing (18S gene). The IIaA16G3R1 subtype was diagnosed in five of the *C. parvum* samples submitted to genotyping (GP60 gene). This is the first world report of *C. parvum* in *M. gouazoubira* and subtype IIaA16G3R1 in cervids.

OA09.04 Mass Mortality in Endangered *Pinna Nobilis* (Linnaeus 1758) Fan Mussels Associated with *Haplosporidium Pinnae*

Dr. Rossella Panarese¹, Dr. Perla Tedesco², Dr. Giovanni Chimienti¹, Professor Maria Stefania Latrofa¹, Professor Francesco Quaglio³, Dr. Andrea Gustinelli², Professor Angelo Tursi¹, Professor Domenico Otranto¹

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The fan mussel *Pinna nobilis* (Linnaeus 1758) is an endemic bivalve of the Mediterranean basin, protected by international legislation as an endangered species. In the early summer of 2018, a mass mortality event (MME) of *P. nobilis* was recorded in the Gulf of Taranto (Southern Italy, Ionian Sea). Moribund specimens of *P. nobilis* were collected by scuba divers and processed by bacteriological, parasitological, histopathological and molecular analyses to investigate the causes of such a MME. Different developmental stages (i.e., plasmodia, spores and sporocysts) of *Haplosporidium* spp. were observed during the histological analysis in the epithelium and in the lumen of the digestive tubules, where mature spores occurred either free or in sporocysts. The spores presented an operculum and an ovoid shape measuring 4.4 µm (±0.232) in length and 3.6 µm (± 0.233) in width. The BLAST analysis of 18SrRNA sequence revealed a high nucleotide similarity (99%) with the reference sequence of *Haplosporidium pinnae* available in GenBank database. Accordingly, at the phylogenetic analysis, 18SrRNA sequence was clustered as a paraphyletic clade with the reference sequence of *H. pinnae*, excluding other haplosporidians (i.e., *Bonamia* and *Minchinia* genera). Based on data reported, *H. pinnae* was the causative agent of MME in the populations of *P. nobilis* sampled in the Ionian Sea, where the conservation of this endangered species is heavily threatened by such a protozoan infection. Further investigations should regard the life cycle of *H. pinnae*, in order to reduce the pathogen spreading and to mitigate the burden of the disease where *P. nobilis* is facing the risk of extinction.

OA09.05 Unravelling the Parasites of Deer

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Parasites are considered to be the most important animal health issue for farmed deer in New Zealand. Deer parasites have seldom been studied in any depth, and deer are host to some unusual nematode species. A better understanding of the infective species present, and their trends, will help inform host-parasite life-cycles, in addition to developing more effective control programs. Our aim is to describe the longitudinal seasonality of egg/ larval output in deer.

Five deer farms were selected on the basis of location, control practices and willingness to participate in the study. Faecal samples were collected from pasture by group (hinds, stags and fawns) every 3-4 weeks. Individual Strongyle faecal egg and lungworm counts were performed, and the remaining faeces pooled by group per farm and cultured to third stage infective larvae (L3). 2000-3000 L3 from each group in time by farm were speciated by deep amplicon sequencing methods.

The results show that farmed deer in New Zealand have very low year-round faecal egg counts, in comparison to other farmed ruminants, with little to no seasonal patterns. Whilst lungworm infections are thought to be the biggest threat to deer farming, the counts were very low for all farms and groups. The speciation data will be presented, once analysis is completed, at the conference. This study will be the first to identify nematode parasites in New Zealand deer by next generation sequencing, and will inform the seasonal pasture contamination and assist with developing control strategies.

There is a disparity in control programs between the farms, generally animals over the ages of 18 months are not drenched, younger animals can be drenched up to 10 times. As the New Zealand deer industry continues to grow, there is an increasing need to better understand the impact of management decisions on sustainable parasite control.

OA09.06 First Record of Possible Life Cycle Stages of a Hepatozoon Blood Parasite Species (Apicomplexa) First Record of Possible Life Cycle Stages of a Hepatozoon Blood Parasite Species (Apicomplexa: Adeleorina: Hepatozoidae) in an Ixodes Tick (Arthropoda: Ixodida: Ixodidae) and an African leopard, Panthera pardus pardus (Linnaeus, 1758)

Mrs. Michelle Van As^{1,2}, Dr. Johann van As², Prof. Oriel Thekisoe¹, Prof. Nico Smit¹

¹North West University, Potchefstroom, South Africa, ²University of the Free State, Phuthaditjhaba, South Africa

Intracellular apicomplexan haemoparasites from the genus *Hepatozoon* Miller, 1908 have been described from a wide range of vertebrate hosts, including wild carnivores. Reports on these haemoparasites from the African Leopard, *Panthera pardus pardus* (Linnaeus, 1758), are scarce and generally non-specific. Furthermore, information on the mode of transmission and descriptions of life cycle stages in infected vectors remains relatively rare. The aim of this study was to explore the role of ticks as potential vectors of a *Hepatozoon* species infecting African leopards in South Africa. Peripheral blood samples and engorged ticks were collected from five wild leopards, three females and two males, while under sedation. Giemsa stained smears of peripheral blood were methodically screened for *Hepatozoon* gamont stages, both extra- and intraleukocytic. Engorged ticks collected from infected leopards were kept alive in a fasting state for seven days before being squashed on clean microscope slides, stained with Giemsa solution, and screened for various possible developmental stages. Sporogonic stages, including microgametes, immature and mature oocysts and infective sporozoites, were observed in a tick (*Ixodes* sp.) collected from a male leopard infected with mature and immature gamont stages of a *Hepatozoon* species. Developmental stages were photographed, differentiated and measured with ImageJ software. This is the first report on the characteristics of different developmental stages of a feline species of *Hepatozoon* in both its potential tick vector and African leopard host.

OA09.07 Sarcosporidiosis: An Emerging Disease in Yaks (*Bos Grunniens*)

Dr Khalid Mehmood^{1,2}, Dr Kun Li¹, Dr Houqiang Luo³, Dr Hui Zhang¹, Dr Muhammad Shahzad², Dr Rao Zahid Abbas⁴

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The sarcocystosis is caused by a coccidian intracellular protozoan belonging to genus *Sarcocystis* (Apicomplexa: Sarcocystidae: *Sarcocystis* tinae). Sarcocystosis is a world-wide distributed parasitic zoonosis. However, scarce information is available about the infection of Sarcocystosis in yaks. Herein, we conducted this study to survey the seroprevalence of Sarcocystosis in yaks on the Qinghai Tibetan plateau (QTP). A total 2549 serum samples were obtained during 2011 to 2017, which were assayed by piloting commercial ELISA kits. The results revealed that the overall seroprevalence of Sarcosporidiosis in QTP yaks was 0.90% (95 CI: 0.6-1.4). The seroprevalence was ranged from 0.20% (95 CI: 0-1.1) to 1.67% (95 CI: 0.8-3.0) in yaks in different areas. The seroprevalence was 0.73% (95 CI: 0.3-1.4) in male yaks and 0.06% (95 CI: 0.6-1.8) in female yaks. In different ages, the seroprevalence were ranged from 0 (95 CI: 0-1.4) to 1.47% (95 CI: 0.6-3.0). In different years, the seroprevalence were ranged from 0 (95 CI: 0-1.4) to 1.86% (95 CI: 0.7-4.0). In the current study, risk factors of region and age were revealed to be the obvious influencing risk factors by piloting conditional step-wise logistic regression. The current study herein first found the emerging infection of Sarcosporidiosis in yaks from high plateaus, which contributes to outline the epidemiological scenario of Sarcosporidiosis in yaks in China. Moreover, our findings highlight the urgent need of Sarcosporidiosis studies in yaks on the QTP for more comprehensive level.

OA10 New Tools and Big Data for Evaluating Intestinal Parasite Infections in Companion Animals

July 8, 2019, 13:30 - 15:30

Breakout Room 5, Meeting Rooms KLOP, Level 4

OA10.01 A Short Review of the 100 Years of Methods Other Than the Direct Smear for Detecting Parasites in Feces

Professor Dwight D Bowman¹

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In 1918, US Army Majors Kofoed and Barber described a brine flotation method they used to examine 100,000 stool samples from service men under diverse field conditions. A skilled examiner could perform 150 to 250 examinations daily, and a staff of 15-20 examiners could process from 2,000 to 3,000 samples daily. The authors stated that "The ova of parasites such as *Ancylostoma duodenale*, *Necator americanus*, *Ascaris lumbricoides*, *Oxyuris vermicularis*, *Trichuris trichiura*, *Taenia saginata*, *Taenia solium*, *Hymenolepis nana*, *Hymenolepis diminuta*, and *Dipylidium caninum* and of trematodes are floated up by the brine into the surface layer of the pool without distortion or noticeable change in appearance during the usual period of examination. Cysts of *Endameba coli* and *histolytica* and of *Giardia intestinalis* are also floated up. Since the ova are at the surface, it is not advisable or necessary to use a cover glass." Additional methods have been developed and compared for the past century, including the Stoll dilution method, the alternate flotation solutions, improved methods with centrifugation, sedimentation methods, the McMaster slide, various stationary flotation devices, centrifugal flotation devices (the FLOTAC and StatSpin Ovatube), the Kato thick smear, the calibrated direct smear, long-distance reading of capture images (FecPak), and potential video recognition software, and many others. The first major methodologic change in veterinary medicine relative to parasite detection came with the development and launch of an assay that could readily detect the presence of cyst wall antigens of *Giardia* in fecal samples. This discovery and other detection methods based on rapid immunologic and molecular methods may provide significant

changes in the future as to how fecal samples are examined. This talk will review the past as we prepare to enter the second century of fecal puddling.

OA10.02 Prevalence of Intestinal Parasites in Pet Dogs in the United States, 2016–2018

Prof. Susan Little¹, Donald Szlosek², DJ McCrann², Donald Martin², Melissa Beall², Troy Goddu²

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To determine the prevalence of parasites in fecal samples from pet dogs in the continental United States and evaluate differences due to age and geographic origin, results from 4,652,566 zinc sulfate centrifugal flotation fecal examinations conducted at IDEXX Laboratories from January 2016 to June 2018 were randomly selected for analysis. Results were grouped by age class and region and analyzed by Chi square and one-way ANOVA with significance assigned at $P < 0.0001$. Parasites identified included *Giardia* (3.3%), hookworms (1.9%), *Eimeria* spp. (1.6%), *Cystoisospora* spp. (1.6%), ascarids (1.5%), and whipworms (0.6%). *Giardia* and *Cystoisospora* spp. were commonly identified in young pups (2–6 months; 12.8% and 6.9%, respectively) but prevalence significantly declined with age (>3 years; 0.2–0.8%). In contrast, detection of *Eimeria* spp. remained fairly constant (1.1–1.7%) throughout all adult age classes (>1 year); by 1–2 years of age, *Eimeria* spp. was the most common protozoa detected. *Giardia* and *Cystoisospora* spp. were most commonly identified in the West (5.8% and 2.7%, respectively), while *Eimeria* was most common in the Northeast (2.3%). Ascarids were most commonly identified in young (5.9%) and older pups (7–12 months; 2.1%), and prevalence significantly declined with age. Older pups were most likely to be shedding hookworm (3.1%) and whipworm (1.5%) eggs; prevalence of hookworms did not fall below 1% until >7 years of age, and whipworms reached its lowest prevalence level >5 years of age. Ascarids were most common in the Northeast, Midwest, and West (1.6%) and least common in the South (1.1%);

in contrast, hookworms were most common in the South (3.0%), while whipworms were most common in the South and Midwest (0.7%). Intestinal parasites, some with zoonotic implications, remain important in pet dogs although prevalence of infection is dramatically reduced compared to that seen in dogs not receiving routine veterinary care.

OA10.03 IDEXX Fecal Antigen Technology: Fecal Dx® Tests

Dr. David Elsemore¹

¹Idexx Laboratories, Inc., Westbrook, United States

The Fecal Dx® panel (antigen detection of ascarids, hookworms, and whipworm) have been on market since 2016. The antigen tests detect excreted or secreted proteins from the young adult to adult stages of each nematode. The discrete protein markers from *Toxocara canis*, *Ancylostoma caninum*, and *Trichuris vulpis* are not associated with reproduction or with eggs and thus provide a novel window into infection in dogs and cats. Experimental infections with the canonical species demonstrate the ability to detect prepatent periods which vary for each nematode life cycle— as soon as 7 days for *T. canis*, 14 days for *A. caninum*, and 46 days for *T. vulpis*. Conservation of the protein markers within each group of nematodes allows breadth of detection beyond the nematode species used to design the assay: ascarid detection includes *T. cati*, *Baylisascaris procyonis*, and *Toxoascaris leonina*; hookworm species include *A. tubaeforme*, *A. braziliense*, and *Uncinaria stenocephala*; and the whipworm test detects *T. felis*. Testing at IDEXX Reference Laboratories has resulted in millions of records that contain both flotation and antigen results. Comparison of egg flotation and the Fecal Dx® panel results shows excellent agreement when egg observations are recorded and when no egg is observed (96.8% to 99%). Disagreement between egg observation and antigen result ranges from 1–3.2% and may reflect fundamental differences between egg flotation and detection of nematode coproantigens.

OA10.04 Agreement of Fecal Antigen Test Results (Fecal Dx®) to Egg Observations Is Influenced by Host and Parasite Parameters—Insights From a Large Field Population of Dogs

Dr. David Elsemore¹

¹Idexx Laboratories, Inc., Westbrook, United States

A data set of 56,512 canine egg positive (ascarid, hookworm, or whipworm) samples with accompanying Fecal Dx® antigen test results was collected providing an opportunity to explore patterns of antigen agreement to egg observations in pet dogs. Various host and parasite parameters could influence the agreement of antigen to egg. Factors that favor patent infections should maximize agreement (egg positive and antigen positive) compared to conditions that disfavor patency (egg positive but antigen negative). To test this idea, antigen to egg agreement for 21,899 *Toxocara* spp. egg positive samples was examined by dog age group. A significant drop in agreement was seen in older dogs consistent with abatement of tracheal-migration in older dogs. This trend was not observed for dogs positive for *Ancylostoma caninum* (n = 12,756). Antigen to egg agreement can also be surveyed across data sets where the dogs were separated into cohorts based on co-observation of pseudoparasites such as *Eimeria*, *Anoplocephala* spp. or strongyle eggs. Antigen to egg agreement should be maximized in dogs without a pseudoparasite co-observation (patent infection more likely) as compared to dogs with a pseudoparasite observation (host behavior(s)/environmental conditions that favor spurious eggs). Significant differences were found for *Toxocara* spp (n = 25,840), *A. caninum* (n = 19,300), and *Trichuris vulpis* (n = 8,028) egg positive dogs between cohorts with and without a pseudoparasite co-observation. The antigen to egg agreement pattern is also different for other species of ascarids or hookworms. *Baylisascaris procyonis* (n = 57) and *Uncinaria stenocephala* (n = 1,970) antigen to egg agreement is significantly lower than observed for *T. canis* or *A. caninum*.

OA10.05 What Is Going On With *Trichuris* in Cats in Florida?

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Data from feral cats in the Miami, Florida region in 2014 indicated a high occurrence of *Trichuris* (>30%). To confirm this level and determine if the same level could be found in other regions of Florida, 143 fecal samples from feral cats (estimated to be 4 months of age or older) in the Miami region, northwest and northeast of Miami and in central Florida were collected in August and October 2018 under an approved IACUC protocol. Samples were analyzed using the IDEXX Fecal Dx® antigen test for whipworm ELISA and centrifugation with zinc sulfate (spg 1.25). Of the 143 samples, 78 (55%) of the cats had at least one parasite infection and 39 (27%) had multiple infections. The most common infection was hookworms (*Ancylostoma* sp. based on location) with 64 (45%) of the cats positive. Twenty six cats (18%) were positive for *Trichuris* sp.: 17 by ELISA and flotation; 8 by ELISA only; and one positive by flotation only. Of these *Trichuris* positive cats, 24 were from the greater Miami region. Occurrence among the tested population from Miami was 42% (24 of 57) while in all other regions was <3% (2 of 86). Cats from one shelter might have been treated; excluding these cats, the occurrence of *Trichuris* outside of the Miami greater region was <2% (1 of 51). While other parasite infections were higher in cats from the Miami greater region, the difference in prevalence was not as extreme. For example, 56% (34 of 57) of the cats were positive for hookworm in the Miami greater region and 46% (28 of 51) from all other areas excluding the suspected treated cats (35% (30 of 86) including the suspected treated cats). The reason for the high occurrence of *Trichuris* in the Miami greater region is unclear and requires further investigation.

OA10.06 Further Comparison of Centrifugation Versus Passive Fecal Flotation for the Recovery of Toxocara Canis, Trichuris Vulpis and Ancylostoma Caninum Eggs

Dr. Michael Dryden¹, Dr. Brian Herrin¹, Dr. Patricia Payne¹, Vicki Smith¹, Debra Ritchie¹, Evan Hegarty²

¹Kansas State University, Manhattan, United States, ²IDEXX Laboratories, Westbrook, United States

Between 2010 and 2018, 239 individuals in 29 classes participated in a week-long clinical parasitology training program. Participants were either veterinarians (233) or persons with advanced training in parasitology (6). As part of the course, attendees participated in a fecal diagnostic wet lab. Fecal samples were collected from dogs at the local animal shelter and verified as positive for various parasite diagnostic stages. While species of parasites and egg counts varied between classes, all classes evaluated samples that contained *Ancylostoma caninum*, *Toxocara canis* and *Trichuris vulpis* eggs. Each participant conducted a direct smear, a commercial passive floatation device (Ovassay[®]) with 1.18 sp. gr. ZnSO₄ solution, a centrifugation procedure using 1.18 sp. gr. ZnSO₄ solution and a centrifugation procedure using 1.27 sp. gr. sugar solution. Participants recovered *A. caninum*, *T. canis*, and *T. vulpis* eggs from 71.9% (172/239), 61.1% (146/239), and 37.7% (90/239) of the samples, respectively when using the Ovassay[®] device. When comparing centrifugation techniques, participants were more likely to recover *T. vulpis* eggs using the higher sp. gr. sugar solution, 96.7% (231/239), than using ZnSO₄, 80.3% (192/239) ($p < 0.001$). Recovery of *A. caninum* and *T. canis* did not significantly differ between the centrifugation methods ($p_{AC} = 0.81$; $p_{TC} = 0.25$). Reliability of the Ovassay[®] technique was significantly inferior to centrifugation for each parasite ($p_{TCZ} < 0.001$, $p_{TCS} < 0.001$, $p_{ACZ} < 0.001$, $p_{ACS} < 0.001$, $p_{TVZ} < 0.001$, $p_{TVS} < 0.001$). *Trichuris vulpis* eggs were recovered by every participant in the class only 5% (1/29) of the time by Ovassay[®] passive flotation, compared to 34.5% (10/29) and 75.8% (22/29) of the time by ZnSO₄ or sugar centrifugation, respectively. This data provides further evidence that passive fecal flotation is an

inferior fecal technique and should not be considered as a reliable diagnostic test in practice, especially in areas where *T. vulpis* infections are common.

OA10.07 US Dog Owner Compliance With Veterinary Recommendations for Heartworm Medication Use in Dogs That Also Receive Flea and Tick Medication

Dr. Robert Lavan¹, Dr. Kathy Heaney¹, Dr. Srinivasan Rajagopalan², Dr. Kaan Tunceli³
¹Merck Animal Health, Perkasie, United States, ²Med Data Analytics, New York City, United States, ³Merck, Inc., Kenilworth, United States

Many flea and tick medications, including some of the newer isoxazoline products, are given to dogs monthly. Many heartworm prophylaxis medications are also re-dosed monthly. If dog owners use an extended duration flea and tick product, dosed at 12-week intervals, then will they also be as compliant with veterinary monthly heartworm medication recommendations as owners of dogs receiving a monthly flea and tick product.

Clinic transaction records for over 200,000 dogs from approximately 650 veterinary clinics in the United States were examined for June, 2014 through November, 2017. Dog owners were identified as “pure users” and put in non-overlapping groups for particular flea and tick medications, meaning that they had purchased at least one dose of a flea/tick medication without switching to another product during a 12-month period. Brands of monthly flea/tick products were compiled as a single comparator group.

The number of doses and treatment duration for purchases of 14 pooled heartworm medications were compared using two flea/tick prevention treatment groups (12-week extended duration versus monthly duration). Dog owners who purchased a longer-acting flea and tick medication purchased slightly more heartworm medication annually for their dogs compared with dog owners who purchase monthly flea and tick medication; however, this increase is not large enough to be biomedically significant.

OA11 Canine Protozoa

July 8, 2019, 16:30 - 18:00

Plenary Hall, Madison Ballroom (ABCD), Level 4

OA11.01 Prevalence of Trypanosoma Cruzi Infection in Domestic Dogs and Kissing Bugs in Oklahoma

Miss Layna Tarpalechee¹, Miss Megan Wohltjen¹, Mrs Alexa Hunter¹, Dr Susan Little¹, Dr Jerry Ritchey¹, Mrs Jana Slaughter², Dr Justin Talley², Dr Kelly Allen¹

¹Department of Veterinary Pathobiology, Oklahoma State University Center for Veterinary Health Sciences, Stillwater, United States, ²Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, United States

Trypanosoma cruzi is the triatomine-borne hemoprotozoan causing Chagas disease in canines and humans in the Americas. Clinical manifestations of the disease range from asymptomatic to acute myocarditis and sudden death to chronic disease with eventual heart failure. Antibody screening of owned or impounded dogs (n = 304) in eastern Oklahoma between years 1996–1997 indicated an overall seroprevalence of 3.6%. Recent surveys estimating *T. cruzi* infection prevalence in domestic dog populations within the state are lacking. In 2018, we tested shelter dogs (n = 170) in eastern Oklahoma (Tulsa County and Le Flore or surrounding county) for *T. cruzi* reactive antibodies by ELISA (Chagas STAT-PAK Assay[®]) and FFPE cardiac tissues from client-owned dogs diagnosed with myocarditis (n=89) between 2007–2017 at the Oklahoma Animal Disease Diagnostic Laboratory (OADDL, Stillwater, OK) for molecular evidence of *T. cruzi* microsatellite DNA. In addition, we analyzed submitted kissing bugs (n = 61) from 15 counties in Oklahoma for evidence of *T. cruzi* DNA in insect abdominal extracts, as well as for the molecular identification of remnant vertebrate blood meal source. Thirty (17.5%) of the 170 canine samples were positive for *T. cruzi* reactive antibodies, which is a significantly higher seroprevalence than previously documented in Oklahoma (p<0.0001), and one (1.1%) of

89 cardiac samples was positive for *T. cruzi* DNA. Fourteen (23%) of the 61 submitted kissing bugs from Oklahoma were positive for *T. cruzi* DNA; vertebrate host remnant blood meal identities could not be determined for the majority of dissected bugs, but seven (11.5%) individual kissing bugs were found positive for human DNA, and one (1.6%) bug was positive for canine DNA. These serologic and molecular data collected from domestic dogs together with molecular data from volunteer submitted kissing bugs suggest that *T. cruzi* may be an under recognized pathogen cycling in eastern Oklahoma.

OA11.02 Black-Backed Jackals and African Wild Dogs Serve As Reservoirs for the Pathogenic Babesia of Domestic Dogs in South Africa

Ms Angy Shabangu¹, Prof Banie Penzhorn¹, Prof Marinda Oosthuizen¹, **Prof Paul Matjila**¹

¹Vector and Vector-Borne Research Programme, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa

Canine babesiosis caused by *Babesia rossi* is a highly pathogenic disease of susceptible domestic dogs. *Babesia rossi* occurs freely in indigenous wild canids and in domestic dogs in South Africa. The aim of this study was to investigate the occurrence of *B. rossi* in asymptomatic indigenous wild canids (black-backed jackals and African wild dogs) and in susceptible domestic dogs.

Wild canids blood samples were collected from (an apparently) healthy populations of free ranging black-backed jackals (n=75) and captive black-backed jackals (n=25). Additional samples from free-ranging African wild dogs (n=52) were also obtained, including 5 samples collected at a zoo from dead wild dogs. Domestic dogs samples (n=75) were collected from dogs suspected with canine babesiosis.

Reverse line blot hybridization assay revealed occurrence of *B. rossi* in 29% of free ranging black-backed jackals, 28% in captive black-backed jackals and 10% in free ranging African

wild dogs respectively. A majority of domestic dogs (88%) were found to be positive for *B. rossi*.

Phylogenetic analysis revealed sequence identity to *B. rossi*, based on the 18S rRNA gene sequences from indigenous wild canids and susceptible domestic dogs. The *B. rossi* that occurs in asymptomatic indigenous wild canids appears to be the same as the one that occurs in susceptible domestic dogs. Thus, this study was able to indicate that since wild canids and domestic dogs share similar pathogenic strain of *B. rossi*, wild canids may serve as reservoirs of *B. rossi*. This highlights the importance of tick control, especially in domestic dogs as they are more susceptible to *B. rossi* infections which appear to be less problematic to wild canids.

OA11.03 Transmission of Anaplasma Phagocytophilum (Foggie 1949, Dumler et al. 2001) by Ixodes Spp. Ticks Feeding on Dogs and Artificial Membranes

Dr Josephus Fourie¹, Mr Alec Evans², Mr Riaan Maree¹, Dr Michel Labuschagne³, Dr Maxime Madder³, Dr Matthias Pollmeier⁴, Dr Bettina Schunack⁴

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The transmission time of *Anaplasma phagocytophilum* (Ap) was evaluated in vivo using *Ixodes ricinus* ticks on dogs, and in vitro using *I. ricinus* and *Ixodes scapularis* on artificial membranes. Infectivity of ticks was confirmed by qPCR on pools and ranged from 21 to 100%.

In vivo study 1: Six groups (G) of 3 dogs each were infested on Day 0 with Ap infected adult ticks (50 in G1 to G5, 60 in G6). Ticks were either removed at 3, 6, 12, 24 and 48 hours (h) post-infestation (G1 to G5), or left until engorged (G6). Starting Day 0 blood was collected weekly for qPCR and serological analysis. Ap specific antibodies and DNA were detected in the 3 dogs in G6 only. No clinical symptoms of Ap were observed.

In vitro studies: Attachment of Ap infected ticks in artificial seeding chambers was assessed and blood pools were sampled using qPCR at 6 hour intervals, up to 72h after first female tick attachment.

In vitro study 1: Sixty artificial feeding chambers were seeded with 5 male and 5 female *I. ricinus* ticks each. Attachment ranged from 20-60%. Ap DNA was detected in 5% of chambers already at 6h. The highest percentage of positive samples (16.3%) was observed at 36h.

In vitro study 2: Artificial feeding chambers (47 for *I. ricinus*, 34 for *I. scapularis*) were seeded with one adult male and female tick each. Ap DNA was detected in 38.24% for *I. scapularis* and 4.26% for *I. ricinus* chambers at 6h. The highest percentage of positive samples was observed for *I. ricinus* at 18h (21.95%) and *I. scapularis* at 24h (65.38%). Conclusion: Transmission of Ap starts within a few hours of tick attachment (within 6h in vitro). However detectable infections of Ap in dogs are apparently dependent on a minimum inoculation dose.

OA11.04 Prevalence of Vector-Transmitted Infections in Dogs in Berlin/Brandenburg

Christina Helm¹, PD Dr. Jürgen Krücken¹, Dr. Jana Liesner¹, Prof. Dr. Manuela Schnyder², Dr. Corinna Weber³, Dr. Elisabeth Müller³, Dr. Roland Schaper⁴, Dr. Stefan Pachnicke⁴, Dr. Hans-Frieder Matthes⁵, Dr. Daniel Schaarschmidt-Kiener⁶, Prof. Dr. Peter Deplazes², Prof. Dr. Barbara Kohn⁷, Prof. Dr. Georg von Samson-Himmelstjerna¹
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Vector-borne diseases are spreading because

of global warming, changing land use and socioeconomy. They pose important animal and human health risks. A previous PCR-based study had revealed low prevalences of vector-borne pathogens in dogs and no evidence for autochthonous transmission of *Babesia* spp., *Ehrlichia canis* and filaroids.

Between May 2016 and April 2018, 1008 serum samples of dogs from Berlin/Brandenburg were tested for presence of antibodies against or circulating antigens of vector-borne pathogens. Samples were tested with IDEXX SNAP®4Dx®Plus for antibodies against *Anaplasma* spp., *Ehrlichia* spp. and *Borrelia* spp. and *Dirofilaria immitis* antigen. Conventional ELISAs were used for detection of antibodies against *Anaplasma* spp., *Babesia* spp., *Leishmania* spp., *Dirofilaria* spp. and *Angiostrongylus vasorum* and *A. vasorum* antigen. Additionally, DNA samples from the previous study from dogs and foxes in Brandenburg were screened for *Hepatozoon canis* by PCR.

While antibodies against *Dirofilaria* spp. were detected in 22/1008 (2.2%) of the serum samples, only 3 (0.03%) were positive for *D. immitis* antigen. Furthermore, 66 (6.5%), 9 (0.1%) and 17 (1.7%) of 1008 samples were positive for *Anaplasma* spp., *Ehrlichia* spp. and *Borrelia* spp. in the SNAP test. In contrast, the complete antigen-based *Anaplasma* ELISA detected 306/1001 samples (30.6%) positive. Regarding *A. vasorum*, 29/1008 (2.9%) samples were antigen-positive, 35 (3.5%) were antibody-positive and 12 (1.2%) were double positive. *Leishmania* spp. and *Babesia* spp. antibodies were detected in 14/991 (1.4%) and 26/1008 (2.6%) samples, respectively. Using the *H. canis* PCR, 46/1050 dogs (4.4%) and 156/201 foxes (77.6%) were positive. Genotyping and travel history of dogs suggest autochthonous transmission of *H. canis* between foxes and dogs in the absence of known vectors. In contrast, autochthonous transmission is so far not a major issue for the Mediterranean and tropical parasite species in local dog populations despite of presence of vectors for some of the pathogens.

OA11.05 Prevalence of *Babesia microti*, *B. Odocoilei* and Co-Infection Rates With *Borrelia burgdorferi* in *Ixodes Scapularis* Nymphs in Wisconsin

Tela Zembsch¹, Dr. Xia Lee¹, Dr. Gebbiena Bron¹, Dr. Lyric Bartholomay¹, Dr. Susan Paskewitz¹

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Babesiosis is a malaria-like disease caused by parasites within the *Babesia* genus. *Babesia microti*, shares a mammalian reservoir, *Peromyscus leucopus*, and a vector, *Ixodes scapularis*, with the Lyme disease spirochete, *Borrelia burgdorferi*. Similarly, *Babesia odocoilei* is transmitted by *I. scapularis* and infects white-tailed deer, *Odocoileus virginianus*. The interactions of these disease agents may allow for either competition or amplification in tick or animal hosts. The two main goals of this study were 1) to calculate the prevalence of *B. microti* and *B. odocoilei* in *I. scapularis* nymphs from multiple locations distributed across Wisconsin and 2) to determine if there is an observable association between these agents in the vector. Over 2,500 nymphs were collected from parks and natural areas throughout the state from 2016-2018 and tested for pathogens via real-time PCR. Prevalence was calculated for the individual pathogens and a Fisher's exact test was used to determine if an association between either of the *Babesia* spp. and *B. burgdorferi* occurred. Overall, there was a strong tendency for ticks infected with *Babesia microti*, but not *B. odocoilei*, to be co-infected with *Borrelia burgdorferi*. Some ticks were co-infected with both *Babesia microti* and *B. odocoilei*, suggesting that the latter parasite is transovarially transmitted from female ticks to their eggs and offspring. Understanding the prevalence and biotic interactions between pathogens enhances veterinarians' and wildlife managers' ability to recognize and respond to disease.

**OA12 Diagnosis and Decision Support
for GI Nematodes in Ruminants II**

July 8, 2019, 16:30 - 18:00

Breakout Room 2, Hall of Ideas E&H, Level 4

**OA12.01 Rapid Assessment of Faecal Egg
Count and Faecal Egg Count Reduction
Using Composite Sampling in Cattle**

Dr Laura Rinaldi¹, Alessandra Amadesi¹, Dr Elaudy Dufourd², Antonio Bosco¹, Marion Gadanho², Anne Lehebel², Dr Maria Paola Maurelli¹, Dr Alain Chauvin², Dr Johannes Charlier³, Dr Giuseppe Cringoli¹, Dr Nadine Ravinet², **Dr Christophe Chartier²**

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Faecal egg counts (FEC) and FEC reduction (FECR) tests for assessing gastrointestinal nematode infection and anthelmintic resistance are rarely carried out on ruminant farms because of the cost of individual analyses. The use of composite (pooled) faecal samples in which equal amounts of faeces from several animals are mixed and analyzed together is a promising method to reduce time and costs, but few studies are available, especially with regard to the evaluation of different pool sizes and its application in FECR test. A total of 29 groups of cattle were investigated in Italy and France (9 to 20 animals/group). In each group, individual faecal samples from heifers (6 to 20 month old) were collected before (D0) and two weeks after (D14) anthelmintic treatment (ivermectin or benzimidazoles). FECs were determined individually and as pooled samples using the Mini-FLOTAC technique. Three pool sizes were used from all the animals of each group: pools of 5 or 10 individual samples or global pool. The mean FEC of individual and pooled samples were calculated as the arithmetic mean and FECR% was $[1 - (\text{arithmetic mean FEC post treatment} \div \text{arithmetic mean FEC pre-treatment})] \times 100\%$. Correlations and agreements between individual and pooled results (FEC and FECR%) were estimated with the Spearman's and the Lin's correlation coefficients respectively (rs and pc). High correlation

and agreement coefficients were found between mean FEC of individual and pooled samples. Values were in the same range for the different pools (0.95 to 0.98 for rs and 0.97 to 0.99 for pc) and indicated that any of pooling strategy was efficient. In contrast, %FECR calculated from individual FECs and from pooled FECs showed lower values for Spearman rs (0.67 to 0.80) and Lin's pc (0.49 to 0.74) meaning that composite sampling has a lack of reliability for estimating FECR%.

**OA12.02 Using Remote Sensing Technologies
for the Detection of Parasitism in Sheep**

Dr Seer Ikurior¹, Professor Bill Pomroy¹, Dr Ian Scott¹, Dr Rene Corner-Thomas¹, Dr Stephan Leu², Dr Nelly Marquetoux¹

¹Massey University, Palmerston North, New Zealand, ²Macquarie University, Sydney, Australia

Animals suffering from parasitism typically display altered grazing behaviour and a voluntary reduction in feed intake. These changes are potentially important as indicators of disease. Recent advances in GPS and accelerometer technologies provide the opportunity to objectively measure animal behaviour while on pasture. GPS tracking collars are suited to monitor and detect changes in movement patterns. Triaxial accelerometers measure body movement in terms of acceleration, which can then be used to estimate physical activity over time. Two preliminary studies using these technologies are presented. In study one, the accuracy and performance of seven commercially sourced GPS receivers were assessed. A motion test was used to assess the receivers' prediction of distance travelled against the inner lane of an athletics track. Stationary tests assessed how dispersed location estimates from the receivers were from a "true" location landmark. Six of seven receivers returned <5 m errors in location estimates. The implication of error rates for measuring distance travelled in parasite-infected and uninfected animals is discussed. In study two, the total activity levels of two groups of crossbred Romney and Suffolk ram lambs were monitored over a period of four days using triaxial accelerometer sensors

after 42-days exposure to treatment. On day zero, all lambs were given anthelmintics. Subsequently, a Suppressive Treatment Group (S) (n=12) was treated with anthelmintics every two weeks. An Untreated Group (U) (n=12) did not receive anthelmintics. Total activity levels were monitored from days' 42 – 46. Activity level was calculated as vectorial dynamic body acceleration (VeDBA). Parasite burdens measured by faecal egg counts were low (mean S = 50 eggs/g; mean U = 500 eggs/g). Activity levels in untreated lambs were significantly less than in the Suppressive Treatment group ($P=0.024$). This small but significant difference in total activity indicates usefulness of this approach in detecting subclinical parasitism.

OA12.03 Motility Based Assays Using Cultured Fourth Stage Larvae Fail to Provide Consistent Discrimination Between Known Avermectin-Resistant and -Susceptible Isolates of *Cooperia* spp. Motility Based Assays Using Cultured Fourth Stage Larvae Fail to Provide Consistent Discrimination Between Known Avermectin-Resistant and -Susceptible Isolates of *Cooperia* Spp.

Kelsey Paras¹, Ray Kaplan¹

¹*University of Georgia, Athens, United States*

The fecal egg count reduction test (FECRT) is the only method commonly used for diagnosing anthelmintic resistance in GIN of cattle, but is time-consuming and potentially cost-prohibitive. Consequently, there exists a need to develop better methods for diagnosing resistance. Assays based on larval motility are used commonly for screening potential drug candidates, but previous work in our lab demonstrated that the L3 stage failed to discriminate between avermectin-resistant and susceptible isolates of *Cooperia* spp. The L4 may be a better stage for this purpose because it is a parasitic and actively feeding life stage without a double cuticle. The L4 were cultured by exsheathing L3, maintaining them in media at 37°C and 20% CO₂, with media changes and observation every 48 hours for nine days. Three avermectin-resistant and two avermectin-susceptible GIN isolates (diagnosed by FECRT) containing >90% *Cooperia* spp.,

were used. Three biological replicates were performed for each parasite isolate using both eprinomectin and ivermectin. Eleven drug concentrations from 0.01µm to 40µm and negative controls were evaluated. Motility readings were taken using the Worminator system before addition of the drug and at 24 and 48 hours post drug exposure. Differences in EC₅₀ for ivermectin were highly variable between susceptible and resistant isolates; resistance ratios (RR) ranged from 0.425-9.47. In contrast, eprinomectin assays had much less variability and consistently yielded similar EC₅₀ for all isolates (RR of 0.958-1.04). Though significant differences ($p<0.05$) in percent inhibition were found at some drug concentrations in some assays, there were no consistent significant differences in the dose-response across comparative assays or for the different drugs. Inhibition was greater for the susceptible isolate in about half of the assays. The lack of consistency in these data indicates that motility of L4 is not a reliable diagnostic phenotype for measuring resistance to avermectin drugs in *Cooperia* spp.

OA12.04 What Are Good Marker Genes for Strongyle Nematode Species Identification and Phylogenetic Reconstruction?

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Species descriptions and identification keys to discriminate closely related strongyle nematodes rely on differences in morphological traits – particularly of the buccal capsule and the male bursa. In the absence of extensive data on intra-species morphometric variation, molecular approaches have mainly focused on the intergenic-spacer (ITS) of the nuclear ribosomal genes, the mitochondrial genome,

particularly the cytochrome oxidase I (COI) and the 12S and 16S rRNA genes. Using data on *Cooperia* species infecting cattle and on equine *Cyathostominae*, this study analysed the suitability of different nuclear and mitochondrial sequences for reliable species identification and phylogenetic reconstruction using maximum likelihood. Using ITS-2 sequences, most but not all species could be differentially identified using phylogenetic analyses but not by simple comparison of identities. In contrast, mitochondrial markers had much better barcoding properties, but resulted in poor phylogenetic resolution, both due to saturated variation in codon position 3. Optimal trees were obtained by partitioned analyses of both nuclear and mitochondrial markers. In *Cooperia*, multi-locus analysis of sequences obtained from individual worms clearly discriminated *Cooperia oncophora* and *Cooperia pectinata*. However, *Cooperia punctata* and *Cooperia spatulata* always formed a common cluster, suggesting that the latter is only a morphotype of *C. punctata*. In the *Cyathostominae*, close relationship between *Coronocylus coronatus* and *Cylicostephanus calicatus* was revealed by virtually identical ITS-2 sequences. Concurrent analysis of nuclear and mitochondrial sequences allowed the discrimination between mitochondrial lineage sorting and potential speciation. Cryptic species were detected in *Cylicostephanus minutus* (3), *Cys. calicatus* (2) and potentially *Cylicocylus nassatus* (3). *Cylicostephanus labiatus* showed diverse mitochondrial and nuclear haplotypes that were mixing with each other, while *Cylicostephanus longibursatus* was comparatively homogenous. In conclusion, combined analysis of nuclear and mitochondrial haplotypes from the same morphologically identified specimens, improved resolution of analyses and should be applied to more species and specimens from various geographic regions.

OA12.05 A Simple Method for Identifying and Quantifying Cattle GI Nematodes

Dr. Dante Zarlenga¹, Dr. Carly Barone¹, Ms. Deborah Hebert¹

¹*Agricultural Research Service, Animal Parasitic Diseases Lab, Beltsville, United States*

Classic approaches for identifying gastrointestinal nematodes (GIN) from fecal eggs require cultivation to infective L3 followed by morphological examination. Over the years, a plethora of molecular techniques have surfaced, most which are predicated upon real-time PCR or PCR amplification followed by gel analysis. While adequate for diagnosis, many techniques fall short of easily quantifying mixed infections without substantial numbers of controls and/or standard curves. Herein we developed a simple and rapid test for differentiating and quantifying mixed infections of GI nematodes using fluorescently-labeled PCR products and a capillary-based sequencer. The assay can be easily scaled for large scale analyses and does not require external controls. Among the common cattle GIN, the ITS2 region is sufficiently distinct in length to delineate among infecting genera. As such, conserved PCR primers that span the ITS2 and bind to the 3'-end of the 5.8S rRNA (forward) and the 5'-end of the 18S rRNA (reverse) were synthesized, one of which was fluorescently-labeled with FAM. DNA from infective L3 was isolated, PCR amplified, diluted directly in HI-DYE sequencing buffer containing LIZ 500 standard, then loaded onto an ABI 3100 sequencer adapted for size fragment analysis. Validation was first performed on monospecific infections of *Haemonchus*, *Ostertagia*, *Cooperia*, *Trichostrongylus* and *Oesophagostomum*. As proof of principle, L3 from *Ostertagia ostertagi*, *Cooperia punctata* and *Haemonchus contortus* were mixed in pairs and in 10% increments, followed by DNA isolation and analysis for relative DNA levels using Gene Marker V1.85. In all cases, experimental data generated a linear response and correlated well with predicted values where $R^2 > 0.95$ with line slopes between 0.90-1.1. The assay was tested on environmentally-derived samples and coincided well with coproculture, PCR gel analysis and deep amplicon sequencing. Data show that primer design and purification are critical to eliminating satellite bands and enhancing quantitative analyses.

OA12.06 Investigating the Diversity and Prevalence of Pathogens in Cattle in the Livestock-Wildlife Interface of Kwale County, Kenya.**Brenda Odhiambo**¹, Dr Shewit Kalayou¹, Dr Dan Masiga¹, Dr Michael Okal¹¹*International Centre For Insect Physiology And Ecology, Nairobi, Kenya*

Livestock keepers in areas bordering wildlife protected areas in Africa bear the brunt of bovine trypanosomosis and a plethora of emerging and re-emerging pathogens. In Kwale, Kenya, bovine trypanosomosis has been the focus of many interventions around the protected wildlife hotspot of the Shimba Hills National Reserve (SHNR). Though some studies have detected tick-borne pathogens in or near the SNHR, the prevalence and overlap of these remains unknown. This study implemented a longitudinal survey in four villages around the Shimba Hills National Reserve in Kwale to determine the diversity of pathogens in cattle. A cohorts of 120 cattle from twelve households within 500 m from the park were screened for bacterial, viral and protozoal pathogens. All households were from villages at least three kilometers apart. Rapid and high-throughput screening of the samples for pathogens was done using the high resolution melting analysis of PCR products. About 30.8% of livestock were infected with *Trypanosoma congolense*. 16.7% of animals were infected with one of four *Anaplasma* sp (An, ovis, An. marginale, An. platys or An. phagocytophilum, 15.5% had *Theileria velifera*, 13.5% *Ehrlichia* sp. and 9.7% *Rickettsia* sp. Viral infections were detected in 37.3% of the sampled livestock. These were predominantly bunya (38.9%), thogoto viruses (40.0%) and phlebo (12.2%) viruses, with a few nairo (5.6%) and alpha (3.3%) viruses also detected. These results highlight the complex disease environment of the wildlife-livestock interface in Kwale and, by extrapolation that of agro-pastoralism near wildlife protected areas in Kenya. The collective potential impact of these on the productivity and production of cattle and their impact on the success of controlling of bovine trypanosomosis, though not investigated here is discussed.

OA13 Flea and Tick Treatment

July 8, 2019, 16:30 - 18:00

Breakout Room 3, Hall of Ideas F&I, Level 4

OA13.01 Efficacy of Oral Afoxolaner (NexGard®) Against *Cochliomyia Hominivorax* (Diptera, Calliphoridae) Larvae Infestation in Naturally Infested Dogs in Brazil**Andre Cutolo**¹, Ingrid Menz², Patricia Thyssen³, Fabiano Silva⁴, Fred Beugnet⁵¹*Boehringer Ingelheim Animal Health Do Brasil Ltda., Paulínia, Brazil*, ²*Ingrid Menz ME, Campinas, Brazil*, ³*Universidade Estadual de Campinas - Unicamp, Campinas, Brazil*, ⁴*Boehringer Ingelheim Animal Health, Fulton, United States*, ⁵*Boehringer Ingelheim Animal Health, Lyon, France*

New World screwworm disease is an infestation with the larvae of the screwworm fly (*Cochliomyia hominivorax*) that lives off the flesh of living warm-blooded animals, including humans. Domestic dogs are among the potential hosts of the fly that lay eggs on pre-existing bleeding open wounds where larvae feed and develop on live tissue. Disease can be fatal if untreated. Treatment with parasiticides must be quick-acting, 100% efficacious and long-lasting, since open wounds can be reinfested. Afoxolaner is a molecule from the isoxazoline family with proven ectoparasiticide action against fleas and ticks infesting dogs. Afoxolaner (NexGard®) was administered from doses ranging from 2.7 mg/kg to 6.7 mg/kg (same dose as to control fleas and ticks) on dogs naturally infested with *Cochliomyia hominivorax* larvae in the municipality of Campinas, São Paulo state, Brazil. From March 2017 to April 2018, 14 dogs having active lesions infested with *C. hominivorax* larvae were included in the study. Infestations were classified according to the number of larvae recovered post-treatment as light (less than 10 larvae), moderate (from 10 to 20 larvae) and severe (more than 20 larvae). No adverse events were observed after treatment. Two hours after treatment intensification of larval movements on the lesions was observed. Six hours post-treatment it was possible to observe that some larvae were expelled from

the lesions. Twenty four hours after treatment all larvae were killed on all treated animals. Total amount of larvae recovered ranged from 3 to 327 larvae per animal (average of 67.7). Afoxolaner (NexGard®) at doses ranging from the minimum active dose of 2.5 mg/kg to the maximum dose 6.7 mg/kg (mean of 3.9 mg/kg) was rapidly efficacious for the treatment of 14 dogs with light, moderate and severe infestations with *Cochliomyia hominivorax* larvae, by eliminating all larvae within 24 hours after a single oral treatment.

OA13.02 Lotilaner Is a Potent Inhibitor of the Novel GABA Receptor of Body Lice *Pediculus Humanus Humanus*

Nicolas Lamassiaude¹, Dr Berthine Toubate², Dr Pierre Charnet³, Dr Cédric Neveu¹, Dr Françoise Debière-Grockieo², Dr Claude Charvet¹, Pr Isabelle Dimier-Poisson²
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Drug resistance in the parasites field, including the cosmopolitan lice (*Pediculus humanus*), and the prevalence increasing despite the marketing of new therapies are an important challenge for our societies. The major pharmacological targets of insecticides like pyrethrins, malathion, spinosad and ivermectin (also used as nematocide and acaricide) are the ligand gated ion channels present in the nervous system of insects. Currently, targets of these molecules remain largely unknown in body lice. Among those channels receptors, γ -aminobutyric acid gated chloride ion channels (GABACl) are the main synaptic inhibitory receptors in insects, making them pertinent pharmacological targets.

In the present study, we identified and characterized the targets of insecticides in lice to decipher the mode of action of insecticides in *Pediculidae*. Research in the genomic databases of *Pediculus humanus* allowed us to identify a GABACl subunit encoded by the Resistance to dieldrin (Rdl) gene. We cloned the corresponding full-length cDNA into a transcription vector and performed in vitro

synthesis of the cRNAs, which were injected in the *Xenopus* oocytes system to reconstitute functional channels. Two-electrode voltage clamp recordings showed that Phh-RDL assemble into a homomeric receptor sensitive to different insecticides like fipronil, picrotoxin and lotilaner, a novel class of ectoparasiticide agent used to treat ticks and fleas of dogs (Credelio™, Elanco). These results correlated with the efficacy of these drugs on lice in vivo. In conclusion, we report the functional characterization of the first GABACl of *Pediculus humanus humanus*. These results contribute to our understanding of the mode of action of insecticide compounds and will allow the development of new therapeutic strategies to control lice infestations.

OA13.03 Early Oral Sarolaner (Simparica™, Zoetis) Acaricidal Activity Against *Ixodes Scapularis* and *Amblyomma Americanum* Adults After Monitored Attachment Intervals on Treated Dogs

Dr. Kelly Allen¹, Dr. Ruth Scimeca¹, Megan Wohltjen¹, Dr. Mason Reichard¹, Nadia Koziar², Deborah Amodie², Dr. Georgette Wilson², Dr. Joyce Login²
¹Oklahoma State University, Stillwater, United States, ²Zoetis Inc., Parsippany, United States

Acaricidal activity of oral sarolaner (Simparica™, Zoetis) against *Rhipicephalus sanguineus sensu lato* and *Dermacentor reticulatus* begins 4 and 8 hours, respectively, after induced infestations on treated dogs. Here, we evaluated *Ixodes scapularis* and *Amblyomma americanum* mortality in response to sarolaner after monitored, documented attachment time intervals within 1–8 hours of infestation. In duplicate, staggered infestation experiments, 6 treated and 6 control dogs were infested with 250 *I. scapularis* and 250 *A. americanum* adults. Ticks were allowed 60 minutes to embed mouthparts into the dermis. A $\leq 50\%$ attachment rate was targeted. Subsets of ≤ 20 ticks were removed from dogs after documented attachment times of 1, 3, 5, and 7 hours or 2, 4, 6, and 8 hours. Live/dead status was assessed at the time of removal and 24 hours post-removal (live ticks incubated at ambient temperature ($\sim 70^\circ\text{F}$ [21.1°C], 80–90%

humidity). Geometric means of combined live tick counts were calculated for attachment intervals of 1–2 hours, 3–4 hours, 5–6 hours, and 7–8 hours, and least squares means were compared by ANOVA with a two-sided significance level set at $\alpha=0.05$. Significantly fewer live *I. scapularis* were removed from treated than control dogs after 3–4 hours of attachment ($p=0.0012$); upon holding 24 hours, significantly fewer *I. scapularis* that had previously been attached to treated dogs for 1–2 hours were alive ($p=0.0049$). Significantly fewer live *A. americanum* were removed from treated than control dogs after 7–8 hours of attachment ($p=0.0254$); upon holding 24 hours, significantly fewer *A. americanum* that had previously been attached to treated dogs for 3–4 hours were alive ($p=0.0003$). These data indicate that acaricidal activity of sarolaner against *I. scapularis* and *A. americanum* begins after 1–2 hours and 3–4 hours of attachment, respectively, on sarolaner treated dogs.

OA13.04 A First Insight Into the Effect of Lotilaner on GABA-Gated Channels From the European Tick *Ixodes Ricinus*

Dr Claude Charvet¹, Clément Auger¹, Dr Claude Rispe², Caroline Hervet², Dr Elise Courtot¹, Pr Steeve Thany³, Dr Olivier Plantard², Dr Cédric Neveu¹

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Ticks are strict blood-feeding arthropods (Acari), which represent a major health issue for wild or domesticated animals and humans, due to their potential to transmit disease agents. Control of ticks is increasingly difficult due to the development of drug-resistant parasites. Ligand-gated ion channels of the tick central nervous system are the primary targets of acaricides. Among those receptors, the γ -aminobutyric acid-gated chloride ion channels (GABACl) are the main synaptic inhibitory receptors. Lotilaner is a recently developed parasiticide from the isoxazoline chemical class that was shown to be a non-competitive antagonist of GABACl from the livestock tick *Rhipicephalus microplus*. In the

present study, we characterized the GABACl from the European tick species *Ixodes ricinus*.

We extracted RNAs from *Ixodes ricinus* nymphs. Taking advantage of the phylogenetic closeness of *I. ricinus* and *R. microplus* in the Arthropoda phylum, we identified the *I. ricinus* GABACl subunit homologue. The cDNA encoding the Iri-GABACl was cloned and the corresponding in vitro synthesized cRNAs were micro-injected into *Xenopus laevis* oocytes to investigate its pharmacological properties. Functional expression and two-electrode voltage clamp studies demonstrated that the GABACl subunit formed a homomeric receptor gated by GABA. Importantly, the insecticides like lotilaner, fipronil and picrotoxin efficiently blocked the GABA currents as previously observed for the *R. microplus* GABACl. Surprisingly, *I. ricinus* GABACl was not sensitive to the pesticide dieldrin, suggesting a potential naturally existing resistance mechanism involving alternative exons.

Here we report the functional characterization of the first GABACl of *I. ricinus* demonstrating that it is an important molecular target for lotilaner. Transcriptomic analysis of *I. ricinus* are in progress to identify new acaricidal targets.

OA13.05 Clinical Efficacy of Afoxolaner in Dogs Naturally Infected With *Sarcoptes Scabiei* and Concomitant Modifications of the Skin Microbiota

Raluca Mindru², Andrei Cristian Lupu², Dr. Constantin Roman², Dr. Charlotte Bernigaud⁵, Dr. Amaury Briand¹, Dr. Frédéric Beugnet³, Dr. Katja Fischer⁴, Professor Liviu Miron², **Professor Jacques Guillot¹**

¹Veterinary College of Alfort, Maisons-alfort, France, ²Ion Ionescu de la Brad, Faculty of Veterinary Medicine, Iasi, Romania, ³Boehringer Ingelheim Animal Health, Lyon, France, ⁴QIMR Berghofer, Medical Research Institute, Brisbane, Australia, ⁵Henri Mondor Hospital, France

Infection by *Sarcoptes scabiei* remains a common disease in dogs, especially in tropical countries where most of stray or non-controlled dogs show cutaneous

lesions evocative of chronic infection. In Europe, cases are also regularly reported in shelters or in privately-owned dog facilities. Isoxazolines are insecticides and acaricides of a new chemical class. The objectives of the present study were to evaluate the efficacy of afoxolaner in twelve dogs naturally infected by *S. scabiei* in Romania and to describe the modifications of the skin microbiota during the acaricide treatment. We performed an open pre-treatment versus post-treatment study enrolling dogs with clinical signs of sarcoptic mange and a positive skin scraping examination. Dogs were mixed-bred, ranging between 4 months and 7 years old, and weighing between 2 and 20 kg. They received oral afoxolaner (2.7-6.9 mg/kg) on days 0 and 28. Treatment efficacy was determined by the reduction of a clinical score based on the skin surface affected by scabies lesions, erythema intensity, and the presence of scales and crusts. The score was calculated on day 0 and subsequently until day 28 for 4 anatomic sites (head, trunk, legs, tail). For 3 random animals, skin scrapings were collected on two sites (ear and hind leg) before (D0) and after (D28) treatments to assess the skin microbiota. DNA was extracted using DNA Midi Kit QiaSymphony (Qiagen) and analyzed by bacterial 16S rDNA (V3-V4) and fungi ITS1 amplicon sequencing (MiSeq Illumina). On day 0, mean clinical scores were 24.7 (4-58). On day 28, all dogs were mite-free and clinical scores declined to 9.5% (1-28). No signs of drug intolerance were noticed. We found changes in the skin microbiota after the initial acaricide treatment.

OA13.06 Ctenocephalides Felis Resistance to Fipronil: Phenotypic and Genotypic Characterization in a Laboratory Model

Dr Emilie Bouhsira¹, Dr Emmanuel Liénard¹, Mr Audric Berger², Dr Shukri Sharif¹, Pr Michel Franc¹, Dr Fabrice Chandre², Dr Sylvie Cornélie²

¹National Veterinary School of Toulouse, Toulouse, France, ²Institute for Research and Development, Montpellier, France

Fleas are considered as one of the major pests of domestic animals, especially *Ctenocephalides felis*, also known as the cat

flea. They are responsible for irritating pets and their owners as their bites can induce pruritus and allergic reactions. They are also responsible for the transmission of pathogens of zoonotic and veterinary importance (i.e. *Bartonella* sp, *Rickettsia* sp, *Dipylidium caninum* ...). Ectoparasiticide products represent the mainstay for preventing and halting insect bites and transmission of arthropod-borne infections in companion animals. Since the late 80s, fipronil has been one of the most used insecticides on pets worldwide. In the past 10 years, veterinarians reported a lack of efficacy when using this molecule for flea control. So far, the resistance of fleas to insecticide has not been properly studied.

The aim of our work was to investigate the mechanisms underlying resistance to fipronil in a laboratory flea strain originated from a field population resistant to fipronil and to compare to a reference susceptible strain. The phenotypic resistance was investigated via tarsal contact using filter papers impregnated with increasing doses of fipronil and dieldrin. The resistant strain showed a resistance ratio at LD50 of 6.03 and 33.23 for fipronil and dieldrin respectively. The activity of detoxification enzymes was investigated in adult fleas and third-stage larvae. The results showed a significant increase in the activity of the α and β esterases in the adults and larvae of the resistant strain. A significant decrease in the cytochrome P450 mono-oxygenases in the resistant larvae was also observed. Phenotypic assays with detoxification enzyme inhibitors are underway to confirm these results. The molecular assays showed that Rdl mutation is established in resistant fleas. Monitoring these mechanisms in natural populations could greatly improve insecticide resistance management and help development of new formulations to control flea infestations.

OA14 Protozoan Parasites

July 8, 2019, 16:30 - 18:00

Breakout Room 4, Hall of Ideas G&J, Level 4

OA14.01 Defining Relapses Attributed to Equine Protozoal Myeloencephalitis**Dr. Siobhan Ellison¹***¹Pathogenes Inc, Reddick, United States*

The consensus opinion (Reed et al 2016) indicate that cycles of clinical improvement followed by relapse often seen in treated cases of equine protozoal myeloencephalitis (EPM) can be due to re-exposure to *S. neurona* (SN) sporocysts or persistent latent but unapparent infections. Persistent *S. fayeri* (SF) infections were linked to clinical disease by Aleman et al (2014). Recurring disease after treating sarcocystosis in horses, post-treatment EPM disease syndrome (PTEDS), and co-infections with *S. neurona*/*fayeri* (SN/SF) may complicate evaluating neuropathies in horses. In this study we investigated the relationship between recurrent clinical neurological disease and seroprevalence of species-specific antibodies of equine-infecting *Sarcocystis* by prophylaxis using decoquinate to identify specific aspects of disease progression. The possibility that clinical signs were due to inflammatory or immune mediated complications after resolution of active infections was examined by the presence of circulating anti-myelin basic P2 (MPP) antibody. Sixty-nine clinically normal horses with a history of chronic relapsing EPM were grouped by seroreactivity (SN, SF +SN, or MPP +SN/SF) and treated daily with oral decoquinate until clinical relapse was apparent. The horses in the SN group did not relapse, three horses in the SF group (10%) relapsed in three months, and nine horses in the MPP group (35%) relapsed in the first three months. The study showed that most relapse cases were found in horses seropositive for MPP antibody and not re-exposure to *Sarcocystis*. Decoquinate prophylaxis was statistically more effective ($p < 0.05$) for preventing sarcocystosis than preventing polyneuritis equi defined by MPP antibody and clinical signs. This study indicates that PTEDS may be unrelated to active infection. Polyneuritis equi was the leading cause of clinical signs

attributed to relapsing EPM in these horses and should be considered in evaluating neuromuscular disease.

OA14.02 Bovine Theileriosis – A New Disease of Cattle in Australasia**Dr. Abdul Jabbar¹***¹Melbourne Veterinary School, The University Of Melbourne, Werribee, Australia*

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 ikeda and/ or chitose of this complex. A recent study showed that bovine theileriosis can lead to significant milk production losses in dairy cattle. Although *Theileria* spp. known to infect cattle were present in Australia since the early 20th century, it is believed that the pathogenic genotypes of *T. orientalis* were recently introduced to the country.

OA14.03 Epidemiology of Theileria Orientalis: Potential Vectors on Northern Tablelands, NSW, Australia**Mr. Biniam Tsegaye Lakew¹**, Professor Nigel R. Andrew¹, Associate Professor Shubiao Wu¹, Mr Steve Eastwood², Professor Stephen Walkden-Brown¹*¹University Of New England, Armidale, Australia, ²Local Land Services, Northern Tablelands, Armidale, Australia*

Theileriosis is an economically important tick-borne disease of cattle in tropical and sub-tropical countries with *Theileria parva* and *Theileria annulata* being the most pathogenic species. Although *Theileria orientalis* is known to cause persistent infections with mild signs, in recent years there has been an increase in severe clinical outbreaks including mortality in Asia-Pacific region. In Australia, such outbreaks commenced in 2006 with new genotypes Ikeda and Chitose apparently responsible for clinical disease despite ongoing widespread prevalence of the nonpathogenic genotype Buffeli. In New South Wales, outbreaks were initially seen in higher rainfall coastal areas with infection now endemic. The situation in nearby inland areas having lower tick populations is less clear although outbreaks do occur. In the Northern Tablelands of NSW clinical outbreaks have occurred since 2013 even during cold winter months when tick activity is low. As part of a study on the abundance and infection status of potential vectors for *T. orientalis* in this region, 89 bovine blood samples from eight farms within 100 km from Armidale were subjected to qPCR assays to quantify and differentiate various *T. orientalis* genotypes. All samples were positive for *T. orientalis* with 84%, 91% and 88% positive for Ikeda, Chitose and Buffeli respectively. With regard to co-infection with different genotypes 70% of samples were positive for 3 genotypes, 24% for 2 genotypes and 7% for a single genotype. Based on published clinical thresholds for parasite counts of the pathogenic Ikeda genotype 4/89 (4.5%), 28/89 (31.5%) and 57/89 (64.0%) fell into the high, moderate and low categories respectively with significant variations between farms. The results indicate that a high prevalence of co-infection with both pathogenic and non-pathogenic genotypes suggestive of endemic status. Investigations into potential biological and mechanical vectors for *T. orientalis* in this region are ongoing.

OA14.04 Evaluation of an ELISA Detecting *S. Fayeri* Antitoxin in Horses With Equine Muscular Sarcocystosis

Dr. Siobhan Ellison¹, Dr. Laura Benedetti¹, Ms Julie Osborne¹, Dr. Steve Duniho², Dr. Craig Reinemeyer³

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²*Talon Pathology Services, Bellingham, United States,* ³*East Tennessee Clinical Research, Rockwood, United States*

Disease due to *Sarcocystis fayeri* is associated with neuromuscular disease in horses and toxin-induced food poisoning in people. Equine muscular sarcocysts are found on postmortem exam and generally considered incidental. We report the presence of circulating *S. fayeri* antitoxin in a group of 32 clinically normal horses that had incidental findings of muscular sarcocysts. *Sarcocystis fayeri* anti-toxin was present in horses with and without sarcocysts. The results of this report show similar sensitivity for detecting *S. fayeri* sarcocysts in horses by histopathology and *S. fayeri* anti-toxin (78% and 74% respectively). These results indicate that *S. fayeri* anti-toxin may be a useful premortem screening test to detect the presence of *S. fayeri* exposure in horses.

OA14.05 Not All Species Are Created Equal: Exploring the Diversity of *Eimeria* Spp. Infecting Domestic Sheep and Goats

Evelin Rejman¹, Dr. John R. Barta¹

¹*Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Canada*

Coccidiosis, caused by the protozoan parasites in the genus *Eimeria*, is a costly disease impacting the global small ruminant industry mainly through reduced animal performance and cost of treatment. Both clinical and subclinical disease cause a decrease in animal performance through decreased feed efficiency and weight loss from gut damage, loss of electrolyte and nutrients from diarrhea and, in severe cases, death of young livestock. There are at least 12 *Eimeria* species that infect domestic sheep and 9 *Eimeria* species that infect domestic

goats but only a few are considered to be highly pathogenic and cause severe clinical disease. The severity of disease is assessed typically using counts of fecal oocysts (i.e. oocysts per gram, OPG) obtained using a modified McMaster method. Less pathogenic species can be excreted at high OPG counts but have minimal clinical impact whereas pathogenic species can cause damage with low OPG counts. Consequentially, conventional enumeration methods may be unreliable for assessing the severity of infections. Traditional identification of *Eimeria* species using morphometrics of oocysts is unreliable due to overlapping measurements and few species-specific morphological features. *Eimeria* species can be identified using sequence based genotyping (eg. Next Generation Sequencing) of mitochondrial CDS or nuclear 18S rDNA loci. The accurate identification of the causative *Eimeria* spp. is a critical step in disease management. This project is focused on using sequencing techniques and PCR with species specific primers to identify *Eimeria* spp. infecting domestic small ruminants from populations that range in complexity from individual farms to regional epidemiological studies that include multi-farm sampling. Combining parasitological parameters, such as identification of *Eimeria* spp. using sequencing or PCR techniques, with animal performance measures (e.g. animal weights) can improve the ability to accurately diagnose the severity of coccidial infections and assess the efficacy of anticoccidial treatments.

Anthelmintic resistance in equine cyathostomin parasites is widespread. A surveillance-based parasite control programs using fecal egg counts (FEC) and fecal egg count reduction tests (FECRT) to decrease anthelmintic use and monitor treatment efficacy is recommended. Information is often distributed through mass media, word of mouth, and veterinarians, but the impact of educational campaigns such as local extension outreach programs are rarely studied. The purpose of this study was to examine shifts equine parasite control program management practices due to a short course presented by the Penn State Extension, and to highlight how data collected from these programs is useful for monitoring anthelmintic efficacy on a large scale. Horse owners were enrolled after participating in a short course, and then filled out questionnaire surveys about their parasite management programs pre and post study, horse information, and farm information. FECs were performed at three time points, and horses above a 200 strongyle eggs per gram cut-off were treated with pyrantel, fenbendazole, or ivermectin. Two weeks post-treatment a FECRT was performed to determine treatment efficacy, which included 29 farms with a total of 513 individual treatments. Prior to the study, only 30.6% of farms used FECs, but after the study 97% of farms said they would use FECs in the future. Horses were given an average of 4.1 anthelmintic treatments per year before the study, and post study 86% of farms were able to reduce the number of anthelmintic treatments used. Fenbendazole was effective on zero farms, pyrantel on 7.4% of farms, and ivermectin on 92.9% of farms. This extension outreach project helped generate information about anthelmintic efficacy levels, causing a shift in practices on participating farms, and collected useful anthelmintic resistance data. Programs such as this could be useful in other states for monitoring resistance and helping shift management practices.

OA15 Education

July 8, 2019, 16:30 - 18:00

Breakout Room 5, Meeting Rooms KLOP, Level 4

OA15.01 The Importance of Anthelmintic Efficacy Monitoring: Results of an Outreach Effort

Jennifer Cain¹, Donna Foulk², Ed Jedrzejewski², Heather Stofanek², Martin Nielsen¹

¹University Of Kentucky, Lexington, United States, ²The Pennsylvania State University, University Park, United States

OA15.02 Teaching Horse Parasitology in the 21st Century: An Adaptive Learning Platform

Ms. Nichola Calvani¹, Dr. Gaby Van Galen¹, Dr. Kris Hughes², Dr. Anne Beasley³, Dr. Shokoofeh Shamsi², Dr. Jan Slapeta¹

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With the increasing uptake of technology, it is important for tertiary educators to move towards providing parasitology teaching and learning outcomes across different teaching platforms. Parasitologists and veterinarians from several universities throughout Australia came together to collaborate on an interactive multi-institution module developed to teach an often neglected aspect of veterinary parasitology to veterinary science students in demanding, outcome-driven curriculums. Our priority was to workshop, build, test, deliver and report on a proof-of-principle flexible teaching module to meet the needs of veterinary parasitology education. The tool chosen was the cloud-based BEST Network/ Smart Sparrow platform, which enables the sharing of purpose-built modules nationally or even internationally depending on content, with the capacity to provide response-triggered feedback at the individual student level. Such a platform enables collaboration on shared resources, with the opportunity for benchmarking and analytics immediately available. The current work showcases our experience with the delivery of a veterinary diagnostics module workshopped in 2018 and developed for deployment to veterinary science students in 2019. The interactive module was designed in collaboration with academics from The University of Sydney, Charles Sturt University and The University of Queensland, with the support of The Australian Society of Parasitology. It presents a theoretical equine parasitology diagnostic scenario that covers themes including diagnosis, short and long term parasite management, communication with clients, and responsible usage of anthelmintic drugs. The platform enables students to learn at their

own pace, supplementing course material with multiple pathways designed to engage critical thinking while supporting students with different levels of knowledge. The result is an equine parasitology diagnostic module that students can repeat as necessary, while still providing tailored feedback according to their level of understanding.

For access to the module, use the following link: <https://aelp.smartsparrow.com/v/ktnsycfu/bu1pjhu3>

OA15.03 Parasites of Zebrafish: Impacts and Potential Models (Lemons and Lemonade)

Dr. Michael Kent^{1,2}, Dr. Thomas Sharpton¹, Dr. Katy Murray², Dr. Justin Sanders¹

¹*Oregon State University, Corvallis, United States,* ²*Zebrafish International Resource Center, Eugene, United States*

The benefit of aquatic models, led by the use of zebrafish (*Danio rerio*), as part of an approach to improving human and veterinary health is being realized by the scientific community. As a laboratory animal model, it is now second only to mice. The field was initially led by investigations in developmental genetics in which experimental end points involved primarily embryos or larval fish. Adult zebrafish are now used extensively as models throughout the biomedical research arena, including infectious disease research. Naturally occurring pathogens in research facilities, including parasites, are a serious problem in many facilities as they cause disease and infections in subclinical fish may cause non-protocol induced variation in experiments. Research laboratories use pathogen-free water as the source for aquaria, but they often acquire zebrafish for studies from pet stores, providing a source for many pathogens. The Zebrafish International Resource Center has provided a diagnostic service to the research community since 2001, and our data from over 100 laboratories and about 15,000 fish shows that two parasites are quite common in research facilities. *Pseudoloma neurophilia* (in about 50% of facilities) infects the central nervous system is vertically transmitted, it may cause emaciation, spinal deformities,

reduction in fecundity, and it alters behavior endpoints in otherwise clinically normal fish. *Pseudocapillaria tomentosa* (reported in about 13% of facilities) causes profound intestinal lesions, emaciation, death, and various lines of evidence indicate that it is a promoter of a common intestinal cancer in zebrafish. The nematode has benefits as a model for high throughput anthelmintic drug discover, elucidating interactions of parasites with bacterial microbiomes, and cancer research. Last, zebrafish can be maintained at human body temperatures, and under these conditions we have shown that we can experimentally infect zebrafish with *Toxoplasma gondii*, and thus providing another model for parasite drug discovery.

OA15.04 Training the Next Generation of African Leaders in Parasitology: USALTI-Afrique @ University of Salford

Dr. Vincenzo Lorusso^{1,2}, Mr. Babagana Mohammed Adam², Mr. Adamu Haruna Mamman², Mrs. Ijeoma Angela Alozor², Mr Michiel Wijnveld³, Abraham Goni Dogo⁴, Dr. Kevin Bown², Prof. Richard J Birtles²

¹*Global Research & Intellectual Property, Vetoquinol, Paris, France*, ²*University of Salford Tick Infections (USALTI)-Afrique, School of Environment and Life Sciences, University of Salford, Manchester, United Kingdom*, ³*Centre for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria*, ⁴*Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Jos, Jos, Nigeria*

Ticks and tick-borne infections (TBIs) majorly undermine livestock health, welfare and productivity in the developing world, including sub-Saharan Africa (SSA). Despite this, there is currently a shortfall of expertise in research and diagnosis of ticks and tick-transmitted pathogens in most of SSA. The University of Salford Tick Infection (USALTI)-Afrique group was established early in 2016 to address this shortage of competences and know-how. USALTI-Afrique provides research, educational and capacity building opportunities to students, scientists and laboratory personnel, focusing on ticks

and TBIs (and, when possible, other major parasites) of economic and public health relevance to SSA.

USALTI-Afrique aims to gather data on the epidemiology and impact of these disease systems, documenting the burden of parasitisms, in several livestock species across several SSA countries (e.g. Nigeria, Uganda, etc.). Findings generated will not only be disseminated through the means of specialised scientific reviews and symposia, but also shared with recipients from the study areas (e.g. veterinary and para-veterinary professionals, entomologists, local pastoralists and livestock keepers, policy makers).

The ultimate goal of the group is to guide the design and roll-out of evidence-based strategic control interventions, designed according to the specific needs of target areas, as well as to help establish contemporary molecular diagnostic capacity in partner laboratories in SSA.

At present, the group encompasses several postgraduate (PhD and MSc) students as well as undergraduate students engaging in summer research projects. Students are trained on the morphological identification of ticks of veterinary and public health importance, especially from the African continent, as well as on the application of a panel of molecular techniques allowing the identification of a plethora of tick-borne infections in biological samples as well as arthropods.

This talk provides an overview of the vision and ongoing research activities of USALTI-Afrique, highlighting potential collaboration opportunities with groups with shared interest.

**OA15.05 Opportunity for Change:
Implementing AAVMC's Competency-
Based Veterinary Education for Veterinary
Parasitology**

Antoinette Marsh¹, Emma Read¹, Andy Moorhead², Dwight Bowman³, Heather Walden⁴

¹College of Veterinary Medicine, The Ohio State University, Columbus, United States, ²College of Veterinary Medicine, University of Georgia, Athens, United States, ³College of Veterinary Medicine, Cornell University, Ithaca, United States, ⁴College of Veterinary Medicine, University of Florida, Gainesville, United States

The American Association of Veterinary Parasitologists (AAVP) Educators Committee organizes an educators meeting every other year. This meeting provides a unique and focused forum for those involved in the veterinary parasitology curriculum and the training of future veterinarians to meet, discuss trends and incorporate various pedagogical methods into teaching this subject. In 2017, we had 39 participants from North American Veterinary Schools or Colleges participate in a day and a half meeting at the Ohio State University College of Veterinary Medicine. Veterinary Parasitology teaching is not a static field. Drugs, diagnostic tests, parasite resistance, and geographic boundaries are ever changing, while student demographics are constantly evolving. To continue to provide a comprehensive veterinary parasitology education program, it is critical to review learning outcomes for veterinary students and day-one veterinarians. The American Association of Veterinary Medical Colleges (AAVMC) developed the Competency-Based Veterinary Education (CBVE) framework in March 2018. This framework provides domains and a series of competencies that describe a day-one veterinary graduate. Veterinary Parasitology Educators need to continuously update their consensus for what constitutes a "practice-ready veterinarian" and AAVMC's CBVE framework provides an opportunity for review. We report on activities at four different USA veterinary schools/colleges implementing the CBVE framework allowing purposeful curricular design related to Veterinary Parasitology. We anticipate additional schools and colleges adopting the CBVE framework.

OA16 Canine Helminths

July 9, 2019, 11:00 - 12:30
Plenary Hall, Madison Ballroom (ABCD), Level 4

**OA16.01 High Risk of False Positive Findings
by Coproscopy in Danish Dogs**

Dr. Stig Milan Thamsborg¹, Dr. A. R. Huelle¹, Dr. H. H. Ebbesen¹, Dr. P. Deplazes², Dr. C.A. Alvarez Rojas², Dr. H. Mejer¹
¹Veterinary Parasitology, University of Copenhagen, Frederiksberg C, Denmark, ²Institute of Parasitology, University of Zurich, Zurich, Switzerland

The aim of this cross-sectional study was to determine the prevalence of endoparasites in healthy Danish family dogs and to assess the relative importance of false positive *Toxocara*-egg findings. Through contacts to training clubs in the Copenhagen area, dog owners volunteered to hand in fresh faecal samples, and 309 dogs ≥ 4 months were examined by a modified McMaster (sensitivity of 10 eggs per g) and a coproantigen (Ag) ELISA (Idexx Laboratories, DK). *Toxocara* eggs were speciated as *T. canis* or *T. cati* by size and PCR. Coproscopy detected *Toxocara* spp. (6.5 %), hookworm (5.2 %), *Cystoisospora* spp. (3.6 %), *Eucoleus* spp. (2.3 %), *Taenia* spp. (1.0 %), and *Strongyloides stercoralis* (0.3 %). Non-canine passengers such as *Eimeria* spp. and *Ascaridia galli* were also found. The copro-Ag ELISA detected *Toxocara* (6.9 %), hookworm (10.2 %), *Trichuris* (0.3 %), and *Giardia* (18.5 %). *Toxocara* eggs from 14 dogs were isolated and characterized by PCR (and size) as *T. canis* (36%) or *T. cati* (64%). Agreement by McMaster and copro-Ag ELISA for positivity was seen in 13 cases while seven dogs were egg(+)/copro-Ag(-), out of which six dogs had eggs identified as *T. cati*. Eight dogs were egg(-)/copro-Ag(+) which could perhaps be related to pre-patent or low level infections. According to the owners, 45 % of the dogs displayed coprophagia. In conclusion, there was a low prevalence of endoparasites in Danish dogs. The high rate of *T. cati* eggs and other non-canine parasites, associated with commonly observed coprophagia, emphasized the importance of false positive results by faecal flotation. False positives may

be reduced by morphological or molecular identification of eggs, or by only walking the dog on a leash for 24-48 hours before sampling.

OA16.02 Combination Anthelmintic Treatment for Persistent Ancylostoma Caninum Ova Shedding in Greyhounds

Lindie Hess¹, Laurie Millward¹, Adam Rudinsky¹, Emily Vincent¹, **Antoinette Marsh¹**

¹*The Ohio State University College of Veterinary Medicine, Columbus, United States*

Ancylostoma caninum is a nematode of the canine gastrointestinal tract commonly referred to as hookworm. This United States study involved eight privately owned adult Greyhounds presenting with persistent *A. caninum* ova shedding despite previous deworming treatments. It is unknown how frequently or widespread drug treatment failure and resistance is in dogs because there is frequently a lack of subsequent routine follow up fecal analysis after normal deworming procedures. Drug resistance in ruminants and equine nematodes is well recognized as a significant problem. Drug resistant nematodes are not thought to be as common in small animals. However, there are reports describing drug resistant *A. caninum* and a drug resistant *Dirofilaria immitis* strain present in the USA. The dogs received a combination treatment protocol comprised of topical moxidectin, followed by pyrantel/febantel/praziquantel within 24 hrs. At 7 to 10 days post-treatment, a fecal examination monitored for parasite ova. Dogs remained on the monthly combination treatment protocol until they ceased shedding detectable ova. The dogs then received only the monthly topical moxidectin maintenance treatment. The dogs remained in the study for 5 to 14 months with periodical fecal examinations performed. During the study, three dogs reverted to positive fecal ova status, with two being associated with client non-compliance. Re-institution of the combination treatment protocol resulted in no detectable ova. Use of monthly doses of pyrantel/febantel/praziquantel and moxidectin appears to be an effective treatment for non-responsive or persistent

A. caninum ova shedding. Follow-up fecal examinations were important for verifying the presence or absence of ova shedding despite the use of anthelmintic treatment. Limitations of the current study include small sample size, inclusion of only privately owned greyhounds, and client compliance with fecal collection and animal care.

OA16.03 Comparison of Different Flotation Techniques Alone or in Combination With ELISA Coproantigen Detection for Gastrointestinal Parasites of Dogs

Dr. Guilherme Verocai¹, Dixie Jarriel², Rita Hanna³, Dr. David Elsemore³
¹*Texas A&M University, College Station, United States*, ²*University of Georgia, Athens, United States*, ³*IDEXX Laboratories Inc., Westbrook, United States*

The objective of this study was to evaluate the diagnostic performance of classical techniques for recovery of parasite eggs and oocysts alone or in combination with a commercial coproantigen ELISAs for detecting common gastrointestinal parasites of dogs. Feces of 132 dogs from shelters and a multidog household were used. Samples were processed using classical copro-parasitological techniques: passive flotation (PF), single centrifugation sugar flotation (SCSF), and double centrifugation sugar flotation (DCSF), and the MiniParasep (MP; Apacor, UK), a commercial kit whose protocol include a centrifugation step. For each test, 2g of feces, and Sheather's sucrose solution (specific gravity = 1.25) were used. Ten minutes was used for each waiting time and/or centrifugation step(s). Approximately 1g of sample of 130 of these dogs was stored frozen for coproantigen ELISA detection of hookworms, whipworms, roundworms, and *Giardia* (IDEXX Laboratories Inc.). Among the classical methods, DCSF detected more animals infected by *Ancylostoma*, *Trichuris*, and *Toxocara* (31.1, 32.6, 19.7%, respectively), followed by SCSF (29.5, 28.0, 18.2%), and PF (28.0, 25.8, 15.2%) and MP (27.3, 25.0, 17.4%). Overall, *Cystoisospora* oocysts, and *Taeniidae* eggs were detected in 15.2, and 3.0%, respectively. The ELISA alone detected more dogs infected with

Ancylostoma (38.5%), but less Trichuris (30.0%) and Toxocara (17.7%) than DSCF. Among the combinations, DCSF + ELISA detected the most infected animals for Ancylostoma, Trichuris, and Toxocara (41.5, 40.0, 21.5%), and found 1, 4, and 3 additional dogs infected for these nematodes, respectively, when compared to the SCSF + ELISA combination. Giardia coproantigen ELISA detected 38.5% of animals positive, but was not assessed using the other techniques. Among the classical techniques, DSCF detected a higher number of dogs infected with Ancylostoma, Trichuris, and Toxocara; and among the combinations, DCSF + coproantigen ELISA was superior in the detection of these three nematodes.

OA16.04 Seasonality and Prevalence of Common Canine Gastrointestinal Nematodes in the U.S.A.

Jason Drake¹, Thomas Carey¹

¹Elanco Animal Health, Greenfield, United States

Parasite prevalence maps created by the Companion Animal Parasite Council (CAPC) provide monthly data on a county, state and national level, enabling evaluation of seasonality and changes in prevalence. The maps have provided 4.3-7.2 million canine fecal sample results per year from 2012-2018. Changes in prevalence and seasonal fluctuations in canine roundworm, whipworm and hookworm from 2012 – 2018 were analyzed. Yearly prevalence for canine roundworm remained between 1.77% and 1.94%. Each year, the highest monthly roundworm prevalence of 2.46-2.70% occurred during the winter months and lowest prevalence of 1.24-1.47% occurred during the late spring / early summer. Yearly prevalence of canine whipworm decreased slightly, starting at 0.83% in 2012 and gradually dropping to 0.67% by 2018. With a seasonal pattern similar to roundworm, the highest monthly whipworm prevalence of 0.75-0.95% occurred each winter and lowest monthly prevalence of 0.60-0.77% occurred during the late spring / early summer every year. Yearly prevalence for canine hookworm from 2012 to 2018 increased, starting at 2.02% in

2012, eventually reaching 2.96% by 2018. The highest monthly hookworm prevalence in 2012 was 2.31%, in May 2012, rising over the 7-year period to a peak summer prevalence of 3.22% in August 2018. Each year from 2013 onward, hookworm prevalence was highest in mid-summer and at the lowest in the winter. Evaluation of gastrointestinal nematode prevalence data from over 39 million fecal samples examined over a 7-year period revealed a subtle, yet significant, increasing prevalence for roundworms ($P < 0.0001$), an increasing prevalence for hookworms ($P < 0.0001$), and a slightly decreasing prevalence for whipworms ($P < 0.0001$). Seasonality was demonstrated for all three nematodes, possibly for the first time for canine whipworms. Evaluating monthly data 2012-2018: roundworm prevalence was highest December-January and lowest May-June ($P < 0.0001$); whipworm prevalence was highest January-February and lowest May-June ($P < 0.0001$); and hookworm prevalence was highest July-August and lowest January-February ($P < 0.0001$).

OA16.05 Exploring the Role of ABCB1 Transporters in Toxocara Canis

Dr. Jeba R J Jesudoss Chelladurai¹, Dr. Matthew T Brewer¹

¹College Of Veterinary Medicine Iowa State University, Ames, United States

Somatic larval migrans is a feature of *Toxocara canis* infection in the canine definitive host and in paratenic hosts. Hypobiotic larval pools in the muscles and somatic tissues of pregnant bitches are reservoirs for transplacental transmission of infection to multiple litters of puppies. These larval stages tolerate therapeutic doses of macrocyclic lactones (MLs) and are difficult to eliminate. The mechanism of tolerance to macrocyclic lactones in hypobiotic *Toxocara canis* larvae is unknown. However, ML resistance in other nematodes of veterinary importance such as *Parascaris*, *Haemonchus*, and *Dirofilaria* is known to be mediated by ATP binding cassette family B1 (ABCB1) transporters. We tested the hypothesis that transport proteins of the ABCB1 transporters, also known as P-glycoproteins, are involved in the efflux

of the macrocyclic lactones in *Toxocara canis*. We describe the pharmacological characterization of the Tca-Pgp-11 transporter and show the localization of Pgp-11 mRNA expression using a novel multiple nucleotide in situ hybridization technique in adult worms. We also describe the expression levels of other ABCB1/P-gp genes in various life stages of the helminth and discuss our observations on the induction of expression of these genes in the presence of various MLs. The presented data will serve as a primer for further research on the mechanism of ML tolerance in *Toxocara canis* and the use of P-gps as putative druggable targets for anthelmintic design.

OA17 Molecular Tools I

July 9, 2019, 11:00 - 12:30

Breakout Room 2, Hall of Ideas E&H, Level 4

OA17.01 A Cysteine Protease Inhibitor RHcyst-1 From Tick *Rhipicephalus Haemaphysaloides* and Its Antitumor Potential

Prof. Jinlin Zhou¹, Dr. Nana Wei¹, Dr. Yujian Wang¹, Dr. Zhengmao Xu¹, Dr. Houshuang Zhang¹, Dr. Jie Cao¹

¹*Shanghai Veterinary Research Institute, Chinese Academy Of Agricultural Sciences, Shanghai, China*

A potent and tight-binding inhibitor of cysteine proteases had been identified from the tick *Rhipicephalus haemaphysaloides*, RHcyst-1, which belongs to the cystatin type 1 family and have a predicted molecular weight of ~11 kDa. An investigation of the RHcyst-1 genes' expression profile showed that it was more richly transcribed in the embryo (egg) stage. The results of proteinase inhibition assays showed that rRHcyst-1 can effectively inhibit the six cysteine proteases' enzyme activities, and especially showed quite a high inhibitory efficiency against cathepsin L and S, which were considered to be effectively controlled targets of tumor cells. To investigate the antitumor effects of RHcyst-1 and to explore the underlying mechanism of these effects, different tumor cells were treated with RHcyst-1 in vitro. Proliferation

activity was evaluated using Cell Counting Kit-8, and migration and invasion were determined by wound healing and Transwell invasion assays. RHcyst-1 significantly inhibited the proliferation, migration, and invasion of all four different tumor cells in vitro. In addition, a mouse tumor therapy model was established by inoculating the left forelimb of mice with B16-F10 cells, and tumor progression was evaluated by assessing tumor volume and survival. The results showed RHcyst-1 inhibited tumor growth and improved survival in vivo. Flow cytometry was conducted to evaluate myeloid-derived suppressor cells (MDSCs), CD4+, and CD8+ T cell levels in PBMCs and spleens. Immunohistochemistry was performed to analyze immune cell infiltration and angiogenesis in the tumors. A decrease and an increase in MDSCs levels were observed in PBMCs and in the spleen, respectively, after RHcyst-1 application. Conclusions: Tick RHcyst-1 has potential antitumor efficacy, and the observed antitumor activities may be partly attributable to changes in cathepsin expression and MDSCs levels in the PBMCs and spleens. The findings of the present study suggest that RHcyst-1 may have the potential to be utilized in cancer treatment.

OA17.02 Evidence of Multiple Point Mutations in *Theileria Annulata* Cytochrome B Gene and Peptidyl-Prolylisomerase Incriminated in Buparvaquone Treatment Failure

Dr Bashir Salim¹

¹*University Of Khartoum, Khartoum, Sudan*

Drug resistance is one of the emerging and re-emerging epidemics affecting both veterinary and public health sectors. Buparvaquone provides the most satisfactory means in the treatment of bovine tropical theileriosis. However, recently there has been widespread reports of development of resistance of *Theileria annulata* to buparvaquone. To investigate the situation in Sudan where bovine tropical theileriosis is endemic, fifty blood samples from *T. annulata*-positive cattle were used for DNA extraction, PCR and cytochrome b gene nucleotide sequencing. Analysis of the two buparvaquone binding site regions Q01 (130-148) and Q02 (244-266),

revealed three non-synonymous mutations at codon 146; alanine (GCT) to threonine (ACT) within the Q01 region across all 50 isolates and the other mutation at codon 129; serine (AGC) to glycine (GGC) in 18 isolates which is very close to the Q01 binding site. However, we documented another mutation at position 227; valine (GTG) to methionine (ATG) close to the Q02 binding site, in three isolates with mutation at codon 129. This result was augmented by sequencing peptidyl-prolylisomerase gene (targeted by buparvaquone) for 67 samples including the above 50 samples. Interestingly, we reported mutation at position 177 in two samples, a mutation responsible for buparvaquone treatment failure and one isolate has the A53P mutation. We showed that this affects Buparvaquone inhibition of Prolyl isomerase activity in vitro. In conclusion, we have one interesting confirmation (A53P), several unexplained SP mutations, and two peculiar mutations in the STOP codon. This study has provided evidence of point mutations in the cytochrome b and peptidyl-prolylisomerase genes of *T. annulata* that is associated with buparvaquone treatment failure in Sudan.

OA17.03 Evaluation of Theileria Orientalis Genome Assembly Methods Using Nanopore Sequencing and Analysis of Variation Between Genomes

Mr. Jerald Yam¹, Dr Daniel Bogema², Dr Cheryl Jenkins²

¹University Of Technology Sydney, Ultimo, Australia, ²NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle, Australia

Theileria orientalis (Apicomplexa: Piroplasmida) is a non-lymphocyte transforming tick-borne haemoparasite of cattle that causes ill-thrift and anaemia. In recent years, clinical outbreaks of *T. orientalis* caused by the pathogenic genotype of this parasite (Ikeda) have been increasingly observed throughout the Asia Pacific. Currently, there are no available vaccines for this disease, although a live vaccine based on a benign genotype (Buffeli) has been proposed to provide cross-protection against Ikeda. However, our recent genomic

studies using Illumina short reads of three *T. orientalis* genotypes, Ikeda, Buffeli and a low pathogenic genotype (Chitose) have revealed substantial genetic divergence, perhaps at the species level. As short read technology is unable to effectively resolve the structure of the genomes, we continued to investigate the isolates using previously generated Illumina short reads combined with nanopore long reads. In this study, we sequenced the three isolates with a R9.4.1 MinION flow cell and tested four different hybrid assembly methods, Flye, Canu, Unicycler and Masurca. Flye and Canu assemblies were further processed with Nanopolish and five iterations of Pilon using Illumina reads. Different combinations of the assemblers were trialed and evaluated in order to determine the best pipeline for *T. orientalis* genome assembly. Evaluations with Quast and MUMmer revealed Unicycler to be the best assembler for *T. orientalis* Ikeda, and Flye for genotypes, Chitose and Buffeli. Alignments to the *T. orientalis* (Shintoku) reference sequence revealed potential structural variation in the apathogenic Buffeli genotype. The detailed methodology and results from this study will be presented and discussed, including the genome annotation and findings of the variation between the pathogenic and apathogenic *T. orientalis* types.

OA17.04 Novel Subunit Vaccine Approaches to Protect Against Bovine Babesiosis

Dr Vignesh Rathinasamy¹, Dr Carlos Suarez^{2,3}, Dr William Poole¹, Dr Heba Alzan², Dr Marta Silva², Prof Brian Cooke¹

¹Biomedicine Discovery Institute, Monash University, Clayton, Australia, ²Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, United States of America, ³Animal Disease Research Unit, United States Department of Agriculture, Pullman, United States of America

Babesia bovis, a major causative agent of bovine babesiosis, is a widespread and economically-important protozoan parasite that causes severe, often fatal disease in cattle. A live vaccine is currently used to partially control bovine babesiosis in endemic areas, however, the variable efficacy

and morbidity associated with live vaccines warrants alternative, more effective, more easily deployable and sustainable control measures such as next-generation sub-unit vaccines. We propose developing novel subunit vaccines that target both, the development of acute clinical disease and parasite transmission as a novel control approach against bovine babesiosis. We are utilising state of the art of genomic, proteomic and cell and molecular biology approaches to define novel potential vaccine antigens, including parasite antigens involved in host cell modification and tick-pathogen interactions as the basis to identify and develop next-generation vaccines. In cattle infected with *B. bovis*, the parasites cause disease by altering the structure and function of the red blood cells (RBCs) that they infect resulting in the unnatural accumulation of RBC's in various vital organs. We have identified a number of uncharacterised parasite-derived exported proteins with predicted functions that are pivotal in pathogenesis of severe babesiosis. Further, we have identified several parasite proteins (HAP2, CCp family and 6-Cys family) that play key roles in sexual reproduction of the parasite in ticks as additional targets for transmission blocking vaccines. Identification and biochemical characterisation of selected vaccine candidates with potential as vaccine future candidates for targeting both the asexual and sexual stage of *B. bovis* will be presented.

OA17.05 Understanding Roles of Genes in Mosquito Melanization Response Against Pathogen Infection by Gene Knock-Down and Gene Knock-Out Technologies

Mr. Lei Zhang¹, Ms Xiaojing Zhu¹, Ms Jing Chen¹, PhD. Chenghong Liao¹, **Professor Qian Han¹**

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Insect melanization plays roles in defense, such as wound healing and native immune response against pathogen infection, as well as roles in cuticle tanning and eggshell formation. Melanization is a process leading to melanin formation and tyrosine is a key substrate for melanin synthesis. In insects, tyrosine is a conditional essential amino acid

that can be obtained from foods, and also can be formed from phenylalanine catalyzed by phenylalanine hydroxylase. Tyrosine is also a key starting substrate for producing sclerotin, a component of insect cuticle that protect insects from pathogen entry.

Many enzymes are involved in the melanization pathway in insects directly or indirectly, including prophenoloxidase, dopachrome conversion enzyme, arylalkylamine N-acetyltransferase, dopa decarboxylase, alpha-methyldopa resistant protein, etc. Mosquitoes have more melanization genes than any other insects, e.g. *Aedes aegypti* mosquito has more than ten prophenoloxidase gene. However, a specific role of each mosquito melanization gene is unclear although some biochemical functions have been revealed.

In this report, we used RNAi, CRISPR-Cas9 and real-time PCR to investigate the roles of some melanization genes in *Aedes aegypti* mosquitoes. Several gene knock-out mosquitoes have been obtained, including a prophenoloxidase, dopachrome conversion enzyme, and arylalkylamine N-acetyltransferase-1 genes. Obvious phenotypes were found in each mutant line. Some other genes functions were investigated using RNAi and real-time PCR, because they are probably lethal genes in the mosquito. The detail findings will be presented in the conference presentation.

OA17.06 PCR-Based Distribution of Plasmodium Species in Mosquito Vectors of Faisalabad District, Punjab, Pakistan

Mr. Khizer Hayat¹, Dr. Muhammad Sohail Sajid^{1,2}, Prof. Dr. Zafar Iqbal¹, **Mr. Muhammad Abdullah Malik¹**, Mr. Haider Abbas³

¹University Of Agriculture, Faisalabad, Pakistan, Faisalabad, Pakistan, ²Center for Advanced Studies in Agriculture and Food Security, University Of Agriculture, Faisalabad, Pakistan, Faisalabad, Pakistan, ³Department of Parasitology, KBCMA, College of Veterinary and Animal Sciences, Narowal Sub-campus of University of Veterinary and Animal Sciences, Lahore, Pakistan, Narowal, Pakistan

Plasmodium (P.), mosquito-borne unicellular parasite, is responsible for “malaria”. Pakistan is at risk of malaria because almost 1.6 million cases are reported every year. The present study was planned to screen mosquito vectors for Plasmodium spp. in Faisalabad district, Punjab, Pakistan using nested PCR. For this purpose, convenient sampling of adult mosquitoes was done from different places including: animal populated areas, lavatories, water storage tank, livestock farms and road side ditches in 70 % ethanol. DNA extraction was done after stereo microscopic identification. Species identification of Plasmodium (P. falciparum, P. vivax, P. ovale and P. malariae) was done through universal forward and species specific reverse primers in the 2nd round of nested PCR. The PCR products were subjected to agarose gel electrophoresis followed by gel imaging. Prevalence of Culex mosquitoes was higher as compared to Anopheles. Plasmodium falciparum and P. vivax were found more prevalent as compared to other species of Plasmodium. The overall prevalence of Plasmodium in mosquito vectors was 46% (14 out of 30 pools for Plasmodium spp.). Results were analyzed through chi-square analyses. Present study may explore the vectorial capacity of mosquitoes which can be an indicator of Plasmodium distribution in an area for large scale metagenomics.

OA18 Leishmania

July 9, 2019, 11:00 - 12:30
Breakout Room 3, Hall of Ideas F&I, Level 4

OA18.01 LeishVet Symposium: Animal Leishmaniosis – Lights and Shadows of Feline Leishmaniosis

Maria-Grazia Pennisi¹

¹University of Messina, Messina, Italy

Cats are infected by Leishmania infantum, Leishmania mexicana, Leishmania venezuelensis, Leishmania amazonensis and Leishmania braziliensis. Leishmania infantum is the most frequently reported species and in endemic areas prevalence of feline infection is not negligible albeit lower than

in dogs. Clinical cases of feline leishmaniosis caused by L. infantum (FeL) are sporadic but increasingly diagnosed. A wide spectrum of clinical signs is described and associated with dissemination of the parasite in skin, mucous membranes, lymph nodes, spleen, bone marrow, eyes, liver, kidney, gastrointestinal and respiratory tract. However, coinfections (immunosuppressive retroviral infections) and debilitating diseases (neoplasia, immune-mediated diseases) are common and may play a role in the progression of Leishmania infection to disease and in clinical signs. However, evidence-based information is still limited. Immunopathogenesis in feline infection is scarcely known. Recently, parasite specific IFN γ was demonstrated in infected cats but the role of T cell function remains still unclear in felines.

Suspected cases can be managed according to LeishVet guidelines formulated by combining a review of evidence-based studies, case reports, clinical experience and critical consensus discussion of LeishVet members. LeishVet guidelines underline the need to confirm a direct role of L. infantum in the clinical manifestations of patients and to make a complete clinico-pathological evaluation for detecting possible complications (chronic kidney disease) that may affect prognosis. Cytology and histology are the first and best options to confirm FeL. Positive PCR proves the infection and may provide speciation. High parasite loads or antibody levels are suggestive of disease, but validated serological tools for cats are not commercially available. Cats are still empirically treated with the same drugs used in dogs however clinical cure is obtained. Importantly, on-host repellents are now available to prevent infection of exposed cats and they may also reduce transmission from infected cats to sand flies.

OA18.02 LeishVet Symposium: Animal Leishmaniosis – Diagnosis for Veterinarians**Dr. Luís Cardoso¹**

¹*Department of Veterinary Sciences, School of Agrarian and Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal*

The process of diagnosing leishmaniosis in domestic dogs and other mammalian species comprises: (a) confirming disease, i.e. evaluating leishmanial infections in clinically suspect animals, but also (b) detecting the presence of *Leishmania* spp. in subclinically infected hosts. Diagnosis is complex due to the variability of non-specific clinical signs and clinicopathological findings compatible with leishmaniosis, and a thorough diagnostic approach needs to be adapted for each situation. The disease suspicion index will be defined by pertinent clinical history, a complete physical examination and include several routine diagnostic tests such as complete blood count (CBC), biochemical profile, urinalysis and serum electrophoresis. The purposes of investigating infection in clinically healthy animals include epidemiological studies, screening apparently healthy individuals living in areas where leishmaniosis is endemic (e.g. dogs prior to vaccination, animals heading towards disease progression or breeding dogs), preventing transmission by blood transfusion from subclinical carriers, avoiding the importation of infected animals to countries where leishmaniosis is not endemic, and monitoring response to treatment. Several techniques have been developed and are available, and it is essential to understand their bases, limitations and appropriate interpretation, in order to be able to choose the best diagnostic tools for each objective and subsequently to interpret their results accordingly. The detection of *Leishmania* infection includes parasitological (cytology, histology, immunochemistry and culture), molecular (conventional, nested and real-time polymerase chain reaction [PCR]) and serological methods (qualitative and quantitative antibody tests). In addition, specific cellular immunity tests have also been developed for *Leishmania* infection, but are currently used only in the research

settings. Different techniques may have to be applied depending on the aim of diagnosing disease or just detecting subclinical infection. Additionally, dogs and other animals with leishmaniosis might be co-infected with further vector-borne pathogens or suffer from other concomitant diseases, making diagnosis more diverse and complicated.

OA18.03 Toll-Like Receptors 2, 4, and 7, Interferon-Gamma, Interleukin 10 and Programmed Death Ligand 1 Transcripts in Skin From Dogs With Different Clinical Stages of Leishmaniosis

Dr Laura Ordeix¹, Dr. Sara Montserrat-Sangrà¹, Dr. Pamela Martinez-Orellana¹, **Dr. Laia Solano-Gallego¹**

¹*Dep. Medicina I Cirurgia Animals, Universitat Autònoma de Barcelona, Bellaterra, Spain*

Canine leishmaniosis is a zoonotic disease caused by *Leishmania infantum* that can have several dermatological manifestations. The type of immune response elicited against the parasite appears to be at the basis for such clinical variability. However, few studies have investigated the immunological response in the cutaneous lesions in *L. infantum* naturally infected dogs with different degrees of disease severity. Therefore, the objective of this study was to determine and compare the transcription of toll like receptors (TLRs) 2, 4 and 7, interferon gamma (IFN- γ), interleukin (IL) 10 and programmed cell death protein ligand (PD-L) 1 in paired clinically-lesioned and normal-looking skin from 25 diseased dogs (mild disease-stage I (n=11) and moderate to severe disease-stages II and III (n=14) as well as in normal-looking skin from healthy dogs (n=10) from a non-endemic area. Moreover, another objective was to correlate the immune response related genes transcripts with clinicopathological, immunological and parasitological findings. Clinically-lesioned skin from mildly affected dogs was characterized by a significantly upregulation of TLR2 ($P<0.0001$) and IL-10 ($P=0.021$) and downregulation of TLR7 ($P=0.004$). On the other hand, normal-looking skin of mildly affected dogs was characterized by a significant lower expression of TLR7 ($P=0.003$), IFN- γ ($P<0.0001$) and PDL-1

($P=0.001$) and a trend for lower expression of TLR2 and IL-10 when compared with more severely affected dogs. The results of the present study provide further insights into the TLR and cytokine profile in clinically-lesioned and normal-looking skin of dogs with different stages of canine leishmaniosis. In this study, TLR7 and PDL-1 transcripts were determined, for the first time, in canine skin of dogs with leishmaniosis and they appeared to be associated with disease severity.

OA18.04 Altered Circulating NK Cell Response During Ehrlichia/Leishmania Co-infection and Potential Role in Progressive Disease

Breanna Scorza¹, Kurayi Mahachi¹, Erin Cox¹, Jennifer Foltz², Dean Lee², Jill Saucier³, Phyllis Tyrrell³, Christine Petersen¹

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Zoonotic Visceral Leishmaniasis (CanL) is driven by transmission of protozoan Leishmania parasites from canine reservoirs to humans. Identifying factors for development symptomatic CanL is crucial to limiting transmission and detecting novel human immune response targets. We recently identified a causal association between tick-borne infection and progression to CanL. Ehrlichia spp., rickettsia transmitted by ticks, were among the most common tick-borne pathogens in dogs with clinical CanL (Toepp 2019). How Ehrlichia co-infection alters Leishmania immunity to lead to CanL progression is unknown. For this study, Natural Killer (NK) cell subsets were compared between endemic controls (EC) and dogs with CanL +/- Ehrlichia co-exposure (L+ and L+E+). We hypothesized Ehrlichia co-infection would be associated with increased activated NK cells. Flow cytometry was performed on PBMCs for NK (CD3-CD94+ lymphocytes) and NKT (CD3+CD94+ lymphocytes) cell populations. Compared to EC, circulating NK cell frequency significantly increased in L+ dogs (~2.6-fold), further increased in dogs co-infected with asymptomatic Ehrlichia (~3.9-fold) and

symptomatic Ehrlichia (~5.5-fold). NKT cell frequency was not modulated. The proportion of NKp46+ NK cells decreased significantly in L+ dogs (~2.3-fold) and in L+E+ dogs (~5.3-fold), which may signal recruitment of this subset to peripheral sites of infection. Similarly, the proportion of NKp46+ NKT cells decreased (~3.5-fold) in L+E+ dogs. Both NK and NKT cells trended to have less intracellular Granzyme B gMFI in L+E+ dogs, indicating granule release. In agreement, preliminary studies show both circulating NK and NKT cell frequencies correlate with PBMC cytotoxic activity. Based on these findings, we hypothesize NK cells from L+E+ dogs will have increased cytotoxicity and cytokine production compared with EC or L+ dogs, effector functions with important implications for anti-Leishmania immunity. Finally, doxycycline treatment of L+E+ dogs with symptomatic Ehrlichia returned NK cell frequencies to near EC levels, indicating treating comorbid tick infections may indirectly benefit CanL.

OA18.05 A Nationwide Survey of Leishmania Infantum Infection in Cats and Associated Risk Factors in Italy

Dr Roberta Iatta¹, Dr Tommaso Furlanello², Dr Viviana Domenica Tarallo¹, Dr Vito Colella¹, Prof Maria Stefania Latrofa¹, Prof Emanuele Brianti³, Prof Paolo Trerotoli¹, Prof Nicola Decaro¹, Dr Eleonora Lorusso¹, Dr Bettina Schunack⁴, Prof Guadalupe Miró⁵, Dr Filipe Dantas-Torres⁶, Prof Domenico Otranto¹

¹University of Bari, Bari, Italy, ²San Marco Veterinary Clinic, Veggiano, Padova, Italy,

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⁵Universidad Complutense de Madrid, Madrid, Spain, ⁶Instituto Aggeu Magalhães, Fundação Oswaldo Cruz (Fiocruz), Recife, Brazil

Since the first description of leishmaniosis in a domestic cat (*Felis silvestris catus*), the number of case reports of Leishmania infantum infection and clinical cases of leishmaniosis in cats increased in several countries of the Mediterranean basin, with large variability in prevalence data. A major limitation in the comparability of these data has been attributed to the differences

in diagnostic techniques employed and cat populations sampled.

The aim of this survey was to assess the prevalence of *L. infantum* infection in owned cats across Italy by serological and molecular tests and the identification of potential risk factors. Blood samples from 2,659 cats from northern ($n=1,543$), central ($n=471$) and southern ($n=645$) Italy were tested for antibodies against *L. infantum*, by an immunofluorescence antibody test and for the parasites' DNA, by real-time PCR. Samples were additionally screened for feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) proviral DNAs.

An overall cumulative *L. infantum* prevalence of 3.9% was recorded by serology (3.3%) and/or by real time-PCR (0.8%), with a higher rate (10.5%) in southern Italy. The risk of *L. infantum* infection in cats was significantly associated to the geographical areas (South vs North and Centre; $p<0.0001$), age class (from 19 months to 6 years old vs ≤ 18 months old, $p=0.0003$), neutering status (not neutered vs neutered, $p=0.0028$) and FIV infection ($p=0.0051$).

Though the role of cats in the epidemiology of *L. infantum* is still debated, our findings indicate that cats are exposed to and/or infected by this protozoan, mainly in endemic regions of Italy. Hence, as future perspectives, the standardization of procedures for a prompt diagnosis of *L. infantum* infection and for screening cat populations is a crucial task for a better understanding of the epidemiology of *L. infantum* infection in cats, and of their potential role in spreading zoonotic leishmaniosis.

OA18.06 Granulocytic Cells in the Intestinal Wall of Dogs Naturally Infected by *Leishmania infantum*

Dr. Wilma Buzetti¹, Mr. Diogo Silva^{1,2}, Ms Maria Luana Alves^{1,2}, Dr. Tricia Oliveira²
¹Unesp, Ilha Solteira, SP, Brazil, ²USP, Pirassununga, Brazil

Visceral leishmaniasis (VL) is a chronic disease caused by the protozoa *Leishmania*

infantum and can cause an inflammatory reaction in the gastrointestinal tract, however the role of granulocytic cells (neutrophils, eosinophils, and mast cells) in the intestine of dogs infected is not fully understood. We performed a quantitative analysis of these granulocytic cells in the intestinal wall of dogs with canine visceral leishmaniasis (CVL). Twenty dogs were assigned to one of three groups: group 1 (G1, $n=8$), dogs with CVL and *L. infantum* amastigotes in the intestine; group 2 (G2, $n=9$), dogs with CVL but without intestinal amastigotes; and group 3 (G3, $n=3$), uninfected dogs (control group). Granulocytic cells were counted in the crypt-villus unit, submucosa, and muscle layer of the intestinal mucosa. It was found that in small and large intestines, the numbers of these cells increased significantly in dogs of the G2 group followed by the G1 group ($p \leq 0.05$), but not in the control group (G3). In G1 there was an inverse correlation between parasite burden of the small intestine and granulocytic cell counts ($r=-0.1$; $p \leq 0.01$). Exception for mast cells in G1 and G2 that were statistically significant only in relation to control group (G3) in the large intestine. The granulocytic cell hyperplasia observed in the intestine of *L. infantum*-infected dogs suggests that these cells may be involved in the cell-mediated immune response for parasite elimination.

OA19 Nematode Molecular Tools, Resistance I

July 9, 2019, 11:00 - 12:30

Breakout Room 4, Hall of Ideas G&J, Level 4

OA19.01 Experimental Validation of Benzimidazole Resistance Alleles and Mechanisms Using Quantitative Assays of Free-Living Nematodes

Clayton Dilks¹, Qicong Sheng¹, Yu-Hsin Huang¹, Erik Andersen¹

¹Northwestern University, Evanston, United States

Parasitic nematodes are a major burden of livestock around the world. Anthelmintic drugs have been the front line to fight these infections, but resistance arose and spread

quickly worldwide. To fight this resistance, a thorough understanding of the genetics and mechanisms of resistance is essential, especially for the key benzimidazole (BZ) class. Three well known BZ resistance variants (F200Y, E198A, F167Y) are found in a beta-tubulin encoding gene in many nematode species. Additionally, two other resistance variants have been discovered in nematode parasites at the 198 position, valine and leucine. In addition to variants discovered in parasites, our lab recently identified a number of other alleles segregating at low frequency within the free-living nematode species, *Caenorhabditis elegans*, which are correlated with BZ resistance. To validate whether any of these alleles underlie resistance to benzimidazoles, we generated CRISPR/Cas9 edited strains introducing each of the alleles discovered in parasites and *C. elegans* into a defined genetic background in *C. elegans*. Using high-throughput assays of fecundity and growth rate, we quantitatively measured resistance conferred by each of these alleles. In addition, we tested both structurally and mechanistically how these alleles affect beta-tubulin function to confer resistance, including tissue-specific promoters driving expression of a susceptible beta-tubulin gene in an otherwise resistant genetic background. These tests enabled discoveries of how the drug acts within the animal and confers resistance. Additionally, we investigated and experimentally validated how the number and diversity of different beta-tubulin genes can influence BZ resistance. To the best of our knowledge, we have characterized the resistance profile of all currently identified nematode benzimidazole resistance alleles.

OA19.02 Genetic Characterisation of Benzimidazole Resistance in UK *N. Battus* Populations Using Next-Generation Amplicon Sequencing and Pyrosequencing Technologies

Lynsey Melville¹, Elizabeth Redman², Pai Chia Rebecca Chen², Russell Avramenko², Alison A. Morrison¹, Sian Mitchell³, Jan Van Dijk⁴, Giles Innocent⁵, John S. Gilleard², **Dave J. Bartley**¹
¹Moredun Research Institute, Penicuik, Scotland, ²University of Calgary, Calgary, Canada, ³Animal and Plant Health Agency, Carmarthen, Wales, ⁴University of Liverpool, Liverpool, England, ⁵Biomathematics and Statistics Scotland, Edinburgh, Scotland

Benzimidazole resistance was recently identified in *Nematodirus battus*, providing an opportunity to create baseline data on the prevalence of resistance in this species. As with many ovine gastrointestinal nematodes, resistance is believed to be conferred by single nucleotide polymorphisms (SNP) within the β -tubulin isotype 1 gene. Pyrosequencing and deep amplicon sequencing assays using the Illumina Miseq platform were designed to identify the polymorphisms at codon F167Y, E198A and F200Y SNPs. The aim of the current study was to detect and quantify BZ-resistant alleles within *N. battus* populations on commercial farms in the UK and compare the two technologies. A total of 192 *N. battus* populations were analysed using each technology. Thirty individual parasites were genotyped per population using pyrosequencing and pooled DNA extracts from 500-1000 parasites were sequenced by MiSeq. F200Y was found to be the most prevalent SNP, identified throughout the UK in around 1 in 4 of the populations tested at a low overall frequency of $2.1\% \pm 0.6\%$ (mean $\pm$ S.E.M.). The F167Y SNP was identified for the first time in this species, in four of the populations tested at a low frequency ($1.3\% \pm 0.01\%$), indicating the early emergence of the mutation. E198A was not identified in any of the isolates tested. Results obtained from pyrosequencing and MiSeq were comparable for F200Y ($r^2=0.96$). The results for F167Y were variable between platforms however, this is likely due to the low allele frequency at this locus (0-13%). Despite low level variation in results, both technologies were highly comparable and could both be used as a diagnostic tool.

OA19.03 Genotypic Characterisation of Monepantel Resistance in UK Isolates of *Teladorsagia circumcincta*

Dr. Frank Turnbull¹, Prof. Eileen Devaney¹, Mrs. Alison Morrison², Dr. Roz Laing¹, Dr. Dave Bartley²

¹*University of Glasgow, Glasgow, United Kingdom*, ²*Moredun Research Institute, Edinburgh, United Kingdom*

The preservation of monepantel (MPTL) efficacy against *Teladorsagia circumcincta*, the predominant veterinary parasitic nematode in UK sheep, is paramount. The first UK case of MPTL-resistance in *T. circumcincta* was reported in 2018, with unconfirmed evidence of further cases. In this study, we had access to a unique resource of three laboratory derived MPTL-resistant strains, three MPTL-susceptible strains from which they were derived, as well as a collection of field isolates representing survivors of Zolvix-treatment in the previous year. Genetic characterisation of individual first and third stage larvae from each *T. circumcincta* population were conducted at the *Tci-mptl-1* loci, β -tubulin loci and at ten microsatellite loci. A particular focus was on polymorphic regions in exons 7 and 8 of the *Tci-mptl-1* gene and at mutations F167Y, E198A and F200Y in the β -tubulin gene.

The *Tci-mptl-1* locus was highly polymorphic both within and between populations. However, comparisons between the MPTL-resistant strains and their respective MPTL-susceptible parental strains showed a reduction in the number of genotypes at the *Tci-mptl-1* gene. This finding was supported by low diversity at the *Tci-mptl-1* locus in the field isolates post-MPTL treatment. Pyrosequencing of the β -tubulin gene showed a low incidence of the F167Y mutation and no evidence of the E198A mutation. A reduction in the number of homozygous benzimidazole-resistant genotypes and an increase in heterozygosity at position 200 was observed in the MPTL-resistant strains compared to their susceptible counterparts.

The reduction in genetic diversity at the *Tci-mptl-1* locus in individuals from the MPTL-resistant strains compared to the MPTL-

susceptible populations suggests that MPTL treatment had applied a purifying selection pressure. Although β -tubulin is not the target of MPTL, the process of selecting for resistance impacts on the benzimidazole-resistant genotypes in individuals based on codon 200 mutations. The results demonstrate the complexity in characterising genetic resistance in *T. circumcincta*.

OA19.04 Diethylcarbamazine Directly Modulates TRP Channels in Filarial Nematodes

Saurabh Verma¹, Sudhanva S Kashyap¹, Melanie Abongwa¹, Alan Robertson¹, Richard J Martin¹

¹*Iowa State University, Ames, United States*

Canine heartworm disease is caused by the filarial nematode *Dirofilaria immitis*, a vector-borne parasitic infection that affects dogs (definitive host), cats, and in very rare circumstances, humans. The disease is endemic in many parts of the world and infections have been reported from every state in United States. The current recommended regimen for prophylactic treatment with macrocyclic lactones (MLs) is not effective at killing adults. To kill adult worms it requires doxycycline and melarsomine along with MLs and/or surgical interventions, which are high risk and costly. There have also been reports of heartworm resistance to MLs. The limited treatment options and development of resistance demands identification of new drug targets and therapeutic agents. One approach is to identify the targets of old drugs, and develop improved compounds for these targets. Diethylcarbamazine (DEC) despite its development in 1947 is still used for treatment of human patients with lymphatic filariasis. However, the molecular mode of action of DEC is undefined with many reports of host-mediated rather than parasite-mediated effects.

We have studied the direct effects of DEC on our filaria model, *Brugia malayi*. We have observed that DEC rapidly produces spastic paralysis, inhibiting adult *Brugia malayi* motility. Our follow up results, with whole-muscle cell patch-clamp recordings, demonstrated that DEC produced a

concentration-dependent inward current followed by outward current (EC₅₀: 11 μ M). We were able to isolate the inward current by blocking K⁺ currents: these currents were sensitive to TRP antagonists and RNAi knockdown.

Our results show that DEC acts by modulating TRP channels of the parasite. We have also tested the effects of TRP antagonists/agonists on adult worm motility. We are presenting here, results that support the hypothesis that DEC directly modulates TRP channels of adult filarial worms.

Supported by NIH grants: NIH R01 A1047194

OA19.05 Emodepside, Macrophilicidal Effects and Filarial SLO-1 K Channels

Sudhanva Kashyap¹, Saurabh Verma¹, Alan Robertson¹, Richard Martin¹
¹Iowa State University, Ames, United States

Heartworm infections caused by *Dirofilaria immitis* are a major concern among dogs and cats worldwide. The current anthelmintic regimen for heartworm includes prophylactic treatment with macrocyclic lactones or multiple doses of doxycycline and melarsomine to kill adults. Treatment to kill adult worms is risky and requires lengthy hospitalization and rarely surgery. There is a therapeutic gap in administration for safe drugs to eliminate adult heartworms. Recently, there have been reports of heartworm resistance to macrocyclic lactones. These issues call for a greater need for identification of new drug targets and develop new and effective interventions that do not develop resistance. Emodepside, a veterinary anthelmintic used for treatment of gastro-intestinal nematode parasites, is reported to have macrofilaricidal effects against filarial nematodes. We explored the mode of action and sensitivity to emodepside using the human pathogen, *B. malayi* as a filarial model. Worminator motility and patch-clamp of single muscle cells showed that emodepside activates voltage-gated potassium channels and that the male is more sensitive than the female. RNAi knock down demonstrated that emodepside targets

SLO-1 channels. We expressed the slo-1 splice variants heterologously in *Xenopus* oocytes and found that the RCK1 slo-1f splice variant, found in muscles of males, is more sensitive to emodepside (EC₅₀=5 μ M) than the RCK1 slo-1a splice variant found in muscles of females (EC₅₀>30 μ M). We also found that emodepside was more potent on worms where slo-1a transcript was knocked down (EC₅₀(slo-1aKD)=242 nM; EC₅₀(control)=617 nM) implying that expression of slo-1a negatively regulates the emodepside sensitivity in female adult *B. malayi*. In silico modelling in filarial nematodes revealed that emodepside binds to the cytoplasmic region in 5 binding loops. RCK1 region where alternative splicing occurs in many filarial species contributes four out of the five binding loops thereby affecting emodepside potency.

Supported by NIH R01A1047194

OA19.06 Lipidome of *Haemonchus Contortus*

Dr Tao Wang¹, Dr Shuai Nie², Mr Guangxu Ma¹, Dr Pasi Korhonen¹, Dr Anson Koehler¹, Dr Ching-Seng Ang², Prof. Gavin Reid³, A/Prof. Nicholas Williamson², Prof. Robin Gasser¹
¹Department of Veterinary Biosciences, Melbourne Veterinary School, The University of Melbourne, Parkville, Australia, ²Bio21 Mass Spectrometry and Proteomics Facility, The University of Melbourne, Parkville, Australia, ³Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Australia

In spite of progress in the area of lipidomics, very little is known about lipid biology in parasites. Employing *Haemonchus contortus* as a model, we characterised the first global lipidome for a parasitic nematode using an advanced LC-MS/MS-based approach. We identified > 550 lipid species representing four categories, and explored lipid profiles in eggs; third-stage (L3) and exsheathed L3s (xL3) and fourth-stage (L4) larvae; female and male adults of *H. contortus*. Lipid composition and abundance in *H. contortus* changed significantly throughout development. The predominant alterations were reflected in: (i) an increase in glycerophospholipids

(principally glycerophosphocholine and phosphatidylethanolamine); (ii) a decrease in triacylglycerol synthesis; and (iii) a modulation of saturated fatty acids and ether-linked lipids. These changes suggest adaptations in terms of nutrient acquisition, metabolism and development, as *H. contortus* transits to the parasitic stage within its host animal. The present lipidomic resources provide a foundation to explore lipid biology in *H. contortus* and related nematodes, and to establish the roles of particular in host-parasite interactions and disease.

OA20 IAFWP Symposium

July 9, 2019, 11:00 AM - 12:30 PM

Breakout Room 5, Meeting Rooms KLOP, Level 4

OA20.01 Emerging Issues With Food-Borne Helminth Parasites: *Trichinella* and *Echinococcus*

Emily Jenkins¹, Caroline Frey^{1,2}, Temitope Kolapo¹, Janna Schurer³, Rajnish Sharma¹
¹University Of Saskatchewan, Saskatoon, Canada, ²Canadian Food Inspection Agency, Saskatoon, Canada, ³Cummings School of Veterinary Medicine and the University of Global Health Equity, Kigali, Rwanda

A recent multicriteria-based ranking of food borne parasites ranked *Echinococcus* spp. cestodes and *Trichinella* spp. nematodes among the top 7 food borne parasites globally from public health and global trade perspectives. Even in developed nations with strong capacity for meat inspection and diagnostic testing, these helminth parasites continue to persist, largely due to wildlife reservoirs. There is evidence that *E. multilocularis* is emerging globally in the circumpolar North (increasing in prevalence and geographic distribution), and that unrecognized genetic diversity within this parasite may be linked to its recent emergence in North America. As well, there is unrecognized diversity within wildlife-reservoired species of *Trichinella*, often of unknown significance for human health. Other factors driving the emergence of these wildlife-reservoired zoonoses include changing interfaces between wildlife and

human communities, human behaviours that favour parasite transmission, loss of traditional protective knowledge, and failure to integrate culturally sensitive messages into well-intended public health risk management. I will discuss challenges in assessing, managing, and communicating risks posed by these neglected helminth zoonoses.

OA20.02 Factors Associated with *Echinococcus Multilocularis* Infection in Coyotes (*Canis latrans*) in Southern Ontario

Mr Jonathon Kotwa¹, Dr. David Pearl², Dr. Mats Isaksson³, Dr. Claire Jardine^{1,4}, Dr. Olaf Berke², Dr. Nicola Mercer⁵, Dr. Eva Osterman-Lind³, Dr. Andrew Peregrine¹

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Echinococcus multilocularis was recently reported in wild canids across southern Ontario, a newly recognized endemic area for the parasite in Canada. In endemic areas, a comprehensive understanding of factors associated with infection in definitive hosts (wild canids) is critical for mitigating risk of transmission to humans and dogs. Evidence suggests that coyotes play an important role for the maintenance of *E. multilocularis* in North America, yet little is known about the transmission dynamics of the parasite in this host. A study was therefore carried out to investigate the association of host-level (sex, body condition), environmental (southern Ontario region, landcover), temporal (season, hunting season, year), and extraneous factors (submitter type) with *E. multilocularis* infection in coyotes in southern Ontario. Between November 2015 and March 2017, 416 coyotes were collected from across the region as part of a study that investigated the prevalence and distribution of the parasite in wild canids; 24% (95% confidence interval

(CI) 20%–28%) of coyotes were positive for *E. multilocularis*. Associations between infection and factors of interest were assessed via a mixed-effects logistic regression model with a random intercept for submitter to account for clustering. Coyotes with poor body condition were at greater odds of *E. multilocularis* infection than those in good condition (odds ratio (OR) 2.09; 95% CI 1.05–4.14; $P=0.036$). A negative association between infection in coyotes and the proportion of natural land was observed (OR=0.69; 95% CI 0.54–0.88; $P=0.003$). The odds of *E. multilocularis* infection were greater in government submitted coyotes compared to those submitted by hunters (OR 2.94; 95% CI 1.01–8.74; $P=0.047$). Coyotes from the western region of southern Ontario had lower odds of infection compared to coyotes from the central region (OR 0.33; 95% CI 0.15–0.72; $P=0.005$). Our findings provide insights into the transmission dynamics of *E. multilocularis* in coyotes in southern Ontario.

OA20.03 An Integrated Approach to Control Cystic Echinococcosis in Southern Italy

Laura Rinaldi¹, Antonio Bosco¹, Maria Paola Maurelli¹, Davide Ianniello¹, Paola Pepe¹, Maria Morgoglione¹, Leucio Camara Alves², Giuseppe Cringoli¹

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²*Department of Veterinary Medicine, Federal Rural University of Pernambuco, Recife, Brazil*

Cystic echinococcosis (CE) is a severe zoonosis, caused by the larval stage of the tapeworm *Echinococcus granulosus*. This helminth infection is of increasing public health and socio-economic concern due to the considerable morbidity rates that give rise to high economic losses in the public health sector and in the livestock industry. Control programmes against *E. granulosus* are considered long-term actions which require an integrated approach and high expenditure of time and financial resources.

The aim of this study was to develop an integrated approach to control CE in an endemic area of southern Italy. The control

programme was based on modern procedures and tools for farm surveillance (by the use of Geographical Information Systems), diagnosis in dogs (using the FLOTAC techniques and molecular analysis), diagnosis in sheep (by necropsy and ultrasonography), information and education (through dissemination of brochures, videos, leaflets) for dogs' owners, farmers and school-aged children.

A ten-year regional initiative addressed the surveillance (active and passive) and control of CE from a holistic perspective based on: 1) geolocation of 306 sheep farms with CE infected animals detected by necropsy and ultrasonography; 2) identification and treatment of 1166 dogs with praziquantel in sheep farms (no. = 306) by using ad hoc confinement cages; 3) education for dogs' owners, farmers and school-aged children. Over ten years, the integrated programme resulted in a significant reduction of the infection rates of dogs, sheep and other livestock species in the Campania region of southern Italy. Therefore, this new approach represented a valid strategy to control CE in endemic regions.

The procedures and tools developed during the programme could be used by regional and national health authorities to target interventions aimed at eliminating CE in animals and humans at fine and large geographical scales.

OA20.04 Control of *Taenia Solium* Transmission by Vaccination of Pigs

Professor Marshall W Lightowlers¹

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Taenia solium is a zoonotic cestode parasite which causes human neurocysticercosis. Pigs transmit the parasite by acting as the intermediate host. A recombinant antigen vaccine, TSOL18, has been developed which has achieved very high levels of protection in pigs against experimental challenge infections with *T. solium*. Field trials have been completed using the vaccine, including a cohort trial undertaken in Cameroon and

an intervention at population level in pigs in the Banke District of Nepal. In Cameroon, young animals were vaccinated and treated once with oxfendazole; controls were given oxfendazole alone. At 12 months of age 194 pigs were examined by slicing muscle and brain tissue to identify and count parasites. Infection was identified in 19.6% (19/97) of non-vaccinated controls while no infection was present among 97 vaccinated animals. In Nepal, all eligible pigs ≥ 2 months of age in the intervention area were vaccinated with Cysvax, the TSOL18 recombinant vaccine recently registered as Cysvax by Indian Immunologicals Limited, every 3 months and, at the same time, given an oral treatment with 30mg/kg oxfendazole. Animals in a region in which no intervention was undertaken served as controls. Cysticercosis was identified in randomly selected, slaughter weight animals at the start and at the end of the intervention period. At the start the prevalence of porcine cysticercosis was 23.6% and 34.5% in control and intervention areas, respectively. Following the intervention, the prevalence of cysticercosis in pigs from the control area was 16.7% (no significant change), whereas no infection was detected after complete slicing of all muscle tissue and brain in animals from the intervention area ($P=0.004$). A three-monthly vaccination and drug treatment intervention in pigs is an effective method for reducing *T. solium* transmission and, if implementation where transmission is endemic, would be expected to reduce the incidence of human neurocysticercosis.

In this study we assessed the shedding of feline lungworms first-stage larvae (L1) and their infectivity to the snail intermediate host (IH), after anthelmintic administration in cats. Thirty-six cats diagnosed with lungworm infections were enrolled in this study and assigned to four groups. In particular, groups A and B were composed of animals infected by *Troglostrongylus brevior* and treated with eprinomectin (Broadline®) and moxidectin (Advocate®) formulations, respectively, whereas groups C and D included cats positive for *Aelurostrongylus abstrusus* and treated with eprinomectin and moxidectin formulations, respectively. Prior to and every day after treatment, faecal samples were analysed by the Baermann technique and the number of larvae per gram of faeces determined. The efficacy of a single administration of the products was finally assessed four weeks after treatment. To evaluate the pre- and post-treatment infectivity of L1 to IH, two snails per cat/day were infected with 100 L1 collected from the faeces of enrolled animals and, then, digested 28 days post-infection. The efficacy of the eprinomectin and the moxidectin formulations at 28 days was 100% for both *A. abstrusus* and *T. brevior*, with a mean number of days for cats to become negative of 7.9 ± 1.2 in group A, 7.8 ± 1.9 in group B, 6.9 ± 1.6 in group C and 8.9 ± 2.0 in group D. Following the artificial digestion, alive third-stage larvae of *T. brevior* and *A. abstrusus* were found in 160 (87.4%) experimentally infected snails. The results of this study demonstrate that a single administration of the two formulations is effective in the treatment of *A. abstrusus* and *T. brevior* infections. Furthermore, during the post-treatment period alive L1 are shed for up to 8.9 ± 2.0 days and these larvae are still able to reach the infective larval stage in the infected snails.

OA21 Cat Parasitisms

July 9, 2019, 13:30 - 15:30
Plenary Hall, Madison Ballroom (ABCD), Level 4

OA21.01 Larval Shedding and Infectivity to Snail Intermediate Host of Feline Lungworms After Anthelmintic Treatment

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OA21.02 Vector-Borne Pathogens in Domestic Cats From Romania: A First Epidemiological Survey

Dr. Luciana Cătălina Rus¹, Dr. Angela Monica Ionică¹, Dr. Ana-Maria Diacu¹, Dr. Ana Maria Boncea², Prof. ECZM Andrei Daniel Mihalca¹
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Different species of bacterial and parasitic vector-borne pathogens can infect felines, but the role of domestic cats in the transmission of various agents to other species remains poorly studied. As the data on the epidemiology of feline parasitic and bacterial agents is scarce, the purpose of the study was to assess the presence of DNA of several vector-borne pathogens in cats from Romania. Three hundred fourteen blood samples from cats from various parts of the country were included in the study: owned (n = 267), feral (n = 27), and stray (n=20). DNA extracted from cat blood samples was tested by conventional or nested PCR assays for Cytauxzoon/Hepatozoon/Babesia/Theileria spp. (18SrRNA), Dirofilaria spp. (cox 1), spotted fever group Rickettsiae (gltA), Bartonella spp. (gltA), Ehrlichia spp. (16S rRNA), Anaplasma phagocytophilum (16S rRNA), Francisella tularensis (17-kDa lipoprotein gene) and Borrelia burgdorferi s.l. (5S-23S rRNA). DNA of blood protozoans was amplified from the blood of 24 cats, Cytauxzoon sp. being found with the highest prevalence (2.9%), followed by Babesia canis (1%), Hepatozoon felis (0.6%) and Hepatozoon sp. (0.3%). Filial DNA was found in 2.6% of cats, while Ehrlichia spp. and Bartonella spp. were detected in 2.2% and 1.6% of the samples tested, respectively. Moreover, the presence of Ehrlichia spp. DNA was found to be more frequent in samples collected from stray cats, compared to owned or feral cats. None of the sampled cats were positive for Rickettsia spp., Borrelia burgdorferi s.l., Francisella tularensis or Anaplasma phagocytophilum. Being the first molecular screening of vector-borne agents in domestic cats from Romania, the present study showed that cats can harbor a wide range of blood-associated parasites and

bacteria. However, further research is required on the implication of various arthropod vector species in their transmission and to understand the pathogenic and zoonotic significance of these infections.

OA21.03 Molecular Confirmation of Zoonotic Strongyloides Stercoralis in Cats

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Strongyloides felis, Strongyloides planiceps, Strongyloides stercoralis, and Strongyloides tumefaciens have been reported in cats worldwide. Differentiation of species has largely been based on geographical location and morphology of larvae retrieved in feces. Location at necropsy has been used to distinguish S. tumefaciens from the other species. However, morphometry of these species overlap, with the exception of S. planiceps, and taxonomic descriptions are poorly detailed for S. felis and S. tumefaciens. In 2013, 2014 and 2018, under approved IACUC protocols, cats euthanized for health reasons and collected as roadside mortalities on the island of St. Kitts were submitted for pathology and parasite collection. Strongyloides parthenogenetic females and larvae were identified in colonic nodules of six cats on histopathological examination. The nodules were consistent with those described in infections by S. tumefaciens. To obtain the first genetic sequences of S. tumefaciens, DNA from paraffin embedded colonic nodules was extracted and subjected to PCR targeting the cytochrome oxidase c subunit 1 (cox1) of the mitochondrial DNA. Phylogenetic analysis revealed that the sequences generated belonged to S. stercoralis and clustered in a clade with global isolates assumed to be zoonotic of humans, dogs, and non-human

primates. These results are the first genetic confirmation of *S. stercoralis* in cats and raise questions about the role of cats in the zoonosis of *S. stercoralis* and the validity of the species *S. tumefaciens*.

OA21.04 Addressing Feline Deworming Guidelines in the Tropics

Filipe Dantas-Torres^{1,2}, Gabriela Pérez Tort^{1,3}, Malaika Watanabe^{1,4}, Gad Baneth^{1,5}, **Jennifer Ketzis**^{1,6}, Peter Irwin^{1,7}, Andrei Mihalca^{1,8}, Tawin Inpankaew^{1,9}, Kamani Joshua^{1,10}, Pablo Castro^{1,11}, Bui Linh^{1,12}, Arumugam Sangaran^{1,13}, Pablo Borrás^{1,14}, Rebecca Traub^{1,15}

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The Tropical Council for Companion Animal Parasites Ltd. (TroCCAP) is a not-for-profit organisation whose mission is to independently and freely inform, guide and make best-practice recommendations for the diagnosis, treatment and control of companion animal parasites in the tropics and sub-tropics, with the aim of protecting animal and human health. In line with this mission, TroCCAP recently finalised the ‘Guidelines

for the Diagnosis, Treatment and Control of Feline Endoparasites in the Tropics’. The development of these guidelines required unique and complex considerations to be addressed, often inapplicable to developed nations. Much of the tropics encompass middle-to-low income countries in which poor standards of environmental hygiene and large populations of stray dogs and cats exist. In these regions, endoparasites pose a significantly high risk to pets, which in turn place their owners at risk of acquiring parasitic zoonoses. These considerations led to the development of unique recommendations with respect to deworming and endoparasite testing intervals for the control of both global and ‘region-specific’ parasites in the tropics. Moreover, the ‘off-’ or ‘extra-’ label use of drugs for the treatment and control of endoparasites is common practice in many tropical countries and many generic products lack manufacturers’ information on efficacy, safety, and quality control. Evidence-based recommendations surrounding use of such drugs and protocols are also addressed in the Guidelines. The formation of these Guidelines is regarded as the first step towards educating and changing veterinarians’ knowledge and perceptions surrounding the veterinary and zoonotic significance, diagnosis, treatment and control of feline endoparasites in the tropics.

OA21.05 Retrospective Fecal Survey of Parasites in 2,323 Client-Owned Cats in Northcentral Oklahoma From 2007 Through 2017

Dr. Yoko Nagamori¹, Dr. Mark Payton¹, Hadley Apple¹, Katherine Herrick¹, Dr. Eileen Johnson¹
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Our study objective was to determine the prevalence and trend of parasitic infection in client-owned cats in northcentral Oklahoma over the past 11 years. All results of centrifugal flotation examination on fecal samples from client-owned cats submitted to the Boren Veterinary Medical Hospital and Oklahoma Animal Disease Diagnostic Laboratory of Oklahoma State University from 2007 through 2017 were included. The impact of sex, age, seasonality, and years on the prevalence of infection were analyzed.

A total of 2,323 cases were included for this study. Either 33% zinc sulfate solution (s.g. = 1.18) or Sheather's sugar solution (s.g. = 1.25) was used to perform a centrifugal fecal flotation test. Majority of cases (76.1%; 1,768/2,323) did not include any parasites, eggs, oocysts, or cysts. The most common parasite observed was *Cystoisospora* oocysts (9.1%; 211/2,323), followed by *Toxocara cati* eggs (7.6%; 176/2,323), *Giardia* cysts (4.1%; 95/2,323), *Alaria* eggs (3.2%; 74/2,323), *Ancylostoma* eggs (1.2%; 28/2,323), *Dipylidium caninum* proglottids/egg packets (1.2%; 27/2,323), taeniid proglottids/eggs (1.1%; 26/2,323), *Demodex* mites (0.9%; 22/2,323), and *Eucolus aerophilus* (0.6%; 13/2,323). Less commonly, *Tritrichomonas blagburni* (0.2%; 4/2,323), *Cheyletiella* mites (0.13%; 3/2,323), *Ollulanus tricuspis* (0.13%; 3/2,323), *Physalopetra* eggs (0.13%; 3/2,323), *Toxascaris leonina* eggs (0.13%; 3/2,323), *Aelurostrongylus abstrusus* larvae (0.09%; 2/2,323), *Platynosomum* eggs (0.09%; 2/2,323), *Sarcocystis* sporocysts (0.09%; 2/2,323), *Trichuris felis* eggs (0.09%; 2/2,323), *Mesocestoides* proglottids/eggs (0.04%; 1/2,323), *Otodectes cynotis* mites (0.04%; 1/2,323), *Spirometra* eggs (0.04%; 1/2,323), and *Toxoplasma*-like coccidian oocysts (0.04%; 1/2,323) were detected.

There was no statistical significance between sexes ($p = 0.06$); however, a significant trend was observed between ages, the younger the cats, the higher the prevalence of parasitic infection ($p < 0.0001$). Statistical analyses also revealed the higher prevalence of infection occurred in summer through fall compared to winter ($p = 0.0005$) and overall prevalence of infection decreased over years ($p < 0.0001$).

OA21.06 *Aelurostrongylus Abstrusus* Infection in the Intermediate Host Mollusk Based on the Recovery of Third-Stage Larvae

Emily Werdal¹, Colleen Sorge¹, Yuri Lee¹, Janice L Liotta¹, Dr. Dwight D. Bowman¹
¹*Cornell University, College of Veterinary Medicine, Ithaca, United States*

Aelurostrongylus abstrusus is a feline lungworm found throughout the world. Its life cycle requires a mollusk intermediate host and can include a transport or paratenic

host such as frogs and rodents. To fully understand the *A. abstrusus* lifecycle one needs to examine infection in the feline final host as well as in the intermediate host, the mollusk. By studying the intermediate host we seek to optimize the infection procedure increasing third-stage larvae (L3) production by increased efficiency of the snail's ability to maintain the infection. Presented here are findings involving the mollusk intermediate host spanning ten years while experimentally infecting 34 cats. *A. abstrusus* positive fecal samples were used to infect aquatic freshwater snails including *Biomphalaria glabrata*, and *Cipangopaludina chinensis*, as well as, several species of terrestrial snails including *Triodopsis albolabris* and *Cornu aspersum*. Third-stage larvae were recovered through pepsin digestion of these snails at various time points. This data was used to assess the infection methods, including the snail species, the infection procedure, and the number of larvae used to infect the snails and recovered from the snails. In all snail species, there is an overall decrease in the recovery of L3 from the snails the longer the snails are maintained after infection. However, L3 were still recovered at 357, 453, 464 days post infection from *T. albolabris*, *C. aspersum* and *B. glabrata* respectively. This data furthers the understanding of the *A. abstrusus* lifecycle in the mollusk, and may provide some insight into what occurs in the mollusk under natural field conditions.

OA21.07 Factors Associated With the Prevalence of *Otodectes Cynotis* in Ambulatory Client Owned Population of Cats in Pakistan

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A study was designed to furnish a base-line data for prevalence and associated risk factors of *O. cynotis* in client-owned cats' population in Pakistan. The samples were collected

from the cats brought on pet's clinics and veterinary hospitals for clinical examination in Lahore and Sheikhpura districts of Punjab, Pakistan. The cats selected for sampling were classified on age, sex, breed, housing and intensity of infection. Ear wax of total $n=450$ cats ($n=234$ from District Lahore and $n=216$ from district Sheikhpura) were sampled. Ear wax were collected by using sterile swabs soaked in liquid paraffin. Superficial skin scrapings were also taken with the help of scalpel blades in those cats that had rashes on skin to check any ectopic mite infestation. Cats were examined through direct smear of ear samples using a stereoscopic microscope. The intensity (counting) of ear mite parasitism was also made by collecting ear canal washings with lukewarm paraffin. Cats were classified on breed, age, sex, housing and intensity of infection. Total 159 out of 450 cats were found positive for *O. cyanotis*, showing prevalence of 89/234 (38.03%) in Lahore district and 70/216 (32.40%) in Sheikhpura district. Results showed that prevalence was highly significantly in cats age of <12months (39.67%) than >12months (29.8%). There was no significant difference present relative to host gender ($p>0.05$), but a greater number of cases were reported in male cats (36.24%) than females (34.39%). No specific breed association to *O. cyanotis* infestation was found. Prevalence was significantly higher in cats that live outdoor (67.35%), both (outdoor & indoor) 46.85% with those cats that live only indoor 12.29% ($p<0.05$). All the superficial skin scrapings were negative to ectopic mite infestation. This is the first study conducted in client-owned cats of Lahore and Sheikhpura district, Punjab, Pakistan that examined the prevalence of *O. cyanotis* infestations.

OA22 Molecular Tools II

July 9, 2019, 13:30 - 15:30

Breakout Room 2, Hall of Ideas E&H, Level 4

OA22.01 Extracellular Vesicles From Hookworms As Vehicles for Vaccine Candidates and Immuno-Therapeutics

Dr Ramon Marc Eichenberger^{1,2}, Dr Javier Sotillo^{1,3}, Prof Alex Loukas¹

¹Australian Institute of Tropical Health and Medicine, James Cook University, Cairns, Australia, ²Institute of Parasitology, University of Zurich, Zurich, Switzerland, ³Carlos III Health Institute, Madrid, Spain

Helminth parasites have evolved a vast array of strategies to manipulate their vertebrate hosts. Extracellular vesicles (EVs) are secreted by all helminth species investigated thus far, and their salient roles in parasite-host interactions are being revealed. Adult hookworms live in the intestine of the host where they release excretory/secretory products, representing a major part of the host-parasite interface. While studies on parasite-host interactions have traditionally focused on soluble proteins, we explored the structure, molecular profile and functional applications of secreted EVs from the rodent parasite *Nippostrongylus brasiliensis*, which has been used as a model for hookworm infection. By proteomics and RNA sequencing, we identified 81 proteins, including a predominance of SCP-like proteins, and moreover, hookworm EVs contain miRNAs that are predicted to bind to murine genes with known roles in the regulation of immunity. Helminths manipulate the host's immune system towards an immunoregulatory phenotype, which can have beneficial effects for both the parasite and the host. Consequently, there is interest in harnessing the immunoregulatory capabilities of helminths to develop novel therapies for autoimmune, allergic and even metabolic diseases. We have shown that hookworm EVs confer protection against inflammation in models of acute and chronic colitis. Helminth EVs are also of interest as candidates for anthelmintic vaccines, and we have begun to evaluate their protective capacity. Vaccinated

mice with purified *N. brasiliensis* EVs show 85.4% reduction in adult worm burden in hookworm challenge infections. We selected and expressed 5 recombinant versions of assumed vaccine candidates, presenting promising but variable efficacies (43.4 – 73.7%). However, vaccination with EVs and EV proteins did not affect the fecundity and morphology of patent worms. Our studies revealed potential applications of EVs as vehicles in anthelmintic vaccine design, as well as in the development of an entirely new generation of therapeutics to treat chronic non-infectious diseases.

OA22.02 Transcriptomic and Genomic Characterization of the P-glycoprotein Gene Family in *Parascaris* sp.

Alexander Gerhard¹, Dr. Jürgen Krücken¹, Professor Dr. Emanuel Heitlinger², Professor Dr. Jianbin Wang⁴, Professor Dr. Richard Davis⁴, Professor Dr. Martin Nielsen⁵, Professor Dr. Georg von Samson-Himmelstjerna¹

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Anthelmintic resistance is on the rise in many parasitic nematodes. However, the mechanisms of anthelmintic resistance are largely unknown. P-glycoproteins (Pgps) have been proposed as putative contributors within a multi-genetic context in several nematode species including an important pathogen of foals, the horse roundworm *Parascaris* sp. The complete Pgp gene family of most nematodes is unknown and to date all studies have focussed on a few available candidate genes. This study aimed to comprehensively characterize the complete Pgp gene family in *Parascaris* sp. at the transcriptomic and genomic level.

One genome and two independent transcriptomes (one with tissue specific data and one obtained from in vitro cultured adult male worms with and without ivermectin exposure) were used as basis for the study. Pgps were characterized using transcriptome-guided RT-PCRs and Sanger sequencing, followed by phylogenetic analysis and relative expression analysis.

In total 10 different Pgps were identified. Among them, an ascarid specific Pgp lineage, Pgp-18, as well as two paralogs of Pgp-11 and Pgp-16. Through re-mapping of transcriptome raw reads on P-glycoprotein cDNAs, relative expression levels were estimated in both transcriptome datasets. Here, no ivermectin specific upregulation was found for any of the Pgps but Pgp-11.1, Pgp-16.2 and Pgp-9 exhibited significantly higher constitutive expression levels compared to low expression Pgps, namely Pgp-3, Pgp-12 and Pgp-18. Interestingly, intestinal Pgp expression was much higher than in any of the other tissues, e.g. in Pgp-11.1 compared to a whole worm sample, over 1000 fold.

The Pgp repertoire of *Parascaris* sp. comprises a diverse gene family and several Pgps were identified as candidates for future studies concerning their potential role in anthelmintic efflux. High level Pgp expression in the intestine suggests a role in intestinal barrier function in nematodes potentially leading to reduced uptake of anthelmintics.

OA22.03 Parasitic Nematode Drug Discovery

Dr Mostafa Elfawal¹, Dr. Sergey Savinov², Dr. Raffi Aoian¹

¹*University of Massachusetts Medical School, Worcester, United States*, ²*University of Massachusetts, Amherst, United States*

Parasitic nematodes are amongst the most prevalent disease-causing agents of human, livestock and companion animals. These parasites have a dramatic impact on the global economy and together perpetuate the poverty cycle via its harmful impact on human well-being, especially hundreds of millions of children and pregnant women. The cost and losses associated with parasitic nematodes

of livestock are estimated to be tens of billions of dollars worldwide due to adverse effects on health and productivity. Parasitic nematodes are controlled via regular administration of anthelmintic drugs. Unfortunately, parasitic nematodes of livestock are currently resistant to almost all classes of anthelmintic drugs available today. Following same track, drug resistance in human nematode parasites is underway with multiple reports of reduced activity and warnings for the foreseen resistance disaster. Clearly, new drugs against these parasites are highly needed. High-throughput phenotypic screening is an absolutely essential tool in finding new leads for drug development. Parasitic nematodes are not typically amenable to high-throughput screens due to their large size and the indispensable need for laboratory animals for parasites maintenance. Thus, screening against a model organism in place of adult parasites has been commonly adopted, including the developmental stages of nematode parasites and, most notably, the free-living nematode *Caenorhabditis elegans*. To answer whether model organisms are of a good representation of adult parasites as a screening tool for anthelmintic drugs, we screened the drug susceptibility of 1280- FDA-approved repurposing drugs to the completion against adult and larval stages of *C. elegans* and the hookworm nematode parasite *Ancylostoma ceylanicum*. Adult whipworms were also tested as part of the protocol. Here we will present the results of our screens, cheminformatics prioritization, in vivo validation, and our proposed pipeline for anthelmintic drug development. We will also present progress towards automation of the pipeline and delineation of a high-throughput platform.

OA22.04 Dynamic Mechanisms Allow Worms to Adjust Their Sensitivity to an Anthelmintic: Levamisole and Brugia Malayi

Dr Richard John Martin¹, Mr Mengisteab Wolday¹, Dr Sudhanva S Kashyap¹, Dr Saurabh Verma¹, Dr Alan P Robertson¹
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Anthelmintics can become ineffective if they develop resistance, which is usually explained as being acquired over generations due to genetic selection. However, individual, initially

sensitive nematode parasites can lose their sensitivity to an anthelmintic and recover when they are maintained in the presence of the anthelmintic.

To explore this phenomenon further, we have been studying effects of maintained concentrations of levamisole on *Brugia malayi*, one of the causes of elephantiasis. We have observed in Worminator motility studies that female *Brugia* initially respond to levamisole with a spastic paralysis; they then progress to flaccid paralysis; and then recovers to normal motility after 4 hours exposure to levamisole. When we have examined effects of maintained applications of levamisole to muscle cells of these worms under patch-clamp, we find that the inward current responses, due to the opening of muscle nicotinic receptors, decline (desensitize) over a period of 5-15 minutes, which may explain the flaccid paralysis that follows the initial spastic paralysis. When we examined the expression of levamisole receptor subunits at 4 hours during the recovery, we found that unc-38 and acr-8 expression was increased, while unc-29 and unc-63 showed little or no change. In *Brugia*, there are multiple subtypes of nicotinic receptors on their body muscle (M-, P-, L, and N-) sensitive to different cholinergic anthelmintics. The increased expression of unc-38 and acr-8 subunits may allow for an increase of acetylcholine nicotinic receptors that contribute to the recovery of motility; but not the recovery of sensitivity to levamisole that requires the presence of receptors composed of UNC-38, ACR-8, UNC-29 and UNC-63 subunits.

Thus, we can see that some worms have dynamic mechanisms that allow them to adjust their sensitivity to an anthelmintic. It will be interesting to determine how widespread these mechanisms are in our parasites.

Supported by NIH R01AI047194

OA22.05 Anthelmintic-like Activity of *Leucaena leucocephala* Aqueous Extract Against *Gyrodactylus* Spp., and Its Effect over the Host Health

Dr Elke von Son-de Fernex¹, MVZ Juan E. Compean-Martínez¹, IAZ Martha Salazar-Ulloa¹, M en C Biol Larisa Chavéz Soriano², M en C Biol. Germán Muñoz-Córdova¹

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The monogean parasite *Gyrodactylus* spp has been considered one of the most aggressive and pathogenic parasites in aquaculture; and the use of formaline baths has been the cornerstone for gyrodactylid control. However, formalin has been reported a carcinogenic and mutagenic agent for both host and consumers. The use of bioactive plant has been recently proposed as an alternative against gyrodactylus infestation. *Leucaena leucocephala* is a tropical plant reported with anthelmintic activity against both internal and external parasites of cattle. Therefore, the objective of this study was to assess the anthelmintic-like activity of *Leucaena leucocephala* aqueous extract (LL-AQ) against *Gyrodactylus* spp., in tilapia fish, and the effect over the host health. Sixty-three naturally infected tilapia fish (± 40 parasites per fish) were divided in 7 experimental groups (n=3) and placed in experimental fish restraint-chutes for a 60 min bath treatment. Five concentrations of LL-AQ were employed: 2, 1, 0.5, 0.25, 0.125 mg / mL -1. Distilled water and formalin (1:4000) were used as negative and positive control, respectively. Three replicates were run for each concentration and control. Parasites were counted pre and post-treatment using a stereomicroscope. Effective concentration (EC99) was calculated through a Probit Analysis using a maximum likelihood estimation method (CI 95%). Finally, the sixty-three animals were humanely slaughter to assess for histopathological changes. The

LL-AQ showed an EC99 of 0.462 ± 0.017 mg mL⁻¹, with a dose-dependent behavior ($P < 0.05$). Histopathological analysis revealed no damage treatment-associated, discarding a negative impact that could jeopardize the life of treated animals. The aqueous extract of *L. leucocephala* showed to be a promising alternative to control gyrodactylus infestation in Tilapia fish.

OA22.06 In Silico Analysis of Potential Vaccine Candidates for *Tritrichomonas Foetus*, the Causative Agent of Bovine Trichomoniasis

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Tritrichomonas foetus is an anaerobic flagellated protist and the causative agent of the venereal disease bovine trichomoniasis. This disease causes spontaneous abortions and, in some cases, infertility in cows and is responsible for decreased calving rates and milk production; infected animals are usually culled. Bovine trichomoniasis is therefore responsible for significant economic losses to farmers in several countries where the disease is endemic, including Australia, Brazil and the USA. Currently there is no vaccine available that can prevent reinfection.

In order to identify potential vaccine candidates for this parasite a reverse vaccinology approach was implemented. The *Tritrichomonas foetus* genome was sequenced on the PacBio platform (147Mb, N50 = 84,706), assembled using SMRTportal and then annotated using multiple automated processes including BRAKER, SNAP and BLAST2GO, integrated with transcriptomic data from both trophozoite and pseudocyst cell types, and improved through manual curation. Cell surface specific genes were identified using in silico prediction of signal peptides, transmembrane domains and GPI anchors.

In our *T. foetus* genome 84,706 genes have been identified, 1,607 of which contain a signal peptide and a transmembrane helix suggesting they are cell surface expressed

and will be further examined as potential epitopes. Once a reduced set of genes has been produced, they will be recombinantly expressed and tested for their immunogenic potential.

We have produced the first fully-annotated T. foetus genome as the first step in a reverse vaccinology approach to this important livestock disease. Preliminary analysis of predicted cell surface proteins has resolved diverse transmembrane proteins as potential vaccine candidates.

OA22.07 Mechanism of the Benzodiazepine Meclonazepam (Ro 11-3128) on Parasitic Flatworms

Dr. John Chan¹

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Parasitic flatworm infections (e.g. tapeworms and fluke worms) are treated by a limited number of drugs. In most cases, control is reliant upon praziquantel (PZQ) chemotherapy. However, PZQ is ineffective against larval/immature worms (cestode cysts and sexually immature lung/liver stage schistosomes), and there have been several reports on the emergence of PZQ-resistant cestodes and trematodes. Given the need for alternative therapies to treat parasitic flatworm infections, we have revisited the anthelmintic benzodiazepine meclonazepam (MCLZ). MCLZ was discovered in the 1970's but not brought to market due to dose-limiting sedative side effects. We were intrigued by observations that MCLZ differs from PZQ in several key aspects and have resumed studies on trematodes to determine its molecular mechanism of action. We show that MCLZ is effective against juvenile, PZQ-refractory *Schistosoma mansoni* infections in vivo. MCLZ also kills parasitic flatworms directly in vitro, while PZQ's effects are rapidly reversible following washout and its mechanism is likely immune-dependent. The targets of PZQ and MCLZ appear to be distinct, which augurs well for the utility of a benzodiazepine based-therapy should reports of PZQ-refractory parasites become more frequent. Binding assays show that MCLZ, but not PZQ, displaces radioligand from L-type

voltage operated Ca²⁺ channels (VOCCs). Involvement of VOCCs in MCLZ's mechanism is supported by data showing that MCLZ is (i) phenocopied by a VOCC agonist, (ii) Ca²⁺ dependent and (iii) inactive in worms with a deletion in a key VOCC subunit. MCLZ also causes collapse of mitochondrial membrane potential and promotes cleavage of proapoptotic caspases, supporting a model whereby VOCC activation increases intracellular Ca²⁺, exceeding mitochondrial buffering capacity to drive permeability-pore transition and programmed cell death. Collectively, these data show that the actions of the benzodiazepine MCLZ on parasitic flatworms are distinct from PZQ and provide a foundation for developing analogs with improved parasite selectivity.

OA22.08 Must Improved Animal Welfare Lead to More Parasites?

Ms. Emelie Pettersson², Dr Marie Sjölund¹, Dr Eva Osterman Lind¹, Professor Johan Höglund², Professor Per Wallgren¹
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The national parasite status of pigs in Sweden has not been investigated since the 1980's. Since then, new animal welfare laws have been implemented, stating that pigs are to be kept loose at all occasions and sows are often housed on deep litter straw beds. Slats are also only allowed to constitute 30% of the pen floor. It has been hypothesized that these examples of more natural housing conditions may increase the parasite load.

The aim of our study was to investigate the status of gastrointestinal parasites in 25 herds with more than 100 sows. In 2017-2018, a total of 962 faecal samples were collected from piglets (n=205), growers (n=207), fatteners (n=177) and sows (n=373) and analysed for nematode eggs and coccidian oocysts using a modified McMaster technique based on 3 g of faeces.

Parasites were found in all but one herd. In total, *Ascaris suum* was detected in 6%, *Oesophagostomum* spp. in 19%, *Trichuris suis* in <1% and coccidian oocysts in 13% of the

samples. Compared to the 1980's, *Ascaris suum* decreased from 27% to 8% in fatteners, but increased from 7% to 11% in sows. Also, *Oesophagostomum* spp. decreased from 6% to 3% in fatteners but increased from 27% to 43% in sows. *Eimeria* spp. were found mainly in sows, 17% compared to 2% in the 1980s. In piglets *Cystoisospora suis* was detected in 16% compared to 20% in previous studies.

Overall our results showed a comparably low parasite load in fatteners, indicating that the changed housing conditions are not associated with increased parasite exposure, despite increased contact with manure and a limited use of chemical disinfectants and antiparasitic drugs. A possibility is that age-segregated rearing, that has been implemented in most herds since the 1980's, decreases especially the spread of *Ascaris suum* from older to younger pigs.

OA23 Leishmania

July 9, 2019, 13:30 - 15:30
Breakout Room 3, Hall of Ideas F&I, Level 4

OA23.01 Splenic Architecture Alterations and Cell Phenotypes between Symptomatic and Asymptomatic US Dogs with Visceral Leishmaniasis

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Dogs are the reservoir species for visceral leishmaniasis, and canine visceral leishmaniasis closely models the course of disease in human patients. Secondary lymphoid organs, particularly spleen, undergo alterations in immune function and microscopic structure as VL progresses from asymptomatic to clinically symptomatic stages. How and why these changes occur are not well understood. We hypothesized that dogs with symptomatic canine visceral leishmaniasis will have fewer splenic follicles, poorly organized follicles and germinal centers, and lower splenic white pulp area when compared to asymptomatic,

Leishmania-infected, dogs. These alterations in follicular organization will be associated with changes in population size and distribution of splenic CD4+CXCR5+T_H cells and CD19+PDL1+ Breg cells.

Dogs were selected from an outbred cohort previously shown to have *L. infantum* infection and determined to be asymptomatic or symptomatic through physical examination. These dogs were humanely euthanized for tissue collection. Light microscopy was used to evaluate splenic structure from H&E stained slides. Staining on slides was secondarily analyzed with digital imaging software to measure follicle area, total white pulp area, and total red pulp area.

From these initial results we conclude that when measured by digital imaging software, adult symptomatic dogs exhibited a greater total follicle area than adult asymptomatic dogs. Adult asymptomatic dogs also had a higher ratio of primary (immunologically dormant) to secondary (immunologically active) follicles. Juvenile asymptomatic dogs exhibited a higher manual follicle count/mm² of splenic area than adult symptomatic or adult asymptomatic dogs. These results suggest greater immunologic activity in adult symptomatic dogs, and age-related differences in follicle size and activation within the cohort.

OA23.02 Safety and Efficacy of Canileish Vaccine in Exposed Dogs

Dr. Manuela Gizzarelli¹, Dr. Valentina Foglia Manzillo¹, Dr. Fabrizio Vitale², Dr. Salvo Sotera³, Dr. Serena Montagnaro¹, **Prof. Gaetano Oliva**¹
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Aims

Vaccination against CanL reduces the parasite load and the risk of progressive infection in dogs. The study aimed to investigate the risk of developing clinical disease in exposed dogs (Paltrinieri et al., 2010) when vaccinated with LiESP/QA-21 (CaniLeish™) and to assess if it is safe in these animals.

Contents

The study was performed during two consecutive transmission seasons in Lampedusa, a small Italian island highly endemic for canine leishmaniasis (Foglia Manzillo et al., 2018). Sixty-nine owned dogs of different sex, breed and age were submitted to clinical examination, blood sample and vaccination. The presence of anti-Leishmania antibodies was assessed by a rapid immunochromatographic test (Speed Leish K™, Virbac BVT) before the prime vaccination. Sera were analysed by IFAT too. Dogs were monitored every six months and after one year they received the booster vaccination. Vaccinated dogs were divided in: group A - 43 negative to the pre-vaccination IFAT test; group B - 26 positive to IFAT at low titer ($<1:160$). No statistical difference was found when the age was compared ($P=0,2696$ - Kendall's Tau). Only 4 animals (2.7%), two for each group, showed high antibodies level and clinical alterations. Kaplan-Meier survival curves demonstrated no statistical difference between the groups ($P=0,5516$). At the end of follow-up 9 dogs (20,93%) of group A remained negative to IFAT while the others showed low antibody titers ($\leq 1:160$). In group B, 1 dog converted to negative serology, the others maintained initial values. Sixty-five dogs had no clinical abnormalities. In both groups few transient adverse events to vaccine were recorded.

Conclusion

Our data seem to demonstrate that: i) the CaniLeish™ vaccine is safe also in dogs classified as “exposed” (Paltrinieri et al., 2010) or “subclinically infected” (Solano-Gallego et al., 2009); ii) in these dogs CaniLeish™ avoids the progression of the Leishmania infection during two consecutive transmission seasons.

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In the New World, *Leishmania infantum* is the agent of visceral leishmaniasis and CanL, which are spread by the bite of infected sand flies. The dog is considered the main reservoir in urban areas of Brazil and the treatment of CanL still represents a challenge. The objective of this study was to evaluate the clinical and laboratory aspects of dogs naturally infected with *L. infantum* submitted to therapy with marbofloxacin associated with allopurinol. To evaluate the efficacy of marbofloxacin 12 domiciled dogs of both genders, varied race and age between 1-7 years were used. The animals were divided into two treatment groups: Group 1 (G1) were treated with oral marbofloxacin (Marbopet® Laboratory CEVA, Brazil) at 2 mg/Kg/day for 28 days in combination with oral allopurinol at 10 mg/kg every 12 hours and Group 2 (G2) treated with marbofloxacin and allopurinol in the same dosage, but the allopurinol was give to dogs from G2 after 28 days of administration of marbofloxacin. After the treatment dogs were assessed during 90 days by performing monthly physical exams, and evaluating red blood count, white blood count, alkaline phosphatase, alanine aminotransferase, urea, creatinine, serum protein, albumin and globulin parameters. Each clinical parameter was classified according to its severity on a numerical scale of 0 to 3. In group G1, the reduction in scores was 76.4%, while the G2 group presented a reduction of 54.8%. The averages of albumin were higher at 90 days in the G1 group. No dogs from both of groups presented increase of urea and creatinine. After the following up of 90 days it can be concluded the marbofloxacin and allopurinol treatment in combination at the same time provided a greater remission of clinical signs, improvement in hematological and biochemical profiles and increase of albumin value.

OA23.03 Evaluation of Clinical, Laboratory Profiles of Dogs Naturally Infected With *L. Infantum* Submitted to Therapy With Marbofloxacin Associated With Allopurinol

Dr. Leucio Alves¹, Dr. Janilene Nascimento¹, Dr. Claudio Rossi², Dr. Leonardo Brandao², Dr. Talita Lins¹, Dr. Maria Vanuza de Meireles¹, Dr. Wagner Andrade¹, Dr. Roseane Feitosa¹, Dr. Winny Silva¹, Dr. Edna Michelly Santos¹

OA23.04 Evaluation of DPP-Leishmania and SNAP-Leish Evaluation of DPP-Leishmania and SNAP-Leishmania Rapid Tests for Diagnosis of Leishmania Infantum Canine Infections

Celeste Souza¹, Professor Valmir Silva², **Dr. Bruno Alberigi**³, Professor Norma Labarthe⁴

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Visceral leishmaniasis is a zoonotic disease of global importance that affects up to 1 million humans every year, causing at least 20,000 deaths worldwide. Besides affecting humans, it also affects wildlife and domestic species. Since dogs play a key role in urban Leishmania spp. transmission, the Brazilian government maintains a Program to Monitor and Control Visceral Leishmaniasis (PVCLV), which promotes awareness campaigns aiming to enhance control of the infection. The PVCLV recommends the DPP® canine visceral leishmaniasis test (Bio-Manguinhos, Brazil) for screening and an ELISA to confirm infection. The DPP® test is produced and distributed by the Health Ministry to the Municipal Health Centers responsible for the local PVCLV; the product is not available to clinics, forcing most veterinarian practitioners to use other rapid tests for screening and diagnosis of this disease in their clinical routine. This study was conducted to compare the performance of the DPP® and the SNAP® Canine Leishmania Antibody Test (IDEXX Laboratories, Inc., USA) tests using sera from dogs with confirmed infections of *L. infantum* as well as sera from dogs residing in non-endemic areas or in areas with a low prevalence of Leishmania infection. The results obtained showed that SNAP® and DPP® tests were virtually equivalent for detection of canine antibodies against *L. infantum*. There was 97.0% agreement between the two tests. The SNAP®

test had sensitivity of 96.3% and specificity of 100%. Agreement between both antibody tests and parasitological detection methods was 96.8%. The DPP® test had sensitivity of 95.8% and specificity of 100%. The SNAP® and DPP® tests demonstrated high and similar levels of sensitivity and specificity. The SNAP test can be used for screening canine samples for Leishmania antibodies.

OA23.05 Canileish Vaccine and Repellents Are Effective Measures to Prevent Canine Leishmaniasis in Field Condition: A 6-Year-Field Retrospective Study

Dr. Antonio Inglese¹, Dr. Manuela Gizzarelli², Dr. Valentina Foglia Manzillo², **Dr. Gaetano Oliva**², Dr. Christelle Speiser Fontaine³

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Medical preventative measures against leishmaniosis includes the regular use of phlebotomine repellents and/or of vaccines (Maroli et al., 2010). The study aimed to assess the efficacy of these measures in long term field conditions.

Data were collected between February, 1st 2012 and April, 30th 2018 in a veterinary clinic located in Grottaglie, Italy. Dogs of ≥ 6 months of age at the time of the leishmaniosis diagnosis or of the first vaccine injection were allocated into 4 groups: no preventative measure (NVNR), repellents only (NVR), vaccine only (VNR), vaccine and repellents (VR). Leishmaniosis diagnosis was confirmed if dogs were symptomatic with ELISA = 1, or, when ELISA > 1 (Solano-Gallego et al., 2009). Cases with uncomplete data were not analyzed.

The analysis was made on 240 dogs: 31/240 (13%) in group NVNR, 63/240 (26%) in group NVR, 63/240 (26%) in group VNR and 83/240 (35%) in group VR. The groups were not different in terms of sex, breed, life style repartition or repellents used when appropriate. The dogs of the VR groups were statistically younger than those of the NVR and the NVNR groups at the beginning of

the follow-up (respectively 1.4 ± 0.8 , 2.9 ± 1.5 and 3.5 ± 2.8 years of age). Diagnosis of leishmaniosis was confirmed in 20/31 (34%) dogs for the NVNR group, 10/63 (15.9%) dogs in the NVR group, 0/63 (0%) in the VNR and 0/83 (0%) in the VR group.

Canileish™ vaccine (Oliva et al., 2014; Martin et al., 2014; Moreno et al., 2014), in addition or not to repellents, prevented the development of an active Leishmania infection in all (146/146) dogs. Unvaccinated dogs treated with repellents were 9.6 time less likely to develop a leishmaniosis infection than untreated dogs. Vaccination and/or the use of repellents decreased the risk of leishmaniosis in at-risk dogs.

OA23.06 Leishmaniosis in Vaccinated Dogs: Clinical Aspects

Rubén Fojí¹, Glòria Polí, Jerónimo Carnés³, Pilar Brazis¹, Lluís Ferrer²

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The approval by the EMA of two vaccines against canine leishmaniosis (CanL) has been a major step in the control of this zoonosis in Europe. In recent years, hundreds of thousands of dogs have been vaccinated. However, the impact of vaccination on the disease epidemiology and on the health of individual dogs has yet to be evaluated. According to published data both licensed vaccines have an efficacy of protection close to 70%, meaning that some vaccinated dogs develop the disease. The characteristics of the disease in vaccinated dogs is of major importance.

The objective of this study was to characterize the clinical aspects of leishmaniosis in dogs vaccinated against the disease with LetiFend® (Protein Q-vaccine).

Forty-six vaccinated dogs were diagnosed with CanL based on clinical signs, clinicopathologic abnormalities (complete blood count, serum biochemistry, urinalysis) and positive serology (ELISA and IFAT). All

dogs were followed for a minimum of six months. Dogs belonged to 20 different breeds and the mean age was 3,9 years. The mean time between vaccination and diagnosis of CanL was 10 months (range: 4 to 16 months) and the majority of cases (41/46 dogs, 89%) had been treated with topical insecticides-repellents. At time of diagnosis, 4 dogs were considered to have the disease in stage I (Leishvet-classification), 33 in stage II, 8 in stage III and 1 in stage IV. Twenty-three dogs (50%) had low-medium IFAT titers, and the rest had medium-to-high titers. In most cases (33/46, 72%), dogs received the standard treatment (N-methyl-glucamine-antimoniate or miltefosine and allopurinol). After six months, 43/46 dogs (94%) were still alive and 78% were free of clinical signs. Anti-Leishmania antibody titers (IFAT) dropped considerably and 33/35 (94%) were negative or had low-medium titers.

Altogether, these data seem to indicate that the disease in vaccinated dogs presents a mild clinical course.

OA24 Nematode Molecular Tools, Resistance II

July 9, 2019, 13:30 - 15:30

Breakout Room 4, Hall of Ideas G&J, Level 4

OA24.01 Identification and Biochemical Characterization of Three Conserved Haemonchus contortus Cathepsin B-like Proteases

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The trichostrongyle, *Haemonchus contortus*, is a blood sucking abomasal nematode that causes significant economic losses due to treatment costs and reductions in animal health and productivity. Incorrect and excessive chemical deworming have resulted in anthelmintic resistance to all major classes of drugs. Consequently, effective vaccine targets continue to be sought as cheap and alternative measures for control. Parasite-

derived proteases and protease inhibitors have been implicated in controlling host responses to infection and therefore make good vaccine candidates. In-silico analyses of protease and protease inhibitor sequences from *H. contortus* showed high degrees of conservation among clade V nematodes. 'Conserved Domain Analysis' (NCBI) was performed followed by homology modeling using SWISS-MODEL to identify a class of cysteine proteases known as Cathepsin B-like (CBP) which are essential parasite virulence factors and therefore attractive vaccine targets. Further, analyses showed that all identified proteins possessed canonical CBP active sites and classic secretory signals. To this end, three novel CBP genes from *H. contortus*, designated Hc-CBP-1, Hc-CBP-2 and Hc-CBP-3, were selected, cloned and expressed. The full-length Hc-CBP-1, Hc-CBP-2 and Hc-CBP-3 genes are 1044, 1041 and 1050 base pairs in length and encode proteins that are 348, 347 and 350 amino acids long, respectively; sequence homology spanned 57-67% among the proteins. When expressed in a prokaryotic system, the protein masses ranged from ~35-37 kDa via gel analysis and Western blots. We investigated the functional and degradative roles of these recombinant cathepsins on various protein substrates over a broad pH range in vitro. Our analyses showed that these enzymes are likely multifunctional and may be involved in nutrition, development and pathogenicity of *H. contortus*. These results suggest that H-CBPs are promising candidates for developing vaccines against Haemonchosis.

OA24.02 Exploring Benzimidazole Resistance in *Haemonchus Contortus* by Next Generation Sequencing and Droplet Digital PCR

Paulius Baltrusis¹, Dr. Peter Halvarsson¹, Professor Johan Höglund¹

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Anthelmintic resistance in gastrointestinal nematode (GIN) parasites of grazing ruminants is on the rise in countries across the world. *Haemonchus contortus* is one of most frequently encountered drug-resistant GINs in small ruminants. This blood-sucking abomasal nematode contributes to massive treatment

costs and poses a serious threat to farm animal health. To prevent the establishment of resistant strains of this parasite, up-to-date molecular techniques need to be proposed which would allow for quick, cheap and accurate identification of individuals infected with resistant worms. The effort has been made in the previous decade, with the development of the pyrosequencing method to detect resistance-predicting alleles. Here we propose a novel droplet digital PCR (ddPCR) assay for rapid and precise identification of *H. contortus* strains as being resistant or susceptible to benzimidazole drugs based on the presence or absence of the most common resistance-conferring mutation F200Y (TAC) in the γ tubulin isotype 1 gene. The newly developed ddPCR assay was first optimized and validated utilizing DNA templates from single-worm samples, which were previously sequenced using the next generation PacBio RSII Sequencing (NGS) platform. Subsequent NGS results for faecal larval cultures were then used as a reference to compare the obtained values for fractional abundances of the resistance-determining mutant allele between ddPCR and NGS techniques in each sample. Both methods managed to produce highly similar results and ddPCR proved to be a reliable tool which, when utilized at full capacity, can be used to create a powerful mutation detection and quantification assay.

OA24.03 Transcriptomic and Genomic Approaches to Ivermectin Resistance in *Haemonchus Contortus*

Dr. Roz Laing¹, Ms. Kirsty Maitland¹, Dr. James Cotton², Dr. Stephen Doyle², Ms. Nancy Holroyd², Dr. Collette Britton¹, Dr. Dave Bartley³, Ms. Alison Morrison³, **Prof. Eileen Devaney**¹

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The BUG consortium is a UK based project aimed at using the *Haemonchus contortus* genomic resources to improve the understanding of anthelmintic resistance, with an emphasis on ivermectin (IVM). Using

a genetic cross between a drug sensitive (MHco3) and a drug resistant isolate (MHco18), a locus on chromosome V was shown to be under selection by pooled whole genome re-sequencing of F3 L3 pre- and post-IVM exposure. Transcriptomic analysis was also carried out using male and female worms from both parental isolates and the F2 generation of the cross, with and without IVM exposure. As expected there were many constitutive differences in gene expression in the parental samples (MHco3 versus MHco18). However, genes that were also differentially expressed in pairwise comparisons of the F2 generation of the cross with and without IVM exposure represent a high confidence set of genes associated with IVM resistance. Many of the differentially expressed genes had orthologues in *C. elegans* and these were significantly enriched for neuromuscular GO terms, including regulation of neuronal differentiation (males) and contractile fiber (females). In an attempt to prioritise candidate genes in the genomic locus under IVM selection, the transcriptomic data was used to identify genes that also showed differential expression in IVM-resistant adults. These included a number of kinases and several genes involved in regulating neuronal/behavioural plasticity. The orthologues of these genes are now being studied in *C. elegans* to determine whether they do indeed play a role in IVM resistance.

OA24.04 Unraveling the Genetic Mediators of Multi-Drug Resistance in *Haemonchus Contortus* Using Forward Genetics

Dr. Stephen Doyle¹, Dr. Roz Laing², Dr. David Bartley³, Dr. Alison Morrison³, Dr. Kirsty Maitland², Dr. Umer Chaudhry⁴, Dr. Nancy Holroyd¹, Dr. Matthew Berriman¹, Dr. John Gilleard⁵, Dr. Neil Sargison⁴, Dr. James Cotton¹, Dr. Eileen Devaney²

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Drug resistance in parasitic helminths is widespread in a number of domesticated animal systems, and represents a genuine

threat to the sustainable control of species that parasitize humans. Unfortunately, the genetic basis of resistance to most anthelmintics remains poorly resolved. As a key aim of the BUG Consortium, we have used a forward genetics approach together with whole genome sequencing to unravel the genetic mediators of multidrug resistance in the gastrointestinal parasite, *Haemonchus contortus*. We performed a genetic cross between susceptible MHco3(ISE) and triple resistant (benzimidazole, levamisole & ivermectin) MHco18(UGA) parasites, after which we sampled the F3 population both pre- and post-treatment of the F2 population in vivo for each of the three drug classes. For each of the drug classes, we identify discrete, non-overlapping regions of the genome linked to resistance. We identified a number of previously characterized genes linked to benzimidazole and levamisole resistance; however, for ivermectin, a more complex signature of selection was apparent, which included a major driver on chromosome V as well as a number of minor effect loci contributing to resistance. Finally, we demonstrate how underdosing primes the genetic landscape, leading to increased frequency of some alleles before higher dosing selects unique combinations of mutations to produce more resistant individuals. These data provide an important genome-wide overview of the genetic response to anthelmintic treatment, and an insight into the evolution and spread of resistance.

OA24.05 How to Detect Genomic Signatures of Selection Associated With Anthelmintic Drug Resistance in Field Populations of Parasitic Nematodes

Dr. Janneke Wit¹, Dr. Matthew Workentine¹, Dr. Umer Chaudhry², Dr. Sam Yeaman³, Dr. Sean Rogers³, Dr. James Wasmuth¹, **Prof. John Stuart Gilleard**¹

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The ability to detect genomic signatures of selection in field populations of parasitic nematodes would allow the genetic basis of

resistance to both current and new drugs to be more rapidly determined and resistance to be detected at an earlier stage. The recent completion of a chromosomal level reference genome assembly for *Haemonchus contortus* now makes this potentially feasible. However, the nature of genomic signatures of selection associated with drug resistance, and the best way to detect them, is poorly understood. Consequently, we have used *H. contortus* as a model to investigate these questions using field populations predicted to have strong genomic signatures of selection associated with benzimidazole resistance. We have undertaken two different genome-wide scanning approaches to compare *H. contortus* populations from government sheep flocks in Pakistan, subject to frequent benzimidazole treatment and closed to animal movement, with pastoral flocks that are rarely treated. We genotyped ~100,000 polymorphic markers across the 300Mb *H. contortus* genome from 15 individual adult worms per population using double-digest Restriction-site Associated DNA (ddRAD) sequencing. We also undertook whole genome sequencing of 15 individual worms from the same populations. A strong signature of selection centred on the isotype-1 β -tubulin locus was visible in the drug resistant populations using nucleotide diversity, linkage disequilibrium and *F_{st}* analysis. Weaker signatures of selection were apparent at a number of other genetic loci. We have used the data to compare the relative merits ddRADseq versus whole genome sequencing and of “Poolseq” versus individual worm sequencing. This work provides a strong proof-of-concept that genome-wide scanning can unequivocally detect anthelmintic resistance associated signatures of selection in field populations of parasitic nematodes and provides important information as to the best approaches.

OA24.06 Investigating the Molecular Epidemiology of Benzimidazole Resistance in Ovine Gastrointestinal Nematodes Using Deep Amplicon Sequencing

Dr. Camila De Queiroz¹, Dr. Russell Avramenko¹, Dr. Elisabeth Redman¹, Dr. Fabienne Uehlinger², Dr. Michel Levy¹, Dr. John Gilleard¹

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Anthelmintic resistance is a threat to sustainable parasite control worldwide. An understanding of how resistance mutations emerge and spread is foundational information for strategies aimed at prolonging the useful life of current drugs. To date, sequence polymorphisms in codons 167, 198 and 200 of the isotype-1 β -tubulin gene have been associated with benzimidazole resistance of several gastrointestinal nematode species. New sequencing technologies now allow us to study the molecular epidemiology of these mutations in the field in unprecedented scale. Deep amplicon sequencing, using next-generation sequencing platforms, has many advantages, including the ability to detect low frequency resistance mutations and haplotypes with a high scalability due to the ability to multiplex hundreds of samples and loci in a single sequencing run. We have undertaken a large-scale screen for benzimidazole resistance-associated mutations of the major gastrointestinal nematode species and applied phylogenetic analysis to study the origins and spread of these mutations in the region. Populations of L1 larvae were harvested from sheep fecal samples, collected before and after treatment with benzimidazoles, from more than 40 farms across western Canada. We sequenced the relevant region of the isotype-1 β -tubulin locus and applied PEAR (paired-end read merger) to the reads obtained from each sample to form contigs. We then used the Mothur bioinformatic tool to assign sequences to nematode species and a pipeline with BWA, VarDict and SnpSift to identify non-synonymous mutations occurring at the three codons. Finally, we calculated the frequency of each of the resistance associated single nucleotide polymorphisms. The F200Y allele was present at high frequency

in many *H. contortus*, *T. circumcincta* and *T. colubriformis* populations. We will present an analysis of the association of this mutation with the resistant phenotype, evidence of selection and haplotype network analysis to investigate the origin and spread of this mutation in Western Canada.

OA24.07 Does Triclabendazole Resistance in *Fasciola Hepatica* Have the Same Genetic Basis in Different Areas of the UK?

Dr. Nicola Beesley¹, Miss Rebecca Hoyle¹, Professor Diana Williams¹, Professor Steve Paterson², Professor Jane Hodgkinson¹
¹*Veterinary Parasitology, University of Liverpool, Liverpool, United Kingdom*, ²*Centre for Genomic Research, University of Liverpool, Liverpool, United Kingdom*

Fasciola hepatica causes parasitic disease in livestock. Emergence of resistance to triclabendazole (TCBZ), the treatment of choice, is a substantial threat to control; yet the mechanism of TCBZ-resistance is unknown. Using an experimental genetic cross of clonal susceptible and resistant *F. hepatica*, we have determined that a single locus, ~3.2Mb in size, can confer TCBZ-resistance and is dominantly inherited. However, we do not know if resistance has the same genetic basis in geographically distinct natural populations.

We sourced parasites from two populations: (i) adult *F. hepatica* collected from six sheep experimentally infected with a TCBZ-resistant population from Wales, UK, (FhLivR4pop); three of which were TCBZ-treated and three untreated (ii) *F. hepatica* eggs collected from Day 0 and Day 21 samples following composite faecal egg count reduction test (cFECRT) on five farms from Wales and England, UK. Following Illumina sequencing, we mapped reads and identified regions with significant differences in SNP allele frequencies between TCBZ-treated and untreated, to highlight genomic regions associated with TCBZ-resistance.

The ~3.2Mb region identified in our genetic crossing experiment was shown to be associated with TCBZ-resistance in adult parasites from FhLivR4pop. This indicates that

one scaffold was under selection in both our experimental system and a natural population. However, it was not the only scaffold under selection and other scaffolds showed a stronger association with TCBZ-resistance in this population. This may mean there are multiple loci associated with TCBZ-resistance or that, given the inherent genetic variation present in field populations compared to our controlled clonal genetic cross, the results are more complex to interpret.

These results provide a basis to define the mechanism of TCBZ-resistance.

OA24.08 A Comprehensive Study of N-Glycosylation of *Haemonchus Contortus*

Dr. Chunqun Wang¹, Dr. Wenjie Gao², Dr. Xin Liu², Dr. Min Hu¹
¹*Huazhong Agricultural University, Wuhan, China*, ²*Huazhong University of Science and Technology, Wuhan, China*

N-glycosylation is one of the most prominent post-translational modifications of proteins that plays pivotal roles in varieties of biological functions and processes. The studies in N-glycosylation of helminths have attracted attentions not just due to the characteristics of composition of N-glycosylation, but also for their potential roles in vaccine and new drug development. *Haemonchus contortus* is an economically important blood-sucking nematode of ruminants with worldwide distribution. Previous evidences have indicated that several bioactive and immunogenic proteins in *H. contortus* are highly glycosylated. However, little is known about the nature of N-glycosylation of *H. contortus*, especially in this area of functional N-glycoproteomics. Here, we comprehensively mapped N-glycosylation of adult *H. contortus* using high-resolution mass spectrometry-based glycomics and proteomics, as well as lectin histochemistry to locate these glycoproteins on the *H. contortus* anatomical sites. Results showed that high mannose glycans were dominated in the N-glycan profiling that were primarily expressed on intestine, gonad of *H. contortus* and lectin localization analysis confirmed the abundance of

N-glycosylated proteins. We further enriched the glycoproteins using hydrophilic interaction chromatography with mass spectrometry and identified 559 N-glycosylated sites on 337 proteins expressed in *H. contortus*. Among these identified proteins, a large proportion of proteins are involved in metabolic processes. According to the sequence alignment analyses, 14 potential vaccine candidates with known immune modulatory properties were detected including aminopeptidases, zinc metallopeptidases, cysteine proteinases, galectin and pepsinogen. In addition, we also revealed the glycosylation characteristics of many key molecules involved in the growth development and invasion processes of this parasite. This study provides a comprehensive insight into the N-glycosylation composition of *H. contortus*, suggesting the importance of further explorations in examining glycosylation in the development of novel interventions against haemonchosis.

OA25 IAFWP Symposium

July 9, 2019, 13:30 - 15:30

Breakout Room 5, Meeting Rooms KLOP, Level 4

OA25.01 The Role of Livestock in the Foodborne Transmission of *Giardia Duodenalis* and *Cryptosporidium* Spp. To Humans

Dr. Brent Dixon¹

¹Health Canada, Ottawa, Canada

Giardia duodenalis and *Cryptosporidium* spp. are common protozoan parasites in humans and domestic and wild animals worldwide, and are important causes of enteric illness. Transmission occurs by means of the fecal-oral route, whereby *G. duodenalis* cysts and *Cryptosporidium* spp. oocysts are ingested directly, through contact with feces, or indirectly by means of a vehicle such as water or food. While person-to-person and waterborne transmission account for most human infections, zoonotic transmission has also generated considerable interest. Both *G. duodenalis* and *Cryptosporidium* spp. are highly prevalent in livestock, particularly young animals, which can shed very large

numbers of cysts and oocysts into the environment. These animals are thought to play a significant role in the contamination of foods, especially fresh produce, potentially leading to illnesses and outbreaks.

Contamination of fruits and vegetables may result through livestock accessing crop lands or surface waters, agricultural runoff, or the use of improperly treated manure as fertilizer. Products of animal origin, such as milk and meat, may also become contaminated due to poor udder hygiene or fecal contamination at the slaughterhouse, respectively. Further work is required to clarify the role of livestock in the contamination of foods with zoonotic isolates of *G. duodenalis* and *Cryptosporidium* spp. A better understanding of the potential sources of contamination will be required in order to develop and implement effective control strategies. This presentation will discuss the potential role of livestock in the contamination of foods with *G. duodenalis* and *Cryptosporidium* spp., and its public health significance. The discussion will be illustrated using the results of studies performed in our laboratory on the prevalence and molecular characterization of these parasites in cattle and swine, as well as in different food commodities, including packaged leafy greens, shellfish, and fresh meats purchased at retail.

OA25.02 Host Adaptation in *Cryptosporidium Parvum*: Genetic Basis and Implications to Epidemiology

Yaoyu Feng¹, Yuanfei Wang^{1,2}, Dawn Roellig²,
Professor Lihua Xiao²

¹Key Laboratory of Zoonosis, Ministry of Agriculture, College of Veterinary Medicine, South China Agricultural University, Guangzhou, China, ²Division of Foodborne, Waterborne, and Environmental Diseases, Centers for Disease Control and Prevention, Georgia, United States

Cryptosporidium parvum is the *Cryptosporidium* species with the broadest host range, responsible for most zoonotic *Cryptosporidium* infections in humans. Host adaptation, however, is known to occur within the species based on sequence analysis of the hypervariable 60 kDa glycoprotein

gene, with IIa, IIc, and IId subtype families preferentially infecting calves, humans and lambs, respectively. To improve our understanding of the genetic basis of host adaptation in *C. parvum*, we sequenced the genomes of 257 specimens of the IIa, IIc, and IId subtype families from various sources using the Illumina 250 × 250 bp paired-end technique. Comparative genomics analyses have identified significant differences in nucleotide sequences among subtype families of *C. parvum*. There are ~13,000 single nucleotide polymorphisms (SNPs) between IIa and IId subtype families and ~20,000 SNPs between IIa and IIc subtype families across the 9.1 Mb genome. Most highly polymorphic genes among the three subtype families are subtelomeric ones encoding secretory proteins, especially the invasion-associated and immunodominant mucin proteins and members of the *Cryptosporidium*-specific SKSR gene family. The three subtype families also differ in the copy numbers of subtelomeric genes encoding the MEDLE family of secretory proteins and insulinase-like proteases, with IIc having significantly fewer copies than IIa and IId. Geographically segregated subpopulations have been further identified in the IIa and IId subtype families at the whole genome level. Thus, host adaptation in subtype families of *C. parvum* is associated with significant genomic differences, and the occurrence of host adaptation in the major zoonotic *Cryptosporidium* species is likely to reduce the occurrence of zoonotic infections in humans and cross-species transmission in farm animals.

OA25.03 Improving *Cryptosporidium Parvum* Subtype Identification Using Next Generation Sequencing

Dr. Monica Santin¹, Dr. Jenny Maloney¹
¹EMFSL/ARS/USDA, Beltsville, United States

Cryptosporidial infection is one of the most common causes of diarrhea in humans and livestock worldwide. The epidemiology of cryptosporidiosis has received significant attention because of the public health and economic importance of this disease in humans and animals. Out of the four species predominantly identified in cattle

only *Cryptosporidium parvum* is a serious pathogen. Prevalence studies indicate a high occurrence of *C. parvum* infections in pre-weaned calves. Molecular methods for species identification are now widely used in epidemiological studies of *Cryptosporidium* infections. Additionally, amplification and sequencing of the 60-kDa glycoprotein (gp60) gene provides the basis for characterization of *C. parvum* at a subtype level. However, to better understand transmission dynamics and to answer many unresolved epidemiological questions, it is necessary to improve detection of mixed subtype infections. Highly sensitive tools with good resolution power are needed to better understand mixed infections. Therefore, a method to study intra-host subtype diversity using next generation sequencing (NGS) of the gp60 gene on the Illumina MiSeq platform was developed and used to expose *C. parvum* subtype composition in fecal samples from calves. The results demonstrate the suitability of this assay to improve our understanding of *C. parvum* subtype diversity. They also denote that subtyping studies able to elucidate intra-host subtype diversity are required to better understand host specificity, source of infection, and transmission dynamics of *C. parvum*.

OA25.04 *Cryptosporidium* Genotypes and Subtypes in Diarrheal Dairy Calves in France

Dr. Mohamed Mammeri¹, Aurélie Chevillot³, Ilham Chenafi¹, Myriam Thomas³, Christine Julien², Isabelle Vallée³, Bruno Polack¹, Jérôme Follet⁴, **Prof. Karim Adjou¹**

¹UMR BIPAR, Ecole Nationale Vétérinaire d'Alfort, ANSES, INRA, Université Paris-Est, Maisons-Alfort, F-94700, France., Maisons-Alfort, France, ²Phileo Lesaffre Animal Care, 137 rue Gabriel Péri, 59 700 Marcq-en-Barœul, France, ³UMR BIPAR, Anses, Ecole Nationale Vétérinaire d'Alfort, INRA, Université Paris-Est, Animal Health Laboratory, Maisons-Alfort, F-94700, France, ⁴Université de Lille, CNRS, ISEN, UMR 8520- IEMN, Lille France 59000, France

Little is known about the genetic of *Cryptosporidium* in calves in eastern regions of France. The aim of this study was to detect and isolate *Cryptosporidium* spp. in faecal samples from naturally infected pre-weaned

calves (≤ 45 days-old) in France. A total of 35 diarrhoeic pre-weaned calves faecal samples were collected from 26 dairy cattle farms with or without diarrhoea in 6 provinces. The screening was established microscopically by the detection of *Cryptosporidium* oocysts using an immunofluorescence (IF) staining method. IF-positive samples were then analysed to determine species by PCR-RFLP and sequencing targeting the 18S rRNA gene. *C. parvum* positive samples were subtyped through the analysis of the partial 60 kDa glycoprotein (gp60) gene. Data were then integrated into a phylogenetic tree analysis. IF revealed the presence of *Cryptosporidium* oocysts in 31 out of 35 (88%) samples. Combining results of 18S rRNA gene analysis, *C. parvum* was detected in 30 samples. Subtyping analysis in 27/30 samples (90%) of the *C. parvum* isolates revealed two zoonotic subtype families, IIa (24/27) and IIc (3/27). Four subtypes were recognized within the subtype family IIa including IIaA15G2R1 (21/27) which is the hypertransmissible subtype the most frequently reported worldwide, IIaA17G3R1 (1/27), IIaA17G1R1 (1/27) and IIaA19G1R1 (1/27). Two subtypes were recognized within the IIc subtype family including IIcA22G1 (2/27) and IIcA27G1 (1/27). These findings illustrate the high prevalence of *Cryptosporidium* in calves in dairy herds and increase the diversity of the molecular characteristics of *C. parvum* isolates with the first description of IIaA17G3R1, IIaA19G1R1 and IIc subtypes in France. The presence of zoonotic subtypes of *C. parvum* species suggests that pre-weaned calves are likely to be an important reservoir for zoonotic *C. parvum*.

OA25.05 *Cryptosporidium* and *Giardia* Species and Genotypes in Sheep in Algeria

Dr. Lynda Sahraoui², Myriam Thomas¹, Aurélie Chevillot¹, Dr. Mohamed Mammeri¹, Dr. Bruno Polack¹, Dr. Isabelle Vallée¹, Dr. Jérôme Follet⁵, Prof. Hacina Ain-Baaziz², **Professor Karim Adjou¹**

¹UMR BIPAR, Ecole Nationale Vétérinaire d'Alfort, ANSES, INRA, Université Paris-Est, Maisons-Alfort, F-94700, France, Maisons-Alfort, France, ²Laboratory of Animal Health and Production, Ecole Nationale Supérieure Vétérinaire (ENSV) d'Alger, Issad-Abbes Oued-Smar, Algiers, Algeria, ³Phileo Lesaffre Animal Care, 137 rue Gabriel Péri, 59 700

Marcq-en-Barœul, France, 4UMR BIPAR, Anses, Ecole Nationale Vétérinaire d'Alfort, INRA, Université Paris-Est, Animal Health Laboratory, Maisons-Alfort, F-94700, France, 5Université de Lille, CNRS, ISEN, UMR 8520-IEMN, Lille France 59000, France Little is known about the presence of *Cryptosporidium* spp. and *Giardia duodenalis* in Algerian sheep, nor their potential role as zoonotic reservoirs. This study aimed to investigate the occurrence of these two protists in lambs. A total of 83 fecal samples were collected from lambs (< 40 days old) on different 14 farms. Samples were screened for *Cryptosporidium* spp. and *G. duodenalis* presence with Immunofluorescence technique (IF). Nested PCR of the small subunit ribosomal RNA (rRNA) gene, followed by restriction fragment length polymorphism (PCR-RFLP) and sequence analyses were used to identify *Cryptosporidium* species. Then, *C. parvum* was further subtyped by sequencing the highly polymorphic 60kDa glycoprotein (gp60) gene. For *G. duodenalis*, nested PCR of the glutamate dehydrogenase (gdh) and triose phosphate isomerase (tpi) genes were applied and then PCR-RFLP was used to determine *G. duodenalis* assemblages. *Cryptosporidium* oocysts and *Giardia* cysts were detected in 36/83 (43%) and 23/83 (28%) of fecal samples, respectively. Of the 21/36 (58%) *Cryptosporidium* samples that were IF-positive, 16/21 (76%) were identified as *C. parvum*, and 5/21 (24%) *C. ubiquitum*. From 15 *C. parvum* isolates, 2 subtypes were identified within the subtype family IIa including IIaA21G2R1 (3/15) and IIaA13G2R1 (1/15), while IIcA16G1 (11/15) was the only subtype within IIc subtype family. Of the 16/23 (69%) *G. duodenalis* IF-positive samples, the most frequent assemblage was the ruminant-specific assemblage E (10/16), followed by assemblage D (4/16), and A + E mixed assemblages (2/16). This study reports for the first time the identification and genotyping of both *Cryptosporidium* spp. and *Giardia duodenalis* from lambs in Algeria. This is also the first description of assemblage D in sheep. The presence of zoonotic *C. parvum* subtypes (IIa, IIc) and *C. ubiquitum*, as well as *G. duodenalis*, indicates that sheep could play an important role as a potential reservoir for zoonotic protists.

OA25.06 Seroprevalence and Associated Risk Factors of Toxoplasma Gondii Infection in Domestic Animals in the Oliver Reginald Tambo District, South Africa

Dr. Whatmore Tagwireyi¹, Prof. Eric Etter², Prof. Luis Neves³

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³Centro de Biotecnologia Universidade Eduardo, Mozambique

Toxoplasma gondii is a protozoan parasite that infects birds and mammals including humans. It is a zoonotic disease of particular concern in rural areas where humans have close contact with domestic animals that can transmit the disease. Serological surveys on *Toxoplasma gondii* have been done in both humans and animals in various parts of the world; however in South Africa literature on this is either outdated or scant. Therefore, a cross-sectional survey was conducted to investigate *Toxoplasma gondii* seroprevalence and associated risk factors in small ruminants, pigs, poultry and cats in the Oliver Reginald Tambo District in the Eastern Cape in South Africa between June 2016 and October 2016. Household-level and animal-level data were collected using a close-ended questionnaire. The Toxoreagent, a latex agglutination test, was used for *T. gondii* antibody detection. Positive samples had agglutination patterns at dilutions of 1:64 or greater, except for chickens, whose cut off titre was 1:32. A household was classified as *Toxoplasma gondii* seropositive if at least one species tested positive. The study revealed that 125 / 150 farms (83.3%), 78 / 121 sheep (64.46%), 69 / 128 goats (53.91%), 36 / 106 pigs (33.96%), 35 / 109 cats (32.11%) and 46 / 137 chickens (33.58%) were seropositive for the parasite. Seropositivity was assessed for association with potential risk factors. Among them, age, municipality, climate, animal production system, rodent control, cat-feed access and cat faecal disposal were found to be statistically significant using the Chi-Squared test or odds ratio, confirmed by the Fisher's exact test. The relatively high seroprevalence of *T. gondii* detected in this study suggests that the infection may pose a substantial public health risk through the consumption of *T. gondii* infected raw meat as well as contact with cat faeces.

OA25.07 Serological Detection of Toxoplasma Gondii in Marine Mammals From Canada

Adrian Hernandez Ortiz¹, Brent Wagner¹, Rajnish Sharma¹, Dr Batol Al-Adhami², Dr Pierre-Yves Daoust³, Enooyaq Sudlovenick³, Dr Sonja Ostertag⁴, Dr Lisa Loseto⁴, Dr Nicholas Pilfold⁵, David McGeachy⁵, Nicholas Lunn⁶, Dr Emily Jenkins¹

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Toxoplasma gondii is an important zoonotic parasite that can be transmitted to humans by contaminated food or water contamination with feline feces containing oocysts, and by consumption of raw or undercooked meat harboring the tissue cysts with the bradyzoites. Wildlife, including marine mammals, are hunted and harvested by different communities in the Canadian North, as part of traditions and culture, and as a critical food resource. In recent years, there has been increasing concern about high rates of exposure to *T. gondii* in northern residents, possibly through foodborne routes. The cycle of the parasite is well known in terrestrial ecosystems; however, the transmission dynamics between terrestrial felids and marine mammals is not well understood, especially in the Arctic where felids are rare above treeline. The aim of this study is to determine the seroprevalence of antibody to *T. gondii* in marine mammals frequently hunted by the northern communities. Serum samples were obtained from grey seal (*Halichoerus grypus*), ringed seal (*Pusa hispida*), beluga whale (*Delphinapterus leucas*) and polar bear (*Ursus maritimus*) from different regions of the Canada. The

samples were tested by a commercial ELISA kit and the Immunofluorescence Antibody Test (IFAT) for detection of antibodies against *Toxoplasma gondii*. We anticipate high seroprevalence in polar bears as they could be infected through carnivory of terrestrial wildlife, as well as through waterborne oocysts, and that exposure to the parasite in seals and beluga whales will be higher in more southern populations due to higher levels of water contamination with the oocysts from felids. We will compare our results to the published literature, and discuss challenges in serosurveillance for *T. gondii* in marine mammals.

OA26 Canine Helminths II

July 9, 2019, 16:30 - 18:00

Plenary Hall, Madison Ballroom (ABCD)

OA26.01 Molecular Detection of Filarial Parasites in Hunting Dogs From Southern Italy

Melissa Beall¹, Laura Pacifico², Francesco Buono², Jennifer Braff¹, Giovanni Sgori², Jesse Buch¹, Benedetto Neola³, Diego Piantadosi², Christian Leutenegger¹, Ramaswamy Chandrashekar¹

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Several filarial parasites are known to infect dogs including *Dirofilaria immitis*, *D. repens*, and *Acanthocheilonema reconditum*. While microscopic exam of circulating microfilaria may allow differentiation based on morphological characteristics, such distinctions are not always possible in veterinary practice. This study investigated the prevalence of these parasites in hunting dogs from Southern Italy using molecular methods. Blood was collected from 1,433 dogs in Napoli, Avellino, and Salerno provinces as part of a Campania region hunting dog health assistance program. Samples were analyzed using SNAP® 4Dx® Plus (IDEXX Laboratories, Inc.) and real-time polymerase chain reaction (PCR) to amplify DNA from *D.*

immitis, *D. repens*, and *A. reconditum*. Two dogs each (0.1%) were identified as PCR positive for *D. immitis* and *D. repens* while 110 dogs (7.7%) were identified as PCR positive for *A. reconditum*. None tested positive for more than one type of filarial parasite. *D. immitis* incidence by antigen was 0.3%. Multiple logistic regression of *A. reconditum* PCR results identified province ($p < .0001$) and *E. canis* serological status ($p = .0011$) as significant predictors of *A. reconditum* infection. Risk factors for positive *A. reconditum* PCR test results included living in Salerno province (OR 4.51 vs. Avellino, 95% CI 2.44-8.32), living in Napoli province (OR 2.67 vs. Avellino, 95% CI 1.18-6.06), and seropositivity for *E. canis* (OR 2.71, 95% CI 1.49-4.92). Hunting dogs in this study were more likely to be PCR positive, and potentially microfilaremic, due to *A. reconditum* than *D. immitis* or *D. repens*. This study identified a significant association between an infection transmitted by fleas, *A. reconditum*, and an infection transmitted by ticks, *E. canis*. These results emphasize the importance of broad-spectrum, year-round ectoparasite control to minimize vector-mediated transmission of pathogens in hunting dogs from Southern Italy.

OA26.02 A Case of Canine *Dracunculus* Sp. Infection from Spain

Irina Diekmann¹, Dr. Alaa Aldin Alnassan², Dr. Majda Globokar², Dr. Nikola Pantchev², Dr. Lina Kurzrock², Dr. Leticia Hernandez³, Dr. Javier Lopez³, Dr. Ricardo Ruano⁴, Dr. Silvia Herrero⁴, M. De Vidales⁴, Prof. Dr. Georg von Samson-Himmelstjerna¹, Dr. Jürgen Krücken¹

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The nematode genus *Dracunculus* contains 14 accepted species, 9 of which parasitize reptiles. Human dracunculosis caused by *Dracunculus medinensis* was an important neglected tropical disease but recent efforts have eliminated the parasite from most endemic countries except of Chad, Ethiopia, South Sudan and Mali. Shortly before its eradication, canine *D. medinensis* infections

have become considerably more frequent and dogs now appear to be important reservoir hosts maintaining the live cycle in endemic areas. However, *Dracunculus lutrae* (host: North American river otter *Lontra canadensis*) and *Dracunculus insignis* (wide host range including raccoons, several mustelids and canids including dogs) infect predominantly North American carnivores. A 2-year-old, podenco-like (local Spanish breed) dog was presented to a veterinarian in Toledo (Spain) due to an oozing ulcer at the hind left limb. The dog had a history of abuse on a farm before being rescued about a year before attendance. From the ulcer, an approximately 12 cm anterior end of a nematode was extracted. The dog had been regularly dewormed, carried a Seresto® collar and was under allopurinol therapy against leishmaniasis. Morphological examination of the nematode excluded a filarioid species and *Dracunculus* sp. was suspected. Following PCRs and sequencing of partial cytochrome oxidase I (COI), 18S and 28S rRNA genes confirmed the allocation to the genus *Dracunculus* but excluded *D. medinensis*, *D. insignis* and *D. lutrae* as well as two undescribed genotypes previously reported from *L. canadensis* as causative agents. Phylogenetic analysis using the COI sequence placed the specimen in a sister position to *D. insignis* within the *Dracunculus* genus but outside of any established species suggesting it represents a species not detected in carnivores before. To the knowledge of the authors, this is the first report of a *Dracunculus* sp. infection of mammals from Europe.

OA26.03 Detection and Prevalence of *Cercopithifilaria baina* Infection in Domestic Dogs and Ticks in Oklahoma

Megan Wohltjen¹, Kellee Sundstrom¹, Susan Little¹, Kelly Allen¹
¹*Oklahoma State University, Stillwater, United States*

Cercopithifilaria baina is a vector-borne filarial nematode of dogs in areas of Europe, Central America, and South America. The subcutaneous parasite is considered largely non-pathogenic, but occasional clinical signs include erythematous papules, polyarthritis, and pruritic dermatitis. Transmission to canid hosts occurs via the blood-feeding of

Rhipicephalus sanguineus sensu lato (s.l.), the intermediate host and vector. Despite the world-wide distribution of *R. sanguineus* s.l., *C. baina* had not been documented in the United States prior to 2017, when a single case report of a dog in Florida with dermatitis was described. To determine the prevalence of *C. baina* in domestic dogs in Oklahoma, client-owned and impounded dogs were screened between January–October 2018 for microscopic and molecular evidence of infection. Dermal biopsies (6 mm) were processed via saline sedimentation; sediments were microscopically examined and, when microfilariae were identified, measurements were taken. Microfilariae positive sediments and skin biopsy sections were tested by PCR to amplify filarioid DNA (12S and 5.8S rRNA gene fragments). Additionally, ticks infesting surveyed dogs, including *R. sanguineus* s.l., were collected and screened for molecular evidence of *C. baina* infection. Microfilariae were observed in 8.0% (20/250) of dogs, with three dogs having organism consistent with *C. baina* by morphometry. *Cercopithifilaria baina* DNA was amplified from skin biopsies of 4.7% (6/127) dogs, 2 of which were positive for *C. baina* microfilariae on skin sedimentation. Two dogs with microfilariae of *C. baina* were noted to have *R. sanguineus* s.l. at the time of skin biopsy collection. To date, *C. baina* has not been detected in any ticks tested (n=112). The present study documented *C. baina* in Oklahoma dogs for the first time, with collective morphometric and molecular data indicating that the parasite is actively cycling within the state.

OA26.04 The Impressive Spread of the Metastrongylid Heart and Lung Nematode *Angiostrongylus vasorum* in Foxes As Cause for Increase of Cases of Canine Angiostrongylosis

Dr. Nina Gillis-Germitsch¹, Dr. Lucienne Tritten¹, Dr. Daniel Hegglin¹, Prof. Peter Deplazes¹, **Prof. Manuela Schnyder**¹
¹*Institute of Parasitology, University Of Zurich, Zürich, Switzerland*

After its discovery in France around 1900, *Angiostrongylus vasorum* (“French heartworm”) was first reported in dog

kennels in Switzerland and in Ireland in the 1960s, and later also in other European countries. A consistent increase of cases of canine angiostrongylosis, however, was only observed in the past decade.

To shed light on this dynamics, 3'954 blood samples collected from Swiss foxes between 1986 and 2017 were tested for circulating *A. vasorum* antigen by ELISA. The samples were allocated to 4 collection timeframes: 1986-1992, 1993-2002, 2003-2012 and 2013-2017. In north-eastern Switzerland, 1.9% (25/1343) and 1.7% (14/833) of foxes were antigen-positive in the first two timeframes, respectively. Antigen-positivity increased in the following two decades to 18.3% (17/93; 2003-2012) and to 62.0% (281/453; 2013-2017). In south-eastern Switzerland comparable prevalences in foxes were determined for 1986-1992 (7.9%; 13/164) and 1993-2002 (6.5%; 45/691).. Between 2003-2012, 38.5% (52/135) of foxes were antigen-positive. In western Switzerland, 1.4% (3/222) of foxes were antigen positive in the first but 53.3% (8/15) in the second time frame. In parallel, necropsy of 467 foxes around Zurich confirmed an increasing prevalence for *A. vasorum* from 20.5% (worm burden [WB]: 1-30, mean 7.3) in 2012 to 75.5% (WB: 1-50, mean 11.3) in 2017.

Our results indicate that *A. vasorum* prevalence in the Swiss fox population was stable at low levels until 2002, whereas it increased significantly since 2003 in all investigated areas. This corresponds to the time in which *A. vasorum* started to be detected in dogs. We hypothesize that the increasing number of cases of canine angiostrongylosis is due to a simultaneous increase of *A. vasorum* prevalence in the Swiss fox population, which constantly increased, particularly in urban areas. As is the case with the zoonotic cestode *Echinococcus multilocularis*, foxes play a crucial role as reservoir hosts.

OA26.05 Multiple Drug Resistance in *Ancylostoma Caninum*: An Emerging Threat to Canine Health

Pablo David Jimenez Castro¹, Daniel Zuluaga², Patricia Wetherly¹, Razan Sheikh¹, Russell Avramenko³, John Gilleard³, Ray Kaplan¹
¹*University of Georgia, Athens, United States*,
²*University of Santa Rosa de Cabal, Santa Rosa de Cabal, Colombia*, ³*University of Calgary, Calgary, Canada*

Over the past two years, we have become aware of multiple cases of recurrent hookworm infections in dogs due to *Ancylostoma caninum* that are relatively unresponsive to usual anthelmintic treatments. We now provide conclusive evidence that the primary cause of these recurrent hookworm cases is the development of multi-drug resistance (MDR) in this parasite. Three suspected-resistant and two susceptible isolates of *A. caninum* were established in laboratory dogs. In vitro bioassays including the egg hatch assay (EHA) and the larval development assay (LDA) were used to measure resistance to benzimidazoles and macrocyclic lactones, respectively, and fecal egg count reduction was measured following successive treatment with fenbendazole, pyrantel and milbemycin oxime in two laboratory dogs. In addition, samples of eggs and L3 were examined using deep-amplicon sequencing to evaluate the beta-tubulin SNP frequency. The EHA IC₅₀ value for our susceptible lab isolate was 0.25µM and for resistant isolates ranged from 2.79-3.73µM, yielding resistance ratios (RR) of 11.1-14.9. LDA IC₅₀ value for the susceptible isolate was 16.62nM and ranged from 91.53-1052nM for resistant isolates, which yielded RR from 5.5-63.3. Following treatment with fenbendazole, percent reduction in faecal egg counts were 63 and 84% (day 23), following treatment with pyrantel were 0 and 72% (day 10), and following treatment with milbemycin were 92 and 58% (day 14) for the resistant Tara and Worthy isolates, respectively. Beta-tubulin SNP were present only at codon 167; all but one sample from a resistant isolate yielded SNP frequencies greater than 88%. These in vitro, in vivo, and molecular data offer pivotal evidence that these *A. caninum* isolates are highly resistant to all anthelmintic classes

currently approved for use in dogs in the United States. Deep amplicon sequencing is a powerful new tool that can be used to study the molecular epidemiology of anthelmintic resistance in *A. caninum*.

OA26.06 Infectivity of Shed *Angiostrongylus Vasorum* and *Crenosoma Vulpis* L3 to Dogs

William Robbins¹, Dr. Spencer Greenwood¹, Dr. Laura Rogers², Dr. Gary Conboy¹

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Metastrongyloid respiratory parasites, *Angiostrongylus vasorum* and *Crenosoma vulpis* infect wild and domestic canids and are important pathogens in dogs. Recent studies indicate gastropod intermediate hosts infected with various metastrongyloids spontaneously shed infective third-stage larvae (L3) into the environment via feces and mucus. Shed L3 retain motility up to 120 days but whether they retain infectivity remains unknown. To assess the infectivity of shed L3, the heart/lungs of 6 red fox (*Vulpes vulpes*) were obtained from trappers in Newfoundland, Canada. Lungs were examined for first-stage larvae (L1) by Baermann technique. A high number of viable *A. vasorum* L1 and a small number of *C. vulpis* L1 were recovered from one fox; these were used to infect laboratory-raised *Limax maximus*. L3 recovered by artificial digestion were fed to two purpose-bred research beagles (100 L3/dog). The L1 shed by these 2 dogs were used to infect 546 *L. maximus* (2,000 - 10,000 L1/slug). Shedding of L3 was induced by anesthetizing slugs in soda water and transferring them into warm (45° C) tap water for at least 8 hrs. Recovered shed L3 were aliquoted on romaine lettuce in 6-well tissue culture plates (80-500 L3/well) and kept at 16° C/75% Relative Humidity. Four research beagles were exposed to 100 L3/dog from the larvae held at 0, 2, 4 or 8 weeks after shedding. All four dogs began shedding *C. vulpis* L1 by 26-36 days post-infection (PI). All four dogs began shedding *A. vasorum* L1 by 50 days PI. Infectivity of L3 for the definitive host was retained in both metastrongyloids

indicating that exposure through environmental contamination may occur in natural infection in dogs. As an exposure route, eating or licking grass or other plant material greatly increases the number of dogs at risk of infection for these parasites.

OA27 Epidemiology

July 9, 2019, 16:30 - 18:00

Breakout Room 2, Hall of Ideas E&H, Level 4

OA27.01 "Tekenscanner": A Novel Smartphone App for Companion Animal Owners and Veterinarians to Engage in Tick and Tick-Borne Pathogen Surveillance in the Netherlands

Prof Frans Jongejans¹, Suzanne de Jong¹, Timo Voskuilen¹, Louise van den Heuvel¹, Rick Bouman¹, Henk Heesen², Carlijn IJzermans², Laura Berger¹
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The engagement of companion animal owners into the process of collecting epidemiological data can be facilitated through smartphone applications. Early 2018, the "tekenscanner" (Dutch for tick scanner) app was launched in the Netherlands with the aim of engaging owners to record ticks removed from their pets and submit these ticks for identification and pathogen testing. A barcode was assigned to each tick for tracking of each submission to our laboratory for taxonomic identification. DNA extracted was PCR amplified, subjected to reverse line blot hybridization (RLB) and screened for pathogens. Results were added to the same app within two weeks.

The app was downloaded 5591 times and resulted in the collection of 1005 ticks from 406 dogs and 268 ticks from 111 cats. *Ixodes ricinus* was the predominant species (90.0%), with all stages found on dogs as well as on cats. *Ixodes hexagonus* (7.3%) female and nymphal ticks were also identified on both hosts, whereas adults of *Dermacentor reticulatus* (2.4%) and *Rhipicephalus*

sanguineus (0.2%) were exclusively found on dogs. Nearly 15% of the ticks recovered from dogs carried one or more pathogens, whereas 13.8% of the ticks removed from cats were infected. *Ixodes ricinus* collected from dogs contained *Borrelia* spp. (1.9%), *Babesia* spp. (0.7%), *Anaplasma phagocytophilum* (1.3%), *Neoehrlichia mikurensis* (2.9%) and *Rickettsia helvetica* (7.3%). *Ixodes ricinus* recovered from cats were infected with *Borrelia* spp. (1.9%), *Babesia* spp. (0.4%), *Anaplasma phagocytophilum* (1.9%), *Neoehrlichia mikurensis* (2.6%) and *Rickettsia helvetica* (6.7%). Three *Rhipicephalus sanguineus*, on dogs from France and the USA imported into the Netherlands, were negative.

The tekenscanner app is a versatile tool to use for submission of ticks and facilitated fast feedback of test results. Community engagement through the app is suitable for identifying hot spots for ticks and pathogens and provided an early warning system for exotic ticks invading the Netherlands.

OA27.02 Detection of Anti-Alpha-Gal Antibodies in Dogs and the Possible Implications in Red Meat Allergy and Protection to Infectious Diseases

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Specific IgE antibodies (Abs) against the carbohydrate galactose-alpha-1,3-galactose (alpha-Gal) has recently been linked to

delayed anaphylactic reaction to mammalian meat in human patients previously exposed to bites of certain tick species. In dogs, alpha-Gal is a self-antigen and their immune system is thus not expected to naturally produce Abs toward the glycan molecule. However, results of this preliminary study demonstrate the occurrence of canine IgG, IgM and IgE serum Abs against alpha-Gal for the first time, and suggests that a tick bite can sensitize dogs to alpha-Gal and potentially trigger delayed allergic reaction to mammalian meat following the induction of specific IgE. The results also suggest the protective role of anti-alpha-Gal Abs against pathogens transmitted by ticks. Our findings open a completely new scientific perspective, which may contribute to a better understanding of the mechanisms involved in the pathogenesis of this unique food allergy, and highlight the possibility that anti-alpha-Gal Abs may help to prevent canine vector-borne diseases.

OA27.03 Diversity of Parasites of Companion Dogs and Cats in East and Southeast Asia. Part 1: Endoparasites

Dr. Vito Colella¹, Mr. Do Yew Tan², Mrs. Na Lu³, D.r Fang Fang⁴, Yin Zhijuan⁴, Jiangwei Wang⁵, Xin Liu⁶, Junyan Dong⁷, Wisnu Nurcahyo⁸, Upik Kesumawati⁹, Virginia Venturina¹⁰, Kenneth Boon Yew Tong¹¹, Yi-Lun Tsai¹², Piyanan Taweethavonsawat¹³, Saruda Tiwananthagorn¹⁴, Thy Anh Dang¹⁵, Thong Quang Le¹⁵, Khanh Linh Bui¹⁶, Malaika Watanabe¹⁷, Puteri Azaziah Megat Abd Ran¹⁷, Giada Annoscia¹, Viet Linh Nguyen¹, Frederic Beugnet¹⁸, Domenico Otranto¹, Lénäig Halos¹⁸
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Asia is the largest continent in the world with an increasing number of dogs and cats kept as companion animals. Nonetheless, in most of Asian countries, there is a lack of knowledge on the etiological agents parasitizing dogs and cats, including those transmissible to humans.

A collaborative field study involving 16 veterinary institutions in 8 regions of East Asia (China, Taiwan) and Southeast Asia (SEA; Indonesia, Malaysia, Philippines, Singapore, Thailand, Vietnam) was set up to assess the occurrence and prevalence of canine and feline parasites.

From June 2017 to June 2018, a total of 2382 client-owned animals (1229 dogs and 1153 cats) were enrolled by veterinary facilities in each country. All animals had a history of regular outdoor access, without any parasiticide treatment in the previous months. Animals were identified and their lifestyle and anamnestic data recorded, along with a complete veterinary examination including parasitological investigations for the diagnosis of both ecto- and endoparasites. In particular, the occurrence of endoparasites was assessed via qualitative coprological tests (flotation, sedimentation and Baermann-Wetzel techniques). Endoparasites were identified in 12.6% and 13.9% of dogs and cats. Nematodes of the families Ancylostomatidae (8.9% and 6.4%) and Toxocaridae (2.5% and 3.9%) were the most frequent gastrointestinal parasites detected.

Ancylostomatidae were diagnosed in 16% of dogs and 12% of cats from SEA, indicating

this geographical area as highly endemic for these pathogens. Coccidia, Strongyloides spp., Trichuris spp., Trichomonas spp., Dyphillobothriidae, Dipylidium caninum and trematodes were also reported in both dogs and cats. Peaks in the occurrence of endoparasites were recorded in selected countries such as Philippines and Vietnam with respectively 32.5% and 23% of the pets enrolled diagnosed with these pathogens.

OA27.04 Diversity of Parasites of Companion Dogs and Cats in East and Southeast Asia. Part 2: Ectoparasites and Vector-Borne Diseases

Vito Colella¹, Do Yew Tan², Na Lu³, Fang Fang⁴, Yin Zhijuan⁴, Jiangwei Wang⁵, Xin Liu⁶, Junyan Dong⁷, Wisnu Nurcahyo⁸, Upik Kesumawati⁹, Virginia Venturina¹⁰, Kenneth Boon Yew Tong¹¹, Yi-Lun Tsai¹², Piyanan Taweethavonsawat¹³, Saruda Tiwananthagorn¹⁴, Thy Anh Dang¹⁵, Thong Quang Le¹⁵, Khanh Linh Bui¹⁶, Malaika Watanabe¹⁷, Puteri Azaziah Megat Abd Rani¹⁷, Giada Annoscia¹, Viet Linh Nguyen¹, Frédéric Beugnet¹⁸, Domenico Otranto¹, **Lénaïg Halos**¹⁸
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¹⁷University of Putra, Petaling Jaya, Vietnam,

¹⁸Boehringer Ingelheim Animal Health, Lyon, France

The 2382 animals enrolled as previously described were examined for ectoparasites and vector-borne pathogens. The body surface of each animal was examined for the presence of ectoparasites (ticks, fleas, lice, and mites), including a thorough examination underneath the third eyelid to detect *Thelazia callipaeda* eyeworms. A deep skin scraping and/or a scotch test was performed only in case of lesions evocative of sarcoptic mange/demodicosis or cheyletiellosis, respectively, and ear wax examined for the presence of *Otodectes cynotis*.

OA27.05 Finding What You Aren't Looking For: A Survey of Parasitic and Infectious Diseases in Dogs in Grenada, West Indies

Dr. Tara Paterson¹, Dr. Marta Lanza Perea¹, Dr. Jancy Hanscom², Dr. Phyllis Tyrrell², Dr. Dave Elsemore², Dr. Christian Leutenegger², Dr. Ramaswamy Chandrashekar²

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Grenada's tropical climate and prevalence of vectors pose significant risk of infectious disease transmission in animals and people. Preventive health care is uncommon in Grenada due to economic limitations and poor understanding of canine diseases, leaving dogs vulnerable to infection. Since heartworm, ehrlichiosis and anaplasmosis are endemic in Grenada, often other differential diagnoses are overlooked when faced with a sick canine patient. The aim of this study was to survey a population of dogs presenting to the St. George's University Junior Surgery & Anesthesia Lab using a comprehensive diagnostic approach. Based on IDEXX SNAP® 4Dx® Plus test results from 166 dogs, 14.5%, 39.2%, 22.9% and 0% of dogs were positive for *Dirofilaria immitis*, *Ehrlichia canis*, *Anaplasma platys*, and *Borrelia burgdorferi*, respectively. Comprehensive infectious disease PCR analysis performed on 163 samples identified the following: *E. canis* 23.95%, *Mycoplasma haemocanis* 22.7%,

Acanthocheilonema reconditum 22.1%, *A. platys* 20.2%, *Mycoplasma haematoparvum* 14.7%, *Babesia vogeli* 5.5%, *Babesia* spp. 4.3%, and *Hepatozoon canis* 3.1%. Fecal samples from 154 dogs were assessed using centrifuged fecal floatation and fecal antigen testing (Fecal Dx®, IDEXX Laboratories) of which 72.1% were infected ≥ 1 parasite and of these, 21.6% harbored ≥ 2 species. More samples were positive for hookworms on fecal antigen (68.8%) versus fecal floatation (58.45%). There was whipworm antigen detected in 14.9% of samples and positive ova identification in 18.8%. The incidence of roundworms was 1.9% on both tests. Based on the results of this study, dogs in Grenada are at significant risk for parasitic and vector-borne disease, several of which have zoonotic potential. This is the first report of *Mycoplasma* spp. infection in Grenadian dogs and highlights the importance for veterinarians to be aware of the infectious organisms threatening their patients. The veterinarian cannot find the underlying etiology of disease if one is not looking for it.

To assess the exposure to vector-borne pathogens in dogs, blood samples were tested for *Dirofilaria immitis*, *Ehrlichia* spp., *Borrelia burgdorferi sensu lato*, *Anaplasma* spp. and *Leishmania* spp. with SNAP® 4Dx Plus and SNAP® *Leishmania* tests. In cats the SNAP® Feline Heartworm Antigen Test Kit was used to detect *D. immitis*. Blood samples were also stored on Whatman® FTA® cards for molecular detection of DNA from filarial parasites, and for species of the genera *Leishmania*, *Babesia* and *Hepatozoon*. Ectoparasite infestations were reported in 30.4 and 27.5% of dogs and cats, respectively. Diversity of flea species included *Ctenocephalides felis* and *C. orientis* and for the ticks *Rhipicephalus sanguineus*, *R. haemaphysaloides* and *R. turanicus*. Exposure to tick-borne pathogens was reported in 18.5% of dogs (14.5% *Ehrlichia* and 7% *Anaplasma*), with 16% and 10% of dogs from Philippines and Taiwan, respectively, scoring positive for *D. immitis*. To our knowledge, we reported for the first time the occurrence of *Leishmania* spp. in dogs from Philippines and Vietnam, and of *D. immitis* in a cat from Indonesia. This multicenter study represents a cornerstone in the knowledge of the

occurrence of parasites infecting dogs and cats kept as companion animals in East and Southeast Asia, including species of zoonotic concern, ultimately leading to the establishment of effective treatment strategies and refined recommendations for veterinarians and pet owners.

OA27.06 Prevalence of Trypanosomosis (Trypanosoma Evansi) of Dromedaries in the Sahel of Tunisia

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An epidemiological study was conducted between 2013 and 2016 in central Tunisia to evaluate the prevalence of *Trypanosoma evansi* infection in camels by the direct agglutination test (CATT Test[®]). A total of 1076 dromedaries of both sexes were examined (96 males and 980 females), aged from 1 to 18 years, belonging to 3 regions of the Sahel of Tunisia were selected for the survey.

262 animals were seropositive (24.3%) with a net prevalence of the disease in autumn (37%) and in animals over 4 years (89%). The Monastir region was the most affected (42%). Appropriate treatment (Cymelarsan[®]) of all seropositive patients resulted in a significant decrease of clinical cases for at least two years in controlled flocks. The clinical signs of high suspicion of trypanosomosis observed in dromedaries were weight loss, anemia, edema in the sloping regions and sometimes locomotors disorders and abortions in females.

OA28 Alternative treatments for Parasites in Ruminants I

July 9, 2019, 16:30 - 18:00

Breakout Room 3, Hall of Ideas F&I, Level 4

OA28.01 The Nordic Seaweeds *Saccharina Latissima* and *Laminaria Digitata* Have Potent Anthelmintic Effects In Vitro

Charlotte Smith Bonde¹, Louis Bornancin², Associate Professor Andrew Richard Williams¹, Associate Professor Helena Mejer¹, Associate Professor Henrik Toft Simonsen², Dr. María Martínez Valladares³, Professor Stig Milan Thamsborg¹

¹University Of Copenhagen, Dept. of Veterinary and Animal Sciences, Frederiksberg, Denmark, ²Technical University of Denmark, Dept. of Biotechnology and Biomedicine, Kongens Lyngby, Denmark, ³CSIC-Universidad de León, Dept. of Animal Health, Grulleros, Spain

Seaweed contains an abundance of bioactive compounds, and some seaweed species have been used as natural deworming agents for centuries in traditional Chinese medicine. In this study, we investigated the in vitro anthelmintic (AH) activity of extracts of seaweed, from Nordic waters. We prepared three different extracts: hexane, dichloromethane:methanol (DCM), and water:methanol (WM), from four seaweed species: *Saccharina latissima*, *Laminaria digitata*, *Ascophyllum nodosum*, and *Palmaria palmata*. The AH activity was assessed using an *Ascaris suum* third stage larvae (L3) mortality assay (1 mg DM/mL dissolved in DMSO). Moving or coiled-up larvae were counted as alive, and immobile or straight larvae as dead. Extracts with more apolar compounds (hexane, DCM) showed higher AH activity than extracts with polar compounds (WM), and the most potent extracts originated from *S. latissima* and *L. digitata*, with an average mortality of >95% after 48 hours. Extracts from *A. nodosum* had significantly lower AH effect after 48 hours. The extracts were further tested for AH effect in vitro against *Teladorsagia circumcincta* L1 (1 mg/mL, 48 h). The results showed a high AH activity of *S. latissima* and very low activity of *A. nodosum* extracts. An egg hatch assay

(EHA), performed using the same extracts (1 mg/mL, 48 h) and *T. circumcincta* eggs, only showed >95% inhibition by the polar *S. latissima* extract. We conclude that the Nordic seaweeds *Saccharina latissima* and *Laminaria digitata* have strong in vitro AH effects against common pig and sheep nematodes, and the AH activity is caused mainly by apolar compounds. Bio-guided fractionation will be used to identify the active compounds, and feeding trials of infected livestock will reveal whether seaweeds as a bioactive forage can play a role in future nematode control.

OA28.02 Microbial Crystals for the Treatment of Veterinary Gastrointestinal Nematodes

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Gastrointestinal nematode (GIN) parasites are one of the leading threats to the animal industry, resulting in decline in reproductive performance, immunity and sometimes death of the animal, amounting to huge economic losses. With the ever-increasing consumer demand for chemical-free animal products and the rise of anthelmintic resistance against current small molecule drugs in these parasites, a need for a new inexpensive and scalable treatment with minimal side effects is on the surge. We have focused on utilizing invertebrate-specific bacterial pore-forming crystal proteins that have evolved naturally to disrupt host membranes, have been

widely used as bio-pesticides and are part of our diet. The crystal protein, Cry5B (FDA approved orphan drug status), from *Bacillus thuringiensis* (Bt) was earlier shown to be efficacious against *Ancylostoma ceylanicum* (hookworm) and *Ascaris suum* (roundworm). However, in these experiments spore-crystal lysates were used to test toxicity to GINs. To avoid the dissemination of live spores and the potential enterotoxicity with Bt, we genetically engineered Bt to form a non-sporulating *Bacillus*. After an inactivation step, the Inactivated *Bacillus* with Cytosolic Crystal (IBaCC) remained highly toxic to hookworms both in vitro and in a hamster infection model. IBaCC significantly reduced *Ascaris* worm burden in infected pigs. To isolate Purified Cry5B Crystals (PCC) for pharmaceutical applications, we devised a two-step scalable process that generated > 90% pure Cry5B protein crystals that was able to cure hookworm infection in hamsters. Additionally, within days of treatment these crystals lowered the *Parascaris equorum* fecal egg count in horses. In a pilot study of African Green Monkeys administered with Cry5B, a decrease in egg and larvae shedding of *Strongyloides* (threadworms) was observed. Taken together, we have developed two drug forms - an encapsulated, inactivated bacteria (IBaCC) and purified crystals (PCC) to meet the ongoing challenge of GIN parasitic infections.

OA28.03 Anthelmintic and Metabolomic Analyses of Chicory (*Cichorium Intybus*) Extracts Revealed an Industrial By-Product With Activity Against Drug-Sensitive and Drug-Resistant Nematodes

Dr. Miguel Peña-Espinoza¹, Dr. Louis Bornancin², Dr. Andrew R. Williams³, Dr. Henrik T. Simonsen², Prof. Stig M. Thamsborg³, Dr. Rodrigo Lopez-Muñoz¹
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Chicory (*Cichorium intybus*) is a bioactive crop rich in sesquiterpene lactones (SL) with reported anthelmintic activity in livestock. Evaluation of SL-extracts from different

chicory materials can help identifying the main antiparasitic compound(s), leading to selection of crops with increased activity and/or the isolation of novel parasitocides. Here, we evaluated the anthelmintic activity and metabolomic profiling of SL-extracts from different chicory materials against drug-sensitive and drug-resistant nematodes.

Fresh leaves from forage chicory cultivated in Denmark (var. Spadona, sampled in 2013, 2015 and 2016) and Chile (var. Choice, sampled in 2016) were used for SL extraction. SL were also extracted from root chicory pulp, a by-product of the industrial extraction of inulin from chicory roots (var. Sativum). The five SL-extracts were tested against a *Caenorhabditis elegans* drug-sensitive strain, and the two most active extracts were evaluated against an ivermectin (IVM)-resistant *C. elegans* strain. The most potent SL-extract was further tested against *Ascaris suum* third-stage larvae (L3). All SL-extracts were analysed by ultra-high-performance liquid chromatography and metabolomic analyses were performed using the Global Natural Product Social Molecular Networking database.

Four SL-extracts induced a dose-dependent inhibition of worm motility in drug-sensitive *C. elegans*. Root pulp and Spadona-2015 extracts were the most active SL-extracts (EC_{50} Root pulp: 72.1 $\mu\text{g/mL}$ vs Spadona-2015: 379 $\mu\text{g/mL}$; $P < 0.0001$). Only the root pulp extract significantly reduced the motility of IVM-resistant *C. elegans* ($P < 0.001$). Furthermore, the root pulp extract induced a marked and dose-dependent inhibition of *A. suum* L3 motility (EC_{50} : 118 $\mu\text{g/mL}$). Metabolomic analyses confirmed the presence of SL in all tested extracts and a distinct SL profile in the root pulp extract.

In conclusion, root chicory pulp showed potent anthelmintic activity against drug-sensitive and drug-resistant nematodes and further research should explore its potential as an antiparasitic by-product. Undergoing bioactivity-based networking analyses could reveal the main anthelmintic compound(s) in chicory.

OA28.04 Scientific Validation of Ethnobotanicals Used Against *Rhipicephalus Microplus* in Pakistan

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¹College of Veterinary and Animal Sciences, Jhang, Pakistan, ²University of Agriculture, Faisalabad, Pakistan

Aims

The objective of the current study was to evaluate the anti-tick, activities of a herbal formulation based on leaves of *Azadirachta indica* and *Nicotiana tabacum*, flowers of *Calotropis procera* and seeds of *Trachyspermum ammi*.

Materials & methods

Rhipicephalus (*Boophilus*) *microplus* ticks were used for evaluation of acaricidal activity. The adult immersion test (AIT) (Sabatini et al., 2001) and larval packet test (LPT) (Luguru et al., 1984) were carried out for in vitro acaricidal activity of extract. For in vivo evaluation of acaricidal activity, 15, 30 and 45% concentrations of extract were prepared in distilled deionized water.

Results

The formulation demonstrated anti-tick activity by inhibiting the egg laying (Adult immersion test), larval mortality (Larval packet test) and reduced tick intensity/infestation on animals. A dose dependent anti-tick effect was observed in all the tests carried out in this study. Egg laying was significantly lower in ticks exposed to different concentrations of herbal extract compared with those exposed to distilled water used as control. Likewise, there was a significant reduction ($p < 0.05$) in the number of ticks exposed to 45% herbal extract compared with control.

Conclusion

Pakistan is rich in indigenous knowledge, diversity of plants and their usage, in traditional veterinary medicine. The herbal formulation demonstrated anti-tick activity by inhibiting the egg laying, larval mortality and reduced tick intensity/infestation on animals. The herbal formulation is suitable for the

resource-poor farmers as a broad spectrum antiparasitic. The contents of the formulation are cheap, commonly available, and easy to use as a decoction. Moreover, it is not a new animal husbandry input as farmers are already using these plants individually in animal health and production.

OA29 Unusual Protozoa

July 9, 2019, 16:30 - 18:00

Breakout Room 4, Hall of Ideas G&J, Level 4

OA29.01 Characterization of Vivaxin, a Novel Bloodstream-Stage, Species-Specific, Cell-Surface Family As Potential Vaccine Candidates Against the Livestock Parasite *Trypanosoma Vivax*

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Trypanosoma vivax is a major animal pathogen causing African Animal Trypanosomiasis (AAT) in livestock across Africa and South America. No vaccine is available for AAT since antigenic variation of the Variant Surface Glycoprotein (VSG) coat displayed on the parasite surface leads to effective immune evasion. However, the *T. vivax* genome contains species-specific, cell-surface genes (TvCSP) expressed during the bloodstream stage, which we believe provide non-VSG and antigenically invariant targets for vaccination. We used a customized peptide microarray to identify

immunogenic epitopes among TvCSP genes. Our analysis revealed that a novel family of *T. vivax* transmembrane proteins, which we called vivaxin, are consistently the most immunogenic non-VSG antigens in natural *T. vivax* infections across the Africa and South America. The vivaxin gene family consists of 44 paralogs, divided into 14 clades, displaying stable polymorphism and constitutive expression in bloodstream infections. Immuno-fluorescent microscopy confirms that vivaxin proteins localize to the entire cell surface. Several vivaxin proteins were expressed recombinantly and used to vaccinate mice; this showed that specific family members produce high antibody titres. Currently, we are conducting challenge experiments in goat to examine the ability of these recombinant antigens to induce protective immunity. This is the first report of a non-VSG TvCSP family being vaccine candidates against AAT. The characterization of these immunogenic but invariant surface proteins will facilitate further study of their biological functions, and represents a new approach to preventing AAT, a goal that would bring profound benefits to animal health and productivity.

OA29.02 Prevalence and Molecular Identification of *Babesia* Species in Asymptomatic Dogs (*Canis Familiaris*) in the Federal Capital Territory, Abuja, Nigeria

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¹University Of Abuja, Gwagwalada, Nigeria,

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Canine babesiosis is a protozoan tick-borne disease caused mainly by several species of *Babesia* with worldwide distribution. Accurate identification of the parasite species is important to the monitoring, management and control of the disease. The aim of this study therefore was to determine the prevalence of *Babesia* infections in dogs as well as identify the species genotype in the Federal Capital Territory, (FCT) Abuja

– Nigeria. Peripheral blood samples were collected from 480 asymptomatic dogs in the six Area Councils (Abaji, Bwari, Gwagwalada, Municipal, Kwali and Kuje) via anti-rabies vaccination campaign and assayed with a light microscope using Geimsa stain. Genomic DNA was extracted from whole blood which tested positive for *Babesia* spp., using DNA extraction kits. DNA amplification of the 18S ribosomal RNA gene was employed using genus specific primers. The amplicons were electrophoresed in 1.5 agarose gel, purified, and then directly sequenced using Big Dye Terminator Cycle Sequencing Kit. The BLAST search analysis was conducted in NCBI database, and the sequences compared with reference data in the GenBank. The prevalence of *Babesia* spp. infection by thin blood smears microscopy showed presence of intraerythrocytic merozoites, in 3.1% (480/15) of the dogs sampled. The PCR assay showed 53.3% (15/8) amplification of *Babesia* sp. at 612bp gene segments on gel electrophorogram. BLAST search analysis of the parasite sequences showed 100% sequence similarity with *Babesia canis vogeli* in the GenBank. This study documents *B. canis vogeli* as the species present in the sampled dog population and to the best of our knowledge, the first molecular identification of the *Babesia* sp. in the FCT, Nigeria. Further studies need to be directed towards utilizing the PCR protocol to confirm parasite species in tick vectors from the region.

OA29.03 Effects of a TrxR Amino Acid Mutation on Enzyme Activity in *Babesia Microti* and the Different Roles of Inhibitors

Houshuang Zhang¹, Jinmiao Lu¹, Jie Cao¹, Yongzhi Zhou¹, Haiyan Gong¹, Jinlin Zhou¹
¹*Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Shanghai, China*

Babesia microti is a zoonotic pathogen, which mainly parasitizes mammalian erythrocytes. The oxidative stress induced by the aerobic environment and immune system causes damage from ROS in the parasite. However, little is known regarding anti-oxidation strategies in *Babesia microti*. In the current study, the expression of TrxR was positively correlated with the ROS level both in vivo and

in vitro. TrxR had a high catalytic activity to the substrate and this activity was strongly dependent on TrxR concentration. Using a combination of homology modeling and domain prediction methods, data suggested that the electron-accepting site of TrxR is the Cys105-VNVGCys110 motif located at the N-terminus and the electron transfer site of TrxR is the Cys547-XXXX-Cys552 motif located at the C-terminus. An inhibitor library was constructed and tested, and several inhibitors were then selected. To reveal the action sites of different inhibitors, a mutant pool was constructed and expressed as His-tagged fusion proteins in *Escherichia coli*, according to the manufacturer's instructions. The reducing activity of the N-terminal mutants Bmi TrxR-C105, Bmi TrxR-C110, and Bmi TrxR-C105-C110 disappeared on the DTNB and thioredoxin (Trx) substrates. The C-terminal mutants Bmi TrxR-C547, Bmi TrxR-C552, and Bmi TrxR-C547-C552 all reacted with DTNB; however, His-tags affected the reaction with Trx. The inhibition efficiency of 1-chloro-2, 4-dinitrobenzene (CDNB) on mutant C547 decreased, and the inhibition of CDNB was attenuated with the cysteine mutation at position 547, indicating that the target of CDNB is 547 cysteine. These results indicate that the site of electron acceptance in the N-terminal plays the same key roles as other homologs and the site of electron transfer in the C-terminal can be affected by the C-terminal His-tag. In conclusion, TrxR is associated with the resistance to oxidative stress and is a promising target for *Babesia microti* therapy.

OA29.04 Identification of East Coast Fever and Corridor Disease Discriminatory Sequence Variations in Allele 1 of the Sporozoite Antigen Gene p67**Dr. Donald Mukolwe Lubembe**^{1,2}, Dr. David O Odongo³, Dr. Sibeko P Kgomo¹¹Vectors and Vector-borne Disease Research Programme, Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort 0110, South Africa., ²Faculty of Veterinary Medicine and Surgery, Egerton University, Kenya., ³School of Biological Sciences, University of Nairobi, Kenya

East Coast fever (ECF) and Corridor disease (CD) caused by the cattle- and buffalo-derived *Theileria parva* respectively are considered to be the most economically important disease syndromes of cattle theileriosis in Africa.

The gene encoding the *T. parva* sporozoite surface antigen, p67, has been explored for the development of a recombinant vaccine. Similar to the current live vaccine, the major limitation of the p67-based recombinant vaccine is the heterogeneity of *T. parva* field isolates. Four p67 alleles have been reported, where allele 1 and alleles 2,3,4 have been associated with ECF and CD respectively. However, allele 1 has also been identified in buffalo-derived *T. parva* in South Africa; it is not clear if parasites possessing this allele can potentially cause the re-emergence of ECF in this country. We assessed the diversity of allele 1 in buffalo- and cattle-derived *T. parva* strains from South Africa, Mozambique, Kenya, Tanzania and Uganda. A 900bp central fragment of the p67-encoding gene was amplified from genomic DNA extracted from blood of *T. parva* positive samples, cloned and sequenced. Analysis of sequence data revealed four p67 alleles with allele 1 present in both buffalo- and cattle-derived *T. parva* strains. Analysis of two p67 B cell epitopes in allele 1 sequences showed that epitope 2 (LQPGKTS) was conserved in both cattle- and buffalo-derived *T. parva* strains. However, CD isolates had unique amino acid substitutions on epitope 1 (TEEEVPPADLSDQVL) and within the gene region in this allele. These results extend earlier data suggesting that p67 is a conserved molecule within cattle-derived parasites and reveal differences between p67 allele 1 variants associated with ECF and CD cases in

eastern and southern Africa. The finding may have implications on the use of recombinant p67 subunit vaccine against a challenge with buffalo-derived *T. parva*.

OA29.05 Differential Gene Expression Response in Domestic Cats (*Felis catus*) Experimentally Infected with *Cytauxzoon felis***Dr. Ruth Scimeca**¹, Dr Mason Reichard¹¹Oklahoma State University, Stillwater, United States

Cytauxzoonosis is a fatal tick transmitted protozoan disease of domestic cats. No study has profiled the gene expression response of cats to infection with *Cytauxzoon felis*. The aim of our work was to determine the host-parasite immune transcriptional elements related with clinical disease in cats experimentally infected with *C. felis*. True Single Molecule Sequencing (tSMS) was used to analyze the whole genome of acutely and chronically infected *C. felis* cats. *Amblyomma americanum* nymphs were acquisition fed on two cytauxzoonosis survivor cats. Replete *A. americanum* nymphs were collected and stored in controlled temperature and humidity chambers until they molted to adults. Adult *A. americanum* were then transmission fed on three naive cats. Controls samples were collected before infestation and infection with *C. felis*. tSMS results showed a total of 1068 differentially expressed genes ($P < 0.0001$, $FC \pm 2$); 480 upregulated and 588 downregulated in the *C. felis* infected samples compared to the non-infected controls. Transcripts involved in leukocyte chemotaxis, innate and cellular inflammatory responses, integrin-mediated signaling pathway, and phagocytosis were upregulated in infected cats, while genes regulating cell division and double-strand break repair via homologous recombination were downregulated. To the best of our knowledge, this is the first report of gene expression in domestic cats in response to experimental infection with *C. felis*. Real time PCR analysis will be conducted to verify tSMS results. Our preliminary results indicate significant shifts in gene expression during *C. felis* infection with relevant processes related to inflammation and mitotic activity.

OA30 IAFWP Symposium

July 9, 2019, 16:30 - 18:00

Breakout Room 5, Meeting Rooms KLOP, Level 4**OA30.01 Unexpected High Toxoplasma Gondii Prevalence in Waterfowl and Seagulls****Dr. Sawsan, Ammar**^{1,6}, Janetta Kelly², Graham Hickling², Justin Brown³, Kyle Van Why⁴, Dr. Chunlie Su⁵, Dr. Richard Gerhold¹

¹Department of Biomedical and Diagnostic Sciences, University Of Tennessee, Knoxville, Knoxville, United States, ²Department of Forestry, Wildlife, and Fisheries, University of Tennessee, Knoxville, United States, ³Pennsylvania Game Commission, Harrisburg, United States, ⁴USDA APHIS Wildlife Services, Harrisburg, United States, ⁵Microbiology Department, University of Tennessee, Knoxville, United States, ⁶Department of Clinical Pathology, Faculty of Veterinary Medicine, Sadat City University, Sadat City, Egypt

Toxoplasmosis is a zoonotic disease caused by the protozoan parasite *Toxoplasma gondii* which infects mammals and birds. Little information is known about the prevalence and epidemiology of *T. gondii* in wild birds. We investigated *T. gondii* prevalence in seagulls and numerous waterfowl species from Tennessee and Pennsylvania to better understand the parasite's host and geographical distribution and zoonotic potential. Numerous waterfowl species are game animals and thus represent a potential *T. gondii* zoonotic risk factor whereas seagulls are broadly distributed opportunistic feeders in areas of human settlements and can indicate environmental contamination. We tested a total of 62 serum samples with modified agglutination test and found a seroprevalence of 72.6% (45/62). Ringed-bill gull (*Larus delawarensis*) samples were collected from Pennsylvania and had a seroprevalence of 78% (18/23) while waterfowl samples were from hunter-killed birds in Tennessee and had a seroprevalence of 69% (27/39). In addition, PCR was performed on heart tissues from all of the above samples as well as ten heart tissues that did not have a corresponding serum

sample. PCR testing disclosed a 14% (10/72) prevalence of *T. gondii* in heart muscle. Our results suggest that *T. gondii* is highly prevalent in seagulls and waterfowl indicating high environmental contamination in the areas of collection. Results also indicate that serology is more sensitive than PCR for detecting *T. gondii* infection in these bird species. Further research is needed to elucidate the transmission dynamics of *T. gondii* in waterfowl, seagulls and other aquatic animals and determine the zoonotic risk.

OA30.02 A Duplex PCR for the Simultaneous Detection of Fasciola Hepatica and Clonorchis Sinensis**Dr. Yi Yang**¹, Dr. Yimin Yang¹, Prof. Aifang Du¹
¹Institute of Preventive Veterinary Medicine, College of Animal Sciences, Zhejiang University, Hangzhou, China

Both *Fasciola hepatica* and *Clonorchis sinensis* are endemic in China, South Korea, Japan and other Southeast Asian countries. Reliable and sensitive diagnostic methods are needed for detecting their infections in humans and animals. Differential simplex and duplex polymerase chain reaction (PCR) methods were developed. The PCRs targeted the second internal transcribed spacer (its2) (408 bp) of *F. hepatica*, and NADH dehydrogenase subunit 2 gene (nad2) (527 bp) of *C. sinensis*. Both simplex PCRs detected as little as 2 pg genomic DNA in one microliter in a 25 µL PCR reaction system. The duplex PCR had similar detection limit as well, and detected as low as one egg in 200 mg feces. These methods were analytical specific with no amplification being observed from the genomic DNA of *Fasciolopsis buski*, *Haemonchus contortus*, *Ascaris ovis* or *Eimeria* spp. Of 158 sheep fecal samples collected from various farms, four and one samples were PCR-positive for *F. hepatica* and *C. sinensis*, respectively. The duplex PCR method described here is time-saving and convenient, and may prove to be an invaluable tool for molecular detection and epidemiological investigation of *F. hepatica* and *C. sinensis* in endemic area.

OA30.03 Who's Who in the Poo: A Taqman Assay for the Differentiation of *Fasciola Hepatica*, *Fasciola Gigantica* and Co-infection in Ruminant Faecal Samples

Ms. Nichola Calvani^{1,2}, Dr. Russell Bush², Dr. Jan Slapeta¹

¹Laboratory of Veterinary Parasitology, Sydney School of Veterinary Science, Faculty of Science, The University of Sydney, Camperdown, Australia, ²Mekong Livestock Research Group, Sydney School of Veterinary Science, Faculty of Science, The University of Sydney, Camden, Australia

Fasciolosis, due to infection with *Fasciola hepatica* and *Fasciola gigantica*, is a neglected zoonotic disease of worldwide importance, with economic impacts estimated to exceed USD \$3 billion/year and over 91 million people considered at risk of infection. Infections are commonly diagnosed by a traditional sedimentation approach, which is time-consuming and prone to sensitivity errors when a large number of samples must be processed, or if the operator lacks sufficient experience. A commercially-available coprological antigen ELISA enables detection of infection prior to the 8-12 week pre-patent period, with increased sample throughput, however species differentiation is not possible in areas of parasite sympatry where co-infection with both species may occur. Diagnosis via real-time PCR offers the combined benefits of highly sensitive species differentiation for medium to large sample sizes. Despite this, there are currently no robust molecular diagnostic assays available for ante-mortem *Fasciola* spp. differentiation from faecal samples.

Our aim was to address this need via the design and validation of two real-time PCR TaqMan assays for *Fasciola* spp. differentiation using either ITS1 or LSU. We used an existing diagnostic workflow for faecal sample preparation and DNA isolation to achieve high analytical sensitivity of <1 egg per gram of faeces. The assays were designed using samples from 13 *F. hepatica*-infected sheep and six *F. gigantica*-infected cattle from Australia and Laos, respectively, which were then used to diagnose infection in 75 cattle from northern Laos expected to have been

exposed to both parasites. We also designed and validated conventional PCR primers for either ITS1 or LSU for use on faecal samples to investigate ambiguous results, or animals identified as co-infected. When combined with the previously-published workflow, the result is two assays that provide the first ante-mortem differentiation between infection with *F. hepatica*, *F. gigantica* or co-infected animals in areas of parasite sympatry.

OA30.04 Study on Zoonotic Parasites in Game in Brandenburg, Germany

Dr. Nadja Bier¹, Dr. Claudia Jäckel¹, Carolyn Kästner¹, **Prof. Karsten Nöckler**¹, Dr. Anne Mayer-Scholl¹, Dr. Annette Johné¹
¹Federal Institute For Risk Assessment, Berlin, Germany

Toxoplasma gondii, *Alaria alata*, and *Cryptosporidium* spp. are endemic parasites in Germany. To better estimate the public health risk mitigating from these three different parasites in wild animals, more data on their presence is needed.

During the hunting season 2017/2018, game was sampled from eight different hunting grounds in the federal state of Brandenburg, Germany. For the detection of *T. gondii* in wild boar, roe and red deer, blood samples from the abdominal cavity were subjected for serological testing by means of a commercial ELISA kit and heart muscle samples were examined by qPCR targeting the 529 bp-repeated element. *A. alata* was detected in wild boar using the mesocercariae migration technique for muscle and fat tissue samples and *Cryptosporidium* spp. in wild boar, roe and red deer was tested in fecal samples by use of 18SrRNA nested PCR.

Serological examination of 194 serum samples from roe deer, red deer and wild boar revealed *T. gondii*-specific antibodies in 18.6% of all tested samples. DNA was detected in 26% of seropositive animals but high Cq values may imply a low parasitic burden of *T. gondii*. Mesocercariae of *A. alata* could be found in 27% of 171 tested wild boar samples and DNA of *Cryptosporidium* spp. could be detected by PCR in 26.7% of 210 wild boar and deer samples.

These results show a high exposure of game to *T. gondii*, *A. alata* and *Cryptosporidium* spp. in the monitored hunting areas in Brandenburg, Germany and may indicate possible transmission routes of these parasites to humans.

OA31 Anthelmintic Resistance in Ruminants

July 10, 2019, 11:00 - 12:30

Plenary Hall, Madison Ballroom (ABCD), Level 4

OA31.01 Development of High Throughput Method for the Analysis of Anthelmintic Resistance Allele Frequencies in Field Populations of Gastrointestinal Nematodes

Umer Chaudhry¹, Madison MacLeay¹, Alison A. Morrison², David J. Bartley², Mike Evans¹, Neil D. Sargison¹

¹University Of Edinburgh, United Kingdom,

²Moredun Research Institute, United Kingdom

Drug resistant helminths have become a major cause of poor health and production in ruminants, and there is a need for diagnostic markers and tools to determine the frequency of resistance alleles in field parasite populations. Gastrointestinal nematode resistance to benzimidazole drugs is caused by a mutation in one of three positions on the isotype 1 β -tubulin locus, and in the absence of markers for resistance to other broad-spectrum anthelmintic classes, these provide a relevant study example. Determination of the prevalence of these single nucleotide polymorphisms in field gastrointestinal nematode populations can be impractical using conventional molecular methods, which may be error prone or lack sensitivity at low levels of resistance. Here, we report the development of a novel method based on an Illumina Mi-seq deep amplicon sequencing platform; to sequence the isotype 1 β -tubulin locus of the small ruminant gastrointestinal nematode, *Teladorsagia circumcincta*, and determine the frequency of the benzimidazole resistance mutations. We validated the method by assessing sequence representation bias in the isotype 1 β -tubulin locus, comparing the results of Illumina Mi-seq and

pyrosequencing, and applying the method to populations containing known proportions of resistant and susceptible L3. Finally, we applied the method to field samples collected from ewes and lambs on over a period of one year on three farms, each highlighting different aspects of sheep management and approaches to parasite control. The results show opportunities to build hypotheses with reference to selection pressures leading to differences in resistance allele frequencies between sampling dates, farms and ewes or lambs, and to consider the impact of their genetic fixation or otherwise. This study provides proof of concept of a practical, accurate, sensitive and scalable method to determine frequency of anthelmintic drug resistance mutations in gastrointestinal nematodes in field studies and as a management tool for livestock farmers.

OA31.02 A 16-Year Retrospective Analysis of Anthelmintic Resistance in *Haemonchus contortus* on Small Ruminant Farms in the United States

Mrs. Sue Howell¹, Mr. Brandon Park², Dr. Anand Vidyashankar², Mr. Bob Storey¹, Mr. James Collins¹, Dr. Ray Kaplan¹

¹University Of Georgia-Dept. of Infectious Diseases, Athens, United States, ²George Mason University-Dept of Statistics, Fairfax, United States

The DrenchRite® larval development assay (LDA) measures resistance to benzimidazoles, levamisole, ivermectin, and moxidectin. From 2000-2016 we performed DrenchRite® LDA on 291 small ruminant farms in 40 US states. We then performed a retrospective analysis of these data to investigate changes in prevalence and levels of anthelmintic resistance in *Haemonchus contortus* over this period. For analyses, time series models were used to identify shifting and emerging resistance trends across time, and data were grouped into three 5-6 year intervals (2000-2005; 2006-2010; 2011-2016). Resistance was highly prevalent to benzimidazoles and ivermectin in goats reaching 100% and 95% prevalence, respectively by 2011-2016. Prevalence of levamisole resistance was consistently the lowest of all drugs on

both goat and sheep farms. The greatest changes were for moxidectin, and these were significantly greater for goat than sheep farms ($p < 0.0001$). Prevalence of resistance to moxidectin progressively increased over the 3 time intervals for goat (28%, 38%, 56%) and sheep (3%, 18%, 40%) farms, with mean IC₅₀ (nM) increasing from 592,225 (2000–2006) to 2433,1761 (2011–2016) for goat and sheep farms, respectively. The time series model for moxidectin on goat farms confirmed that low resistance was initially detected, followed by full resistance during the later years. For sheep farms, an increasing trend was demonstrated where susceptibility was shown initially, which then moved to suspected resistant in the middle-years, and is currently moving toward full-resistance. Total anthelmintic failure (resistance to all 3 classes plus moxidectin) increased over time from 3% to 35% and 3% to 10% for goat and sheep farms, respectively. These data and the time series analyses performed provide interesting insights into changes occurring in resistance levels over time, and differences in changes between drugs and hosts. Though worse on goat than on sheep farms, these data indicate a severe situation exists in the USA with regard to anthelmintic resistance.

OA31.03 Evidence of Widespread Anthelmintic Resistance in Cattle in Ireland

Ms. Anne Kelleher^{1,2}, Dr. Orla Keane², Dr. Barbara Good³, **Assoc. Prof. Theo de Waal¹**
¹University College Dublin, Belfield, Ireland,
²Teagasc, Animal and Bioscience Department, Grange, Ireland, ³Teagasc, Animal and Grassland Research and Innovation Centre, Athenry, Ireland

Irish beef production is predominantly pasture based with the result that grazing cattle are exposed to a number of different gastrointestinal nematodes. Irish farmers rely heavily on anthelmintic treatments to control helminth infection. Although anthelmintic resistance (AR) is more commonly reported in sheep it has been increasingly reported in cattle worldwide. In 2014 AR was reported for the first time on two cattle farms in Ireland. During 2017 and 2018 a more extensive survey was conducted in Ireland to determine the

extent of AR in the Irish cattle population. A total of 24 dairy-to-beef farms were recruited for the study and on each farm groups of 20 first grazing season calves were treated with either benzimidazole (BZ), levamisole (LEV), ivermectin (IVM) or moxidectin (MOX) (not all drugs were tested on every farm). Faecal egg count reduction was determined from pre- and post-treatment faecal samples. Faecal egg counts were determined using the mini-FLOTAC technique (sensitivity of 5 eggs per gram). Pooled pre- and post-treatment faecal cultures were also established. Reduced BZ efficacy (range 15%–93%) was recorded on 71% (12/17) of farms; 25% (3/12) of the farms recorded reduced efficacy for LEV (range 79%–90%). All the farms tested (100%; 17/17) showed reduced IVM efficacy (range –228%–89%) and 75% (9/12) of the farms recorded reduced efficacy against MOX (range 1%–93%). *Cooperia* and *Ostertagia* were the predominant nematode genera present on all farms. Surprisingly, *Ostertagia* was identified as the main nematode species (> 50%) surviving treatment in 89% BZ, 33% LEV, 23% IVM and 12.5% MOX resistant farms. Results from this study indicates that AR is widespread on cattle rearing farms in Ireland and that strategies to manage and minimise AR are urgently needed.

OA31.04 Nicotine-Sensitive Acetylcholine Receptors Are Relevant Pharmacological Targets for the Control of Multidrug Resistant Parasitic Nematodes

Dr. Elise Courtot¹, Fabrice Guégnard¹, Jacques Cortet¹, Dr. Cédric Neveu¹, Dr. Claude Charvet¹
¹INRA, Université de Tours, UMR1282, 37380, Nouzilly, France

The control of parasitic nematodes impacting animal health relies on the use of broad spectrum anthelmintics. However, intensive use of these drugs has led to the selection of resistant parasites in livestock industry. In that respect, there is currently an urgent need for novel compounds able to control resistant parasites. Nicotine has also historically been used as a de-wormer until modern anthelmintics were marketed. The pharmacological target of nicotine has been identified in nematodes as acetylcholine-

gated ion channels. Nicotinic-sensitive acetylcholine receptors (N-AChRs) therefore represent validated pharmacological targets than remain largely under-exploited. In the present study, we developed an automated larval migration assay (ALMA) and showed that nicotinic derivatives (anabasine/nornicotine) efficiently paralyzed a multiple (benzimidazoles/levamisole/pyrantel/ivermectin) resistant field isolate of *Haemonchus contortus*. Additionally, using *Caenorhabditis elegans* as a model, we confirmed that the N-AChRs subtype contributes to the anthelmintic effect of nicotinic analogs. Interestingly, the functional expression of the homomeric N-AChR from *C. elegans* and the distantly related horse parasite *Parascaris equorum* in *Xenopus* oocytes highlighted some striking differences in their respective pharmacological properties towards nicotine derivative sensitivity. Noteworthy, nicotine and anabasine were more potent than ACh in activating the *P. equorum* N-AChR as revealed by their respective EC₅₀ values ($2.9 \pm 0.5 \mu\text{M}$ and $1.7 \pm 0.1 \mu\text{M}$ versus $6.4 \pm 1.1 \mu\text{M}$, respectively), unlike nornicotine ($34.9 \pm 7.2 \mu\text{M}$) whereas the potency series for the *C. elegans* N-AChR was Nic > ACh = Ana > Nor. Taken together these results validate the exploitation of the N-AChRs of parasitic nematodes as targets for the development of resistance-breaking compounds.

OA31.05 Anthelmintic Combinations: A Sustainable Strategy to Optimize Parasite Control on Commercial Cattle Farms?

Dr. Candela Canton¹, Dr. Laura Ceballos¹, Dr. Laura Moreno¹, Veterinarian María Paula Domínguez¹, Ms. Lucila Canton¹, Veterinarian Miguel Buffarini², Dr. Carlos Lanusse¹, Dr. Luis Ignacio Alvarez¹

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In an attempt to minimize therapeutic failures following anthelmintic treatments and to delay the development of resistance, the combined use of nematocidal compounds

with different mechanisms of action has been proposed. A pharmaco-parasitological assessment of different nematocidal combinations was performed at different cattle commercial farms in Argentina. The Ivermectin(IVM)-ricobendazole(RBZ), IVM-levamisole(LEV) and RBZ-LEV combinations were assessed in calves naturally infected with susceptible/resistant gastrointestinal nematodes. The observed pharmacokinetic(PK) data demonstrated that the co-administration of two anthelmintics did not modify the plasma PK behaviour of either drug in cattle. In fact, no adverse PK interactions were observed after each combined treatment. Similar PK parameters($P>0.05$) were obtained between the single-drug and the combined-based strategies. The IVM-RBZ, IVM-LEV and RBZ-LEV combinations were the only treatments achieving 100% clinical efficacy, even when a highly IVM-resistant *Haemonchus* spp. isolate was present(45% efficacy for IVM alone). In fact, the combination IVM-RBZ maintained its 100% efficacy against an IVM-resistant *Haemonchus* spp. population over the last five years. Additionally, the weight gain was significantly higher in calves treated with the IVM-RBZ combination. Overall, after the combined treatments only a therapeutic additive effect is achieved. However, the efficacy of IVM-RBZ against nematodes resistant to IVM and RBZ was greater than an additive effect. In fact, in two farms with multiple resistance to IVM and RBZ, while the efficacies during the first year of study were 54%(IVM), 84%(RBZ) and 98%(IVM-RBZ) in Farm A, the egg reductions were 40%(IVM), 64%(RBZ) and 90%(IVM-RBZ) in Farm B. During the second year of study, the high efficacy of the combination IVM-RBZ could be maintained in Farm A(97%), but not in Farm B(67%). Overall, the potential advantages of anthelmintic combinations should be carefully assessed before being extensively recommended. Their rational use should be strongly supported by pre-treatment diagnosis and considering the epidemiological situation of each individual farm.

OA32 Avian Parasites

July 10, 2019, 11:00 - 12:30

Breakout Room 2, Hall of Ideas E&H, Level 4

OA32.01 Resistance to Fenbendazole in *Ascaridia Dissimilis* and Its Economic Impact of the Production of Turkeys

Mr. James Collins¹, Dr. Brian Jordan¹,
Dr. Luke Baldwin¹, Dr. Justin Fowler¹, Dr.
Claude Hebron², Dr. Kelsey Paras¹, Dr. Anand
Vidyashankar³, Dr Ray Kaplan¹

¹University of Georgia, Athens, United States,²Prestage Farms, Clinton, United States,³George Mason University, Fairfax, United
States

Ascaridia dissimilis is the most prevalent and one of the most economically important gastrointestinal nematodes of turkeys. Infections are most often subclinical, producing reduced feed conversion efficiency, with heavier infections causing clinical symptoms such as lethargy, intestinal blockage, diarrhea, and in some cases death. Currently, fenbendazole is the only drug approved by the United States Food and Drug Administration for use against this parasite in turkeys. We recently tested the efficacy of fenbendazole against 4 field isolates of *A. dissimilis* acquired from commercial farms. Three of the four isolates (Wi, Ow, Po) demonstrated greater than 99% efficacy, with the fourth (Sn) yielding only 63.89% efficacy, indicating that this isolate is resistant to fenbendazole. Having proven fenbendazole resistance in *A. dissimilis*, we wanted to determine the economic impact that resistant worms have on growth and productivity. We infected 384 turkey poults with either the Sn resistant isolate or the Ow susceptible isolate and divided them into treated and untreated groups. Both the treated and untreated groups for each isolate had 8 replicates of 12 birds each. Turkeys were grown for an 18-week growing cycle, changing feed types at the recommended time points as per normal commercial growing practices. Birds were infected via a trickle dosage of 50 eggs/bird/week sprinkled onto their feed. At 4, 8, 12 and 16 weeks, birds in the treated groups were administered fenbendazole in

the water (SafeGuard® Aquasol, 1mg/kg) for five consecutive days. Water consumption, feed intake, and bird weights were measured weekly. Differences between treated and untreated groups of an isolate, as well as between the two isolates, are being analyzed to determine the impact of resistant parasites feed consumption, water consumption and feed conversion.

OA32.02 Methodology for the Assessment of Parasitic Infections Caused by *Allopsoroptoides galli* (Acari, Psoroptoididae) in Laying Hens

Edna Clara Tucci¹, Nilce Maria Soares², **Paulo Henrique Selbmann Sampaio**¹

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Allopsoroptoides galli is a parasitic mite found in 2010 when it shifted from wild avian hosts to battery caged layers in the state of São Paulo, Brazil. Clinical findings include intense pruritus and severe dermatitis, with dry scaling skin and yellowish exudate on the calami. Necessity prompted standardizing a methodology to study the infection. It is based on grading dermatitis of the apteria and counting live mites on skin scrapings. To grade dermatitis, a mean score of the ventral alar, dorsal and lateral apteria is calculated. Scores range from 0 to 5 (0 = no dermatitis; 1 = mild dermatitis; 2 = mild to moderate dermatitis; 3 = moderate dermatitis; 4 = moderate to severe dermatitis and 5 = severe dermatitis). Microscope slides are used to scrap each apterium until a hemorrhage occurs. Scrapings are clarified with potassium hydroxide, coverslips are placed and acari are immediately counted under a microscope. To validate the methodology, 22 naturally infected layers were randomly assigned to treatment with a 3% emulsion of a botanical acaricide (70% *Azadirachta indica*; 2.5% *Melaleuca alternifolia* and 2.5% *Melaleuca leucadendron* essential oils) or to the control group. 0.3% neutral detergent was added to aid skin contact. Control fowl were sprayed with water and 0.3% neutral detergent. Both groups were sprayed on experimental days 0,

+7 and +14. Hens were checked for dermatitis and mite counts on days +7; +14; +28; +35; +42 and +72. A significant reduction of acari was achieved after the first treatment, but mites were recovered until day +35. Treated layers presented mild dermatitis until the end of the trial. Dermatitis does not imply *Allopsoroptoides* infection. Therefore, checking apteric skin for mites is fundamental. The standardized methodology presented in the current abstract is effective and can be useful in further studies, under experimental and field conditions.

OA32.03 On-Animal Sensors to Assess Chicken Body Louse Effects on Poultry Behavior and Welfare

Dr. Amy Murillo¹, Dr. Richard Blatchford², Mr. Alireza Abdoli¹, Dr. Eammon Keogh¹, Dr. Alec Gerry¹

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The chicken body louse (CBL), *Menacanthus stramineus*, is a common poultry pest in the United States, especially on small backyard or hobby flocks. It is an obligate ectoparasite that primarily feeds on feathers, but will also blood-feed. Severe infestations can cause economic damage to egg-laying hens.

Increasing awareness of animal welfare in livestock has led to the development of standardized metrics for assessing animal welfare. One example, the Welfare Quality® Assessment (WQA) protocol has been widely adopted in North America as a research and on-farm tool. The WQA uses both environment- and animal-based measures, but only the presence or absence of ectoparasites is noted and there is no qualitative or quantitative evaluation. The degree of impact ectoparasites have on hen health or welfare is likely to vary based on ectoparasite species and severity of infection. In this study, animal welfare metrics were evaluated as a function of louse infestation using two groups of birds; louse infested flocks and louse-free control flocks.

Louse infestations may also hinder or alter normal chicken behaviors, another welfare

component. On-animal sensors, which measure magnitude of force ('movement') along 3-axes, were used to identify and quantify chicken behaviors before and during ectoparasite infestation compared to uninfested control birds. The behaviors measured were pecking, preening, and dustbathing.

During a pre-infestation period (week 1), both WQA measures and bird behavior data (sensor readings) was recorded to provide baseline data for bird welfare and behavior. Lice were subsequently introduced to 2 of 4 flocks, and WQA and sensor readings were repeated during Week 7 and Week 12 after louse populations had established in the infested flocks.

Variation of WQA and behaviors among individuals is high, though preliminary results indicate trends that relate to louse populations on birds.

OA32.04 More Effective Control of House Flies in Poultry Facilities Using the Fungus *Beauveria Bassiana*

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Control of house flies and other muscids are a significant challenge for poultry producers, especially in layer facilities. Most egg production occurs indoors with accumulating poultry waste thus creating perfect habitat for breeding flies. To control pest flies, the poultry industry spends 20 million dollars annually on pesticides alone. This estimate doesn't include the cost of animal loss due to house fly vectored pathogens causing disease, the cost of labor for pesticide application, or litigation that can be taken by residents living near production facilities due to increased fly numbers. However, regulatory restrictions, fly resistance to current active ingredients, and lack of options labeled for pest control in poultry facilities make fly control challenging. Finding a new and effective tool for fly control is needed by the poultry industry. Biological control using the entomopathogenic fungi *Beauveria bassiana* may be an effective

method for fly control. One product is currently available for use in poultry facilities, but efficacy has been unsatisfactory. In addition, *B. bassiana* takes 6 or more days to kill an adult fly which limits the ability to control house fly outbreaks. The goal of this project is to develop a more effective *B. bassiana* product and develop it into a more practical tool that can be easily adopted into both Integrated Pest Management programs and organic farming practices. The objectives are to 1) identify isolates of *B. bassiana* from house flies collected from poultry facilities in Pennsylvania, 2) select successful isolates for quick kill times, and 3) assess the compatibility of these isolates on other common biological control agents (e.g., parasitoid wasps). House fly collections were made on 8-layer house facilities in Pennsylvania bi-weekly from June to August. To date, five isolates of *B. bassiana* have been identified from fly collections. Selection and safety results will be discussed.

OA32.05 Effect of a Herbal Complex Against Common Parasites of Animals and Poultry in Pakistan

Dr. Muhammad Arfan Zaman¹, Professor Zafar Iqbal², Dr. Rao Zahid Abbas², Professor Muhammad Fiaz Qamar¹

¹College of Veterinary and Animal Sciences, Jhang, Pakistan, ²Department of Parasitology, University of Agriculture, Faisalabad, Pakistan

The objective of the current study was to evaluate the anti-tick, anthelmintic and anticoccidial activities of a herbal formulation (HF) based on complex of water extracts of leaves of *Azadirachta indica* and *Nicotiana glauca*, flowers of *Calotropis procera* and seeds of *Trachyspermum ammi*. The HF demonstrated anti-tick activity by inhibiting the egg laying, larval mortality and reduced tick intensity/infestation on animals. Anthelmintic activity of herbal formulation was evident from the in vitro mortality of *Haemonchus contortus*, ovicidal effects in egg hatch test and fecal egg count reduction in sheep naturally parasitized with gastrointestinal nematodes. Anticoccidial effects of herbal formulation were confirmed by reduction in the oocyst counts in feces,

oocyst scores, bloody diarrhea and FCR in chicks treated with herbal extracts compared with infected unmedicated chicks. The survival rate and weight gain was higher in chicks treated with herbal extract compared with infected unmedicated chicks. According to the findings of this study, the HF is suitable for the resource-poor farmers as a broad spectrum antiparasitic. The contents of the formulation are cheap, commonly available, and easy to use as a decoction. Incorporation of this herbal formulation in integrated parasite management practices will add to the sustainability and thus, income of the farmers. The HF seems promising as a broad spectrum antiparasitic. Large scale controlled studies are, however, recommended for standardization of the doses and applications of the product. Future experiments may be planned to understand the mechanism of absorption as well as developing an appropriate delivery agent for the herbal formulation inside the tick body. Studies on shelf life of the plant extract and its residual activity need to be carried out.

OA32.06 Georeferenced Database of Avian Wild Parasites in Mexico

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Georeferenced analysis techniques emerged, among other causes, to relate environmental, biological and abiotic variables, with the epidemiology of diverse infectious diseases. There is a great diversity of documented avian parasites in Mexico. However, the information is dispersed in different documents, making the research even more variable.

The main objective of this work consisted of collecting, purifying, organizing and analyzing the information about wild avian parasite occurrences and presences from bibliographic and diagnostic data all over the country for the development of a georeferenced database illustrated with several maps to show the parasite occurrences of these registered hosts in Mexico from 1990 to 2018, as well as the projected distribution to the year 2050

considering the effects of climate change. A total of 982 records were integrated into the data base and 7 maps of parasites occurrences were generated corresponding to 279 municipalities, as well as 23 maps of the projected distribution to 2050 according to the Maxent program. The bioclimatic variables that contributed to the parasite distribution are BIO19, BIO15, BIO2 and BIO4. The knowledge of the environmental variables involved in the spatial distribution of the parasites will support the proposal of parasite infection risk in avian wildlife to apply One Health guidelines an approach to design and implement preventive and conservation strategies and programs.

OA33 Equine Cyathostomes I

July 10, 2019, 11:00 AM - 12:30 PM
Breakout Room 3, Hall of Ideas F&I, Level 4

OA33.01 A Coprological Survey on Internal Parasites of Horses in Italy

Dr. Francesco Buono¹, Dr. Laura Pacifico¹, Dr. Diego Piantedosi¹, Giovanni Sgroi¹, Dr. Domenico Rufrano², Dr. Cristina Roncoroni³, Dr. Giuseppe Mazzeo⁴, Dr. Francesco Chiarotto⁴, **Dr. Vincenzo Veneziano¹**
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Internal parasites are ubiquitous in horses and cyathostomins are the most important due to the development of anthelmintic resistance. A national survey was conducted from December 2013 to January 2019 to assess the prevalence of the main endoparasites in 338 horse farms in Italy. Faecal samples were collected from 3,092 horses (764 males, 1592 females, 736 geldings), mean age 9.9 (1 month–34 years) and examined using the Mini-FLOTAC technique (detection limit: 5 eggs per gram) and a Sheather's sugar solution (specific gravity:1.250). A centrifugation/flotation technique and a sedimentation technique were used for the

diagnosis of Anoplocephalidae and Fasciola hepatica, respectively. Faecal cultures were performed for each horse with strongyles faecal egg count (FEC) >200 EPG. Intestinal strongyles were the most common parasitic species found with a prevalence of 54.5%. Infection intensity was lower than 200 EPG in 27.5% of horses (low contaminators); 11.3% were moderate contaminators (200–500 EPG), 15.7% were high contaminators (>500 EPG); 45.5% had negative strongyle egg count. Faecal cultures revealed only the presence of cyathostomins. Other parasites were Parascaris spp. (9.7%), Anoplocephala spp. (4.1%), Oxyuris equi (3.2%), Eimeria leuckarti (0.6%) and Strongyloides westeri (0.1%). No eggs of Fasciola hepatica were detected. Prevalence of strongylid infection was significantly different for horse ≤1 year (OR=2.02, p<0.0001) and 1–4 years (OR=1.99, p<0.0001) versus older horse (>4 years). Prevalence of ascarid infection was significantly different for horse ≤1 year (OR=56.95, p<0.0001) and 1–4 years (OR=11.62, p<0.0001) versus older horse. Considering the high prevalence of horses with FEC<200 EPG (73%) the responsible use of equine anthelmintics is mandatory. In practice it is necessary to determine the EPG using a quali-quantitative coprological method and treatments should be made only after a diagnosis. It is crucial that veterinary play an active role in planning and monitoring effective and appropriate equine worm control programs.

OA33.02 Precision, Sensitivity, and Specificity Analysis of an Automated Parasite Fecal Egg Counting System in Comparison to McMaster and Wisconsin Methods

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Fecal egg counts are the cornerstone of equine parasite control programs. Previous

work developed an automated image analysis based parasite egg counting system that reduces operator error, and should thus increase method precision. The system has been further developed to include an automated reagent dispenser unit and a standalone digital imaging unit generating higher resolution images. The aims of this study were to conduct a comprehensive comparison of method precision between two different imaging units as well as the traditional McMaster and Wisconsin manual techniques, and to perform a Bayesian analysis for estimation of method sensitivity and specificity. Feces were collected from horses, screened with triplicate Mini-FLOTAC counts, and placed in very high (>1000 eggs per gram (EPG)), high (500-1000 EPG), and medium (>500-200 EPG) egg count categories. Ten replicates per horse were analyzed for each technique: the automated system using both a smartphone and standalone camera model, McMaster, and Wisconsin. Although not statistically significant from McMaster (76.1%), the standalone camera produced the numerically highest (78.8%) precision for the high counts. Wisconsin had the lowest precision at 63.7%, and the smartphone camera had a precision of 68.7%. Wisconsin tended to produce lower egg counts than the other methods, whereas the older camera system tended to produce higher counts than the others. The Bayesian analysis also included low (> 200 - < 0 EPG) and negative (no eggs seen) counts, and results for sensitivity and specificity will be presented at the conference. Overall, the automated counting method is a promising new development in equine parasitology, and continuous improvement to the counting algorithms will help increase precision in the future.

OA33.03 A Parasite for all Seasons: Investigation of Seasonality in Strongyle Egg Shedding and Strongylus Vulgaris

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Strongylus vulgaris is widely recognized as a major pathogen in horses, causing non-strangulating intestinal infarctions, peritonitis, and death. In addition, cyathostomin (small strongyle) parasites are ubiquitous in grazing horses and are main targets of parasite control in mature horses. Historic studies conducted in Northern Europe have suggested seasonal fluctuation in strongyle egg shedding as well as the occurrence of patent *S. vulgaris* infection; however, these epidemiological patterns have not been investigated in recent years or in other climatic locales. In this study, we analyzed the seasonal fluctuation of strongyle fecal egg counts, presence of *S. vulgaris* larvae on coproculture, and concentration of antibodies specific to migrating *S. vulgaris* larvae in a research herd of horses maintained at the University of Kentucky that has not received anthelmintic treatment since 1979. Fecal and serum samples were collected from all horses (N=20) twice monthly during 2018. Individual strongyle fecal egg counts and coprocultures were carried out for every time point. Similarly, a validated ELISA was run to determine the concentration of anti-*S. vulgaris* antibodies. Data were analyzed with mixed linear models. There were no seasonal differences in strongyle egg shedding and anti-*S. vulgaris* antibody levels; however, the number of *S. vulgaris* third stage larvae identified in coprocultures was significantly higher ($p > 0.0001$) in the spring compared to summer and autumn. Furthermore, horses aged 15-18 years of age had significantly higher *S. vulgaris* larval counts than horses aged 8-10 years ($p < 0.0009$). The lack of seasonality in strongyle egg shedding is in contrast with studies performed in the United Kingdom several decades ago. Possible reasons could be climatic influences, cyathostomin species composition, stocking density, and the absence of anthelmintic treatment. It was surprising to see the oldest horses demonstrate the highest *S. vulgaris* larval counts, as this parasite is generally considered to be immunogenic.

OA33.04 It's All About the Mucus? Evaluation of the Immune Response to Larvicidal Treatment of Equine Cyathostomin Infection

Dr. Ashley Steuer¹, John Stewart¹, Day Barker¹, Dr. Amanda Adams¹, Dr. Martin K. Nielsen¹
¹*University Of Kentucky, Maxwell H. Gluck Equine Research Center, Lexington, United States*

Cyathostomins are omnipresent parasites of equids worldwide. In rare cases, they can cause life-threatening larval cyathostominosis, which is characterized by a generalized typhlocolitis when synchronous mass emergence of the larvae from the large intestine occurs. Little is known about the immune response to these parasites in horses. Goblet cell hyperplasia has previously been noted as an important component with cyathostomin infection; however, its role is unclear. This study was to evaluate the local and systemic inflammatory response, with particular interest in the role of goblet cells, to cyathostomin infections following larvicidal treatment. 36 ponies with naturally acquired cyathostomin infections were evenly divided into three groups: fenbendazole (10mg/kg PO 5days), moxidectin (0.4mg/kg PO once), and untreated control. Blood was collected weekly and tissue collected from the cecum and dorsal and ventral colon at 2 and 5 weeks post treatment (WPT). Real-time PCR was used to evaluate the gene expression of IL-4, IL-5, IL-6, IL-10, IL-13, IL-22, IFN γ , Resistin-like Molecule beta (RELM β), Mucin 2 (MUC2), and TNF α . MUC2, RELM β , and IL-22 were decreased in circulation at 3-5 WPT ($p < 0.0001$), suggesting a role and spillover involving goblet cells across all groups. Pro- and anti-inflammatory markers were significantly different between treatment groups in tissues. These results suggest that larvicidal treatment can affect the mucosal immune response itself, as well as immune modulation elicited by cyathostomin larvae. We also found evidence that the large intestinal organs respond differently to treatment and to the encysted larvae themselves. These results may have implications for unraveling parasitic disease processes and consequences of larvicidal treatment.

OA33.05 Strongylus Vulgaris and Colic in Swedish Horses: A Case-Control Study

Ass Prof, Eva Tydén¹, Dr. Ylva Hedberg-Alm¹, Dr. Miia Rhimäkii¹, Dr. Martin Nielsen², Professor Arvid Uggla¹, Eva Osterman-Lind³
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Strongylus vulgaris is re-emerging in the Scandinavian horse population with a prevalence of about 60% on farm level. In recent years an increase in parasite-related injuries, such as higher frequency of colics and parasite related damages at autopsy, have been noted at the clinics of the Swedish University of Agricultural Sciences (SLU). The aim of this study was to evaluate if *S. vulgaris* is more prevalent in horses with clinical signs of colic. This was investigated in a case-control study conducted from February 2017 to February 2018 at the equine clinic of SLU. Cases were horses with signs of colic and controls were horses that attended the clinic on the same week but for other reasons. Blood and faecal samples were collected from 142 cases and 142 controls. Sera were analysed by a *S. vulgaris* antigen ELISA and faecal samples for strongyle eggs with a modified McMaster method and for *S. vulgaris* with an ITS-2 specific PCR on larval cultures. No significant associations were found between colic and positive serology, level of faecal egg output or *S. vulgaris* positive larval cultures. Eight horses with colic underwent surgery of which one had suspected signs of migrating *S. vulgaris* larvae due to inflamed mesenteric arteries. To be highlighted, four horses with colic were euthanized and after necropsy two of these were confirmed to have intestinal infarction caused by migrating *S. vulgaris* larvae in the mesenteric arteries. Both these horses excreted 0 strongyle eggs and were negative on the ITS-2 PCR but positive in the ELISA. To conclude, from this study we could not demonstrate a higher occurrence of *S. vulgaris* in horses with clinical signs of colic than in control horses without colic.

OA33.06 Development of a Diagnostic Test for Small Strongyle Infections in Equines – Supporting Treatment Decisions in Practice

Dr. Kathy Geyer¹, Dr. Kirsty Lightbody², Dr. Tom Tzelos³, Prof Martin Nielsen⁴, Dr. Corrine Austin², Prof Jacqui Matthews¹
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Small strongyles (cyathostomins), comprising a group of approximately 50 different species, are universally regarded as the most prevalent and potentially pathogenic helminth parasite of equines. Cyathostomin early third stage larvae (L3) can undergo a period of developmental arrest by encystment in the mucosa/submucosa of the gut wall where they can remain inhibited for months to years. The clinical syndrome, larval cyathostominosis, is caused by en masse development and emergence of encysted larvae from the gut wall and has a case fatality rate as high as 50%. A diagnostic test that informs on cyathostomin burden, especially levels of encysted larvae, is lacking. In some regions, an annual moxidectin (MOX) treatment in autumn/winter is the current recommended approach to target cyathostomin larvae in all horses. Due to extensive use of anthelmintics in interval-treatment programmes, drug resistance is a major problem, as evidenced by reported widespread resistance of cyathostomins to fenbendazole and pyrantel compounds. As reduced cyathostomin egg reappearance period (ERP) after MOX treatment is now commonly reported, it is paramount to preserve efficacy of this anthelmintic, the only remaining effective larvacidal compound. With the aim of developing a serum-based ELISA for diagnosis of cyathostomin infection, we previously identified diagnostic antigens from the common cyathostomin species, *Cylicostephanus longibursatus*, *Cyathostomum catinatum* and *Cylicocyclus nassatus*. Following the production of recombinant proteins for use in ELISA and immunoblotting, these antigens have been shown to be targets of a specific IgG(T) response. The ELISA demonstrates great diagnostic potential (93% sensitivity/88%

specificity). Following optimisation of recombinant antigen expression and purification, we are now validating a commercial version of the test.

The availability of such a test for detection of cyathostomin infection will provide an important tool to inform veterinarians on treatment decisions and contribute toward sustainable control of equine small strongyle infections.

OA34 Flies and Fly Control in Ruminants

July 10, 2019, 11:00 - 12:30
Breakout Room 4, Hall of Ideas G&J, Level 4

OA34.01 Impacts of Long-Term Insecticide Treatment Regimes on SKRD and KDR Pyrethroid Resistance Alleles in Horn Fly Field Populations

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We evaluated the effects of four different six-year duration control strategies on the resistance levels and frequency of the pyrethroid target site resistance alleles, superkdr (skdr) and kdr, at four field populations of *Haematobia irritans irritans* (Linnaeus, 1758) (Diptera: Muscidae) in Louisiana, USA. Consecutive use of pyrethroid ear tags for six years caused a significant increase in the resistance ratio to pyrethroids as well as the frequencies of both skdr and kdr resistance alleles. After three years of consecutive use of pyrethroid ear tags, followed by one year with no treatment, and followed by two years with organophosphate ear tags, the resistance ratio for pyrethroid was not significantly affected, the %R-skdr significantly dropped while the %R-kdr allele remained relatively high and stable. Similar results were observed when pyrethroid ear tags were used for three consecutive years,

followed by one year with no treatment, and followed by two years with endosulfan ear tags; however, this treatment resulted in a slight increase in the resistance ratio for pyrethroids. In a mosaic, the resistance ratio for pyrethroids showed a 2.5-fold increase but the skdr-kdr genetic profiles did not change, as the %R alleles (skdr and kdr) remained low and stable through the six years. Lack of exposure to pyrethroid insecticides for three years significantly affected the skdr mutation but not the kdr mutation, preventing re-establishment of susceptibility to pyrethroids. SS-SR (skdr-kdr) individuals were responsible for the maintenance of the kdr mutation in two of the populations studied, and fitness cost seems to strongly affect the SR-RR genotype. None of the four treatment regimens evaluated in the study had satisfactory results for the management of skdr and kdr resistance alleles.

OA34.02 Cypermethrin Resistance in Stable Fly Populations from Central Brazil

Dr. ATM Barros¹, VD Rodrigues², Dr. PHD Cançado¹, **Dr. Luisa Domingues³**
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In the last decade, large-scale proliferation of stable fly (*Stomoxys calcitrans*) (Diptera: Muscidae) in organic residues and byproducts of ethanol production has become an unprecedented problem for cattle producers in some locations of the Southeast and Midwest regions of Brazil. As with other livestock pests, insecticides have been commonly used by ranchers to control this pest in cattle herds nearby sugarcane mills. However, there is limited knowledge about the effects of insecticides on immature and adult stages in the field, and almost no information on the susceptibility of stable fly populations to the insecticides commercially available. The present study aimed to evaluate the susceptibility to cypermethrin of two *S. calcitrans* colonies kept at Embrapa Beef Cattle (Campo Grande,

Mato Grosso do Sul state, Brazil) and three wild populations collected at sugarcane mills in the state of Mato Grosso do Sul. Wild flies were collected at sugarcane mills with Nzi traps and immediately used in bioassays. Susceptibility to cypermethrin was assessed after a 2-hour exposure to impregnated filter papers. All tested populations were resistant to cypermethrin, with resistance factors ranging from 6.8 to 38.6 in the wild populations, showing that pyrethroid resistance is currently present in stable fly populations in the Brazilian Midwest. To our knowledge, this is the first report of insecticide resistance in stable flies in Latin America.

OA34.03 Selected Insecticide Delivery Devices for Management of Horn Flies (*Haematobia irritans*) (Diptera: Muscidae) on Beef Cattle

Dr. Sonja Swiger¹

¹*Texas A&M AgriLife Extension, Stephenville, United States*

The horn fly, *Haematobia irritans* (L.) (Diptera: Muscidae), is one of the most important pests of the beef cattle industry. Horn fly adults are blood feeders that remain in constant contact with cattle, providing management opportunities via insecticide-impregnated ear tags. Controlling horn flies in the United States is time consuming and costly, but failure to implement management can lead to weight loss and decreased weight gain of calves and yearlings. In the past decade, new chemical combinations have been impregnated into ear tags for pest management. The objectives of this project were to 1) evaluate the efficacy of ear tags against horn fly populations and 2) determine if reduced fly density results in economic return. Several years of data compiled by insecticide class show significant reductions in horn fly populations with the use of macrocyclic lactone treatments, pyrethroid treatments, and organophosphate treatments compared with untreated animals. In Texas, this reduction has been shown to be more effective when tagging of cattle is delayed.

OA34.04 Examining Cattle Producer Management of Horn Flies, *Haematobia irritans* (L.) (Diptera: Muscidae)

R. T. Trout Fryxell¹, K. Lewis¹, S. Schexnayder¹, A. P. Griffith¹, D. B. Taylor², P. Olafson², L. McKay¹

¹University Of Tennessee, ²505 EJ Chapman Dr, United States, ²USDA-ARS

Horn flies (*Haematobia irritans* (L.)) are not only an economic concern to cattle producers, but also a health and welfare concern because of their blood-feeding behaviors. Economic losses to the cattle and dairy industries from this obligate behavior include decreased weight gain, loss in milk productivity, and transmission of bacteria causing mastitis in cattle. Horn fly management strategies are labor intensive and can become ineffective due to the horn fly's ability to develop insecticide resistance. Tennessee and Texas cow-calf producers were surveyed to examine cattle producer management of horn flies. While consumer surveys are prevalent in agricultural economics literature, producer surveys are less common given a producer panel is less assessable than a consumer panel. This paper will describe the results and the potential for introducing novel horn fly management strategies.

OA34.05 Back From the Dead: Resurrection of the USA Horse Fly *Tabanus Variegatus* Fabricius 1805

Bradley Mullens¹, Rebecca Fryxell², Paul Masonick¹, Travis Davis²

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Tabanidae (horse and deer flies) are major pests of livestock and sometimes people. The blood-feeding North American tabanid fauna is well known taxonomically overall. However, the highly variable species *Tabanus sulcifrons* Macquardt, which is widespread and abundant in the eastern USA, has been difficult to resolve using morphology alone. In the southern USA a slightly smaller and darker-colored variant has been suspected of being different, but there is enough overlap

in morphological features that it has defied separation. In certain areas such as eastern Tennessee, typical *T. sulcifrons* fly distinctly earlier (June to August) than the darker form, which flies later (August to October). Dual seasonal peaks are suspicious for a group that generally is univoltine. We have done morphological, molecular (16S and COI mitochondrial genes), and quantitative morphometric studies using *T. sulcifrons* s.l. from across a wide geographic range. The late-flying and darker form, also called the "Carolina form" by some tabanid taxonomists, is indeed distinct from typical *T. sulcifrons*. The range of the late form extends from southern New Jersey to northern Florida, and west at least to about western Tennessee. The late-flying form is the most abundant large horse fly attacking livestock in much of the mid-South (North and South Carolina, Georgia, eastern and central Tennessee, and Alabama). The taxonomic situation in the western portion of their apparent range is less clear, and ongoing studies are addressing that. After much detective work in the old literature, and despite heavy damage on the type specimen, the late-flying species is probably *T. variegatus* Fabricius 1805. That species name has not been used since a *Tabanus* revision in 1938. We will present the scientific and historical evidence for this conclusion, and a paper resurrecting and redescribing *T. variegatus* is in preparation.

OA34.06 Adult Population Dynamics of the Stable Fly (*Stomoxys Calcitrans*, L.) on Dairy Farms of Manitoba, Canada

Ms. Gina Karam¹, Dr. Kateryn Rochon¹

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The stable fly (*Stomoxys calcitrans* L.) is one of the most important livestock pests in North America. Fly bites are painful and host energy is diverted to avoidance behaviours, reducing weight gain and decreasing milk yields in dairy cattle. Environmental conditions such as temperature, precipitation and substrate suitability vary throughout the duration of the fly season and affect the number of stable flies that successfully emerge and reproduce. Six Coroplast® sticky traps were deployed weekly at three dairy farms from June 17 to

October 21 in 2017 (n=53,540 flies) and May 23 to October 3 in 2018 (n=42,585). In 2017, stable flies were first trapped on June 17 and population distribution was unimodal with the highest population recorded between July 14-27. In 2018, stable flies were first captured on June 6 and population distribution was bimodal with peaks in July 24-August 2 and August 30-September 6. The sex ratio was determined and used to uncover any trap biases. Females removed from sticky traps were dissected to determine ovarian development (stage 0-4, nulliparous and uniparous), which was used to determine changes in the population age structure throughout the season. Relationships between adult stable fly abundance as functions of environmental conditions over time were analyzed using multiple linear regression models and ANOVA F-tests, revealing relative humidity, maximum air temperature, or soil temperature to be the best predictors of fly abundance, but the parameters changed between years. Very few studies on stable fly biology have occurred in in Manitoba. Knowledge of stable fly population dynamics provides critical information on the timing of life events linked to environmental conditions, and can aid in predicting outbreak patterns and lead to strategic management plans.

OA35 Ruminant Trematodes I

July 10, 2019, 11:00 - 12:30

Breakout Room 5, Meeting Rooms KLOP, Level 4

OA35.01 Diagnosis of Albendazole Resistance in *Fasciola Hepatica*

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The increasing widespread development of drug resistance in the liver fluke *Fasciola hepatica* has motivated the need for alternative diagnostic tools. The work reported here describes the validation of an egg hatch test (EHT) as an in vitro technique to detect albendazole (ABZ) resistance in *F. hepatica*. The validation includes the intra-assay, inter-assay and intra-herd variations, and the comparison of results obtained after performing the EHT and a controlled efficacy test. Additionally, the development of the protocol included the adjustment of different critical factors to improve the simplicity of the assay. The greatest uniformity between results within the assay and over time until 8 weeks after gallbladder eggs collection (the deadline proposed for egg analysis), was obtained after incubation with an ABZ concentration of 0.5 µM. The length of exposure to ABZ was shown to be critical, as prolonged (15 days) ABZ incubation led to a reversal of drug resistance. There was a close agreement between the outcome of the EHT and that obtained for the in vivo assays. Moreover, the same level of resistance was observed when eggs and faeces were collected from animals of four (4) different farms and analyzed with both the EHT and the faecal egg count reduction test. A 0.5 µM drug level is confirmed as the discriminating concentration to predict ABZ resistance by the EHT in *F. hepatica*.

OA35.02 On Farm Risk Mapping of Liver Fluke (*Fasciola Hepatica*): Current Evidence and Future Directions

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Fasciolosis is a disease affecting approximately 50 countries worldwide and resulting in an estimated US\$3.2 Billion due to loss of production. Distribution and transmission of liver fluke is dependent on the presence of lymnaea, the intermediate host, which is limited to particular geographic regions. The snail and the free-living stages of the parasite require narrow environmental conditions to survive. Geographic information system (GIS) mapping provides a unique tool to identify high risk grazing areas for stock infection. While GIS mapping has been validated at a regional level, it has not yet been done at a farm level.

The initial study investigated potential indicators of metacercariae presence, on a commercial sheep and cattle property in the New England region of New South Wales, Australia. Measurements utilised included an electromagnetic induction survey to ascertain soil apparent electrical conductivity (ECa), a proximal normalised difference vegetation index (NDVI) survey and a digital elevation model (DEM) was created using a differentially corrected geographical positioning system (dGPS).

The sampling locations were randomly selected from 3 management zones created by interpolating the three spatially measured parameters (ECa, NDVI and Elevation) individually and applying an unsupervised K-means clustering algorithm. Metacercariae counts were coupled with static ECa, NDVI and elevation measurements for calibration purposes at these sample sites. Metacercariae density was positively associated with pH ($P < 0.0001$), ECa ($p = 0.0109$) and NDVI ($p = 0.0039$). Examination of the cluster means showed that one of the identified management zones aligned directly with the hypothesised higher risk parameters. Overall, this one-off scoping study suggests that this management zoning approach and conversion, with ground-truthing, to a “risk map” could be implemented as a tool to improve sampling programs for research purposes and guide site-specific parasite

management on property. These results are discussed as well as future directions to improve the accuracy of this mapping technique.

OA35.03 Evaluation of Anthelmintic Efficacy of Silver Nanoparticles against Fasciola Hepatica, Parasite of Ruminants, Lahore, Pakistan

Dr. Asma Abdul latif¹

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Parasitic diseases affect lots of people world widely, particularly in developing countries. Fasciola hepatica infects humans as well as ruminants, it causes Fasciolosis and it is also worldwide in distribution. Fasciola hepatica is prevalent in different areas of Pakistan in ruminants. Synthetic anthelmintics/Vaccines are not effective for the control of Fasciolosis due to parasitic resistance, therefore, new therapies are being explored for the control of parasites. Various Nano particles are being examined on wide scale as antiparasitic drugs. The objective of the present study was to evaluate the anthelmintic activity of silver nanoparticles against Fasciola hepatica. Adult Fasciola hepatica were isolated from 100 liver samples of freshly slaughtered ruminants. The efficacy of seven different concentrations (mg/ml) of silver nanoparticles from 0.17, 0.085, 0.043, 0.021, 0.011, 0.0053 to 0.0027 on adult flukes of Fasciola hepatica was checked in adult motility Assay. Albendazole drug (12 mg/ml) and PBS were used as positive and negative control respectively. It was revealed that the degree of immobilization got delayed as the concentrations of silver nanoparticles were reduced. At highest concentration of silver nanoparticles the mean time (min) for paralysis and mortality was recorded as 1.67 ± 0.234 and 1.83 ± 0.24 respectively. At lowest concentration of silver nanoparticles 0.0027mg/ml mean time for paralysis and death of flukes was recorded as 19.33 ± 1.41 and 21.67 ± 1.25 respectively. Total time recorded for death and paralysis of Fasciola hepatica flukes in positive control of Albendazole 12mg/ml drug was recorded as 8.56 ± 0.16 and 6.77 ± 0.32 respectively. The Present study showed substantial anti-parasitic effect of

silver nanoparticles on adult flukes of *Fasciola hepatica* and hence emerged as dynamic and effective drug with minimum side effect as compared to other drugs.

OA35.04 Assessment of Factors Influencing Faecal Diagnostic Outcomes and Predictive Modelling of Endemic *Fasciola Hepatica* on Australian Sheep and Cattle Farms

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The diagnosis, monitoring and flukicide efficacy testing of fasciolosis on-farm is reliant on non-terminal methods. The coproantigen ELISA (cELISA) has been recommended for diagnosis of fasciolosis and associated flukicide efficacy testing as an alternative to fluke egg counts (FFEC) for monitoring parasitism.

Experimental multi-age *F. hepatica* infection in a controlled study¹ and subsequent monthly monitoring on endemic sheep and cattle farms over 12 months² indicated greatest diagnostic sensitivity when cELISA and FFEC were utilised in parallel. To analyse factors influencing diagnostic outcomes on endemic farms linear models were applied for each diagnostic alone and as binary data, both in parallel and series. Variables examined were of climatic origin, locality, treatment status and species with fixed effects being sample time and individual animal included as a random effect. Additionally, the suitability of the Ollerenshaw Index as a predictive model for fasciolosis under Australian conditions was examined.

Linear regression models demonstrated the results of the two diagnostics utilized in parallel were explained substantially ($R^2=0.91$) as were series data ($R^2=0.88$) when the respective models were fitted. In contrast, the fitted models for FFEC ($R^2=0.54$) and cELISA ($R^2=0.58$) were poor explanations for test outcomes. The outcomes of these models support previous findings that suggest that the two diagnostic tests are best utilized together, particularly in parallel. The application of the Ollerenshaw Index

to Australian conditions requires further investigation.

1. George et al. (2017) *Veterinary Parasitology*, 246, 60-69.
2. George et al. (2019) *Vet Parasitology*: X, <https://doi.org/10.1016/j.vpoa.2018.100001>

OA35.05 *Fasciola Hepatica*: Secreted Molecules Associated With Liver Pathogenesis

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The liver fluke *Fasciola hepatica* is an economically important worm pathogen of humans and their livestock worldwide. Infection of the mammalian host involves parasite activation from an encysted stage followed by penetration through the intestinal wall and migration to the liver. The major pathogenesis associated with infection results from the extensive liver damage caused by the tunnelling and feeding activity of migrating immature flukes coupled with the pathology associated with host immune responses. Within the liver, the parasite's growth advances rapidly, doubling in size approximately every two weeks, alongside the development of the digestive and reproductive structures. Here, we report our transcriptomic and proteomic approach to determine the factors associated with parasite development and the resulting liver pathogenesis. Investigation of gene transcription demonstrated that the immature flukes up-regulates >8000 transcripts once within the liver. Specifically, genes associated with liver fluke metabolism and the mechanisms associated with active feeding and immune evasion, were found to be enriched. These analyses were further corroborated by proteomic analyses, which highlighted that the parasites secrete a plethora of proteins to negotiate the range

of different host molecules, tissues and micro-environments they will encounter and to effect the degradation of the liver tissue. This study provides information vital to our understanding of fluke/host interactive biology that can be exploited for the development of control strategies that target this pathogenic stage.

OA35.06 Dynamic Transcriptomic Analyses of the Entire Life Cycle of *Fasciola Gigantica* Identify Key Processes Through Vastly Different Environments

Dr. Xiao-Xuan Zhang¹, Dr. Krystyna Cwiklinski², Mr. Rui-Si Hu¹, Mr. Wen-Bin Zheng¹, Mr. Zhao-An Sheng³, Mr. Fu-Kai Zhang¹, Dr. Hany Elsheikha⁴, Professor John Dalton⁵, **Professor Xing-Quan Zhu¹**

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Fasciola gigantica is a digenetic trematode and causative agent of fasciolosis, an economically important livestock disease and zoonosis. In this study, we reveal the dynamic transcriptional changes that occur throughout the parasite lifecycle. A total of 58,422 transcript clusters (unigenes) were assembled and re-mapped to the eight transcriptomes representing the various lifecycle stages. 7445 unigenes were transcribed by all the lifecycle stages, with the remaining unigenes (n=50,977) exhibiting stage-specific expression. The free-living miracidia transcribe a myriad of genes indicating this stage is prepared to seek out and invade the snail intermediate host. Consistent with previous studies of *F. hepatica* metacercariae, *F. gigantica* metacercariae are metabolically active, though they display up-regulation of transcripts involved in regulating gene expression. By parsing the data derived from the stages that infect and develop within the snail (miracidia, rediae and cercariae) with the buffalo-specific stages (juvenile 42dpi, juvenile 70dpi and adult parasites), molecules that are important for each stage of the

lifecycle were elucidated. Particularly, we found an abundance of transcripts associated with the immune response for both hosts, indicating that the parasite manipulates the immune system of snail and mammal to ensure its own survival. 70% of the *F. gigantica* transcripts share homology with *F. hepatica* genes; however, the profile of the abundantly expressed transcripts within the various lifecycle stages (egg, metacercariae and adult) imply species-specific regulation that could reveal specific adaptations that have taken place since these two parasites diverged 25 million years ago. This is the first analysis of the transcriptional profile of the *F. gigantica* lifecycle, which identified key processes the parasite must undergo as it progresses through vastly different environments. The comparative analysis with *F. hepatica* has provided further insight into *Fasciola* biology, crucial for the development of novel control strategies against both *Fasciola* species.

OA36 Treatment and Control of GI Nematodes in Ruminants

July 10, 2019, 13:30 - 15:30
Plenary Hall, Madison Ballroom (ABCD), Level 4

OA36.01 Performance Characteristics of the Adequacy of Infection Criteria Recommended in VICH GL 7 for Anthelmintic Efficacy studies

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Veterinary International Committee for Harmonization Guideline 7 (VICH GL7) "Efficacy of Anthelmintics: General Requirements" recommends criteria for adequacy of infection in anthelmintic studies. The criteria include the option to use statistical confidence limits based on the geometric mean or the median worm burden in the control group.

Using simulation studies, we investigated the performance of the statistical criterion given in VICH GL7 for determining the adequacy of

infection in anthelmintic studies. The results show that under certain circumstances the statistical criterion is useful in safeguarding against overestimating the adequacy of infection as the sample size increases. However, the statistical criterion may be overly restrictive for samples with zero counts and may not protect against overestimating adequacy of infection when the sample contains no zero counts.

OA36.02 Effects of Anthelmintic Treatment on Production Performance, Carcass Quality, and Predominant Nematode Species in Feedlot Cattle from Western Canada

Mr. Eranga De Seram¹, Dr. Gregory Penner¹, Dr. John Gilleard², Dr. John Campbell¹, Dr. Elisabeth Redman², Dr. Colleen Pollock³, Mr. Samantha Ekanayake¹, Dr. Fabienne Uehlinger¹
¹University of Saskatchewan, Saskatoon, Canada, ²University of Calgary, Calgary, Canada, ³Merck Animal Health, Kirkland, Canada

Changes in climate and anthelmintic susceptibility are changing the epidemiology of gastrointestinal nematodes in livestock. However, the production impact of gastrointestinal nematodes in beef cattle from western Canada has not been studied in decades. We determined the effects of currently used anthelmintics on production performance, carcass quality characteristics, and species diversity in western Canadian feedlot calves. A randomized controlled trial was conducted with 234 auction market-derived, weaned, fall-placed steer calves. Calves were assigned to three treatment groups: control; injectable ivermectin; combination of injectable ivermectin and oral fenbendazole. Each group contained replicates of 6 pens with 13 animals per pen. Calves were treated according to individual body weights and manufacturers' specifications. Individual fecal samples were collected to determine the pre- and post-treatment fecal egg counts by modified Wisconsin sugar flotation. The predominant parasite species in each treatment group was determined using a deep-sequencing nemabiome assay. Monthly body weights and pen level feed intake were used to

determine the average daily gain and feed efficiency. Carcass quality information was obtained at slaughter. Pens were considered the experimental unit. Anthelmintic treatment had no effect on the average daily gain and feed efficiency during backgrounding or finishing. However, compared to the control group, some superior carcass quality traits (quality grade, yield grade, and marbling score) were significantly more frequent in anthelmintic treated calves. *Ostertagia ostertagi* was the predominant parasite species in all calves before anthelmintic treatment and in control calves 14 days post-treatment. After treatment, *Cooperia oncophora* was the predominant parasite species in ivermectin-treated calves while no parasite eggs were recovered from calves given the combined treatment. Anthelmintic treatment improved some carcass quality traits in these calves, the economic significance of which remains to be determined. *Ostertagia ostertagi* in control calves may suppress superior carcass characteristics and this requires further research.

OA36.03 Relative Effectiveness of a Slow Release Drug Versus Strategic Administration of a Short Acting Anthelmintic for Controlling Nematodes in US Beef Cattle

Dr. Louis Gasbarre¹, Dr. William Epperson², Dr. Dante Zarlenga³, Dr. Paul Beck⁴, Dr. Harold Newcomb⁵
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There has been an increase in the use of a slow release long acting macrocyclic lactone in beef cattle operations in the United States. Given the potential for development of anthelmintic resistance in such a program, a study was designed to ascertain if older strategic anthelmintic programs using a product with no residual activity could be as effective on parasite control and animal productivity. Two hundred calves were acquired from sale barns throughout the southeastern US. Calves were divided into

two groups; one receiving slow release eprinomectin as per label instructions (Group 1) while the other received an initial combination treatment with oral fenbendazole and injectable ivermectin followed by a topdressed mineral containing fenbendazole in the daily feed supplement on days 28-31 and 56-59 (Group 2). Calves from each group were slaughtered and parasites were recovered, enumerated, and identified to species at days 10, 66, and 114. Total mean worm recoveries day 10 were: Group 1 - 5,247 worms consisting of *Cooperia punctata* (Cp) and *C. oncophora* (Co), *Haemonchus* sp (Hae), and *Oesophagostomum radiatum* (Or); Group 2 - 3 larvae in the abomasum. At day 66: Group 1 - 16,973 worms - Cp, Co, Hae, Or, and *Ostertagia ostertagi* (Oo); Group 2 - 40 worms - Oo and larval *Cooperia*. At day 114: Group 1 - 7,737 worms and Group 2 - 3,532 worms, with all parasite species previously identified in both groups. A representative sample of recovered Hae were subjected to PCR and all were shown to be *H. placei*. There was no significant difference in weight gains between the 2 groups at the termination on the experiment on day 123. Previous studies have indicated the *Cooperia* sp are increasingly surviving macrocyclic lactone treatments. This study indicates that this may now be true of *H. placei* and *O. radiatum*.

OA36.04 Longitudinal Study on the Effect of Moxidectin Administration With and Without an 'Exit' Drench on Production and Parasitological Parameters in a UK Sheep Flock

Dave Bartley¹, Leigh Andrews¹, Arundhati Rao¹, Alison Morrison¹

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Long-acting anthelmintics, like moxidectin (MOX), are powerful endectocides used for the treatment of both gastro-intestinal nematodes and sheep scab mites. Recent studies in the UK suggest an increase in MOX resistant nematodes. Due to the perceived production benefits afforded to lambs later in the grazing season many UK farmers drench ewes with MOX around lambing. The use of MOX in ewes around

lambing has been suggested as one factor that may increase selection for MOX resistance in gastrointestinal nematodes. Recommendations to counter this potential problem suggest that the use of a short acting anthelmintic (exit-drench) at the end of the persistent period of activity can remove survivors from the MOX treatment and reduce the selection pressure for MOX resistance, but no empirical data exists to back up these recommendations in Temperate Europe. A replicated field trial was undertaken over the grazing seasons of 2017 and 2018 assessing the impact of different treatment protocols on lamb productivity and parasitological parameters (faecal egg count, species composition). Ewes with twin lambs were grazed on 12 paddocks (n=5 and 10 respectively) between spring and late autumn. Ewes were treated at turn out with MOX (Cydectin 0.1% Oral drench)±monepantel (Zolvix; 5 weeks post MOX), lambs were treated at weaning and mid/late season with either MOX or ivermectin (Oramec) or moxidectin+monepantel (as above) at weaning. Results at the end of the trials showed that the difference between the poorest responding group (MOX administered to ewes and lambs) and the other treatment strategies were; Live-weight gain differences were between 3-6Kg heavier over 140 days and worm burdens were greater 2x lower. Moxidectin efficacy based on worm burdens ranged between 98%-100% and 85%-100% for years 2017 and 2018 respectively. The results demonstrate that an exit drench strategy may have a positive impact, in the short-term, when administering moxidectin.

OA36.05 Anthelmintic Efficacy and Pharmacokinetics of Topical 0.5% W/V Eprinomectin (EPRINEX®) Administered at 1 Mg per Kg Body Weight in Lactating Dairy Goats With Induced Nematode Infections

Dr. Dietmar Hamel¹, Dr. Steffen Rehbein¹, Valerie Kvaternick², Dr. Hailun Wang³, Dr. Michael Kellermann¹, Sandra Mayr¹, Renate Rauh¹, Martin Visser¹, Thea Wiefel¹, Becky Fankhauser³

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INTRODUCTION: Lactation is discussed as a physiological covariate which may influence the exposure characteristics of systemically acting drugs including macrocyclic lactones and potentially alter their pharmacological response. Therefore, efficacy and pharmacokinetics of topical 0.5% w/v eprinomectin, which has been recently authorized in Europe as EPRINEX® Multi as a broad-spectrum anthelmintic with zero hours milk withdrawal in sheep and goats, were evaluated in a GCP and VICH anthelmintic testing guidelines compliant, blinded study using lactating dairy goats with induced nematode infections.

METHODOLOGY: Twenty lactating German White Noble goats, harboring induced infections of adult gastrointestinal nematodes and lungworms were ranked on pre-treatment bodyweight and allocated at random either to remain untreated (control) or to be treated with EPRINEX® topically at 1 mL per 5 kg body weight. Plasma concentrations of eprinomectin B1a were determined in blood samples collected prior to treatment and at specific times up to necropsy. A necropsy was performed 14 days after treatment and nematode counts made to determine efficacy.

RESULTS: Treatment was well accepted and no health problems were observed throughout the study. Counts of adult *Dictyocaulus filaria*, *Haemonchus contortus*, *Teladorsagia circumcincta* (pinnata/trifurcata), *Trichostrongylus axei*, *T. colubriformis*, *Cooperia curticei*, *Nematodirus battus* and *Oesophagostomum venulosum* were significantly ($p < 0.0070$) lower in the treated goats (>95% reduction relative to controls). Basic pharmacokinetic parameters of eprinomectin (B1a component) were: AUClast, 23.8 ± 9.7 day*ng/mL, Cmax, 5.55 ± 2.27 ng/mL, MRTlast, 3.59 ± 0.60 days, and T1/2, 3.57 ± 0.77 days; individual maximum plasma concentrations were observed from 8 to 48 hours (median of 12 hours).

In conclusion, eprinomectin administered topically at 1 mg per kg body weight to

lactating dairy goats was highly efficacious against gastrointestinal and pulmonary nematode infections. These results as well as the pharmacokinetics in lactating dairy goats of eprinomectin are similar to that observed in young growing and adult female dry goats.

OA36.06 Nemabiome Analysis Reveal a Change in Parasite Fauna After Years of Anthelmintic Treatment

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Parasitic nematode infections are abundant in grazing livestock and they put a constraint in maintaining animal health and welfare, being the most important factor that limits livestock production. Since the advent of anthelmintic drugs, heavy infections can be treated effectively. Recently, a buildup of resistance to anthelmintics has been observed all over the world. In this study we are investigating the effects of anthelmintic treatments on parasitic nematode communities in 35 Swedish sheep farms between 2007 and 2016. The first cases of anthelmintic resistance (to Valbazen) were reported in 2007 and recommendations for treatment changed to Ivermectin. However, Ivermectin resistant *Haemonchus contortus* are now present on many farms. Larval fecal cultures from 50 samples were collected before and after anthelmintic treatment respectively. The conserved ITS-2 region of the nematode rRNA was PCR amplified and sequenced using PacBio® SMRT cell technology. Using this strategy, the nemabiome of each sample was characterized for the whole nematode community. In the period from 2007 to 2016, *H. contortus*, the nematode considered the most pathogenic for sheep, showed a 100% increase in frequency of all ITS-2 sequences, while the two other common species, *Teladorsagia circumcincta* and *Chabertia ovina* showed 56% and 80% decrease of the ITS-2 sequences respectively. Furthermore, after treatment, *H. contortus* showed a decreased sensitivity for Ivermectin and Valbazen. In contrast, treatment with Levamisole remained effective against *H. contortus* on the investigated farms.

OA36.07 Boron-containing Compounds Are Rich Source of Future Antiparasitic Agents

Yong-Kang Zhang¹, Tony Chun Yu Liu¹,
Yasheen Zhou¹, Chunliang Liu¹, Marissa
Aubrey¹, Jacob J. Plattner¹

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Finding new chemical entities (NCEs) with novel mechanisms of action is a common approach to combat parasitic resistance and improve treatment efficacy. Boron chemistry provides an emerging area of rich NCEs demonstrating broad antiparasitic activities. For examples, it has been reported that boron-containing compounds have excellent activities against tick and flea^{1,2}, against malaria-causing parasite *P. falciparum*^{3,4}, against GI worms⁵, against Animal African Trypanosomiasis (AAT) parasites *T. congolense* and *T. vivax*⁶, and against Human African Trypanosomiasis (AAT) parasite *T. brucei*⁷. This presentation will provide an overview of boron chemistry antiparasitic application and predict possible future research direction.

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OA36.08 New Benzimidazole Derivates As Potential Anthelmintic Compounds

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Gastrointestinal nematode infections cause direct and indirect losses of great economic impact worldwide by decreasing productions yields and increasing health care cost in sheep. Thus, the aim of this study was to test the nematocidal effect of a new collection of benzimidazole derivatives against *Teladorsagia circumcincta* by in vitro methods. Compounds were tested in eggs, first stage larvae (L1) and third stage larvae (L3) by means of the Egg Hatch Assay, L1 Mortality Assay and the Larval Migration Inhibition Assay. An initial screening was done at a single concentration of 50 μ M; those molecules that showed an activity higher than >90% were selected to determine their half maximal effective concentration (EC50) and were tested again against a resistant strain. Finally, the anthelmintic effect was compared with their cytotoxicity in two digestive established cell lines, Caco-2 and HepG2, and their corresponding concentrations required to reduce cell viability by 50% (CC50) were determined in order to establish a selective index (SI) for each molecules. Only 5 of the 18 compounds were able to arrest the hatching of eggs in the susceptible strain in more than 99% at 50 μ M and 4 of them were also able to produce a significant effect on the resistant strain used in the current study. Considering only those compounds that have a significant effect, their CC50 values were very homogeneous between Caco-2 and HepG2, which makes that SI values were comprised between 1.84 to 3.60 for Caco-2 cells and 1.53 and 2.73 for HepG2 cells. These results indicate that they could be good candidates for further studies to determine their in vivo activity. Study funded by AGL2016-79813-C2-1-R, Junta de Castilla y León co-financed by FEDER [LE020P17], EVG by FPU17/00627 and MMV by RYC-2015-18368.

OA37 Poultry Coccidia, Aquatic Infections

July 10, 2019, 13:30 – 15:30

Breakout Room 2, Hall of Ideas E&H, Level 4

OA37.01 Hemato-Biochemical and Histopathological Investigations Following Concurrent Coccidia and Clostridia Experimental Infection in Broiler Chickens Vaccinated With Clostridia Toxoid and/or Coccidia VaccineDr. Ahmed El-Shemy¹, Professor Safaa Yassin², Professor Hamdy Soufy¹, Professor Alaa Ahmed², **Professor Soad Nasr¹**¹Department of Parasitology and Animal Diseases, National Research Center, 33 Bohouth Street, Dokki, P.O. Box 12622, Giza, Egypt, ²Department of Clinical Pathology, Faculty of Veterinary Medicine, Cairo University, P.O. Box 12211, Giza, Egypt

Concurrent infection with Coccidia and Clostridia is one of the major diseases affecting chickens causing economic losses. This experiment aimed to evaluate the effect of concurrent experimental infection with *Eimeria tenella* and *Clostridium perfringens* type A -local isolates- on hematological and biochemical parameters and histopathology of the internal organs of chicks vaccinated with Clostridia toxoid and/or Coccidia. A total of 225 -one day old- broiler chicks were divided into 5 equal groups; (G1): normal control, (G2): infected, (G3): vaccinated with Coccidia vaccine on the 3rd day old, (G4): treated with Clostridia toxoid at the 5th day old, and (G5): vaccinated with Coccidia vaccine on the 3rd day old and treated with Clostridia toxoid at the 5th day old. All groups were challenged orally after 21 days post vaccination (dpv) with Coccidia oocysts at a dose of 5×10^4 and then with *Clostridium perfringens* type A at a dose of 0.5 ml of 24 h cooked meat broth 1×10^7 CFU at the 23rd dpv. Blood samples and tissue specimens from liver, spleen, gall bladder, intestine and kidney were collected from all groups at the 5th, 13th and the 21st dpv and at the 5th, 7th, 15th and the 21st day post challenge. Results revealed that (G2) showed anemia, leukopenia, decrease levels of iron, glucose, total

cholesterol, total protein and A/G ratio with increases in AST, ALT, ALP and GGT activities and creatinine, uric acid, and total globulins levels. Histopathological examination revealed in the (G2) post challenge; severe changes in the liver, gall bladder, spleen, intestine and kidney. While, less histopathological changes were recorded in the (G3) and (G4) and non-significant changes were recorded in the (G5). In conclusion, using the Coccidia vaccine beside Clostridia toxoid minimized the severity in hematological and biochemical parameters and histopathology of the internal organs in infected chicks.

OA37.02 The Role of Parasitic Crustacea as Vectors of Aquatic Diseases**Dr. Nico J Smit¹**, Dr. Kerry A Hadfield¹¹North-West University, Potchefstroom, South Africa

In assessing the role of parasitic crustaceans as vectors, it is interesting to note that the relevant literature appears limited to a handful of species. Isopods of the genus *Gnathia* (family Gnathiidae) likely act as definitive hosts and vectors of fish blood parasites of the genus *Haemogregarina*. They may also transmit fish viruses (such as viral erythrocytic necrosis), and may be intermediate hosts for nematode larvae. Furthermore, cymothoid isopods (family Cymothoidae) may transmit lymphocystis virus to fishes. Recent studies show barnacles (subclass Thecostraca) on the carapace and gill filaments of crabs could be potential reservoir hosts for shrimp viruses. Copepods of the genus *Caligus* and *Lepeophtheirus* (family Caligidae) are noted as potentially important mechanical vectors or alternative hosts of a number of viral diseases between fishes and *Lepeophtheirus* can transfer pathogenic bacteria between fishes. Ergasilids (family Ergasilidae) parasitic on the gill filaments of fishes can support the replication of shrimp viruses, and likely act as viral vectors and transmit lymphocystis. Branchiurans, specifically from the genus *Argulus*, are thought to serve as mechanical vectors of several viruses to fishes, especially carp, as well as acting as intermediate hosts for dracunculoid and skrjabianid nematodes of fishes. All of these vector examples are

further discussed within the presentation, and areas of possible future research are identified.

OA37.03 Processing of Bovine Faecal Samples by New Parasitological Technique and Perspectives of Automation in the Diagnosis of Cryptosporidium SPP.

Sandra Valéria Inácio¹, Professor Jancarlo Ferreira Gomes², Professor Alexandre Xavier Falcão², Saulo Hudson Nery Loiola², Bianca Martins dos Santos² Celso Tetsuo Nagase Suzuki², **Professor Katia Bresciani¹**

¹São Paulo State University (UNESP), School of Veterinary Medicine, Araçatuba, Brazil, ²Laboratory of Image Data Science (LIDS), Institute of Computing, University of Campinas (UNICAMP), Campinas, Brazil

Cryptosporidiosis arouses great interest in the scientific community due to its significant zoonotic potential. The usual techniques of concentration and permanent staining for the parasitological diagnosis of *Cryptosporidium* spp. oocysts, present limitations in the existing protocols, mainly related to the composition of the chemical reagents, low productivity, lack of coverage and high costs. In view of these problems, we made possible a new parasitological technique called TF-Test Coccidia, that was evaluated and validated in an intralaboratorial study, aiming to identify this protozoan in temporary slides which were assessed under conventional light microscopy. For this purpose, 68 faecal samples of calves from the city of Araçatuba, São Paulo/Brazil, were processed by the new technique just as centrifuged-sedimentation with negative staining of malachite green. The cases were confirmed by Nested-PCR. The Kappa index was used to measure the strenght of agreement between both procedures. Thus, we noted the success of TF-Test Coccidia for detection of oocysts of *Cryptosporidium* spp. which showed positivity in 34 samples and almost perfect agreement (Kappa = 1,000). We considered as advances: the low amount of debris in the fecal smear, the speed of preparation of the slide with a good concentration of oocysts without morphological deformation and the adjustment of a temporary staining solution

that highlighted the sporozoites. From these results, it will be possible to work towards the automated diagnosis of these structures. Therefore, studies on the adequacy of TF-Test technique have been conducted (FAPESP PROCESS: 2014/12236-1), to make possible the computerized identification of these parasites by building an image database that will serve to train the classifier in the pattern recognition module of the system wich will take into account inner structures of oocyst and then offer better alternatives to the procedure and reduce interpretation errors improving consequently the efficiency in the laboratories routine.

OA37.04 The Effect Of Long-Term Cell-Culture Passage On Sarcocystis Neurona Genome Content And Predicting Genes Necessary For Completion Of The Parasite Life Cycle

Jamie Norris¹, Dr. Sriveny Dangoudoubiyam¹, Dr. Daniel Howe¹

¹Department of Veterinary Science - University of Kentucky, Lexington, United States

Sarcocystis neurona is an obligate intracellular parasite that causes equine protozoal myeloencephalitis (EPM). The *S. neurona* strain SN3 was isolated from an EPM horse in 1991 and has been passed in cell culture almost continuously for nearly 30 years. To aid molecular and genetic studies of *S. neurona*, a reference genome for the SN3 strain was produced in 2013. Next generation sequencing (Illumina) was used subsequently to obtain genomic sequence data from three additional *S. neurona* isolates that had not been passed extensively in cell culture (SN4, SN-OT1, SN138). Interestingly, alignment of the SN4, SN-OT1, and SN138 sequences to the SN3 reference genome revealed significant genomic regions (up to ~120 kb) that are seemingly absent from the SN3 strain. These genomic regions are largely devoid of gene annotations, partly due to a paucity of transcript evidence from all stages of the parasite's life cycle. We hypothesize that these regions have been deleted from the SN3 genome because they contain genes that are deleterious to asexual parasite growth in vitro but potentially important during other stages

of the parasite's life cycle (i.e., bradyzoites and/or sporozoites). Gene prediction models based on the closely-related parasite *Toxoplasma gondii* will be used to determine whether the deleted regions contain genes not identified in the original annotation effort for the SN3 genome. Putative functions associated with these sequences may provide insights into the natural lifecycle of *S. neurona* and related parasites.

OA37.05 Biological and Molecular Characterization of a Pathogenic Unnamed *Eimeria* Species Causing Clinical Coccidiosis in Commercial Chukar Partridge (*Alectoris Chukar*) Flocks in Ontario

Jessica Rotolo¹, Dr. John Barta¹, Rachel Imai¹, Perryn Kruth¹, Ryan Snyder¹

¹*University Of Guelph, Guelph, Canada*

A local commercial producer of Chukar partridge (*Alectoris chukar*) has had recurrent issues with frequent clinical coccidiosis and high flock mortalities. Traditional live vaccination using a few infective oocysts given to day-of-age birds to elicit protective immunity has been unsuccessful. Partridge chicks are apparently unable to acquire protective immunity fast enough to prevent parasite replication and intestinal tract damage. In preparation for exploring alternate methods of vaccination for coccidiosis management in commercial Chukar partridge flocks, the responsible pathogenic *Eimeria* species was isolated and characterized. The morphology and life cycle of this *Eimeria* species was examined in vivo and sequence-based genotyped at two genetic loci. Morphometrics of oocysts and sporocysts were measured using light microscopy with computerized image analysis. Experimental infections with coccidia free chukar partridges were used to describe the complete endogenous development and daily fecal collection post inoculation was used to determine the prepatent period and duration of shedding. Endogenous development was determined histologically from samples collected at 8 locations along the intestinal tract every 8 hours throughout prepatency. The parasite had 5 asexual generations prior to oocyst formation over its

120 hour prepatent period; oocyst shedding persisted until 10 days post-inoculation. To complement biological data, the complete mitochondrial genome and partial nuclear 18S rDNA were sequenced. Molecular and biological observations confirm that this *Eimeria* species has not been reported from partridges nor any other galliform bird to our knowledge. We suggest that it will need formal description as a new species. Understanding of the biology of this, as yet, unnamed *Eimeria* sp. will inform development of vaccination methodologies designed to elicit protective immunity, such as: 1) live oocyst vaccination followed by a carefully monitored 2-step partial house brooding; and, 2) a 'bioshuttle' (vaccination/anticoccidial combination). Effective coccidiosis control will enhance flock health and increase profitability for commercial chukar producers.

OA37.06 When Minor Species Are Not So Minor: *Eimeria Innocua*, Far From Innocuous

Rachel Imai¹, Ryan Snyder¹, Jessica Rotolo¹, Dr. John Barta¹

¹*Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Canada*

Historically, only *Eimeria meleagritidis* and *Eimeria adenoides* were considered highly pathogenic to turkeys. Recently, all other *Eimeria* spp. that infect turkeys (*Eimeria dispersa*, *Eimeria gallopavonis*, *Eimeria innocua* and *Eimeria meleagridis*) were shown to be present within flocks in commercial turkey facilities. Detection of these 'minor' species has only become possible recently through application of a nested, species specific PCR assay that can differentiate all 6 species in fecal samples. The last of the species added to this assay was *Eimeria innocua*; this parasite was considered relatively non-pathogenic but preliminary research suggested that this may not be the case. To clarify the pathogenic potential of *E. innocua*, an in vivo infection trial was conducted. Hens were reared coccidia-free and infected at 14, 23, 30 or 40 days-of-age. Poults were inoculated by oral gavage with 100 to 1,000,000 oocysts per bird. Five days post-inoculation (DPI), turkeys were

necropsied to permit description of the macroscopic lesions at the various doses. Tissue samples were taken from each bird at fixed positions for histopathology. Body weights of each bird were taken immediately before inoculation and again at necropsy. *Eimeria innocua* produced macroscopically obvious pathological changes that, at higher doses, extended from the duodenal loop into the ileum (20cm or more beyond Meckel's). Bleaching, ballooning and thinning of the intestinal mucosa were evident; unsurprisingly, lesion severity and length of affected intestinal tract both increased with higher numbers of oocysts inoculated. However, dose-dependent increases in lesion severity were not consistent for the youngest poults; maturation of the turkey gut could potentially be a mitigating factor affecting the impact of infections and must be considered when challenge experiments are conducted. Body weight gains during infections with the highest doses were up to 30% less than uninfected sham controls. Pathogenicity of *Eimeria innocua* may have been grossly underappreciated in past literature.

larval cyathostominosis, a potentially fatal condition. Other, putative effects of cyathostomin infection on horse productivity and performance are largely unsubstantiated. Regardless, the compulsion to deworm is sacrosanct among horse owners, even if implementation is based more on tradition and myth than scientific evidence.

To date, effective cyathostomin control measures share the common strategy of reducing environmental contamination with eggs. However, application of this strategy is complicated by numerous practical questions, including which horses to treat, what drugs to use, and timing of administration in relation to seasonal and management factors. These persistent questions will remain unresolved without a better understanding of the regulatory mechanisms of cyathostomin population dynamics. Accordingly, future efforts must investigate the effects of host age, immunity and genetics, intensity of exposure, density-dependent mechanisms, and climate and season of infection on establishment of EL3s, sequential development and survival of L4, L5, and adult stages, and associated egg production.

OA38 Equine Cyathostomes II

July 10, 2019, 13:30 - 15:30
Breakout Room 3, Hall of Ideas F&I, Level 4

OA38.01 Equine Cyathostomins 1: Relative Importance and Challenges to Sustainable Control

Dr. Craig Reinemeyer¹, Dr. Dave Leathwick², Dr. Christian Sauermann², Dr. Martin Nielsen³
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Cyathostomin nematodes are ubiquitous parasites of horses and constitute the major target of helminth control for mature, managed equids. Cyathostomins are usually minor pathogens, but large burdens of encysted stages may cause

Anthelmintic therapy is widely considered to be an essential tool for successful strongyle control in horses. But currently, the sustainability of this approach is threatened by almost universal resistance among cyathostomins to benzimidazoles, prevalent and expanding resistance to pyrimidines, and indications that macrocyclic lactones may be in early decline. Egg counts are the current, gold standard measure of success, but the overall benefits of control in terms of reducing infection intensity, promoting host immunity, and slowing the development of anthelmintic resistance remain nebulous and perhaps even counter-intuitive. Future cyathostomin control strategies must address the dual challenges of significantly decreasing environmental contamination and minimizing concurrent selection for anthelmintic resistance.

OA38.02 Equine Cyathostomins 2. What Does Building and Running the Model Tell Us?

Dr. Dave Leathwick¹, Dr. Christian Sauermann¹, Dr. Craig Reinemeyer³, Dr. Martin Nielsen⁴
¹AgResearch, Palmerston North, New Zealand,
²AgResearch, Palmerston North, New Zealand,
³East Tennessee Clinical Research Inc, Rockwood, United States, ⁴M.H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, United States

A model for the dynamics of equine cyathostomins requires not only an understanding of the basic life-history, but also of the factors which drive population change. The dynamics of free-living stages is reasonably straight-forward because these respond to temperature and moisture similarly to other parasites. Modelling the parasitic stages was more difficult due to limited data on what drives worm dynamics. The completed model includes 1) a declining establishment of ingested infective stage larvae as horses age, 2) constant development rates for all the parasitic stages except the encysted early third stage larvae (EL3), for which development rates are variable to reflect the sometimes extended arrestment of this stage, 3) negative feedback from adult worms to the L4 stage which prevents L4 maturing to adults and results in larval mortality. In the absence of anthelmintic treatments, the life span of adult worms is approximately 12 months. Anthelmintic treatment, which removes the adult worm burden, allows development of L4 to replace the removed worms and an acceleration in the development of EL3.

Model performance is strongly influenced by the rate and seasonal pattern of ingestion of L3 from herbage. While the adult worm burden remains relatively stable within a year the numbers and proportions of larval stages increase with the numbers of infective larvae ingested. Further, the seasonal rise and fall of encysted stages is largely driven by the seasonal pattern of infective larvae on pasture. Because of this, the model reproduces the contrasting seasonal patterns of mucosal larvae, typical of temperate and tropical environments, using only the appropriate seasonality of larvae on pasture.

Thus, the model reproduces output typical of different climatic regions and suggests that observed patterns of arrested development may simply reflect the seasonality of free-living stages on pasture as determined by different weather patterns.

OA38.03 Equine Cyathostomins 3. The Importance of Genetic and Fitness Parameters When Modelling Anthelmintic Resistance

Christian Sauermann¹, Martin Nielsen², Dave Leathwick¹
¹AgResearch, Palmerston North, New Zealand, ²M.H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, United States

In order to model the development of anthelmintic resistance in cyathostomin parasites it is necessary to incorporate genetic mechanisms for resistance into the models. However, very little is currently known about the genetics of resistance to anthelmintics in these parasites. The first use of this model was to compare the effect of different assumptions regarding the inheritance of resistance on model outputs. Comparisons were made between single and two-gene inheritance, where the heterozygote survival was dominant, intermediate or recessive under treatment, and with or without a fitness disadvantage associated with the resistance mechanism. Resistance developed fastest when the heterozygotes survived anthelmintic treatment (i.e., were dominant) and slowest when they did not (i.e., were recessive). Resistance was slower to develop when inheritance was poly-genic compared to a single gene, and when there was a fitness cost associated with the resistance mechanism, although the latter variable was the least influential. Importantly, while these genetic factors sometimes had a large influence on the rate at which resistant genotypes built up in the model populations, their order of ranking was always the same when different anthelmintic use strategies were compared. Therefore, while the described model is unlikely to be a useful at predicting the timing of resistance development, it should be useful for comparing different treatment and management strategies on their propensity to select for resistance.

OA38.04 Equine Cyathostomins 4: The Impact of Climate and Seasonality on Anthelmintic Resistance Development

Martin Nielsen¹, Christian Sauermann², Dave Leathwick²

¹University Of Kentucky, Lexington, United States, ²AgResearch, Palmerston North, New Zealand

Anthelmintic resistance is widespread in equine cyathostomin populations across the world, and the equine industry is forced to abandon traditional parasite control regimens. Current recommendations aim at reducing treatment intensity and identifying high strongylid egg shedders in a targeted treatment approach. But, virtually nothing is known about the effectiveness of these recommendations, nor their applicability to different climatic regions. This study made use of a computer model to evaluate the influence of treatment intensity, climate, and seasonality on the development of anthelmintic resistance in cyathostomin parasites. All simulations evaluated the use of a single anthelmintic (e.g., ivermectin) over the course of 40 model years. The study made use of weather station data representing four different climatic zones: a cold humid continental climate, a temperate oceanic climate, a cold semi-arid climate, and a humid subtropical climate. Initially, the impact of time of the year was evaluated by simulating a single anthelmintic treatment administered once a year in any of the twelve months. We then evaluated the impact of treatment intensities varying between 2 and 6 treatments per year. And finally, we evaluated treatment schedules consisting of a combination of strategic treatments administered to all horses and additional selective treatments. Month of treatment had a large effect on resistance development in colder climates, but little or no impact in the subtropical climate. Resistance development was affected by treatment intensity, but was also strongly affected by climate. Selective therapy delayed resistance development in all modelled scenarios, but, again, this effect was climate dependent with the largest delays observed in the colder climates. This study is the first to demonstrate an impact of climate and seasonality on anthelmintic resistance

development and these results call for more field research to better understand the dynamics behind anthelmintic resistance in cyathostomin parasites.

OA38.05 Characterization of the Cyathostominae Community within the Equine Large-Intestine “Nemabiome”: Morphological Identification and Next-Generation Sequencing.

Jennifer Bellow¹, Dr. Jocelyn Poissant², Dr. John Gilleard³, Dr. Martin Nielsen¹

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Cyathostominae are prevalent equine nematodes, comprising ~40 distinct species in horses, nearly all of which are identifiable only by examination of adult morphology upon equine necropsy. This study characterized distinct Cyathostominae communities within the equine large intestine, utilizing and comparing traditional morphologic identification and a recently developed next-generation sequencing assay (NGS). Cyathostominae recovered from the cecum, ventral colon, and dorsal colon of 26 horses were identified by morphology or NGS. NGS identified 18 species, while morphological identification yielded 15 species and “immature” and “unidentifiable” categories. *Cyathostomum catinatum*, *Cylicocyclus nassatus*, and *Cylicostephanus longibursatus* together comprised more than 50% of the entire Cyathostominae community. Immature stages contributed ~11% to the total morphologically identified population. Most species exhibited spatial niche preferences with ~80% or more specimens recovered from a single gut compartment. Species diversity varied significantly between gut compartments; the cecum was dominated by *Coronocyclus coronatus*, ventral colon by *Cya. catinatum*, and the dorsal colon by *Cys. longibursatus*. Diagnostic statistics and method agreement were explored for

11 species. Statistically significant Cohen's Kappa values ($p \leq 0.05$) were interpreted as fair (0.21-0.40) for two, moderate (0.41-0.60) for six, and substantial (0.61-0.80) agreement for three species. For seven species, McNemar χ^2 p -values ($p > 0.05$) showed no test disagreement. However, for the three most abundant species, *Cya. catinatum*, *Cor. nassatus*, and *Cys. longibursatus*, McNemar's test showed significant disagreement ($p \leq 0.05$). Sensitivity and specificity were calculated for NGS against gold standard morphologic identification. Sensitivity and specificity were variable across species, both ranging from ~0.43-0.95% with means of 77%. Positive and negative likelihood ratios ranged from 1.7-11 and 0.07-0.60, respectively. Poor test agreement, low specificity, and high negative likelihood ratios are influenced by a high rate of "false positives," resulting from NGS identifying immature or otherwise morphologically unidentifiable specimens. Further development and validation of NGS with previously morphologically identified specimens is warranted.

OA38.06 Equine Cyathostomins 5: Benefits of Selective Anthelmintic Therapy are Age and Climate Dependent

Martin Nielsen¹, Christian Sauermann², Dave Leathwick²

¹University Of Kentucky, Lexington, United States, ²AgResearch, Palmerston North, New Zealand

Selective anthelmintic therapy has been recommended as a sustainable strategy for cyathostomin control in horse populations for several decades. By determining strongyle fecal egg counts (FEC) for all horses, and only treating those exceeding a predetermined FEC threshold, the aim is to achieve a reduction of overall egg shedding, while leaving a proportion of the herd untreated. This is based on the overdispersed distributions of fecal egg counts across herds and the consistent shedding levels maintained by individual horses across time. We used the cyathostomin model to evaluate the influence of selective treatment strategies with between 1 and 4 yearly treatment occasions, where either 1) all horses were

treated or 2) horses were treated if they exceeded thresholds between 100 and 600 strongyle eggs per gram. We imported weather data representing four different climatic zones: a cold humid continental climate, a temperate oceanic climate, a cold semi-arid climate, and a humid subtropical climate. Additionally, we evaluated equine herds with three different age structures; 1) all yearlings, 2) all mature horses 10-20 years old, and 3) a mixed age structure of 1-20 years of age. Results indicated a clear effect of age structure, with resistance developing quickest in the yearling group and slowest among the mature horses. Resistance development was affected by treatment intensity and selective therapy generally delayed resistance. However, this effect was more pronounced in the mature horses compared to the two other age structures. Finally, the effects of selective therapy on resistance development were also climate dependent with resistance being delayed least in the subtropical climate and most in the other climates. These results suggest that defining an optimal selective treatment strategy is more complicated than simply applying an arbitrary threshold across all horses and environments, and more work is needed to provide meaningful recommendations.

OA38.07 Outbreak of Acute Larval Cyathostominosis After Anthelmintic Treatment: Investigation of the Clinico-Pathological Parameters and the Faecal Microbiome Changes in Twelve Horses

Ms. Nicola Walshe¹, Professor Grace Mulcahy^{2,4}, Dr. Fiona Crispie³, Dr. Paul Cotter³, Dr. Raul Cabrera Rubio³, Associate Professor Vivienne Duggan¹

¹School of Veterinary Medicine, University College Dublin, Ireland, ²School of Veterinary Medicine, University College Dublin, Ireland, ³Teagasc Moorepark/Alimentary Pharmacobiotic Centre, University College Cork, Ireland, ⁴Conway Institute of Biomolecular and Biomedical Research, Belfield, Ireland

Cyathostomins are the most common and pathogenic endoparasites in horses. Within the cyathostomin life cycle, hypobiosis of

encysted larvae in the large intestinal mucosa can occur, allowing for accumulation of large burdens. One clinical presentation is a severe local and systemic inflammatory syndrome – acute larval cyathostominosis (ALC). The risk factors and pathophysiology of ALC are incompletely understood, but it can be precipitated by administration of anthelmintics.

We have previously documented systemic and local inflammation and decreased diversity of the faecal microbiota, in clinically normal horses hosting encysted larval cyathostomins. We hypothesised that similar, but greater, changes in these parameters would be seen in horses which develop ALC. Here, we document the clinico-pathological and faecal microbiota changes during an ALC outbreak in Ireland.

The outbreak of ALC in a herd of twenty-three horses began in mid-November approximately three weeks after treatment with ivermectin/praziquantel. Clinical signs included sudden weight loss, diarrhoea, dull demeanour, pyrexia, colic and vasculitis. Clinico-pathological changes included neutrophilic and lymphocytic leucocytosis, hyper-fibrinogenemia, hyper-globulinemia, hypo-albuminemia and an albumin:globulin reversal.

Faecal bacterial microbiota were characterised by 16sRNA sequencing in longitudinal samples taken throughout the outbreak, in both affected and clinically normal horses.

Affected horses were treated with moxidectin and corticosteroids. Supportive therapy was administered according to clinical status. Broad spectrum antimicrobial therapy was administered to horses with persistent pyrexia.

Of the twelve clinically-affected horses, ten recovered and two were euthanised. Postmortem findings showed a marked ulcerative typhlocolitis consistent with the mass emergence of cyathostomin larvae and evidence of opportunistic bacterial pathogenesis.

This study provides further evidence of an interplay between cyathostomin infection, systemic inflammation and gut homeostasis in the horse.

OA38.08 Cyathostomin Infection Influencing Growth Rate and Performance of Thoroughbred

Dr. Marcelo Molento¹, Dr. Luciana Dias de Castro¹, Carolina Abrahao¹, Dr. Paulo Guimaraes¹, Joaquim Antunes²

¹UFPR, 157, Brazil, ²Private Practitioner, Sao Jose dos Pinhais, Brazil

Cyathostomin infection are ascribed to cause impairment to body development and performance of foals, but the actual effect of the disease remains unclear. The aim of this study was to determine the growth rate and racing performance of 31 naturally infected Thoroughbred horses, and to correlate with gender, month of birth, age, number of offspring per mare, fecal egg count (FEC) and packed cell volume (PCV), from birth to 3.8 years of age. Contents: Body weight (BW) and withers height (WH) were conducted monthly, PCV was measured from 12-21 months of age, and FEC from 9-21 months of age. Performance was determined by the Spearman's correlation, considering the number of races between Dec/2015 and Mar/2017, and previous FEC. Correlation between all variables was analyzed using Spearman's rank correlation. All animals were positive for cyathostomins, and FEC ranged from 389 – 1.635. Average BW at birth was 56.55 kg for males and 51.9 kg for females, and at 18 months' males and females weighed an average of 435.89 and 441.65 kg, respectively. Average WH at 18 months the mean height was 154.78 and 153.5 cm, respectively. Cyathostomin infection did not appear to have affected the growth rate of the animals, as the correlations between FEC, BW and WH were not significant. The number of offspring per mare influenced the weight of the animals at birth ($p = 0.03$) and at 6 months of age ($p = 0.026$). The racing history ($n=30$) data revealed that animals with high FEC showed poorer ($p<0.002$) performance when compared to those with low FEC with a coefficient of -0.580. Conclusions: The current

study provides strong evidence regarding the expected growth rate of Thoroughbred foals in Brazil, and the racing performance associated FEC, which may have important breeding implications.

OA39 Insecticide and Acaricide Resistance in Ruminants

July 10, 2019, 13:30 - 15:30

Breakout Room 4, Hall of Ideas G&J, Level 4

OA39.01 Macrocyclic Lactones Side-Resistance in Isolates of *Rhipicephalus Microplus* From Rio Grande Do Sul, Brazil

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The Rio Grande do Sul (RS) state plays a major role in Brazilian cattle industry. The parasitism by the cattle tick, *Rhipicephalus microplus*, causes great economic losses to the producers in the area. In order to minimize damage and optimize the chemical control, it is fundamental to monitor the acaricide resistance status. The resistance of *R. microplus* to macrocyclic lactones has been detected in RS for 17 years. Side-resistance among the drugs of this class is an important condition, considering its implications in resistance management and cattle tick control strategies. The aim of this study was to evaluate the occurrence of side-resistance among avermectins (ivermectin and doramectin) and milbemycins (moxidectin) in *R. microplus*. Engorged female ticks were sampled in 26 premises and their larvae were used in larval immersion tests with ivermectin, doramectin and moxidectin. Mortality data was submitted to probit analysis to calculate the lethal concentrations (LC50) and 95% confidence intervals (CI). Resistance ratios (RR) were calculated in

relation to a susceptible reference strain. Fisher's test was used to determine the correlation of resistance among the drugs. There was correlation in the resistance between ivermectin and doramectin ($p=0.04$) in 19 samples, and there was 2.3-fold (95%CI: 1 to 5.4-fold) more chances of a tick population to be resistant to doramectin if the same is resistant to ivermectin. There was no correlation of resistance between ivermectin and moxidectin ($p=0.24$) nor between doramectin and moxidectin ($p=0.67$). This is the first study to demonstrate side-resistance among avermectins in *R. microplus*. The results obtained here have an impact on cattle tick control strategies, since side-resistance between avermectins limits the availability of drugs to be used. Contrarily, moxidectin can still be rationally used as a control measure. Further studies should be performed to elucidate the mechanisms of resistance and side-resistance among different macrocyclic lactones.

OA39.02 Detection of Resistance to Chemical Acaricides in *Rhipicephalus (Boophilus) Microplus* Ticks From Selected Cattle Farms in Luzon, Philippines

Dr. Remil Galay¹, Dr. Sherwin Alota¹, Dr. Tisha Rogelle Edquiban¹, Dr. Jayvee Evangelista¹, Mr. Fred Gio Rean Valera¹, Dr. Kristina Andrea Sandalo¹, Dr. John Michael Bernardo¹, Dr. Tetsuya Tanaka²

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Tick infestation and tick-borne diseases cause great economic losses in the cattle industry worldwide. The cattle tick *Rhipicephalus (Boophilus) microplus* is one of the most widely distributed ticks, especially in tropical countries including the Philippines. Tick infestation remains to be a burden to many cattle raisers, leading to health problems, decreased productivity and economic losses. Tick control is mainly through application of chemical acaricides such as amitraz and synthetic pyrethroids. Ivermectin is also widely used to control

gastrointestinal nematodes, and partly for tick control. Acaricide resistance has been reported in several countries, but there are no published reports in the Philippines. Hence, this study aimed to determine whether there is resistance already to various chemical acaricides that are being used. Engorged or nearly engorged *R. (B.) microplus* female ticks were collected from selected cattle farms in provinces in northern and southern Luzon island of the Philippines, which were allowed to lay eggs in the laboratory to produce larvae. The resulting larvae were exposed to various concentrations of amitraz, cypermethrin and ivermectin through the larval packet test (LPT). The concentrations included discriminating dose (DD), half of DD, and double of DD. Larval mortality was determined after 24 hours. LPT for amitraz showed more than 90% larval mortality for all farms tested. Meanwhile, LPT for cypermethrin showed more than 90% larval mortality in DD and double DD in all except for three farms tested, wherein the mortality for DD was below 80%, suggesting presence of resistance to cypermethrin. In case of ivermectin, the mortality of tick larvae from five farms was lower than 80% even in double of DD, suggesting resistance. Additional farms from other provinces are currently being tested. This study provides the first scientific evidence of *R. (B.) microplus* resistance to cypermethrin and ivermectin in the Philippines.

OA39.03 Monitoring the Resistance of *Rhipicephalus Microplus* to Conventional Acaricides (Coumaphos, Amitraz and Flumethrin) and Ivermectin on Cattle Farms in Mexico

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Rhipicephalus microplus is widely distributed in tropical and subtropical regions of

the world where livestock is a principal activity with great veterinary and economic importance. One of the main tick control measures is the use of acaricides and macrocyclic lactones (ML). In Mexico no information on the distribution of level of acaricide and ML resistance in *R. microplus* field populations is available. For this study field tick populations were collected from 42 farms in 12 different states of Mexico and level of resistance to different acaricides and macrocyclic lactone determined. The dose-response bioassays were carried out using the modified larval packet (coumaphos and flumethrin) and larval immersion test (amitraz and ivermectin) against *R. microplus*. Mortality data were subjected to probit analysis to calculate lethal concentrations at 50%. A logistic regression model was used to evaluate the relation between resistance and possible associated factors. Phenotype was defined as susceptible, low resistance or high resistant. The overall prevalence of cattle farms with *R. microplus* resistant to coumaphos, amitraz, flumethrin and ivermectin were 19.0%, 52.4%, 45.2% and 85.7%, respectively. For coumaphos, 80.9%, 14.3%, and 4.8% were susceptible, low resistance and high resistance, respectively; for amitraz, 47.6%, 26.2%, and 26.2%, respectively; for flumethrin, 54.8%, 23.8%, and 21.4%, respectively; and for ivermectin, 14.3%, 61.9%, and 23.8%, respectively. We identified that cattle farms without acaricide rotation program (OR: 9.16, CI95%: 1.70-48.40, P: 0.009) had higher probability of developing *R. microplus* resistant to amitraz. It is concluded that amitraz, flumethrin and ivermectin resistance in *R. microplus* is common, but mainly at low level in cattle farms of Mexico. Besides the intensive use of coumaphos for many years in the control *R. microplus* in cattle from Mexico, this acaricide can still be used as a tool to control the cattle tick.

Research funded by Bayer Animal Health, Germany.

OA39.04 Mapping Acaricide Resistance of the Three Major Cattle Tick Species *Amblyomma Variegatum*, *Rhipicephalus Appendiculatus* and *Rhipicephalus Microplus* in East and West Africa by Using the Larval Packet Test (LPT)

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Acaricide resistance is a major constraint in the control of ticks on production and companion animals all over the world. A variety of reasons contribute the development of resistance, among which long-time use, under- or overdosing or inappropriate use. In this study the degree of acaricide resistance of three economically most important cattle tick species (*Amblyomma variegatum*, *Rhipicephalus appendiculatus* and *R. microplus*) collected in resource-poor farmer communities in East and West Africa were determined. In seven countries (Benin, Ethiopia, Ghana, Nigeria, Tanzania and Uganda) and at two localities in each country known for its high livestock density and occurrence of ticks and tick-borne diseases, engorged females belonging to these species, if present, were collected. Their subsequent offspring was used in larval packet tests to determine LD50 and degree of resistance, when compared to a susceptible tick strain. For *A. variegatum*, six different tick stocks were analysed (one from each country); for *R. appendiculatus* five (three from Uganda and two from Tanzania) and for *R. microplus* 10 different stocks (two from each country). To determine the degree of resistance, a susceptible strain of *R. appendiculatus* and *R. microplus*, both from South Africa, were included in the tests.

Five different acaricides were tested: chlorfenvinphos, alpha-cypermethrin, fipronil, ivermectin and amitraz. The results of the LPT indicate an intermediate and high level of resistance for *R. microplus* and *R. appendiculatus* against chlorfenvinphos and amitraz. *Rhipicephalus microplus* was

also resistant to alpha-cypermethrin. Only LD50 for *A. variegatum* could be determined because of the absence of a susceptible strain.

The degree of resistance and use of the different acaricides in each country is discussed.

OA39.05 Efficacy of a New 2.5% Fluazuron, 7% Chlorpyrifos, 6% Cypermethrin, 6% Piperonyl Butoxide-based Pour-On Formulation Against *Rhipicephalus (Boophilus) Microplus* in a Cattle Population under Field Conditions in Brazil

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In Brazil *Rhipicephalus microplus* in cattle causes serious economic losses. The objective of this study was to test the efficacy of a single pour-on treatment with a new formulation 2.5% Fluazuron, 7% Chlorpyrifos, 6% Cypermethrin, 6% Piperonyl Butoxide at 1 mL/10kg/Bw on the elimination of a natural tick infestation in a Brazilian cattle population.

For this study, 22 male or female cross-breed animals *Bos indicus* x *Bos taurus*, 24 to 36 months old, 190-340 kg live weight were selected from the experimental herd of Universidade Federal Rural do Rio de Janeiro. Animals were kept on pastures, without parasiticide treatment and were naturally infested by *Rhipicephalus microplus*. On D0, these animals were randomized to the control group (C, n=11) or the treatment group (T, n=11) with consideration to the average number of engorged females ticks ($\phi \geq 4.5$ mm) collected on D-3, D-2, D-1. On D0, 1 mL/10 kg/Bw of this new formulation containing 2.5% Fluazuron, 7% Chlorpyrifos, 6% Cypermethrin, 6% Piperonyl Butoxide

(Virbac) was poured on the back of group T animals, from the base of their horns to the base of their tails. Once a week, from day D+7 until D+70, engorged females ticks (≥ 4.5 mm) were counted on animals.

Between D-3, D-2, D-1 the average tick counts in geometric means were 70.6 and 69.8 in groups T and C, respectively. In treated animals, the tick population decreased significantly ($p < 0.05$) in comparison with the control group for all time points from days D+7 until D+70, and in geometric means, by 97.4, 98.6, 96.7, 98.7, 97.5, 96.4, 98.6, 97.15, 92.2, and 81.1% respectively.

In this study, this new pour-on formulation was found to be effective against *Rhipicephalus Microplus* with a reduction of tick counts by more than 95 % until 56 days after the treatment.

OA39.06 In Vivo and In Vitro Parameters for Acaricide Efficacy of Macrocyclic Lactones Against *Psoroptes Ovis* in Cattle

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Psoroptic mange is an important disease in beef cattle, and Belgian Blue cattle are particularly susceptible. Treatment failure of macrocyclic lactones (ML) against *Psoroptes ovis* has been reported, but clear evidence for resistance of *P. ovis* against ML in cattle is lacking. This study was conducted to investigate ML efficacy in 16 beef farms in Belgium and The Netherlands in vivo and in vitro.

On each farm a group of animals ($n = 7-14$) with clinical psoroptic mange was treated with two subcutaneous injections of a short-acting ML with 7-10 days interval ($n = 15$) or a single injection with a long-acting ML ($n = 1$). In vivo efficacy was assessed by the reduction

in mite counts and the cure rate after the first treatment round and the number of treatment rounds needed to cure all animals. In vitro knock-down and mortality was evaluated in a contact assay based on Brimer et al., 1995 (Vet Parasitol 59, 249-255).

All farms needed ≥ 2 treatment rounds (2-8) to obtain full efficacy. Cure rates varied from 0%-80%. Only three farms had a mite count reduction of $>90\%$, two of which had a lower limit of the confidence interval of $<90\%$. All other farms had a mite count reduction $<90\%$ (-41%-81%). LD50 values in vitro varied from 2,951-36,867 g/mL and 0.3-58.3 g/mL at 24h and 120h, respectively. No significant correlation was found between in vitro LD50 values and any of the parameters for in vivo efficacy.

In conclusion, unambiguous treatment failure was detected on 13/16 beef farms, confirming the presence of ML resistance in Belgian Blue beef farms. Tentative in vitro parameters could not detect ML resistance. The potential use of different in vitro and in vivo parameters to evaluate acaricide efficacy and to detect acaricide resistance will be discussed.

OA39.07 Acaricidal Activity of Nanoscale ZnO Encapsulated Piperine Formulation Against *Rhipicephalus Microplus*

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A study was undertaken with an aim to synthesize and evaluate the acaricidal activity of the nanoscale zinc oxide piperine formulation (NZPF) against *Rhipicephalus microplus* ticks infesting cattle. NZPF was prepared by employing encapsulation technique using 0.1% zinc oxide nanoparticles (ZnONPs) and 2% piperine solution. The synthesised NZPF was characterized by subjecting UV-Vis spectroscopy, Fourier Transformed Infrared analysis, X-ray Diffraction, Dynamic Light Scattering, Scanning Electron Microscopy and Energy Dispersion Spectroscopy analysis. Acaricidal activity of deltamethrin, piperine, ZnONPs and NZPF on *Rhipicephalus microplus* was evaluated by two bioassays viz., Larval

Packet Test (LPT) and Adult Immersion Test (AIT). LPT with a discriminating dose of deltamethrin (75ppm) showed a mortality of 59% of *R. microplus* larvae. Mortality of *R. microplus* larvae was 100% at concentration of 9 ppm, 8 ppm and 7 ppm with piperine, ZnONPs and NZPF, respectively. AIT with a discriminating dose of deltamethrin (75ppm) against adult *R. microplus* showed a mortality of 40%, oviposition inhibition of 78.309% and the lowest egg mass weight with 17.8 ± 1.31 mg. Mortality rate and oviposition inhibition of *R. microplus* were 100% whereas egg mass and reproductive index were completely nil with both piperine and ZnONPs at a concentration of 20 ppm and NZPF at a concentration of 15 ppm. NZPF showed a potent ovulation inhibitory activity with significantly ($P < 0.05$) lower IC₅₀ and IC₉₉ values compared to ZnONPs and piperine.

Both LPT and AIT results indicated the development of resistance in *R. microplus* ticks against deltamethrin. NZPF, ZnONPs and piperine were found to have significantly ($P < 0.05$) higher acaricidal activity. However, NZPF had high acaricidal efficacy at lower concentrations than pure phytochemical piperine, ZnONPs and deltamethrin. NZPF could be potential alternative to routine chemical acaricides for control of tick infestation of cattle in the wake of the development of acaricidal resistance, residual effect and environmental pollution.

OA39.08 Failure of Macrocyclic Lactones to Control Psoroptic Mange Infection in Feedlot and Grazing Beef Cattle

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The current work assessed the relationship between pharmacokinetic behavior and clinical efficacy of ivermectin (IVM) or doramectin (DRM) against natural *Psoroptes ovis* var. *bovis* infection in cattle. The study involved two trials (I and II) carried out on different beef cattle production systems, a feedlot (Trial I) and a grazing (Trial II) system. In Trial I, 40 mange-infected steers were allocated into 4 groups (n=10) and treated with a single (day 0) or repeated (days 0 and 7) subcutaneous injection of two different formulations of IVM (1%) at 0.2 mg/kg. In Trial II, 20 grazing calves with active mange infection were allocated into 2 groups (n=10) and treated with a single subcutaneous injection of either IVM (1%) or DRM (1%) at 0.2 mg/kg. Blood and skin samples were collected from 8 animals of each group to measure IVM/DRM concentrations by HPLC. Skin scraping samples were collected from each animal and mites were counted. In Trial I, the repeated administration of IVM increased the systemic availability and skin drug exposure compared to the single treatment ($p < 0.05$). However, both formulations failed to achieve a clinical mange cure at either single or repeated treatment. Efficacy of IVM was 10% (single dose) and 50% (repeated treatment) at day 14 post-treatment. The non-cured animals remained with active mange 28 days post-treatment. No differences ($p > 0.05$) in the *P. ovis* scores density were observed after single or repeated treatments. In Trial II, there was also a positive correlation between IVM or DRM concentrations in plasma and skin samples. Although IVM and DRM failed to obtain a complete parasitological cure, the efficacy of DRM was higher (80%) than those obtained by IVM (10%) ($p < 0.05$). Additional studies are needed to confirm the presence of *P. ovis* populations resistant to macrocyclic lactones, and to enhance the control of psoroptic mange in cattle.

OA40 Zoonoses

July 10, 2019, 13:30 – 15:30

Breakout Room 5, Meeting Rooms KLOP, Level 4

OA40.01 Zoonotic Malaria Parasites Among Non-Human Primates in Sarawak, Malaysian Borneo

Ms. Khamisah Abdul Kadir¹, Dr. Thamayanthi Nada Raja¹, Dr. Paul Cliff Simon Divis¹, Mdm Dayang Shuaisah Ahmad Mohamad¹, Mr Ahmad Matusop¹, Professor Mohd Tajuddin Abdullah³, **Professor Balbir Singh¹**
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Human infections with *Plasmodium knowlesi*, a malaria parasite of long-tailed and pig-tailed macaques (*Macaca fascicularis* and *M. nemestrina* respectively), were thought to be extremely rare until a large focus of human infections were reported in the Kapit division of Sarawak, Malaysian Borneo in 2004. Macaques are natural hosts of *P. knowlesi* (Pk), *P. cynomolgi* (Pcy), *P. inui* (Pin), *P. coatneyi* (Pco), *P. fieldi* (Pfi) and *P. simiovale*. In Sarawak, only macaques in the Kapit division have been examined so far and found to harbor these malaria parasites. The objective of this study was to determine the prevalence and distribution of simian malaria parasites among non-human primates in other divisions in Sarawak, Malaysian Borneo. From 7 out of the 9 administrative divisions in Sarawak, blood samples were obtained from 84 non-human primates: *Macaca fascicularis* (n=45), *M. nemestrina* (n=28), *Presbytis cristata* (n=2), *Nasalis larvatus* (n=2), *Macaca arctoides* (n=3), *Hylobates muelleri* (n=4). They were screened with nested PCR assays using primers specific for Pk, Pcy, Pin, Pco and Pfi. Only macaques were found to be infected with malaria parasites and they originated from all 7 Sarawak divisions sampled. A total of 31 macaques (20 *M. fascicularis* and 11 *M. nemestrina*) were malaria-positive. All 5 *Plasmodium* species were detected, with macaques harbouring either single (n=12), double (n=8), triple (n=7) or quadruple (n=4)

infections. Pk, Pcy and Pin were detected either singly or in combination in all the malaria-positive macaques. This study indicates that the simian malaria parasites that can infect humans, namely *P. knowlesi*, *P. cynomolgi* and *P. inui*, are found in macaques throughout Sarawak, Malaysian Borneo. Travelers to forested areas in Sarawak and similar areas in Southeast Asia should be made aware of the potential risk of acquiring zoonotic malaria.

OA40.02 Neuroinflammation and Neurodegeneration during *Toxocara Canis* and *T. Cati*-Induced Neurotoxocariosis in Mice

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Migrating larvae of *Toxocara* spp. may cause disease in humans, including neurotoxocariosis (NT). Previous studies on *T. canis*- and *T. cati*-induced NT in the model organism “mouse” have indicated distinct differences between both *Toxocara* species regarding larval migration patterns and clinical symptoms. The aim of the present study was to investigate patterns of neuroinflammation and neurodegeneration during *T. canis*- and *T. cati*-induced NT in the mouse model. Histopathology was carried out on cerebra and cerebella of *Toxocara*-infected and uninfected C57Bl/6 mice at 7, 14, 28, 42, 70 and 98 days post infection (dpi). In addition to haematoxylin-eosin and luxol-fast-blue staining, immunohistochemistry was employed to visualize microglia and beta-amyloid precursor protein (beta-APP) as an indicator of axonal damage. Furthermore, a comprehensive multiplex bead array assay profiling was conducted to elucidate patterns of immunomodulatory

cytokines and chemokines in affected mouse brains. Haemorrhages, eosinophilic vasculitis and activated microglia were detected in both infection groups starting 7 dpi, followed by eosinophilic meningitis 14 dpi. Neurodegenerative processes (demyelination, beta-APP accumulation, gitter cells) occurred earlier during *T. canis* than *T. cati* infection, and affected significantly more *T. canis*- than *T. cati*-infected mice, especially regarding the cerebrum. In both infection groups, a continuous decrease of certain pro-inflammatory cytokines, including TNF- α , IFN- γ and IL-12, was detected in the cerebrum over the course of infection, while differences were detected regarding Th2-cytokines. *T. canis* infection was characterised by significantly elevated IL-4 and IL-5 levels in the cerebrum in the acute and subacute phase of the disease, which were not detected in *T. cati*-infected mice. Further differences were observed, for example, regarding eotaxin. Earlier and more severe neurodegeneration during *T. canis*- than *T. cati*-induced NT may explain the differences in behavioural alterations observed in previous studies. In addition to larval migration preferences, immune regulatory mechanisms may contribute to these patterns.

OA40.03 Accurate Diagnosis of Lesions Suspected of Being Caused by *Taenia Solium* in Body Organs of Pigs with Naturally Acquired Porcine Cysticercosis

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The definitive method for diagnosis of porcine cysticercosis is detection of cysticerci at necropsy. Cysts are typically located in the striated muscle and brain. Until recently *Taenia solium* cysticerci have not been definitively identified in other tissue locations, despite several comprehensive investigations having been undertaken which included investigation of body organs other than muscle and brain. Recently a study conducted in Zambia reported 27% infection with *T. solium* in the liver of pigs with naturally acquired porcine cysticercosis, as well as some *T. solium* infection in the lungs and

spleen of some animals. We investigated the cause of lesions in sites other than the muscle or brain in a total of 157 pigs from *T. solium* endemic regions of Uganda and Nepal which were subjected to extensive investigations at necropsy. Lesions which had the potential to be caused by *T. solium* were characterised by macroscopic and microscopic examination, histology as well as DNA characterisation in PCR-RFLP and sequencing. Lesions were confirmed as being caused by *Taenia hydatigena* (both viable and non-viable), by *T. asiatica* and *Echinococcus granulosus* (in Nepal) and nematode infections. No cysticercus or equivocal lesion was found to have been due to *T. solium* in any tissue location other than muscle and brain. It is recommended that future evaluations of porcine cysticercosis in aberrant tissue locations involving DNA analyses take appropriate care to avoid the possibility of contamination of tissue specimens with DNA from a different tissue location or a different animal, and the use of appropriate control samples in order to confirm the absence of cross sample contamination.

OA40.04 Prevalence of Ticks Parasitizing Human Beings in the Nilgiris Hills of Southern India

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Ticks act as a vector to transmit varieties of pathogenic organisms to human beings viz., Kyasanur forest disease and Congo haemorrhagic fever. A study was conducted to know the prevalence of tick species on 92 human beings (56 male and 36 females) working in the tea estate, fire wood collectors, reserved forest, coffee /pepper pickers, persons residing in home and also on the premises at Pallipadi, Irumbupalam, Karalikandi, Devala, Pandiyar Tan tea 2B, Sussex (Pitheri), Genepool (Nadukani),

Erumadu and Mundakunnu of Gudalur, the Nilgiris district of Tamil Nadu, India from January 2018 to February 2019. Overall prevalence of tick infestation on human beings was 53.26%. The collected ticks were identified as larval and nymphal stages of *Haemaphysalis spinigera*, *H. megalaimae*, *Haemaphysalis* Spp., *Amblyomma* Spp., *Anomalohimalaya* Spp. and nymphal stage of *Amblyomma varanense* and *Hyalomma isaaci* by stereo zoom and scanning electron microscope (SEM). Ticks collected from the premises by tick drag and tick flag methods were identified as larval and nymphal stages of *H. spinigera*. Persons infected with ticks showed fever and vomiting until removal of ticks and reluctant to work, severe itching, tiredness, inappetence and the skin lesions persist from a week to more than two years. Erupted, thickened brownish gray scar (2 to 5 mm) on chest region and wide-spread multifocal lesions on the back from the neck to hip region, abdomen, shoulder and hands were seen. The affected persons working in the reserve forest region revealed multifocal erythematous nodular eruptions in the leg and thigh region. The prevalence ticks found to be more on the persons working in tea estate (42.86%) followed by persons residing in home (20.41%), fire wood collectors (18.37%), reserved forest (18.37%) and no ticks were observed on coffee /pepper pickers.

Key words: Ticks – human – prevalence – lesions – southern India

OA40.05 Molecular Characterization of *Enterocytozoon Bieneusi* in Pallas's Squirrels (*Callosciurus Erythraeus*) from Kanagawa Prefecture, Japan

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Pallas's squirrel (*Callosciurus erythraeus*) was introduced from Taiwan to Japan in the 1930s and eventually established itself in several areas across the country. The species is designated as a regulated organism under the Invasive Species Act by the Ministry

of the Environment, Japan, and municipal governments, including Kanagawa Prefecture, are implementing control measures to reduce their numbers. *Enterocytozoon bieneusi* is a common microsporidian species, frequently reported from immunocompromised humans and from a wide range of domestic and wild animals. More than 240 genotypes have been identified in both humans and animals worldwide; however, information regarding *E. bieneusi* in mammals in Japan is scarce. We aimed to determine the occurrence and genotype of *E. bieneusi* in Pallas's squirrel from Kanagawa, Japan for the first time. Genomic DNA was extracted from a total of 168 feces samples from Pallas's squirrels that were captured in Hayama City between January and June 2018. Nested PCR was performed targeting the ribosomal internal transcribed spacer (ITS) region. The overall prevalence was 16.7 % (28/168), and sequence analysis showed 100 % homology to genotype SCC-2 for all 28 samples. This genotype was recently reported from pet chipmunks (*Eutamias sibiricus asiaticus*) in Sichuan Province, China, which phylogenetically clustered into a novel group (Group 10). It is not known whether this genotype poses a risk of zoonotic transmission and can cause human microsporidiosis. As Pallas's squirrels in Kanagawa Prefecture live in close proximity to humans, it is important to be aware of the potential risk from a public health perspective. Continuing studies on the distribution of *E. bieneusi* genotypes in the squirrel population in Japan are necessary to further assess the effect of the pathogen on both wildlife and human populations.

OA40.06 Improved Diagnostics for *Echinococcus Multilocularis* in Wild and Domestic Canid Species.

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In recent times, there has been an increasing detection of alveolar echinococcosis (AE) in humans and dogs in Canada, which is

a serious and potentially fatal “parasitic tumour” originating in the liver. *Echinococcus multilocularis*, the tapeworm responsible for this infection, normally occurs in wild canids (foxes, coyotes and wolves) as its definitive hosts and rodents as the natural intermediate hosts. Wild canids are sources of infection for humans and dogs serving as aberrant intermediate hosts. Since humans have close interaction with dogs and with the increasing density of wild canids in urban areas, it is important to adequately diagnose the infection in the canid definitive hosts so as to prevent or control spread of infection to humans. The gold standard test (adult cestode recovery) for the detection of *E. multilocularis* is laborious and fatal for the canid; hence, there has been a shift to molecular methods of diagnosis. Fecal, heart blood, chest fluid, and adult taeniid cestodes were recovered from two hundred coyotes trapped in Saskatchewan winter of 2018. We compared prevalence based on adult cestode recovery (73%) with taeniid eggs on fecal flotation and centrifugation (27%), and two new copro-PCR assay (73%). Our findings suggest that PCR based methods offer promising results for detection of intestinal infections with taeniid cestodes in both wild and domestic canids. Finally, it is important to detect cases of AE in dogs as rapidly as possible for a more favorable clinical outcome. Therefore, we will also test coyote blood samples with a novel serological test for canine AE to ensure that the test does not detect adult cestode infection. It is important to be able to distinguish the two possible states in the canid hosts, as one has public health implications and the other has animal health implications, and both require prompt intervention.

OA40.07 Screening of dogs for *Echinococcus granulosus* infection by Copro-Polymerase Chain Reaction

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Echinococcosis, a cyclozoonotic helminthosis caused by the dwarf dog tapeworm *Echinococcus granulosus*. It is distributed worldwide and common in areas where hygienic conditions are poor and literacy is low. In India, high prevalence of cystic echinococcosis has been reported but even though dogs are disseminators of infection as definitive hosts, very few studies have conducted. Therefore, the present study was undertaken to detect the copro-DNA of *E. granulosus* in dogs by PCR in Karnataka state, South India. Totally 300 faecal samples, which includes 100 samples of dogs collected during post-mortem examination and 200 from stray dogs were screened by microscopy. Out of 100 dogs examined on necropsy, only ten were found positive for *E. granulosus* worms along with nineteen showed mixed infection with *Ancylostoma caninum*, *Dipylidium caninum*, *Toxocara canis* and *Taenia hydatigena*. Among 200 faeces of stray dogs screened, forty-nine showed eggs of *Ancylostoma caninum*, *Taenia* sp. *Dipylidium caninum*, *Toxocara canis*, *Spirocerca lupi*, *Trichuris vulpis*, *Diphyllbothrium latum* and *Isospora canis*. Since, the morphology of taeniid eggs including *Echinococcus* resembles each other and it’s very difficult to differentiate by microscopy alone, the positive samples were subjected to Copro-PCR for species specific identification of *E. granulosus* eggs from *Taenia* species of dogs using two sets of *E. granulosus* specific primers of Eg 1f, Eg 1r and JB 3f, JB 4.5r. The Eg 1f, Eg 1r primers yielded a single amplicon of 255 bp whereas, JB 3f, JB 4.5r yielded 440 bp amplicon which were specific to *E. granulosus* in twenty-one dog samples. The Copro-PCR was found more sensitive in detection and differentiation of *E. granulosus* from *Taenia* sp. of dogs and can be used effectively as a confirmatory diagnostic tool for detection of *E. granulosus* in dogs at field level.

OA41 Biology and Pathology of GI Nematodes in Ruminants

July 10, 2019, 16:30 - 18:00

Plenary Hall, Madison Ballroom (ABCD), Level 4

OA41.01 Demonstration of Overwintering of Ostealgia Ostertagi and Cooperia Oncophora L3 Larvae on Pasture in Western Canada

Dr. Tong Wang¹, Ms. Dawn Gray², Dr. Libby Redman¹, Dr. Russell Avramenko¹, Dr. Janneke Wit¹, Ms. Cailey Patullo¹, Ms. Bruna Salles¹, **Dr. John Gilleard**¹, Dr. Doug Colwell²

¹University Of Calgary, Canada, ²Agriculture and Agri-Food Canada (AAFC), Canada

Gastro-intestinal nematodes (GIN) have a negative economic impact on beef production in pastured cattle. Current control recommendations in western Canada are based on the assumption that the cold winters in this region (lowest temperature -30°C) prevent overwintering of infective larvae (L3) on pastures. However, there is no published information on the ability of cattle GIN species to overwinter on pastures in western Canada. Consequently, we are investigating the overwintering capability of bovine trichostrongylid L3 larvae on western Canadian pastures.

A study was conducted on three organic farms over two consecutive years. Two experimental approaches were taken: First, environmental samples (grass, feces and soil) were collected before and after each winter followed by L3 enumeration in the laboratory. The species composition of L3 populations was quantified using nemabiome sequencing, a novel deep amplicon sequencing approach. Second, L3 overwintering was further investigated using tracer calves, a more sensitive approach. Parasite-free tracer calves were placed on pasture for 3-4 weeks in spring to allow ingestion of overwintered L3 from pasture before adult worm burdens were determined for each GIN species. Detailed weather data at farm level was monitored by solar powered weather stations. We found that a proportion of both *C. oncophora* and *O. ostertagi*, but not *H. placei*, L3 larvae survived on pasture over the winter on all

three Alberta farms. Survival of *C. oncophora* was proportionally greater than *O. ostertagi*. Although less than 5% of L3 present on the pasture in the fall were able to survive the subsequent winter, these were able to infect the tracer calves and establish spring infection.

These results to date suggest that overwintered larvae play an important role in the epidemiology of GIN infection of beef cattle in western Canada and needs to be considered when designing strategic parasite control regimes.

OA41.02 Outbreak of Calf Sudden Death Associated with Strongyloides Papillosus Infection in a Western New York Dairy Farm

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¹Department of Population Medicine & Diagnostic Sciences, College of Veterinary Medicine, Animal Health Diagnostic Center, Cornell University, United States

Strongyloides papillosus are slender hair-like worms that have a predilection for the proximal small intestine of ruminants worldwide. These worms have a unique life cycle in which the parthenogenetic females are the only adult stage in the host. They shed larvated eggs in feces and susceptible hosts are infected percutaneously by the filariform L3. *Strongyloides papillosus* are generally considered nonpathogenic, as apparently healthy animals tolerate heavy infection. Although there are sporadic reports of major outbreaks with sudden death of calves from East Asia, similar outbreaks have not been reported in North America. In this report we document an outbreak of sudden death of calves on a NY dairy farm attributed to *S. papillosus* infection. In early September 2018, 15 calves died over a period of 14 days. Calves were recumbent with signs of respiratory distress prior to death, or found dead. All female calves also had precocious udder development. Necropsy and histopathologic findings revealed severe mammary congestion and ductal hyperplasia without other significant lesions. Testing for various viral and bacterial agents yielded inconclusive

results. An ancillary finding of 18,800 EPG of *S. papillosus* in a fecal float from a dead calf raised suspicion of a helminth-associated outbreak. Subsequent fecal testing of 7 other calves yielded EPG ranging between 1,370 and 8,300. Shortly after deworming with Dectomax and extensive cleaning of the barn, death loss stopped abruptly and the udder enlargement resolved. A similar mortality event on this dairy had occurred the previous year (July– August, 2017) resulting in the sudden death of 30 calves. Ionophore toxicity was suspected but feed ionophore levels were within safe limits. Unfortunately, parasite testing was not performed in 2017. This report highlights the importance of including *S. papillosus* in the list of differentials for sudden death in young calves.

OA41.03 Nematode Exsheathment 2: Abomasal Species Differ in Their Response to Exsheathment Triggers

Dr. Kiliana Bekelaar¹, Dr. Tania Waghorn¹, Dr. Michael Tavendale¹, Catherine McKenzie¹, Dr. Dave Leathwick¹

¹AgResearch Ltd, Palmerston North, New Zealand

Infection of ruminants by gastrointestinal nematodes is initiated by the exsheathment of the infective third stage larvae. Both carbon dioxide (CO₂) and heat shock have been shown to play crucial roles in triggering *Haemonchus contortus* exsheathment in vitro. This study set out to investigate how relevant these triggers are in rumen fluid and whether this data can be extrapolated to other species.

In CO₂-saturated rumen fluid, all species showed an efficient exsheathment response (> 80 % in under 4 hours) following exposure to heat shock, which was significantly higher compared to slow temperature changes. In the artificial buffer, the effect of heat shock was species-dependent. For *H. contortus* and *Ostertagia leptospicularis* the response was similar to that in rumen fluid, but exsheathment of *Ostertagia ostertagi* and *Teladorsagia circumcincta* was significantly lower and/or slower, and with no benefit of heat shock.

In CO₂-depleted rumen fluid exsheathment of *H. contortus* could not be achieved, whereas *O. ostertagi*, *T. circumcincta* and *O. leptospicularis* all showed some degree of exsheathment (respectively 46 %, 22 % and 15 %). These findings were confirmed in a CO₂-free artificial buffer. Interestingly, the ability to exsheath in the absence of CO₂ was dependent on the composition of the buffer, indicating the involvement of co-factors.

Overall, even though these species all exsheath in the rumen and reside in the abomasum, there appear to be significant differences in their response to exsheathment triggers. The data suggest an important role for both heat shock and CO₂ in vivo, but in vitro their effects are dependent on the nematode species as well as the surrounding medium.

OA41.04 Studies on Gastrointestinal Nematodes of Australian Alpacas

Mr. Harun Rashid¹, **Dr. Abdul Jabbar¹**

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Gastrointestinal nematodes (GINs) can cause significant economic losses in alpacas and llamas. Very little is known about prevalence of GINs, control practices and the use of anthelmintics. This study aimed to (i) assess the worm control practices used by Australian alpaca farmers (ii) assess the prevalence of GINs and (iii) assess the efficacy of commonly used anthelmintics against GINs of alpacas. An online questionnaire survey was conducted to assess current worm control practices. A longitudinal and cross-sectional study was conducted on 13 and 91 selected alpaca farms, respectively, located in different climatic zones of Australia. For the longitudinal study, a total of 1,692 faecal samples were collected and analysed using faecal egg counts (FEC) and faecal cultures. The mean faecal egg count was 167 eggs per gram (epg), with a highest burden of 15,540 epg for strongyle type nematodes. In the cross-sectional study, the mean egg count was 291 epg of 1545 samples analysed. Over one hundred of gastrointestinal tracts were also examined to determine the worm burdens and their spectrum. Total worm counts revealed the

mean worm burden as 1,291, with a maximum count of 29,000 worms in one alpaca. The main five genera/species identified were *Camelostrongylus mentulatus*, *Haemonchus contortus*, *Trichostrongylus* spp., *Cooperia* spp. and *Nematodirus* spp. Finally, the efficacy of commonly used anthelmintics was assessed by faecal egg count reduction test. In addition, above three studies, a molecular diagnostic kit has been developed which can identify seven different nematodes of alpacas from faecal DNA. Results of this study showed that shared-GINs are prevalent in Australian alpacas and they are resistant to most widely used anthelmintics. This study provides invaluable information on the prevalence of GINs of Australian alpacas, control practices and efficacy of commonly used anthelmintics which could be used to develop control strategies against GINs in alpacas.

OA41.05 Differences Within Churra Breed Sheep in the Early Immune Response to the Infection by *Teladorsagia circumcincta*

Verónica Castilla Gómez De Agüero¹, Maria Martinez Valladares¹, Elora Valderas García¹, Beatriz Gutierrez Gil¹, Juan José Arranz¹, Jorge F. González², Julia N Hernandez²

¹Universidad de Leon, Leon, Spain,

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In the present study, the mechanisms by which Churra breed sheep can present different resistance phenotypes were studied in adults during the early stage of the infection by *Teladorsagia circumcincta*. For that, 12 animals were classified as resistant (6) or susceptible (6) to the infection by *T. circumcincta* based on their cumulative faecal egg count after an initial experimental infection. A negative correlation was found between the cumulative FEC at the end of the infection and the level of IgA in serum at day 3 pi ($r = -0.764$; $P = 0.004$), when IgAs reached the highest values in the resistant group. Sheep were dewormed and were infected again to be slaughtered at day 7 post infection. At slaughter, level of IgA in serum and gastric mucus was higher in the resistant group although only showing slight significant differences in serum samples

($P = 0.1$). At the necropsy, abomasum tissue samples were collected for histological and immunohistochemistry analysis. A positive correlation was found between CD4+ and $\gamma\delta$ + T cells ($r = 0.714$; $P = 0.04$), suggesting that both cell populations could participate in the early immune response. Moreover, we found an association between the number of $\gamma\delta$ + T cells and eosinophils ($r = 0.600$; $P = 0.05$); this association was even stronger in the resistant group ($r = 0.900$; $P = 0.037$), but absent in the susceptible animals, suggesting that the activation of this mechanism could play an important role in the resistance to the infection. On the other hand, the susceptible group showed a negative correlation between globule leukocytes and $\gamma\delta$ + T cells ($r = -0.812$; $P = 0.05$) but also slight with CD4+ T cells ($r = -0.800$; $P = 0.1$). Therefore, it is possible that in the susceptible group the immune response was not strong enough to activate the recruitment of a higher number of globule leukocytes to protect against the infection.

OA42 Diagnostic Techniques

July 10, 2019, 16:30 - 18:00

Breakout Room 2, Hall of Ideas E&H, Level 4

OA42.01 The Babesia Caballi Spherical Body Protein 4 (SBP-4) Is Recognized by Antibodies in Sera from B. Caballi Infected Equids in Egypt and Has Potential for Developing Novel Serological Diagnostic Method

Dr. Mona Mahmoud¹, Dr. Omnia Kandil¹, Dr. Nadia Abu El Ezz¹, **Dr. Seham Hendawy**¹, Bassma Mahmoud¹

¹National Research Centre, Veterinary Research Division, Parasitology and Animal Diseases Department, Dokki, Cairo, Egypt

Equine piroplasmiasis is caused by two intra-erythrocytic haemoprotozoan, *Babesia caballi* (*B. caballi*) and *Theileria equi* (*T. equi*), in most tropical and subtropical areas of the world where competent tick vectors are present. A competitive inhibition ELISA diagnostic kit based on a monoclonal antibody to Bc48 (79/17.18.5), reactive with a member of the *Babesia* sp. rhoptry-associated protein

(RAP)-1 family lacked the ability to detect specific antibodies of *B. caballi* strains in Egypt, Israel and South Africa. The Spherical Body Protein 4 (SBP-4) was had shown an excellent performance when used as a new serological antigen in several distinct ELISA diagnostic assay for *B. bovis*. This study describes investigations aimed to defining the SBP4 of *B. caballi* as an alternative specific antigen that could be used for the detection of *B. caballi* strains circulating in Egypt. The *B. caballi* recombinant SBP-4 (r-SBP4) protein derived from an Egyptian isolate of *B. caballi*, was purified by immunoaffinity and immunologically tested. The purified r-SBP4 shows a single 37 kDa band upon coomassie blue staining of SDS-PAGE gels, which reacts with anti-HIS antibodies in Western blot analysis. The pattern of reactivity of the purified r-SBP4 against antibodies in the sera of equids infected with *T. equi* and *B. caballi* from an equine population was studied. No cross-reactivity was detected when using *T. equi* positive serum, suggesting that the r-SBP4 reacts specifically with *B. caballi* infected sera. A total of 191 equids sera were tested by *B. caballi*- IFA slides of VMRD Inc. and a novel SBP-4 I-ELISA. IFAT slides detect *B. caballi* specific antibodies in Egyptian equine's samples with low incidence (38.21 %) in contrast to SBP-4 I-ELISA (61.78%). Hence, r-SBP4 could be considered as a candidate for developing a reliable serological test that can be used to detect *B. caballi* infection in Middle East and Africa.

against *Sarcoptes scabiei* mites. Motile stages (larvae, nymphs and adults) of *S. scabiei* mites were collected from experimentally-infected pigs maintained at the veterinary College of Alfort, France. Petri dishes filled with Columbia agar supplemented with pig serum were used for the bioassays. Each molecule to be tested (beauvericin versus dimpylate and ivermectin as positive controls) was incorporated into the medium following the method described by Brimer et al. (1993, 1995) with slight modifications. For each molecule, the efficacy of 3 different concentrations (10, 100, and 1000 µg/g) was evaluated. All tests were carried in quintuplicate with 10 mites/plate at room temperature. Plates were examined after 1, 2, 3, 4, 5, 6, and 24h for survival assessment. Mites were considered dead when no movement occurred under the microscope. Final data of all treatments were analyzed by Kaplan Meier survival curves. The present study clearly demonstrated the activity of beauvericin against motile stages of *S. scabiei*. After 1h of exposure, the mortality rate was 8%, 20% and 56% at 10, 100, and 1000 µg/g, respectively. After 6h of exposure, the mortality rate was 42%, 86% and 94% at 10, 100, and 1000 µg/g, respectively. The concentration effect was notable at 10 µg/g and the mortality recorded with ivermectin was significantly higher then that recorded with dimpylate and beauvericin. The survival capacity seems to be different according to the evolutive stage of the mites. Larvae and nymphs exposed to beauvericin were killed more rapidly than adults.

OA42.02 In Vitro Evaluation of the Activity of a Mycotoxin Produced by the Entomopathogenic Fungus Beauveria Bassiana Against Sarcoptes Scabiei

Charbel Al Khoury², Dr. Nabil Nemer², **Prof. Jacques Guillot¹**

¹Veterinary College of Alfort, France, ²Holy Spirit University of Kaslik, Lebanon

The entomopathogenic fungus *Beauveria bassiana* is known to produce beauvericin, a secondary metabolite with many biological effects including insecticidal/acaricidal, antitumor, antibacterial, and antifungal activity. The objective of this study was to evaluate the in vitro efficacy of beauvericin

OA42.03 Improved Patient Care by Combining Diagnostic PCR Panels With Fecal Antigen and Fecal Flotation Tests in Dogs With Diarrhea

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¹Idexx Laboratories, Inc, United States

Diarrhea is a very common problem in dogs presenting to veterinary practices with GI clinical signs. Diagnosing the etiologic cause of diarrhea in dogs and selecting the proper treatment at presentation is challenging due to the many potential causes for an infectious GI syndrome.

Real-time polymerase chain reaction (PCR), also known as quantitative PCR (qPCR), offers improved sensitivity, specificity, safety, speed and scalability compared to other fecal tests alone for the detection of pathogens in fecal samples. Improved, rapid detection of the often multifactorial causes of canine diarrhea may allow the initiation of early etiologic treatments based on the detected pathogen(s) that are causing disease. This will help prevent unnecessary antibiotic use, facilitate improved management practices, minimize the severity of outbreaks, and improves pet owner satisfaction. In addition, early etiologic treatment reduces the potential of zoonotic pathogen transmission to humans, other pets or food animals. In this study, fecal flotation, fecal antigen ELISA (Fecal Dx™) for ascarids, hookworms, and whipworms were combined with a comprehensive canine diarrhea RealPCRTM panel. Of 157 included samples, 51% tested positive for at least one infectious agent by qPCR. More than 90% of all positive samples were positive for one or more pathogens by qPCR, with the majority (82%) positive by only qPCR. Fecal flotation detected a gastrointestinal pathogen in 15.5% of samples. The Fecal Dx panel was positive for one or more parasites in 4.8% of samples. The addition of qPCR allowed the detection of 8 times more infectious agents.

This study highlights the benefits of a syndromic qPCR panel directed at common gastrointestinal pathogens in combination with fecal antigen testing and the fecal flotation tests for the evaluation of canine diarrhea.

OA42.04 Phenotypic and Genotypic Characterisation of Canine Filarid Nematodes of Karnataka, India

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Filariasis is one of the major parasitic diseases of animals and human beings caused by a small group of filarid nematodes throughout the world. Canine filariasis

is widely prevalent and the larval stages of *Dirofilaria*, *Dipetalonema* and *Brugia* species are identified as causative agents among canines. Although, canine filariasis is widespread in the temperate and tropical countries of the world, only few reports are available in India. Accurate identification of canine filarid nematodes is important, because of zoonotic concerns and therapeutic implications. Presently, there is no conclusive data available on confirmatory diagnosis of *D. repens* in canines and its zoonotic implication in Karnataka state. In the present study, a total of 100 canine blood samples suspected for microfilariosis were collected from different regions of Karnataka. Thirty-eight samples were found positive for microfilaria by modified knott's method (MKM) and quantitative buffy coat (QBC). Morphologically, the microfilariae were unsheathed with blunt head and a tapering tail. The nerve ring and excretory cell at the excretory pore region of the microfilaria could be well appreciated and tail was long with hook like posterior end. The micrometry of microfilaria recovered by the modified knott's method had length of $334.9 \pm 4.573 \mu\text{m}$ and width of $6.862 \pm 0.133 \mu\text{m}$. Based on the morphometry, the microfilariae were identified as *D. repens*. To confirm the species, the samples were subjected to polymerase chain reaction (PCR) targeting the cytochrome oxidase subunit I (COI) genes of *Dirofilaria repens*. The amplicons were sequenced and analysed. The microfilariae recovered from canines of different regions of Karnataka were confirmed as *D. repens* based on PCR targeting COI genes. The phylogenetic analysis of the COI gene nucleotide sequence obtained in the present study showed no more than 95% homology with *D. repens* sequences existing for comparison.

OA42.05 Contemporary Status of Equine Trypanosomosis in Punjab, India**Lachhman Singla¹***¹Department Of Veterinary Parasitology, Guru Angad Dev Veterinary and Animal Sciences University, India*

Equine trypanosomosis or Surra elicited by the first pathogenic obligate, eukaryotic, intercellular haemo-flagellate protozoan parasite *Trypanosoma evansi*, is an unsystematically reported disease of equids in India. The dearth of information regarding the epidemiological status, role of hemato-biochemical indices including oxidative stress indicators on pathogenesis and the need of field oriented diagnostic test prompted us to undertake this comprehensive cross-sectional survey, using a combination of parasitological (classical) and sero-molecular diagnostic techniques together with analysis of risk factor associated with them, to unravel the exact position of epidemiological status of equine trypanosomosis covering all equid species from different agro-climatic zones of Punjab. The investigation established the endemic stability of Surra in all agro-climatic zones of Punjab. The risk factors evaluated indicated that the donkey/mules, unorganized farms, commercial purpose equids and farm without application of any fly repellent/insecticides were more vulnerable to get infected from *T. evansi*. Oxidative stress parameters indicated that though microscopically positive animal (patent infection) were more vulnerable, however, latent infection (PCR positive) was also producing pathogenicity by induced anemia and stress. Thus, diagnosis of latent infection is important to save the animal from induced stress and spread of infection to other susceptible animals. As all the samples positive by microscopy and PCR were also positive by LAMP assay, indicated LAMP assay to be a promisingly sensitive and specific technique for the diagnosis of *T. evansi* under isothermal conditions in field situations. Mapping of endemic area based on GIS predictive prevalence can act as alarming forecast model for establishment of control programs.

OA42.06 Detection of Blastocystis Mixed Subtype Infections in Dairy Calves Using Next Generation Amplicon Sequencing**Dr. Jenny Maloney¹**, Dr. Monica Santin¹*¹USDA, ARS, EMFSL, United States*

Blastocystis is a ubiquitous intestinal parasitic protist found in humans and many animals worldwide and is transmitted through fecal contamination of food or water. Nine of 22 *Blastocystis* subtypes have been found in both humans and animals, suggesting zoonotic transmission. Epidemiological data on *Blastocystis* in food animals is limited, and within-host *Blastocystis* diversity remains largely unexplored. Therefore, a method to study intra-host *Blastocystis* communities using next generation amplicon sequencing (NGS) was developed and compared to Sanger sequencing. Fecal samples from cattle were screened with a PCR that amplifies a fragment of the SSU rDNA gene followed by direct Sanger sequencing. If a mixed infection was suspected, amplicons were cloned. Seventy-five samples were analyzed by NGS using the same SSU rDNA gene region. Sanger sequencing indicated mixed infections in 18 of the 75 samples tested, but only three were successfully confirmed through cloning. NGS identified 49 mixed infections and revealed 14 subtypes, ten previously reported (ST-1 to ST-5, ST-10, ST-11, ST-14, ST-17, and ST-21) and four novel (named ST-23 to ST-26); ST-1, ST-2, and ST-11, were not observed by Sanger sequencing. Subtypes 1 through 5 are potentially zoonotic, and one or more were found in 79% (59) of the specimens using NGS. Subtype 3, the most common subtype found in humans, was found in 37% (28) of specimens tested by NGS and in only four specimens using Sanger. *Blastocystis* mixed subtype infections may be far more common than previously thought due to the limitations of current detection methods. Better characterization of within-host *Blastocystis* subtype diversity in infected humans and animals is needed to improve our understanding of *Blastocystis* epidemiology. The role of cattle as reservoirs of infection for humans and other animals through either direct contact or contamination of food and water should be explored.

OA43 Equine Parasites

July 10, 2019, 16:30 – 18:00

Breakout Room 3, Hall of Ideas F&I, Level 4

OA43.01 Cross-Sectional Analysis of Infectious Disease Agents in Relation to Helminth Parasites of Healthy Equidae in an Exhibition Setting

Ms. Hayley G. Hofmar-Glennon^{1,4}, Ms. Nicole M. André¹, Dr. Alison Stout^{2,5}, Dr. Renata Ivanek², Dr. Gary R. Whittaker^{1,4}, Dr. Laura B. Goodman^{2,3}, Dr. Belinda S. Thompson^{2,3}, Dr. Manigandan Lejeune^{2,3}, Dr. Erin L. Goodrich^{2,3}

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Helminth infections are known to modulate the host immune response, which is thought to either protect or injure the host against other infectious agents. In equines, cyathostomins (small strongyles) are considered the most common helminths, however, their role in modulating host immune response is not clearly known. Horses are predisposed to being infected with strongyles at either high, moderate or low levels. The spectrum of strongyle burden may influence the prevalence of infectious pathogens (bacterial or viral) in a healthy population. Moreover, testing for infectious disease agents in feces from healthy equids is rarely performed and it is unclear which pathogens may be present in the general population. Understanding the prevalence of infectious agents in healthy populations would help determine the relationship between helminth burden and the occurrence of infectious agents. This will also aid in the interpretation of diagnostic test results in animals with confirmed clinical disease. In this study, 100 fecal samples were collected from apparently healthy equids at an exhibition in NY State. The feces were examined for helminths and other parasites through morphological

testing for parasite stages, and were further analyzed for bacteria, bacterial toxins and viruses by PCR. All animals were categorized for their helminth egg shedding potential as high, moderate or low according to AAEP Guidelines. Each individual infectious agent was then compared to the helminth burden present in the sample to test for an association, using Chi-squared or Fischer's Exact Test. This study provides initial baseline data on the role that helminth burden may play in modulating the occurrence of infectious agents in healthy equid populations.

OA43.02 Expansive Chemosensory, Vision, Immunity, and Insecticide Resistance Gene Families in the Stable Fly: A Functional Genomics Tale

Pia Olafson¹, Joshua Benoit², Stomoxys Genome Consortium

¹U.S. Department of Agriculture - Agricultural Research Service, Livestock Arthropod Pests Research Unit, United States, ²Department of Biological Sciences - McMicken College of Arts and Sciences, University of Cincinnati, United States

Stable flies are a global pest of livestock production, having economic and social impacts on affected communities. Integrated pest management tools are currently employed for population suppression, but increasing worldwide outbreaks require development of supplementary and/or improved control approaches. Understanding those biological pathways regulating critical stable fly behaviors would inform such development. Here, we report the high quality sequencing and community curation of the stable fly genome. Coupled with transcriptome and bacterial community analysis, this effort revealed expansions in gene families that may enable the stable fly to persist in microbe-rich environments, localize hosts, and detoxify xenobiotic compounds. These insights enhance our understanding of this important pest and provide a framework for identifying novel control targets.

OA43.03 Surveillance of Equine Strongyle Infections and the Efficacy of a Larvicidal Dose of Fenbendazole in Horses Across the United States

Dr. Brian Herrin¹, Dr. Duane Chappell², Dr. Bryant Craig², Dr. Craig Barnett², Dr. Earl Gaughan², Dr. Wendy Vaala²
¹Kansas State University, United States, ²Merck Animal Health, United States

Equine strongyles are a common parasite of grazing equids, and the small strongyles are considered to be the parasite around which all adult horse deworming protocols are built. The current study enrolled 410 horses on 27 farms (197 weanlings; 213 horses >1 year old) from across the United States, to determine the strongyle burdens by fecal egg count (FEC) using the Modified Wisconsin technique, as well as the efficacy of fenbendazole as judged by fecal egg count reduction test (FECRT) from horses with >150 strongyle eggs on the first FEC. Horse owners were also asked to fill out a survey on their normal deworming and management strategies. Horses under 1 year of age received one dose of fenbendazole (10 mg/kg), and the older horses received the 5 consecutive days of treatment at 10 mg/kg. The average FEC for all animals with a strongyle-positive fecal was 185 eggs per gram (Range: 1 – 1,286 EPG), with horses older than 1 year of age having a significantly higher FEC (244.6 EPG) than weanlings (95.28 EPG, p -value < 0.0001). Deworming reduced overall strongyle egg counts by 71.8%, but the efficacy of fenbendazole was highly variable between farms (Range: 41.6 – 100%). There was no significant difference in reduction of strongyle egg counts between the drug regimen used for the older horses versus the young horses (p -value = 0.1345). There were no significant management strategies that effected the pre-treatment FEC or percent reduction. The data highlight the fact that most horses (398/410; 97%) fall into the low or moderate egg shedding category for strongyles. While fenbendazole treatment was >90% effective in reducing strongyle egg counts at some farms, the variability highlights the importance of FEC and FECRT in the routine care for horses to determine the efficacy of the deworming program at each barn.

OA43.04 Etiology and Treatment of Buffalo Fly-Associated Lesions

Dr. Peter James¹, Ms. Hayley Kidd², Dr. Con Constantinoiu³, Prof. Michael McGowan²
¹University Of Queensland, Australia, ²University of Queensland, Australia, ³James Cook University, Australia

Buffalo flies (*Haematobia exigua*), which are very closely related to horn flies (*Haematobia irritans*) and are sometimes considered a sub species, are a major pest in Australia's northern cattle industries. Buffalo flies (BF) are consistently ranked by northern cattle producers as amongst their main cattle health issues with the welfare aspects of BF-associated lesions often key amongst their concerns. These lesions can vary from dry and alopecic to open suppurating wounds, found most frequently on the medial canthus of the eyes, the neck, dewlap, abdomen and flanks of cattle. An unnamed species of filarial nematode, *Stephanofilaria* sp., vectored by BF, is frequently found associated with these lesions in northern herds. However, in the more southern parts of the Australian BF range, where lesions are prevalent and can be severe, detection of *Stephanofilaria* has not been reported from either lesions or BF dissections. A similar species, *Stephanofilaria stilesi*, is transmitted by *H. irritans*, but lesions are usually much less severe, ventral in distribution and most commonly found on the abdomen or near the udder of dairy cows. Determining the etiology and relative role of BF feeding and *Stephanofilaria* sp. in the development and persistence of BF lesions is critical to determining optimal control and treatment regimes. A study towards this end will be described.

OA43.05 Species Composition, Seasonal Incidence and Relative Abundance of Biting Midges at Horse Boarding Facility Located Near Cedar Key, Florida With a Case of Sweet Itch

Daniel Kline¹
¹USDA-ARS, CMAVE, United States

For the past decade an ongoing research project has been conducted at a horse

boarding facility located near Cedar Key, Florida, on the species composition, seasonal incidence, and relative abundance of biting arthropod populations. This study site has gradually been transformed from a scrub oak forest into a full fletched horse boarding facility with several extensive pastures. Changes in fauna and flora HAVE BEEN DOCUMENTED. Various trap types and sampling techniques have been utilized to monitor the changes in populations of biting midges, blackflies, mosquitoes, deerflies, horseflies and stable flies. Since a case of Sweet Itch has been attributed to the biting of the horse by biting midges, this presentation will focus on the biting midges. Over 13 species of Culicoides were collected, but only six consistently from year to year. Three species seemed to bother the horses the most. Culicoides furens was the main nuisance species from late spring through October. Culicoides mississippiensis was the main pest species from mid-October through May. Culicoides insignis was abundant from November through December and seemed to bother the horses a lot. The details of the Sweet Itch case will be presented.

OA44 Canine Arthropods

July 10, 2019, 16:30 - 18:00
Breakout Room 4, Hall of Ideas G&J, Level 4

OA44.01 Shall We Go for a Walk? – Tick Migration Behavior on Dogs

Heinz Sager¹, Denis Mosimann², Regina Lizundia¹, Christian Epe³
¹Elanco Animal Health, Switzerland, ²Idorsia Pharmaceuticals, Switzerland, ³Boehringer Ingelheim Animal Health, United States

The objective of this study was to investigate the migration behavior of different tick species between hosts and within the same host on experimentally infested dogs. Different sets of experimental infections were conducted using three tick species: Dermacentor variabilis, Rhipicephalus sanguineus and Ixodes ricinus. The ticks were color coded to allow identification of the host origin of the respective males and females.

Dogs were experimentally infested with 50 ticks and housed individually for 3 to 6h to allow tick-attachment. Then dogs were co-housed in pairs in biosafety rooms throughout the duration of the experiments. Color coded ticks were counted and recorded for each dog at 48h and 96h after each infestation.

Our results show that each tick species and gender had different migration behavior. R. sanguineus females migrate in higher proportion than males when their gender is the only one present in the room. However, when both genders are present in the same room, R. sanguineus males migrate more than females. D. variabilis females migrate more than males whether only one or both genders are present in the room. Interestingly, only I. ricinus males migrate. Individually marked ticks also showed that migration within the same host (on-host migration) occurs frequently.

In conclusion, our data show species-dependent and high mobility of ticks after experimental infestation of dogs. This migration behavior may influence the transmission of tick borne diseases, as pre-feeding of ticks on one dog prior to attachment to a second dog would be expected to accelerate transmission of pathogens to the other dog. This underlines the importance of tick prevention in dogs, to reduce the risk of exposure for pets and humans.

OA44.02 Assessment of an Activity Monitor (Vetrax®) and Effect of Pulicidal (Trifexis®) Treatment on Pruritic Behaviors in Flea-Bite Sensitive Dogs

Anthony Rumschlag¹, Marcel Sarzen², William Ryan³, David Young⁴
¹Elanco Animal Health, United States, ²AGL Technology, United States, ³Ryan Mitchell Associates LLC, United States, ⁴Young Veterinary Research Services, United States

Activity monitors have potential for guiding veterinarians and owners to treat and to track canine responses to treatments. A study investigated the potential for the Vetrax monitor to track canine pruritic behaviors in flea infested, flea-bite sensitive dogs, including following treatment with a combination product of spinosad and

milbemycin oxime (SMO) (Trifexis). VetraX units were attached to the collars of 10 mixed breed dogs on Day -14. On each dog, flea infestations of 100 fleas were placed on Day -13, 30 fleas on Days -10, -7 and -4, and 15 fleas were placed daily on Days 7 to 12 and 21 to 27. Video recordings of dogs were scored for pruritic behavior by blinded observers over a 4-hour period on Days -14, -3 (for randomization), 3, 13, 20 and 27. Dogs were randomized to be either untreated controls or to receive SMO on Day 0. Fleas were combed from all dogs and counted on Days 5, 14 and 28. Comparisons of video-recorded with monitor-recorded pruritic behaviors were completed for each dog, combining data from each four-hour period over which observations were made. For individual dogs, correlations between video- and monitor-recorded behaviors ranged from 0.48 (an outlier dog that was compulsively paw-licking) to 0.91. Overall mean correlation was 0.70 ($P \leq 0.0001$), or 0.75 adjusting data from the outlier dog. No fleas were detected in any SMO-treated dog, while control dogs remained infested (efficacy 100%). Relative to controls, based on analysis of video observations, in the SMO group there were significant reductions in mean pruritic behavior duration, overall ($P = 0.0242$) (54.4%) and on Day 27 ($P = 0.0031$) (66.2%). SMO reduces or eliminates the reaction of sensitive dogs to flea bites arising from challenges through the month following treatment. VetraX appears to be suitable for assessing pruritic responses to pulicidal treatment of flea-bite sensitive dogs.

OA44.03 Out-of-Africa, Human-Mediated Dispersal of the Common Cat Flea, *Ctenocephalides felis*: The Hitchhiker's Guide to World Domination

Prof. Jan Slapeta¹

¹*Sydney School of Veterinary Science, University of Sydney, Australia*

The cat flea (*Ctenocephalides felis*) is the most common parasite of domestic cats and dogs worldwide. Due to the morphological ambiguity of *C. felis* and a lack of — particularly largescale — phylogenetic data, we do not know whether global *C.*

felis populations are morphologically and genetically conserved, or whether human-mediated migration of domestic cats and dogs has resulted in homogenous global populations. To determine the origin of the species and to investigate the potential drivers behind the establishment of regional cat flea populations, we characterised a global collection of fleas from cats and dogs across six continents. Using a multigene approach combining two mitochondrial (*cox1* and *cox2*) and two nuclear (Histone H3 and EF-1a) gene markers, as well as a *cox1* survey of 516 fleas across 56 countries, we demonstrate out-of-Africa origins for the genus *Ctenocephalides* and high levels of genetic diversity within *C. felis*. We define four bioclimatically limited *C. felis* clusters (Temperate, Tropical I, Tropical II and African) using maximum entropy modelling. This study defines the global distribution, African origin and phylogenetic relationships of global *Ctenocephalides* fleas, whilst resolving the taxonomy of the *C. felis* subspecies and related taxa. This study reveals the drivers behind the establishment and success of the cat flea as a global parasite. Through synanthropic host hitchhiking, the cat flea is successfully achieving global dominance.

OA44.04 Ectoparasite Control on Pets: Who Did It, With What and Where? A Comparison Between Pet Owners from Two High-Risk Lyme Regions in the United States

Dr. Gebbiena Bron¹, Dr. Maria del Pilar Fernandez², Dr. Maria Diuk-Wasser², Dr. Susan Paskewitz¹, Dr. Lyric Bartholomay¹

¹*University Of Wisconsin - Madison, United States*, ²*Columbia University, United States*

Many pet owners know that outdoor pets are the proverbial canary in a coal mine for tick activity. Dogs and cats readily pick up ticks, which inspires owners to seek ectoparasite control measures for their pet. However, the initiation and adherence to recommended ectoparasitic treatment frequencies can be dependent on the owner's perceived risk of disease. Lyme disease (LD) and black-legged ticks (*Ixodes scapularis*, also known as deer ticks) are highly abundant in the United States Northeast (NE) and Midwest (MW).

As part of a broader human behavioral study on tick encounters, we asked participants about pet-tick encounters and details on pet tick control, using a smartphone application as a survey tool (The Tick App). We aimed to understand if there are differences in the implementation of tick control on pets between the MW and NE, and if controls increase with the owner's personal protective measures? The majority of dog owners treated their dog at least once during the summer (91.4% of 574 respondents). There was no significant difference in treatment frequencies (never, once, every 2-3 months, every 4-6 weeks, more frequent than every 4 weeks) between the NE and MW; about half of the dog owners treated their dog every 4-6 weeks (51.7%). Interestingly, this included 17 dog owners who used a product with a recommended use frequency of every 8-12 weeks, implying overuse of the product. Dog owners who reported more tick prevention measures for themselves were also treating their dogs more frequently, and dog owners were also more likely to report daily tick checks of their pet if they had found a tick on their pet(s) the previous summer. In summary, the implementation of tick checks and ectoparasite control on pets was correlated to the owner's experience with prevention strategies and finding ticks on pets.

OA44.05 Pathogens in Fleas Collected from Cats and Dogs: Distribution and Prevalence in the UK

Dr. Swaid Abdullah¹, Dr. Chris Helps², Prof. Séverine Tasker², Ms. Hannah Newbury³, Prof. Richard Wall²

¹The University of Queensland, Australia,

²University of Bristol, United Kingdom, ³MSD Animal Health, United Kingdom

Fleas (Siphonaptera) are the most clinically important ectoparasites of dogs and cats. Rising levels of pet ownership, climate change and globalisation are increasing the importance of understanding the endemicity and prevalence of flea-borne pathogens. The study recruited veterinary practices around the UK, asked to follow a standardised flea inspection protocol on a randomised selection of cats and dogs. A total of 326 practices

participated and 812 cats and 662 dogs were examined. Overall, 28.1% of cats and 14.4% of dogs were flea infested. More than 90% of the fleas on both cats and dogs were *Ctenocephalides felis felis*. Fleas of the same species from each infested host were pooled. DNA was amplified from 470 of the pooled flea samples using conventional PCR, 66 of which (14% $\pm$ 95% CI 3.14%) were positive for at least one pathogen. Fifty-three (11.3% $\pm$ 95% CI 2.85%) of the pooled flea DNA samples were positive for *Bartonella* spp., 35 were from cats and 4 from dogs. Seventeen of the *Bartonella* spp. samples were found to be *Bartonella henselae*, 27 were *Bartonella clarridgeiae*, 4 samples were *Bartonella alsatica* and one was *Bartonella grahamii*; 4 samples could not be identified. Fourteen (3% $\pm$ 95% CI 1.53%) of the flea DNA samples were found to be positive for *Dipylidium caninum*, 10 of which were collected from cats and one from a dog, the other 3 positive flea samples had no host species record. Only 3 flea samples were positive for *Mycoplasma haemofelis* or *Mycoplasma haemocanis*; 2 were collected from cats and one had no host species record. Three flea samples had were positive for more than one pathogen. This study highlights the need for ongoing flea control, particularly given the relatively high prevalence of *Bartonella* spp., which is of concern for both animal welfare and human health.

OA44.06 Tick Biting Trends on Pets Revealed by Crowdsourced Data: Some Surprises and Implications for Prevention

Heather Kopsco¹, Steven Engborg¹, Roland Duhaime¹, Dr. Thomas Mather¹

¹University Of Rhode Island Center for Vector-borne Disease & Tick Encounter Resource Center, United States

Citizen science is increasingly utilized to track important vectors of human and companion animal disease, providing a scalable, cost-effective, and highly-sensitive strategy for identifying new foci, changing phenology, and associated disease impacts across wide geographies. We describe a digital tick surveillance program that provides photograph-based tick identification and public health messaging services free to

the public and share important insights into pet tick encounters and pressing tick bite prevention needs.

From 2014-2016, the University of Rhode Island's TickSpotters program received 17,906 tick photograph submissions from all 50 U.S. states and Canadian Provinces. More than half (55.5%) were from Lyme endemic states, but submissions where pet was identified as host were more evenly distributed between endemic (49.2%) and non-endemic (50.1%) states. Pets were identified as hosts in 23% of all submissions while 10% were ticks found loose and wandering on humans, pets, or in the home. *Ixodes scapularis* (43.7%) were the most common species found attached to pets, followed by *Dermacentor variabilis* (26.7%), *Amblyomma americanum* (6.6%), and *Rhipicephalus sanguineus* (5.9%). These ticks were mostly adults (88%), and ticks found on pets had a longer estimated engorgement time (median three days) compared to humans (median one day). Ticks were spotted year-round; during spring and summer, ticks from pet hosts made up 20.1% and 16.8% of all submissions, respectively, while pet host submissions increased to 34.8 and 39.1% during autumn and winter, respectively.

Crowdsourced data reveal that mostly adult ticks are spotted on pets, and that they are detected later in the blood-feeding process than humans, putting pets at heightened risk for tick-borne disease transmission. The doubling of reports of ticks found on pets during autumn and winter may reveal a critical knowledge gap regarding seasonal activity of *Ixodes scapularis*, vector of Lyme disease, providing an opportunity for prevention-education to pet owners.

OA45 Ruminant Trematodes II

July 10, 2019, 16:30 – 18:00

Breakout Room 5, Meeting Rooms KLOP, Level 4

OA45.01 Is There An Interplay Between *Fasciola hepatica* and MAP Infection in Cattle?

Ms. Amalia Naranjo Lucena¹, Laura Garza-Cuartero¹, Dr. Conor McAloon², Dr. Alan Wolfe¹, Dr. Guy McGrath³, Dr. David Graham⁴, José Pérez⁵, Prof. Grace Mulcahy¹, Dr. Annetta Zintl¹
¹*Veterinary Pathobiology Section, UCD School of Veterinary Medicine, Ireland*, ²*Herd Health and Animal Husbandry Section, UCD School of Veterinary Medicine, Ireland*, ³*UCD Centre for Veterinary Epidemiology and Risk Analysis, Ireland*, ⁴*Animal Health Ireland, Ireland*, ⁵*Department of Anatomy and Comparative Pathology, Faculty of Veterinary Medicine, University of Córdoba, Spain*

Co-infection with *Fasciola hepatica* interacts with *Mycobacterium bovis* infection in cattle, altering the response to tests for the diagnosis of bovine tuberculosis and reducing bacterial load. The importance at an epidemiological scale is still unclear. We hypothesized that *F. hepatica* might also interact with the clinical course and/or epidemiology of infection with *Mycobacterium avium* paratuberculosis (MAP), the cause of Johne's disease, and used epidemiological and histopathological methods to investigate.

A database of MAP ELISA test results and *F. hepatica* liver scores reported in abattoirs from 62,447 cattle in Ireland was analyzed. A model for Johne's disease infection was developed using both herd and environmental data. We found that Johne's positive herds were less likely to be positive for *F. hepatica* infection ($\chi^2 = 13.895$, $df=1$, $P < 0.002$, $OR = 0.75$, 95% CI: 0.62–0.9). However, multivariate analysis showed that the main risk factors for Johne's disease were herd size, soil, and rainfall with *F. hepatica* co-infection ranking at position 17.

In addition, histological sections were obtained from the ileum of 17 animals with clinical Johne's disease, with and without

concurrent or recent *F. hepatica* infection. Lesions were classified, and numbers of immune cells and apoptosis quantified by immunohistochemistry. The two animals with current liver fluke infection only developed diffuse intermediate lesions, whereas animals which were either not infected with *F. hepatica* or had historic infections also developed multifocal, diffuse multibacillary and diffuse lymphocytic lesions. Moreover, there was less evidence of apoptosis in animals with concurrent fasciolosis than in animals which had no concurrent liver fluke infection ($P=0.026$).

Our results show that while *F. hepatica* infection appears not to be a major factor in the epidemiology of Johne's disease, it may affect lesion development in the intestine and hence its clinical course. Further *in vitro* studies are warranted to investigate these effects in more depth.

OA45.02 Development of a Nest-PCR for the Detection of Fasciola Hepatica in the Intermediate Snail Host, Radix Cucunorica, and the Evaluation of Transmission Risk in Northwestern China

Si-yang Huang¹

¹*College of Veterinary Medicine, Yangzhou University, China*

Fasciolosis, a foodborne zoonotic disease, caused by *Fasciola hepatica*, which is considered an important problem for human health and livestock husbandry development. Snails are intermediate hosts of *F. hepatica*, the epidemiological surveillance of snails can evaluate the transmission risk of this disease in human and livestock. The aim of this study was developing a nest-polymerase chain reaction (nest-PCR) to detect the *F. hepatica* infection in *Radix cucunorica*, a prevalent intermediate host of this parasite in northwestern China. The nest-PCR was used to amplify a 208 bp fragment of the second internal transcribed spacer (ITS-2) of *F. hepatica* with two pairs of primers, respectively. To evaluate the transmission risk of this disease, 409 snail samples collected from different areas of Gansu province China, were used to detect and analyze the

transmission risk of *F. hepatica* in this area. The method was able to detect up to 10-4 pg genomic DNA in a 25 µL PCR reaction system even with high concentrations of snail DNA, and no cross reaction being observed from the genomic DNA of *Paramphistomum cervi*, *Clonorchis sinensis*, *Orientobilharzia*, *Metorchis orientalis*, *Dicrocoelium chinensis*. Of 409 snail samples, the overall *F. hepatica* prevalence is 43.76%. However, the *F. hepatica* infection of snails was 92.75 % in the Tibetan Autonomous Prefecture of Gannan, while no snail was detected in 216 samples collected in Hui Autonomous Prefecture of Linxia. The nest-PCR was firstly used to detect the infection of *F. hepatica* in snail. It is a novel, useful and convenient method with high sensitivity and specificity. This is the first report about the epidemiological surveillance of *F. hepatica* infection in *Radix cucunorica* in northwestern China, which will help to evaluate the transmission risk of *F. hepatica* in this area.

OA45.03 Molecular and Morphological Characterization of Liver Fluke Intermediate Host Lymnaeids (Gastropoda: Lymnaeidae) Snails From Selected Regions of Okavango Delta of Botswana, Kwazulu-Natal and Mpumalanga Provinces of South Africa

Prof. Samson Mukaratirwa¹, Ms. Pulane Malatji¹, Prof. Jennifer Lamb¹

¹*University of KwaZulu-Natal, School of Life Sciences, South Africa*

The study aimed at identifying populations of Lymnaeidae snails from selected sites of the Okavango Delta (OKD) in Botswana, and sites located in the KwaZulu-Natal (KZN) and Mpumalanga provinces of South Africa using a combination of shell morphology and molecular approaches. Lymnaeidae snails were collected from 8 locations from the Okavango delta in Botswana, 9 from KZN and one from Mpumalanga provinces and were identified based on phylogenetic analysis of the internal transcribed spacer (ITS-2). Principal component analysis (PCA) was performed to assess if Lymnaeidae species determined by analysis of the ITS-2 marker can be distinguished through shell morphometry measurements. Analyses based on the ITS-2 marker identified the presence

of a well-supported Radix clade containing Radix auricularia, R. (Lymnaea) natalensis and R. rubiginosa, which were not well resolved. Experimental samples from the OKD and KZN present in this clade were referable to these species. An unidentified experimental taxon from the OKD formed a well-supported sister clade to the Radix clade, although it was not possible to identify it. Galba truncatula was well supported in a sister relationship to a well-supported Pseudosuccinea columella clade which included samples from Mpumalanga and KZN provinces of South Africa. We observed that P. columella shared the same habitats with R. natalensis and R. auricularia in KZN. Shell morphometric studies were not able to distinguish among the above-mentioned genera and species. The study further identifies the species which are likely to co-exist in the same environment and this information will be of use to those designing control programs for fasciolosis. This is the first study reporting the presence of R. auricularia in the OKD of Botswana and KZN province of South Africa.

OA45.04 Characterization of the Mitochondrial Genome Sequences of the Liver Fluke Amphimerus (Trematoda: Opisthorchiidae) From Ecuador and Phylogenetic Implications

Dr. Jun Ma¹, Dr. Jun-Jun He¹, Miss Cheng-Yan Zhou¹, Dr. Miao-Miao Sun¹, Dr. William Cevallos², Prof. Hiromu Sugiyama³, Prof. Xing-Quan Zhu¹, Prof. Manuel Calvopiña⁴

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Amphimerus Barker, 1911 is a liver fluke infecting several animal species and humans. Being a digenetic trematode of the Opisthorchiidae family, Amphimerus is closely

related to the genera Metorchis, Clonorchis and Opisthorchis. Recently, a high prevalence of Amphimerus infection in humans, cats, and dogs had been demonstrated in a tropical Pacific region of Ecuador. Hence, we determined and characterized the entire mt genome sequences of adult liver flukes, morphologically identified as Amphimerus, collected in the endemic region of Ecuador, and examined its phylogenetic relationships with flukes in the Opisthorchiidae family using Bayesian inference (BI) based on the concatenated amino acid sequences and partial cox1 sequences. The complete mt genome sequence (15,151 bp in length) of the Amphimerus sp. contains 35 genes, including 12 protein-coding genes (PCGs, without atp8), two rRNAs (rrnL and rrnS) and 21 tRNAs, lacking trnG. The gene content and arrangement of the Amphimerus sp. mt genome was similar to those of other trematodes in the Opisthorchiidae family. Genetic distances between Amphimerus sp. and other genera in Opisthorchiidae were rather high, ranging 26.86% to 28.75% at nucleotide level and 29.37% to 31.12% at amino acid level. Phylogenetic analysis placed the Ecuadorian Amphimerus within the branch of Opisthorchiidae, but it was not gathered with flukes from Opisthorchis. Our results indicate that the disputable liver fluke Amphimerus from Ecuador does not belong to the genus Opisthorchis, and that it should be assigned under the valid genus Amphimerus. This study provided a new genetic marker for future studies on taxonomy and molecular epidemiology of Opisthorchiidae trematodes. Also, the determination of mt genome sequences of the Ecuadorian Amphimerus has implications for diagnosis, drug design, control and prevention of amphimeriasis.

OA45.05 Global Serum Proteomic Changes in Water Buffaloes Infected with Fasciola Gigantica

Dr. Jun-Jun He¹, Dr. Fu-Kai Zhang¹, Dr. Rui-Si Hu¹, Prof. Hany Elsheikha², Dr. Zhao-An Sheng³, Prof. Wei-Yu Zhang³, Dr. Wen-Bin Zheng¹, Prof. Xing-Quan Zhu¹

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Science, University of Nottingham, Sutton Bonington Campus, United Kingdom, 3College of Animal Science and Technology, Guangxi University, China

The liver fluke *Fasciola gigantica* modulates several signaling pathways in infected buffaloes to facilitate its survival and establishment of persistent infection, while the buffalo host activates immune responses to counter the infection. Isobaric Tags for Relative and Absolute Quantitation (iTRAQ)-based quantitative proteomic analysis of the serum of *F. gigantica* infected buffaloes can reveal serum protein changes that are linked with *F. gigantica* infection process. In present study, a total of 313, 459 and 399 proteins were identified; of these 92, 93 and 138 were differentially abundant proteins at 3, 42 and 70 days post infection (dpi), respectively. Differentially abundant proteins were involved in key biological processes, such as the complement system, coagulation and platelet activation, lymphocyte binding on liver epithelium and lysozyme hydrolysis. Other important markers of infections were six proteins, which were significantly upregulated in infected serum at all three time points after infection. These findings provide novel insight into the serum proteomic signature of buffaloes during the course of *F. gigantica* infection.

OA45.06 Identification of T Cell Epitopes Within Cathepsins L1 and L3 From *Fasciola Hepatica* Associated with Cell Proliferation and IFN γ Production

Dr. Lauren M. O'Neill¹, Dr. Laura Garza-Cuartero¹, Dr. Thomas Geurden², Dr. Suman M. Mahan³, Dr. John M. Hardham³, Professor John P. Dalton⁴, Prof. Grace Mulcahy¹

¹*School of Veterinary Medicine, University College Dublin, Ireland,* ²*Veterinary Medicine Research and Development, Zoetis Inc, Belgium,* ³*Veterinary Medicine Research and Development, Zoetis Inc., United States,* ⁴*School of Biological Sciences, Medical Biology Centre, Queen's University Belfast, United Kingdom*

Fasciola hepatica, the common liver fluke, has a geographically-widespread distribution, resulting in significant economic losses in

ruminants worldwide. With the emergence of resistance to anthelmintic treatments, efforts have focused on the development of a safe and effective vaccine against this pathogen. Various studies have explored the use of many different antigens from this parasite within a range of hosts. Although promising results have been obtained, further efforts to increase the level and consistency of protection would be useful. Furthermore, it is proposed that the parasite may use decoy epitopes in an effort to misdirect the host immune response. We hypothesised that lymph node samples from vaccinated and infected animals could be used to identify protective and non-protective T cell epitopes within antigens of interest, with the ultimate aim of developing smart designer vaccines to protect ruminants against fasciolosis.

Lymph node cells were isolated from non-vaccinated infected cattle and from vaccinated infected cattle. These cells were stimulated with overlapping peptides from two antigens, FhCL1 and FhCL3 that have been shown to be useful in vaccines. Cell proliferation was measured using the BrdU assay and IFN γ production quantified by ELISA. Cattle vaccinated with FhCL1 and FhCL3 demonstrated higher levels of cell proliferation than cattle vaccinated with FhCL1 alone while, in general, vaccinated cattle demonstrated higher cell proliferation than control non-vaccinated infected cattle. IFN γ production was found to be stimulated by a region within the signal peptide sequence of CL1 (amino acids 3-15) along with the region within the active enzyme (amino acids 200-280) which has also been shown to be a B cell epitope. This work demonstrates the potential of sub-unit vaccines to target T-cell responses towards specific T-cell epitopes and this, together with identification of protective B-cell epitopes, could aid in the development of protective effective anti-fluke vaccines.

OA46 Gastrointestinal Protozoa in Ruminants

July 11, 2019, 8:30 - 10:30

Plenary Hall, Madison Ballroom (ABCD), Level 1

OA46.01 Comparing Different Metabarcoding Approaches to Investigate Eimeria Species Diversity and Community Structure in Cattle

Dr. Libby Redman¹, Dr. Berit Bangoura², Dr. Bruna Palmeira¹, Ms. Nicolette Shaw³, Ms. Jill De Rijke³, Ms. Rebecca Chen¹, Dr. Matt Workentine¹, Prof. John Gilleard¹

¹Faculty of Veterinary Medicine University of Calgary, Canada, ²Department of Veterinary Sciences, University of Wyoming, United States, ³University of Waterloo, Canada

Bovine coccidiosis, caused by protozoal parasites of the genus *Eimeria*, is an important cause of clinical disease and sub-clinical production loss in cattle with control being largely dependent on the routine administration of anticoccidial drugs. Whilst *E. zurneii* and *E. bovis* are considered to be the most important, over 20 different species of *Eimeria* have been described in cattle and relatively little is known regarding their pathogenesis, prevalence, drug sensitivity or interactions with other pathogens. We have undertaken a metabarcoding approach to explore bovine *Eimeria* species diversity and community structure. Paired-end short read sequencing (Illumina Miseq) was used to target three variable regions of the SSU 18S rRNA coding sequence as well as the ITS-1 rDNA region. The full length SSU 18S rRNA coding sequence was also targeted with long-read sequencing (PacBio Sequel SMRT). The different approaches were compared to evaluate their ability to successfully estimate the species proportions on a variety of different samples. These included mock communities of known *Eimeria* species proportions and field samples from beef and dairy cattle across Western Canada in which ~100 sporulated oocysts had been morphologically identified per sample. The species proportions determined by metabarcoding were broadly similar to the morphological data but differences between the various methodologies occurred. The

strengths and weaknesses between the different loci and between short- and long-read metabarcoding approaches is discussed.

OA46.02 A New Real-Time PCR Method for Identification of Two Pathogenic Eimeria Species in Sheep, *E. ovinoidalis* and *E. Crandallis*

Lea Bordes¹, Dr. Christelle Grisez², Francoise Prevot², Didier Marcon³, Philippe Jacquet²
¹UMR INRA/ENVIT 1225 IHAP, France, ²National Veterinary School of Toulouse, ENVIT, France, ³Domaine expérimental Bourges-La Sapinière INRA, France

Coccidiosis in lambs, caused by intestinal parasites of the *Eimeria* genus, has high medical and economic impacts throughout the world. Diagnosis of lamb coccidiosis is currently performed in laboratory by microscopic examinations of fecal material but this method is time-consuming. Moreover, the species identification, based on microscopy, requires experienced personnel for accurate identification as oocysts of many different species are difficult to distinguish morphologically. As an alternative to morphological approach, a real-time Polymerase Chain Reaction using Taqman probes has been developed to quantify all *Eimeria* oocysts in the sample and to identify and quantify more specifically oocysts of *Eimeria ovinoidalis* and *Eimeria crandallis*, the species associated with clinical ovine coccidiosis. As available genomic data are scarce, a strategy of sequencing was performed on different *Eimeria* spp oocysts, isolated by micromanipulation. Sequences of 18S were used to designing primers and probes for three PCR test: a Pan-*Eimeria* test for estimate the total number of oocysts, a *Eimeria crandallis* test and a *Eimeria ovinoidalis* test for the amount of oocysts of each pathogenic species present in a fecal sample. The Locked Nucleic Acid technology was used to increase the specificity of the PCR reactions. Thanks to a modified pUC57 plasmid from *Escherichia coli* and an estimation of the average of gene's copy number within and between species, the standard curve accurately quantifies all oocysts in the sample and the proportion of

each pathogenic species. This new diagnostic tool could be used in sheep farms for monitoring ovine coccidiosis and for an early detection of pathogenic species.

OA46.03 *Cryptosporidium Parvum* gp45 Protein Is a Promising Vaccine Candidate to Prevent Bovine Cryptosporidiosis

Dr. Karine Sonzogni-Desautels^{1,2,3}, Dr. Timothy Geary³, Dr. Momar Ndao^{1,2,4}

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Bovine cryptosporidiosis is a scourge in the dairy industry because newborn dairy calves can get infected as soon as they are born and can die during the first two weeks of age due to *Cryptosporidium parvum*-related severe diarrhea and dehydration. Our goal is to immunize pregnant cows to transmit neutralizing antibodies through colostrum to the newborn calf. We examined several candidates for immunization and selected *C. parvum* gp45 as the most promising antigen. We established by liquid chromatography/tandem mass spectrometry that *C. parvum* gp45 is secreted during excystation of sporozoites from oocysts. Confocal microscopy confirmed that gp45 is expressed by *C. parvum* during excystation, most likely to promote parasite adhesion to host intestinal cells. We are currently investigating the ability of recombinant *C. parvum* gp45 to bind intestinal cells in vitro. *C. parvum*-infected interferon gamma receptor knock-out (IFNgammaR-KO) mice present clinical signs of cryptosporidiosis similar to those of *Cryptosporidium* spp.-infected newborn calves. Adult IFNgammaR-KO mice were immunized with recombinant gp45 and challenged with *C. parvum* and vaccination reduced intestinal parasite burden by up to 80%. To determine if neutralizing antibodies against *C. parvum* gp45 can be transmitted by colostrum, we immunized female IFNgammaR-KO mice twice and bred them after the last immunization. Colostrum extracted from the stomach of the newborn

pups is analysed by ELISA. The presence of anti-gp45 antibodies in the mouse colostrum would support the possibility to passively immunize newborn calves against cryptosporidiosis. Preliminary data suggest that *C. parvum* gp45 is a promising vaccine candidate and support further investigation in a cow/calf model of infection.

OA46.04 New Leads and Drug Targets in the Zoonotic *Cryptosporidium Parvum* by Phenotypic Drug Screening

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Among the 26 or more *Cryptosporidium* species, *C. parvum* is the major zoonotic agent infecting humans and other mammals. Currently, there is a lack of fully effective drugs to treat cryptosporidiosis in both humans and animals. In the anti-*Cryptosporidium* drug discovery campaign, we developed an in vitro assay suitable for high-throughput screening of anti-cryptosporidial activity. Using this assay, we have screened 1200 existing drugs and 800 natural products with defined molecular structures, and discovered a number of hits or leads with excellent efficacy against the growth of *C. parvum* in vitro (i.e., low nM to sub- μ M EC50 values) and large safety intervals (i.e., much higher cytotoxicity TC50 values than EC50 values). These hits and leads are serving as templates for developing more efficacious and safer compounds. Additionally, the phenotypic screening data also revealed and confirmed several drug targets in *Cryptosporidium*. Some of the targets are being characterized and explored for target-based drug discovery against cryptosporidiosis. These findings provide basis for developing more selective and potent inhibitors, and for studying the mode/mechanism of action of leads on the parasite.

OA46.05 Cryptosporidium Parvum Animal Model in Neonatal Calves

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A *Cryptosporidium parvum* model was set up for in future efficacy studies of veterinary products. Nine calves were included from which six were inoculated and three were negative controls. The calves were orally inoculated with 10^6 (one animal 10^7) commercially available viable oocysts at day of enrollment. Health parameters were scored twice a day and feces was sampled once a day (mixed). Calves were weighted once a week. Health parameters included: overall clinical appearance, body temperature, feed (milk) intake and signs of dehydration. In daily feces, consistency was scored on a 3 point scale, and fecal volume, fecal dry matter content and fecal oocyst count were determined.

All calves from the inoculated group shed *C. parvum* oocysts and no control calves shed *C. parvum* oocysts. When estimated with PCR, shedding started 3 to 5 days after inoculation and stopped between 14 and 17 days after inoculation. All calves showed signs of diarrhea, both clinically and estimated by dry matter content. Based on dry matter content, the duration of diarrhea differed between the calves from 1 to 10 days. When a score of 2.5 per day was regarded as diarrhea, all inoculated calves but no control calves experienced diarrhea for more than three days. All inoculated calves showed some signs of general clinical illness, ranging from mild in some calves to more severe in others. With this model, generating shedding of *C. parvum* and diarrhea in all calves while evoking only moderate symptoms of general illness, we have a model that is suitable for testing Investigational Veterinary Products.

OA46.06 Evaluating the “Protobiome”: A Novel Diagnostic Tool for the Sustainable Control of Protozoan Parasite Communities in Ruminants

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Piroplasmosis is caused by tick-borne haemoprotozoa of the genera *Theileria* and *Babesia*, that impose a serious impact on animal production and human health. While multiple haemoprotozoan species can infect a single host, there is a lack of reliable molecular diagnostic tools with which to understand the composition of these complex parasite communities. Haemoprotozoa vary in their epidemiology, drug sensitivity, pathogenicity and interaction of co-infected species in the host, while common features include that animals become the persistent carriers of these pathogens after primary recovery from infection and can play a significant roles as a reservoir hosts. In the present study, we developed for the first time, the use of deep amplicon sequencing using an Illumina Mi-seq platform to identify haemoprotozoan communities and to establish the concept of a “protobiome”. Haemoprotozoa of ruminants were used to develop the concept, because mixed species infections are common. First, four phenotypically verified species of *Theileria* and *Babesia* were used to prepare mock pools with random number of parasites, and amplified with four different numbers of PCR cycles to assess the species representation bias. Second, we evaluated the threshold of the deep amplicon sequencing assay for each of the four species present at different levels of parasitemia and to confirm the accurate relative quantification of all four species. Finally, we applied the assay to the field samples to afford insight of the species composition of haemoprotozoan communities in small and large ruminants in the Punjab province of Pakistan. The “protobiome” concept has a wide range of potential applications in veterinary and human research including responses to drug

treatment, parasite epidemiology/ecology, parasite interactions during mixed infections and parasite control strategies.

OA46.07 Cryptosporidiosis in Pre-Weaned Graded Murrah Buffalo Calves of Coastal Districts of Andhra Pradesh State, India

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Cryptosporidium is an obligate intestinal coccidian parasite, which infects a wide range of hosts. In the present study, Cryptosporidium in pre-weaned buffalo calves of coastal districts (Guntur, Krishna, West Godavari and East Godavari) (Krishna-Godavari river basin) of Andhra Pradesh was studied. Dung samples (n=505) from pre-weaned buffalo calves under one month old were screened for Cryptosporidium infection. In modified cold strong Ziehl-Neelsen (mZN) staining technique 54 (10.69 %) dung samples were found positive for Cryptosporidium oocysts, whereas in FEA sedimentation coupled mZN staining method detected 51 (12.14 %) positives. Randomly selected 267 dung samples were subjected to nested PCR for detection of Cryptosporidium and 71 (26.59 %) positives were recorded. Based on the results of mZN staining method, the prevalence of Cryptosporidium infection was higher (15.30 %) in the Godavari river basin (West Godavari and East Godavari districts) and lowest in the Krishna river basin (Guntur and Krishna districts) (4.91 %). Nested PCR detected 14.91 (17/114) and 35.29 percent (54/153) of Cryptosporidium infection in Krishna and Godavari river basins, respectively. DNA sequence analysis revealed that, the majority of the buffalo calves has *C. bovis* infection, followed by *C. ryanae* and *C. parvum* infection. The zoonotic species, *C. parvum* was recorded both from the Krishna river basin and the Godavari basin areas of the Andhra Pradesh state. In conclusion, this is the first report on the Cryptosporidium in pre-weaned buffalo calves of the Krishna-Godavari river basin region of Andhra Pradesh state.

OA47 Wildlife Helminths

July 11, 2019, 8:30 - 10:30
Breakout Room 2, Hall of Ideas E&H, Level 4

OA47.01 Occurrence, Prevalence and Intensity of Internal Parasite Infections of African Lions (*Panthera Leo*) in Enclosures at a Recreation Park in Zimbabwe

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A coprological survey was conducted to determine the types, prevalence, and intensity of infection of internal parasites in a population of captive African lions at a recreational game park in Zimbabwe. Fecal samples were collected over a 4-month period from each of 30 lions (55%) out of 55 animals held. The samples were examined using flotation and sedimentation techniques as well as larvae identification. The overall prevalence of helminth infections was 100% (30/30), and 80% (24/30) of fecal samples also were positive for protozoan parasite forms. Eggs of *Ancylostoma* spp. were found in the feces of 23 (76.7%) lions, *Physaloptera* sp. in 14 (46.7%) lions, *Toxascaris leonina* in 13 (43.3%) lions, *Toxocara cati* in 12 (40%) lions, and *Gnathostoma spinigerum* and *Toxocara canis* in 2 (6.7%) lions. Furthermore, eggs of *Cylicospirura subequalis*, *Gnathostoma* spp., *Lagochilascaris major*, *Acanthocephalan*, *Linguatula* spp. and larvae of *Aelurostrongylus* spp. were identified in the feces of one lion. Oocysts of five apicomplexan parasites and cysts of one mastigophoran protozoan parasite were recorded, namely, *Cystoisospora leonina* in 11 (36.7%) lions, *Cystoisospora* spp. in 9 (30.0%) lions, *Cystoisospora felis* in 5 (16.7%) lions; *Toxoplasma*-like spp. in 5 (16.7 %) lions, and *Giardia* spp. in 8 (26.7%) lions. The majority of lions (28/30) showed mixed infections with different internal parasite. *Eimeria* spp. oocysts, identified were spurious and probably originated from prey species. Among the parasites identified were some of zoonotic importance that have health implications for at-risk personnel and visitors who get into contact with the animals.

OA47.02 Clinical Crenosomosis Lungworm Infection in a Black Bear (*Ursus Americanus*)

Ms. Haifaa Mahjoub¹, Nicole Murphy¹, Dr. Paula-Marie Mather², Dr. Spencer Greenwood¹, Dr. Gary Conboy¹

¹University of Prince Edward Island, Canada,

²Maritime Animal Hospital, Canada

An orphaned 9-month-old underweight black bear was evaluated at a wildlife rehabilitation facility for chronic cough and wheezing. Fecal samples were submitted to the AVC Diagnostic Services for parasitological examination for detection of GI helminths. Baermann examination was performed due to the detection of first-stage nematode larvae (L1) on centrifugal flotation; large numbers (>8000 L1/gm) of L1 were recovered and identified as *Crenosoma* spp. based on morphology. Three species have been reported from black bears (*Crenosoma petrowi*, *Crenosoma potos*, *Crenosoma vulpis*). Based on larval length measurements, the L1 were tentatively identified as *C. petrowi*. The length of L1 ranged from 253.4 to 276.7 µm. Further molecular characterization using Polymerase Chain Reaction (PCR) and DNA sequencing of the small subunit (SSU) rRNA gene and two regions of the large subunit (LSU) rRNA gene did not match any submissions in GenBank, but were most similar to *Crenosoma mephitis*. The only three sequences available from the GenBank database are *Crenosoma vulpis*, *Crenosoma mephitis*, and *Crenosoma striatum*. There is a paucity of molecular data for members of the genus *Crenosoma*. Unfortunately, no sequence data on *Crenosoma petrowi* or *Crenosoma potos* are currently available in GenBank. Therefore, lungs were obtained from black bears during the following spring hunting season. Analysis of partial SSU and LSU rRNA gene sequences from adult-male *C. petrowi* recovered from the black bear was identical to that obtained from the bear cub L1 confirming the morphological identification as *C. petrowi*. Following treatment with Panacur® (fenbendazole), clinical signs resolved. This is the first report of clinical chronic respiratory disease due to *Crenosoma* infection in a black bear.

OA47.03 Fecal Examinations to Inform Deworming Protocols of Raccoons (*Procyon lotor*) Entering a Rehabilitation Center in Upstate New York

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A local veterinarian working in collaboration with a local wildlife rehabber in Upstate New York state, was interested in developing a tailored deworming protocol for raccoons (*Procyon lotor*) entering the facility. To determine the presence, identity and prevalence of parasitic infections, fecal samples from raccoons under his care were examined over a 1-year period (September 2017 through September 2018). A total of 37 samples were collected from raccoons of various ages, sometimes housed as litters. Fecal samples were stored under refrigeration, and processed within 7 days of collection. Each sample was examined by 1.18 spg zinc sulfate and 1.3 spg sugar centrifugal flotation. Parasites were detected in 30 of the 37 fecal samples processed. These infections consisted of three genera of protists and at least four genera of nematodes and one trematode; no cestode infections were diagnosed. Single parasite infections were diagnosed in 18 samples, while 10 had 2 parasites present, and 2 samples had more than 2 parasites detected (3 and 5, respectively). Protistan infections were the most common, followed by nematode, and trematode infections. The most common parasite detected was *Cryptosporidium*, and the most common co-infection was with lungworms. Details of the findings of the investigation and potential contributing factors will be presented and discussed.

OA47.04 Occurrence of a Zoonotic Tapeworm, *Taenia Crassiceps* (Zeder, 1800) Rudolphi, 1810 in a Muskrat (*Ondatra Zibethicus*) in New York State, USA

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Taenia crassiceps is a widely distributed parasite across the Holarctic, cycling between canine and rodents as definitive and intermediate hosts, respectively. In contrast to other *Taenia* species, *T. crassiceps* has proliferative cysticercus that develops asexually by budding, thus ingestion of one or a few eggs can result in massive infection. In this report, we describe a case of cysticercosis attributable to the taeniid cestode *T. crassiceps* in the carcass of an adult male muskrat from New York State. Gross inspection revealed numerous round to square pearly white soft structures (1-2 mm in diameter) present on the serosal surface of all abdominal viscera and the peritoneal surface of the diaphragm. These structures were identified as taeniid cysticerci and based on the characteristic rostellar hook morphology were confirmed as that of *Taenia crassiceps*. Histopathological examination revealed cysticerci with distinct rostellar hooks, lateral suckers, and numerous calcareous corpuscles. Remnants of hooks associated with small mineral deposits were occasionally observed in the adipose tissue of the retroperitoneal space. Molecular investigations with PCR amplification using a universal primer set targeting a partial sequence of mitochondrial cytochrome c oxidase subunit 1 (COX1) gene of taeniid yielded amplicons which were Sanger sequenced and blasted to confirm *T. crassiceps*. This reported case emphasize the zoonotic threat pose by *T. crassiceps* in New York State and the need for continued surveillance in domestic dogs to reduce the risks to humans.

OA47.05 Investigating the Potential Impact of *Heterobilharzia Americana* on Endangered Florida Panthers (*Puma Concolor Coryi*)

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Dr. Bambi Clemons³, Dr. Mark W.
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Heterobilharzia americana is a trematode parasite of mammalian wildlife in the southern United States that is related to the human schistosome, *Schistosoma mansoni*. The most common definitive host is the raccoon (*Procyon lotor*), but it is found in many other hosts such as coyote (*Canis latrans*), opossum (*Didelphis virginiana*), nutria (*Myocastor coypus*) and less frequently in dogs or other domestic animals.

In the mammalian host, adult worms live in the mesenteric veins, and eggs migrate through the intestinal wall to be excreted in feces. Eggs can enter the circulation and become trapped in other organs often causing granulomatous inflammation. The impact of this infection in dogs can range from asymptomatic to fatal, and clinical findings may include hemorrhagic enteritis, diarrhea, weight loss, hepatic disease, and hypercalcemia.

In a historical report, *H. americana* was found in a small number of Florida panthers (*Puma concolor coryi*), an endangered subspecies of puma restricted to a small geographic area in Florida, USA. In this study, archived tissues collected opportunistically from 18 panthers (12 male, 6 female, age range 4 months-4 years, median age 2 years) necropsied between 2004-2016 were evaluated histologically for *H. americana* infection. Sections of small intestine and liver were examined for all 18 animals, and additional tissues including pancreas, lung, and kidney were examined in some cases. Despite poor fixation and advanced autolysis of many tissues, trematode eggs were observed in the small intestine of 5 animals, and also in the liver of one of those. Eggs were most commonly observed in the intestinal mucosa and were not associated with inflammation in most cases. Molecular techniques were attempted to confirm the identity of parasites in selected tissues from multiple animals. To preserve this endangered population, it is vitally important to determine the impact of infectious diseases such as *H. americana*.

OA47.06 Mammomonogamus N. Sp. From the Nasal Cavity of River Otter (*Lontra Canadensis*) in Canada

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The nasal cavities of 308 river otter (*Lutra canadensis*) obtained as a by-product after pelting from trappers in Eastern Canada and the Maritimes were examined for helminth infection. The trematode, *Clinostomum marginatum* was found in 4.2% (13/308) of the otter with a mean infection intensity of 2.4 flukes/infected animal (range = 1-16 flukes). A nematode identified as a species of *Mammomonogamus* (Family Syngamidae; Subfamily Syngaminae) was recovered from 8.8% (27/308) of the otter. The mean infection intensity was 6.6 worms/infected animal with a range of 1-22 worms recovered. In most instances adult female worms were recovered with male worms attached (=“Y” worms). Female worms were 11-22 mm in length (Mn=15.4 mm) and red in color; male worms were 2-5 mm in length (Mn=3.7 mm). Adult worms had a large buccal cavity with longitudinal ribs visible on the inner wall. Eggs in the uterus of the female lacked opercula and the rays in the male genital bursa were rounded. All previously described species of *Mammomonogamus* have been reported from tropical or subtropical regions of the world infecting the larynx, nasal cavities and middle ear in cervids, felids, ruminants and the elephant. Endemic *Mammomonogamus* infection has not been reported in river otter or in a temperate region previously.

OA47.07 The Unseen Side of a Bio-Invasion: A Unique Spatiotemporal Study of Infection Dynamics in a Natural Large-Scale Perturbation Experiment

Peter Stuart¹, Karen Loxton², Nathalie Charbonnel³, Maxime Galan³, Heikki Henttonen⁴, Joeseeph Jackson⁵, Colin Lawton⁶, Celia Holland¹
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The introduction of the bank vole (*Myodes glareolus*) to Ireland in the 1920's presents a unique opportunity to investigate how bio-invasions alter the parasitic dynamics of invasive and native species. Such models are required due to the association between translocations of hosts and parasites and emerging infectious diseases. Bank voles and native woodmice (*Apodemus sylvaticus*) were trapped in Ireland at the point of introduction of the vole, at the expansion front and in areas where only woodmice are found. Parasite surveys were carried out and 16SrRNA sequencing was used to detect major genera of pathogenic bacteria. Results demonstrate that the influence of the bio-invasion on pathogen dynamics is dependent on where the animals are located across the expansion range. Overall voles trapped in Ireland have less helminth parasites than those elsewhere in Europe. Also at the expansion front they have significantly less parasites than at the core population. Helminth parasites of the native woodmice are also diluted, with a significant decrease in Brillouin's Index of Diversity, where the vole has been established longest. Metagenomic results show voles and mice share pathogenic bacteria in their endemic range (France) and the pathobiome of mice from France and Ireland is dominated by the same pathogenic bacteria. However voles in Ireland have a unique pathobiome to that of voles in their endemic range. This unique pathobiome may be related to the changes in the host parasite community.

OA47.08 The Role of Wildlife in Parasitic Zoonoses: The Case of Philippine Wildlife

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Wildlife ecosystem has been subjected to environmental and anthropogenic impacts such as deforestation and climate change. Many wildlife species are being displaced and unable to adapt to environmental alterations leading to biodiversity loss in areas of urban development. However, some wild animals are attracted to human habitations due to abundant food supply and presence of structures where animals could shelter. Macaques are common sights in some urban,

agriculture, and tourist areas in South East Asia, including the Philippines. The same is true for rodents, wild cats, wild pigs, and other wild animals in some residential, agriculture and agroforestry areas in the country. Unfortunately, some of these wildlife species are also hosts for a number of parasites of public health and veterinary importance. Due to the complexity of many parasitic life cycles involving several host species, the interactions between wild animals, domestic animals and humans could enhance transmission of these parasites. Our recent studies confirm the presence of five simian *Plasmodium* spp. in macaques, eight parasite species in Palawan native leopard cats, 12 helminth parasite species in wild rats, and 15 parasite species in wild native pigs. In addition, hemoparasites of birds and reptiles were recorded. Moreover risk factors of zoonotic transmission due to animal-human conflicts will be discussed. Since more than 75% of human diseases are of zoonotic origin, it is important to understand the dynamics of wildlife, domestic animal, and human nexus, as well as conduct more interdisciplinary researches, to include socio-economic factors, for control strategies of zoonotic transmissions.

OA48 Equine Ascarids

July 11, 2019, 8:30 - 10:30
Breakout Room 3, Hall of Ideas F&I, Level 4

OA48.01 Long Live the Worms: In Vitro Maintenance for Intestinal Stages of *Parascaris* Spp. And a Method for Assessing Viability

Jessica Scare¹, Dr. Ashley Steuer¹, Dr. Carrie Shaffer¹, Dr. Paul Slusarewicz², Dr. Angela Mousley³, Dr. Martin Nielsen¹

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Parascaris spp. is a clinically important parasite infecting young horses and has widespread anthelmintic resistance. Maintenance of helminth parasites in vitro facilitates the elucidation of numerous processes, such as

metabolism, interactions with host immune cells, and xenobiotic defense mechanisms. Numerous studies report the maintenance of adult *Ascaris suum* employing a variety of nutrients, media, and environmental conditions. However, no studies have determined the requirements of the equine ascarid, *Parascaris* spp. Helminth longevity is traditionally assessed on an alive/dead basis, and some reports subjectively assessed viability. Currently, none of the published methods allow for a viability assessment of adult *Parascaris* spp. while accommodating their large size. The aims of this study was to establish the in vitro requirements of *Parascaris* spp., and to develop an objective viability assessment method. In total, 1045 worms were maintained over 212 cultures. Worm longevity and viability was assessed. Worms obtained from naturally infected foals at necropsy were maintained in culture flasks containing 200 mL of culture media. Various media, nutrients, and environmental conditions were examined. A scoring system (0-7) based on muscle tone and motility was used to assess worm viability. Worms were evaluated every 12 hours. The addition of glucose to ascaris ringers solution resulted in a significantly longer lifespan (84 hours) than any of the other added nutrient supplements, but glucose did not improve viability. Of all the media and nutrient supplements tested, worms maintained in the Roswell Park Memorial Institute-1640 medium had significantly better viability ($P < 0.0001$) and a maximum lifespan of 168 hours. The use of a platform rocker also significantly improved viability ($P = 0.0305$). The CO₂ incubator did not influence worm longevity or viability. This is the first study to examine the in vitro requirements for maintenance of *Parascaris* spp. intestinal stages and to objectively evaluate their viability over time.

OA48.02 Hatching Protocol and in Vitro Exposure to Ivermectin in *Parascaris* Univalens

Dr. Eva Tydén¹, Frida Martin¹, Sofia Jonsson¹
¹Swedish University of Agricultural Sciences, Sweden

The equine roundworm, *Parascaris univalens*

is a pathogenic parasite of foals and yearlings and infection can result in impaired growth and intestinal obstruction. Drug resistance to macrocyclic lactones is spread worldwide and in recent years resistance to pyrantel has been reported in the U.S and Sweden and to benzimidazoles in Australia. Little is known about molecular mechanisms linked to anthelmintic resistance (AR) and currently no in vitro model is available for *P. univalens*. The aim of this project is to understand the molecular mechanisms of AR by developing an in vitro hatching protocol for *P. univalens* and to study gene expression after drug exposure of the larvae. *P. univalens* eggs were isolated from foals naturally infected with the parasite. The external chitinous layer was removed from unembryonated eggs using 2% sterile sodium hypochlorite solution and incubated in 25 °C for 10 days for development of larva. Hatching was induced according to a protocol for *Toxocara canis* with slight modifications of additional three strokes in a glas homogenizer to improve number hatched larva. Hatched larva were cultured for 16 days in RPMI (containing 10% FBS 1% Penicillin and Streptomycin, 1% L-glutamine) in 37 °C with 5% CO₂. The hatching ratio of this protocol was approximately 28% and the survival rate of hatched larva 85% after 16 days. In addition, expression of genes involved in the drug metabolizing pathways were studied in hatched *P. univalens* larva after 24 h of exposure to ivermectin. Our hope is that this in vitro method will help us gain more knowledge about AR and the genes involved in different pathways responding to anthelmintic treatment in ascarid worms. The situation with development of resistance to all available drug classes is an increasing threat for the equine industry and animal welfare.

OA48.03 Expression of Drug Metabolizing Genes in *Parascaris Univalens*

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Anthelmintic resistance (AR) in the equine

roundworm *Parascaris univalens* is an increasing threat to equine welfare as resistance to all available drug classes have been found in recent years. Despite the first report of treatment failure to ivermectin 17 years ago the mechanisms for drug metabolism and drug resistance in this species are still largely unknown. The aim of this project is to gain knowledge about the molecular mechanisms of drug metabolism and resistance in *P. univalens*. We have investigated the expression of a number of candidate genes believed to be involved in the drug metabolism of *P. univalens* by qPCR. Adult *P. univalens* were obtained from two 6 month old Icelandic horses that had never been treated with anthelmintic drugs. The worms were collected at an abattoir, transported to the laboratory and in vitro exposed to ivermectin, pyrantel or thiabendazole for 24 h. RNA was then extracted from the anterior end of the worm, converted to cDNA and used as template for PCR and quantitative real time PCR.

Results show that drug metabolizing enzymes such as the phase I cytochrome P450 family, phase II glutathione S-transferases and UDP-glycosyltransferases and transport proteins such as ABC transporters are expressed. Preliminary results also show that phase I and phase II enzymes are differentially expressed after drug exposure.

This is the first time phase I and II drug metabolizing enzymes have been shown to be expressed in *P. univalens*. Results from this study will hopefully help us gain more understanding of the mechanisms of drug metabolism and AR.

OA48.04 Differential Transcriptome Analysis of *Parascaris Univalens* After In Vitro Exposure to Ivermectin, Pyrantel and Thiabendazole

Dr. Frida Martin¹, Dr. Oskar Karlsson Lindsjö¹, Dr. Matthías Eydal², Prof. Johan Höglund¹, Dr. Tomas F. Bergström¹, Dr. Eva Tydén¹

¹Swedish University of Agricultural Sciences, Sweden, ²University of Iceland, Iceland

Anthelmintic resistance in *Parascaris univalens*

is an increasing threat to equine welfare as resistance to all available drug classes have been found in recent years. Despite this fact, the genetic and molecular background of drug metabolism and resistance in *P. univalens* is still largely unknown. The aim of this study is to gain deeper knowledge about drug metabolizing genes in *P. univalens*. To achieve this a whole genome approach was applied, which is an unbiased and systematic method to identify genes involved in different pathways responding to anthelmintic treatment. We have used RNA-Seq to compare the transcriptomes of adult *P. univalens* after in vitro exposure to three different drug classes.

Adult *P. univalens* was obtained after slaughter from two 6-month old Icelandic horses that had never been treated with anthelmintic drugs. In the laboratory the worms were in vitro exposed to ivermectin, thiabendazole and pyrantel, each at three different concentrations, for 24 h. RNA was extracted from the anterior end of the parasite and three biological replicates per concentration were sequenced using Illumina NovaSeq.

Sequencing of 36 samples (27 exposed and 9 controls) generated 20,462,622 to 35,338,763 paired-end reads with a length of 100bp per sample. A mapping index was created using the previously published genome (GenBank: NINM000000000.1), transcript expression were quantified using Salmon and differential gene expression analysis was performed using R package DESeq2.

Preliminary results show that between 35 and 474 genes are significantly differentially expressed ($p < 0,05$) in response to exposure depending on drug and concentration. Ongoing analysis of KEGG pathways will provide insights about the function of these genes and should help us gain more understanding of drug metabolism in *P. univalens*.

OA48.05 Effect of Macrocyclic Lactones on *Parascaris* Sp. Glutamate-Gated Chloride Channels

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Parascaris sp. is the largest parasitic nematode of horse causing digestive and respiratory disorders to the animal. The control of equine ascaridiosis relies on anthelmintic treatments including macrocyclic lactones (MLs) as the gold standard. However, control of infestation is increasingly difficult due to the emergence of resistant parasites throughout the world. In the free-living model nematode *Caenorhabditis elegans*, glutamate-dependent chloride channel receptors (GluCl) were identified as the main targets of MLs. However, in *Parascaris* sp, the mode of action of MLs remain poorly understood. Here we identified the *Parascaris* sp. GluCl and characterized the effect of a wide range of MLs.

Using a candidate gene approach, we identified the orthologs of 6 genes encoding GluCl subunits in *Parascaris* sp. The complete cDNAs encoding these subunits were amplified by PCR and cloned into a transcription vector. The corresponding cRNAs were synthesized in vitro and then microinjected into *Xenopus laevis* oocytes. Two-electrode voltage-clamp experiments were performed on recombinant GluCl to investigate their pharmacological properties. Thus, the expression of a single subunit and combination of different subunits in *Xenopus* oocytes allowed us to obtain the functional homeric and heteromeric GluCl of *Parascaris* sp. The receptors were both sensitive to glutamate and ivermectin but the effect of seven different ML compounds revealed striking differences. The physiological function and the impact of MLs on these receptors in vivo are in progress.

This study, provides a better understanding of the pharmacology of GluCl as well as the mode of action of MLs in nematode parasites.

OA48.06 Ascarids Exposed: In Vitro Drug Exposure and Gene Expression Analysis of Anthelmintic Naïve Parascaris Spp.

Jessica Scare¹, Dr. Pouya Dini¹, Jamie Norris¹, Dr. Jianbin Wang², Dr. Ashley Steuer¹, Dr. Kirsten Scoggin¹, Holli Gravatte¹, Dr. Daniel Howe¹, Dr. Paul Slusarewicz³, Dr. Richard Davis², Dr. Martin Nielsen¹

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Ascarid parasites infect a variety of hosts, and control measures rely heavily on anthelmintics raising concerns for anthelmintic resistance. *Parascaris* spp., the equine ascarid, is the only ascarid with documented substantial drug resistance. Elucidating drug responses of susceptible parasites may lead to further understanding of anthelmintic resistance mechanisms, identifying alternative drug targets, and parameters for slowing the development of resistance among other ascarid species. The purpose of this study was to examine the response of anthelmintic naïve *Parascaris* spp. to in vitro drug exposure using transcriptomic analyses and quantitative PCR. Adult *Parascaris* spp. worms were obtained at necropsy from foals born into a herd kept without anthelmintic treatment. Adult worms were maintained in Roswell-Park Memorial medium 1640. The drugs employed were oxibendazole (OBZ) and ivermectin (IVM), and 10-fold serial dilutions were prepared. Optimum drug concentration and exposure length were determined using a worm viability assessment method. Subsequently, RNA-seq analysis identified genes with significantly different expression between drug treated and control groups. Finally, a selection of the significant genes was further investigated using qPCR in a repeat drug exposure trial. Following initial viability assessments, OBZ (10 µg/mL) for 24 hours and IVM (1 µg/mL) for three hours were the conditions employed. RNA-seq analysis revealed a total of 88 transcripts with significantly ($\alpha=0.01$) different expression between all drug treated and control worms. The top five genes selected for further analysis were snap25, cytochrome p450, kelch10, sup9, and ferm.

Gene functionalities are related to muscle contraction, detoxification and exocytotic events, and regulatory processes. qPCR did not identify any significant differences between groups, but this may be due to the lack of method sensitivity. The results of RNA-seq analysis and gene function warrant further investigation and their role in ascarid xenobiotic responses.

OA48.07 Gastrointestinal and Respiratory Nematodes in Feral Horses of Sable Island – A Living Experiment in Equine Parasite Epidemiology

Dr Emily Jenkins¹, Amber Lynn Backwell¹, Alice Liboiron¹, Julie Colpitts¹, Christina Tollett², Sarah Medill³, Jennifer Bellaw⁴, Todd Shury^{1,3}, David McRuer³, John Gilleard⁵, Jocelyn Poissant⁵, Philip McLoughlin¹
¹University Of Saskatchewan, Canada, ²Queen's University, Canada, ³Parks Canada, Canada, ⁴Gluck Equine Research Center, United States, ⁵University of Calgary, Canada

Sable Island National Park Reserve is a small sandy island located approximately 275 km east of Halifax, Nova Scotia, Canada. Horses (about 500 head) are the only terrestrial mammals on the island, and survive despite high pasture densities and absence of human interventions such as supplemental feeding and deworming. Strongyle fecal egg counts, monitored in all live horses every summer, are high (mean 1500 eggs per gram - EPG), vastly exceeding the guidelines to deworm at values greater than 200 EPG in companion horses. Larval culture has demonstrated that small strongyles (cyathostomes) had the highest relative abundance amongst Sable horses, followed by the large strongyles *Strongylus equinus*, *S. edentatus*, and *S. vulgaris*. In April 2017, we examined 30 natural mortalities and found evidence of cyathostomiasis, pathology associated with larvae of *S. vulgaris* in the cranial mesenteric artery of yearlings, and pancreatic damage associated with larvae of *S. equinus*. We also found high prevalence of *Dictyocaulus arnfeldi* in airways, a lungworm not normally thought to circulate in horses in the absence of donkeys, the normal maintenance host. Ten of 12 (83%) yearlings were infected, and

lungworms were also found in 8 of 18 (44%) adult horses. Dictyocaulus larvae were present in 3 of 31 (10%) of fecal samples collected from live horses in early August. Identification as *D. arnfeldi* was based on DNA characterization of the ITS 2 region of both adult and larval nematodes. In April 2018, all fecal samples examined from live yearlings (n=8) had larvae of Dictyocaulus (mean of 17 larvae per gram -LPG) while 13 of 36 (36%) of adult horses were shedding larvae (mean 3 LPG). Transmission of this lungworm is enigmatic, and further investigation is needed to determine significance as a respiratory pathogen, especially in yearlings which had the highest intensities of infection.

OA49 Ticks on Cattle

July 11, 2019, 8:30 - 10:30

Breakout Room 4, Hall of Ideas G&J, Level 4

OA49.01 Detection of Winter Tick Infested Cattle Using Near Infrared Reflectance Spectroscopy of Bovine Feces

Ms. Samantha Hays¹, Dr. Pete Teel¹, Dr. Sonja Swiger², Dr. Jeffery Tomberlin¹, Dr. Thomas Hairgrove³, Dr. David Anderson³

¹Texas A&M University, United States, ²Texas A&M AgriLife Research and Extension, United States, ³Texas A&M AgriLife Research and Extension, United States

External parasites annually cost the U.S. beef cattle industry \$2.4 billion through the direct effects of parasitism, and an even greater cost when animal handling and treatment expenses are included. Tick parasitism of range cattle can occur year-round. While most species are active in spring and summer months, certain species such as the winter tick, *Dermacentor albipictus*, are active in fall and winter when forage quality and quantity are low. *Dermacentor albipictus* is recognized as a one-host tick economically important in large ungulates and is a known vector that has previously been shown to transstadially transmit *Anaplasma marginale*, causal agent of bovine anaplasmosis. Direct production losses accrue from tick parasitism through bloodloss, irritation, weightloss, and

diminished reproductive capacity. Integrated tick management (ITM) strategies have been developed that have defined tactics including habitat and wildlife management, fencing, grazing rotations and cattle treatments with acaricides. Tick management requires producers to gather and physically inspect animals on a regular basis to determine tick presence and abundance, and make informed decisions regarding management tactics. Animal stress, time, labor, facilities wear and expense are disincentives to ITM adoption. Near infrared reflectance spectroscopy (NIRS) has previously been used to detect tick infested cattle and monitor success of ITM tactics. Ticks modulate the immune system of cattle in order to obtain a blood meal over a period of days to weeks. This modulation of the immune system results in cascading effects through the endocrine system to the digestive system producing changes in fecal chemistry that are detectable by NIRS. This presentation will evaluate if NIRS analysis of bovine feces (fNIRS) can discern different levels of winter tick infestations on cattle, further testing the sensitivity and feasibility of fNIRS technology as an alternative method expected to improve ITM adoption, decision-making, and efficacy for tick management.

OA49.02 Modeling Cattle-Nilgai-Deer Interactions to Assess Impact of Nilgai on Cattle Fever Tick Eradication Efforts in the U.S.A.

Kimberly Lohmeyer¹, Hsiao-Hsuan Wang², William Grant², Denise Bonilla³, Hallie Hasel⁴, Andy Schwartz⁵, Pete Teel², Adalberto Perez de Leon¹

¹USDA-ARS, United States, ²Texas A&M University, United States, ³USDA-APHIS-VS, United States, ⁴USDA-APHIS-VS, United States, ⁵Texas Animal Health Commission, United States

Abundant nilgai, *Boselaphus tragocamelus*, in South Texas pose significant challenges to cattle fever tick (*Rhipicephalus microplus* and *R. annulatus*) eradication efforts. Modeling cattle-nilgai-white-tailed deer interactions provides a tool to assess the impact of nilgai on the efficacy of standard cattle fever tick eradication protocols. To meet this need, we

revised our spatially-explicit, individual based, tick simulation model that simulates cattle fever tick population dynamics in response to tick control measures in the presence of cattle and/or white-tailed deer on hypothetical heterogeneous landscapes in southern Texas. The updated model includes nilgai as an alternative cattle fever tick host species and refines information on rangeland landscapes in and around the permanent quarantine zone in south Texas along the U.S.-Mexico border. Information on nilgai life history and ecology, including patterns of habitat use and efficacy as a tick host were analyzed and used to describe movement rules quantitatively, and to estimate the spatial and temporal dynamics of host seeking larvae in the system. Updates on the development of the cattle-nilgai-white-tailed deer model and how it can be utilized to adapt cattle fever tick suppression tactics utilized by the Cattle Fever Tick Eradication program will be presented.

OA49.03 Detection of Fecal Chemistry Changes in Cattle Infested With the Southern Cattle Tick, *Rhipicephalus (Boophilus) Microplus*

Mr. Brian Rich¹, Dr. Pete Teel¹, Dr. Donald Thomas², Dr. Jay Angerer¹, Dr. Douglas Tolleson¹, Dr. Adalberto Perez-DeLeon³
¹Texas A&M University, United States, ²USDA ARS, United States, ³USDA ARS, United States

Bovine babesiosis, a highly fatal tick-borne disease of cattle, was eliminated from the US in the last century through the federal and state Cattle Fever Tick Eradication Program against the cattle fever ticks (CFT) *Rhipicephalus microplus* and *R. annulatus*. The threat to the US cattle industry continues through CFT introductions from Mexico. The standard method to detect infestation by the Program is physical examination of restrained cattle to find CFT. New methods of detecting CFT-infested cattle could improve reliability and reduce animal stress. This study determined whether changes in fecal chemistry induced by *R. microplus* infestation was detectable using near-infrared reflectance spectroscopy (NIRS). Fecal samples were collected daily from 6 stanchioned *Bos taurus* yearling heifers (initial mean weight 163.3 kg

+/- 4.7 kg) at the USDA-ARS Cattle Fever Tick Research Laboratory, in Edinburg, TX, before, during, and after an infestation with 5000 *R. microplus* larvae. Cluster analyses were conducted using GRAMS IQ for NIR spectra in the 576-1126 nm range to test for fecal chemistry changes different from pre-infestation condition, and coincident with the biological phases of *R. microplus* infestation. The first three factors of spectral variation accounted for 87.87% of spectral variation among all samples. Factors 1, 2 and 3, had F-Ratios for the Reduced Eigenvalues of 941.59, 387.44 and 221.79, respectively. Three-dimensional analysis for these 3 factors shows shifts in sample clusters away from pre-infestation and coincident with progressive *R. microplus* blood-feeding and post-infestation recovery. We conclude that fecal NIRS may provide a tool for detection of *R. microplus*-infested cattle. However, further testing is needed to determine the sensitivity of detection on cattle with varying levels of *R. microplus* burden, and a protocol developed and evaluated for fecal sampling under field conditions.

OA49.04 Interaction Between a Systemic Acaricide and Immunological Control of *Rhipicephalus (Boophilus) Microplus*

Charluz Arocho Rosario¹, Robert Miller², Pete Teel¹, Felix Guerrero³, Adalberto Perez de Leon³
¹Texas A&M, United States, ²USDA-ARS, United States, ³USDA-ARS, United States

The southern cattle fever tick *Rhipicephalus (Boophilus) microplus*, causes large economic losses in cattle production, particularly in tropical and subtropical parts of the world. In the United States losses were estimated to be ~\$130.5 million in the late 1800's before the eradication program began. Ectoparasites develop easily in tropics and sub-tropics and are responsible for large economic losses in the dairy and meat industry.

The southern cattle tick *R. microplus* is a one-host tick species considered the most important ectoparasite of livestock in the world because of its association with high financial loss due to direct feeding and in the

transmission of the hemoparasites *Babesia bovis*, *B. bigemina*, and *Anaplasma marginale*, the causative agents of babesiosis and anaplasmosis, respectively.

Rhipicephalus microplus has a high potential for population growth due to its relatively short life cycle and preference for cattle reared in large numbers. Unfortunately, ticks in many parts of the world have evolved resistance to all pesticides available on the market, driving the development of new technologies to control this species.

Vaccination against ticks using the protein Bm86 has been shown to be effective against acaricide-resistant ticks. This technique has been successfully implemented in Puerto Rico for the control of *R. microplus* on dairy and beef cattle. Observations from Puerto Rico indicate a potential interaction between anti-tick vaccination in conjunction with systemic acaricide use. Controlled animal studies were completed directly comparing the efficacy of vaccination with and without systemic acaricide. Additionally, *in vitro* feeding of ticks with immunoglobulin-G from vaccinated animals with several combinations of acaricides was used to screen antigen/acaricide combinations and to confirm results of field tests using animals. The results show that the vaccine had a synergistic interaction with the acaricide. Better and longer control was achieved with the combination than when either treatment was applied alone.

OA49.05 Diagnosis of Acaricide Resistance in Cattle Tick in Three Districts of Punjab, Pakistan

Dr. Zia ud Din Sindhu¹, Mr. Muhammad Usman Naseer¹, Talha Zafar¹, Rehman Hafeez¹, Rao Zahid Abbas¹, Muhammad Kasib Khan¹, Bilal Aslam², Zafar Iqbal¹

¹Department of Parasitology, University of Agriculture, Pakistan, ²Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Pakistan

The one host tick, *Rhipicephalus* (*R.*) *microplus* is the most important species, which causes many obstacles in a profitable livestock business of bovines. Chemical

acaricides are the main strategy of tick control, however, there are reports of development of resistance from various parts of the world. Thus present study was designed to check the cypermethrin resistance against cattle tick at three different Govt. livestock farms and their surrounding areas in Punjab, Pakistan by using larval packet test. A total of 33 farms were screened for this purpose from three different districts, namely Sargodha, Okara and Attock located in different geographical areas of Punjab, Pakistan. The overall prevalence of resistance was 24.2% of the samples collected from these farms. The percentage of susceptible and tolerant strains from all the studied farms was 36.5 and 39.3%, respectively. The results of this study revealed that when the RF50 was considered resistant, 45.5, 16.7 and 10% strains were found to be resistant in Districts of Sargodha, Okara and Attock, respectively. On the other hand, when the RF99 was used, the percentage of resistant strains was raised to 100% at all farms. It was concluded that *R. microplus* strains collected from different farms of Punjab and their surrounding areas have great variability in their cypermethrin resistance and susceptibility level, depending on the particular condition at each farm.

OA49.06 Comparison of *Ixodes Ricinus* Populations in Adjacent Habitats on a Pasture-Based Dairy Farm

Mr. Taher Zaid¹, Ms. Sorcha Brosnan¹, Prof. Olaf Schmidt¹, Dr. Jason Newton², Dr. John Browne¹, Ms. Tiphaine Dubourdieu⁵, Prof. Jeremy Gray¹, Mr. Jack O'Connor³, Dr. John Mee⁴, **Dr. Annetta Zintl**¹

¹University College Dublin, Ireland, ²NERC Life Sciences Mass Spectrometry Facility, United Kingdom, ³MSD, Ireland, ⁴Moorepark Research Centre, Ireland, ⁵Paul Sabatier University, France

The distribution of *Ixodes ricinus* ticks in the environment tends to be highly overdispersed with greatly divergent densities in adjacent habitats. In order to (1) investigate the mechanisms that underlie this overdispersion and to (2) assess the animal and public health risks associated with the different habitats, we compared tick populations in adjacent

habitats with regard to tick abundance, the most recent engorgement host and the prevalence of tick-borne pathogens (TBPs). Five adjacent sites on a dairy farm in Co Kerry were sampled for the presence of ticks using standard blanket dragging methods. From each site, 15 nymphs were analysed for C and N stable isotope compositions and 40 were screened for the presence of TBPs using Taqman qPCR analysis.

Both nymphs and adult ticks were most abundant in hedges bordering the pasture, somewhat less common at the edge of the path and in the woodland and absent from the centre of the pasture. Isotope analysis indicated that the nymphs that quested in the woodland had fed on the broadest range of hosts, while qPCR analysis suggested that nymphs collected from the hedge that separated the woodland from the pasture had the highest prevalence of TBPs (*Anaplasma phagocytophilum*, *Babesia* (cattle or deer spp) and *Borrelia* spp).

While there was some overlap in the feeding guilds of hosts parasitized by nymphs in the different habitats, nymphs in the hedge bordering the woodland seemed to be chiefly feeding on one guild (possibly ruminants) while nymphs in the woodland fed on a broader range of hosts (probably rodents and birds in addition to ruminants). The results also suggest that ticks that detach from livestock or deer on pasture move into the hedge, boosting the tick numbers and increasing the prevalence of livestock TBPs in ticks in this site.

OA49.07 Multi-Acaricide Resistant Tick Population: Problem and Mitigation Strategy

Dr. Srikant Ghosh¹, Dr. Rajesh Kumar², Dr. Satyanshu Kumar³, Dr. Sharad Srivastava⁴, Dr. Sanis Juliet⁵, Dr. Binod Kumar¹, Dr. Manjunathachar V¹, Mr. Sonu Sharma³, Ms. Ankita Verma², Dr. Anil Kumar Sharma¹, Dr. Gaurav Nagar¹, Dr. Gajanan Chigure¹, Dr. Shanmuganath C.¹, Dr. Parthasarathi BC¹, Dr. Mukesh Shakya¹

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Due to sanguivorous nature, *Rhipicephalus microplus*, causes severe economic loss to the dairy industries globally. Repeated application of different generations of chemical acaricides has led to the development of multi-acaricide resistant tick populations. Along with organophosphates, synthetic pyrethroids and amidines, the tick population in India is developing resistance to ivermectin. Consequently, tick management has become a challenge to sustain the animal productivity. Phyto-formulation and strategic use of cross-protective vaccine were targeted to mitigate the situation. By combining empirical and modern approaches, a highly potent, widely available plant having anti tick activity was identified. Chemoprofiling of the active solvent guided extract resulted in identification of 22 compounds, of which two potent, safe and stable molecules were selected. Integration of solvent guided extracts and active compounds in delivery matrix resulted in the development of one natural and another nano-formulation having >80% anti-tick activity against experimental challenge infestations. The formulations are working through multiple routes resulted in damage of cuticle, gut and reproductive organs with significant up and down regulation of multiple genes involved in physiological process of the tick species.

Conserved tick proteins, Subolesin, Calreticulin, Cathepsin, Ferritin-2 and Tropomyosin of *Hyalomma anatolicum* and Subolesin of *R. microplus* were identified using RNAi and in silico analysis. The identified proteins are expressing significantly ($P < 0.001$) high level in all the stages of the tick species. The recombinant version of the proteins conferred 65.4% (Subolesin), 41.3% (Calreticulin), 30.2% (Cathepsin), 51.2% (Ferritin-2) and 64% (Tropomyosin) protection against *H. anatolicum* and 65.4% (Subolesin) against *R. microplus* experimental challenge infestations. The possibility of development of multiantigenic antigen formulation for conferring cross-protection against the most economically important ticks was explored. Simulation of both the strategies in field

situation against multi-acaricide resistant tick infestations is discussed.

OA50 Alternative treatments for Parasites in Ruminants II

July 11, 2019, 8:30 - 10:30

Breakout Room 5, Meeting Rooms KLOP, Level 4

OA50.01 Anthelmintic-like Activity of Four Polyphenolic Compounds and Their Interactions Against the Cattle Nematode *Cooperia punctata*

Dr. Elke von Son-de Fernex¹, Dr. Selene Escareño-Díaz¹, Dr. Miguel Ángel Alonso-Díaz¹, Dr. Pedro Mendoza-de Gíves², Dr. Epigmenio Castillo-Gallegos¹

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Polyphenolic compounds (PCs) have been proposed as one of the most bioactive secondary metabolites group occurring in nature, and have been associated to anthelmintic (Ah)-like activity of plants against cattle nematodes. However, little is known regarding their synergetic/antagonistic interactions. This study assessed the in vitro anthelmintic-like activity of commercial PCs: quercetin, caffeic acid, rutin and coumarin and their combinations against the egg hatching and larval exsheathment of *Cooperia punctata*; one of the most prevalent nematodes affecting grazing cattle in tropical regions. The molecules selected for the in vitro analysis were previously identified in bioactive plants through bio-guided fractionation. To estimate mean effective concentrations (EC₅₀) five increasing concentrations were used for both Egg hatching (EHIA) and larval exsheathment (LEIA) inhibition assays (0.6 to 9.8 mg / mL-1 and 0.15 to 2.4 mg / mL-1, respectively). From the four molecules, only rutin did not affect egg hatching; while quercetin, showed no bioactivity against eggs or larvae ($P > 0.05$). Best-fit EC₅₀ estimated through the

EHIA was considered for PCs classification as bioactive (coumarin and caffeic acid) and non-bioactive (quercetin and rutin). Phytochemical interactions were subsequently assessed combining bioactive:non-bioactive PCs (8:2 ratio), and the nature of their interaction was classified using the fractional inhibitory concentration index (FICindex). All four combinations had a synergetic interaction against larval exsheathment (FICindex < 0.5), and only coumarin:rutin had no interaction against egg hatching (FICindex > 0.5). Quercetin and rutin acted as PCs AH-like activity enhancers, reducing EC₅₀ of bioactive molecules in a range of 43% to 64% and 68% to 83% for EHI and LEI, respectively. Furthermore, coumarin and caffeic acid bioactivity against free-living stages of *C. punctata* makes them suitable candidates as markers for anthelmintic-like activity in bioactive forages.

OA50.02 Effect of Cry5B From *Bacillus Thuringiensis* on *Haemonchus Contortus* in Experimentally Infected Sheep

John Sanders¹, Dr. Raffi Aroian², Dr. Gary Ostroff², Dr. Ambily Abraham², Dr. Kelly Flanagan², David Gazzola², Dr. Yan Hu², Tasia Kellogg², Dr. Hanchen Li², Dr. Anne Zajac¹
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The worldwide rise of anthelmintic resistance in trichostrongyle nematodes of ruminants has led to the urgent need for alternative methods of control. Previous research has shown significant anthelmintic effect in vitro and in vivo of *Bacillus thuringiensis* (Bt) Cry 5B crystal protein on several intestinal nematode parasites of rodents, pigs, and dogs.

The effect of Cry5B on *Haemonchus contortus* was examined in vitro using larval development and adult motility assays. Exposure of *H. contortus* eggs and larvae to Cry 5B significantly reduced the number of third stage larvae produced. Additionally, in a motility assay, adult *H. contortus* collected from sheep abomasa and incubated in medium containing Cry5B

showed significantly reduced motility compared to control worms. To investigate the effect of the compound in vivo, five female and five castrated male lambs aged approximately 7-8 months were removed from pasture and confined for the duration of the study. All were dewormed to remove existing trichostrongyle infection. Sheep were subsequently each administered 10,000 third stage *H. contortus*. Following patency of infection, sheep were divided into two groups with similar mean FEC (determined by Modified McMaster test, detection limit 25 epg). Treatment group sheep were orally administered 200 mL of a suspension of genetically engineered non-sporulating *Bacillus* producing Cry5B daily for 4 days. Control group sheep received 200 mL water. FEC were determined daily for 7 days post infection (PI) and thereafter every two days until necropsy at 14 days PI. After 24 hours, mean FEC of treated animals was reduced by 78% compared to controls and by 94% after 72 hours. Of the few eggs produced by treated animals, only 27% developed to the L3 compared to >90% of eggs from control lambs. These results suggest that *B. thuringiensis* Cry5B protein may show promise as a treatment for *H. contortus*.

OA50.03 Control of Gastrointestinal Nematodes in Wool Sheep Using Novel Formulations Based on a Colombian Strain of the Nematophagous Fungi *Duddingtonia* Flagrans: Reduction in Eggs per Gram of Feces Indicator

Jaime Andres Cubides-Cardenas¹, **Dr. Diego Cortes-Rojas¹**, Elizabeth Céspedes-Gutierrez¹, Prof. Jimmy Jolman Vargas-Duarte², Dr. Martha Izabel Gómez-Alvarez¹

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Gastrointestinal nematodes (GIN) can reduce or limit sheep production in Colombia. Nematophagous fungi are natural enemies of GIN and a new alternative for its biological control. The aim of this study was to evaluate the effectiveness of three novel formulations (E3, E16 and CS) based on *Duddingtonia*

flagrans (strain CIL1A) against GIN in a controlled field trial. The experiment was carried out under Colombian Andes tropical conditions (Mosquera, Colombia). Four different groups of lambs naturally infected with GIN were allocated in separate paddocks and received daily different fungi formulations at a dosage of 1×10^6 spores/kg of body weight for 18 weeks. Control group was not treated with fungi. Parasitological criteria such as the number of eggs per gram of feces (EPG), the percentage of larvae recovered from coprocultures and some others clinical and zootechnical indicators were evaluated weekly. The results were compared using a repeated measures across time model. The estimated mean reduction of EPG of the developed formulations compared with the control group were 62.7%, 79.2% and 62.3% for E3, E16 and CS respectively. There was a significant reduction ($P < 0.05$) between treated groups and control group. No significant differences were observed between E3 and E16 formulations. The main nematode genera identified were *Haemonchus* spp., *Trichostrongylus* spp., and *Teladorsagia* spp. (resistant Moxidectin-benzimidazole strains) and reductions were demonstrated for each of these species. It is considered that the reduction of EPG will lead to the reduction of infective larvae in the grass and would result in productivity increases in grazing sheep and reduce the use of anthelmintic chemicals. Therefore, use of these formulations will provide an alternative option to control GIN parasites on pasture.

OA50.03 Association of Phytominerals with the Serum Trace Elements Profile and Quantitative Gastrointestinal Parasitic Burden in Grazing Sheep

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The grazing meadows and forages are important components of the animal production. Variety, composition, distribution and nutritive value of forages in grazing areas depend upon the soil type, season, and climate. We studied the role of mineral supplementation in animals thru selected forages in mitigating the gastrointestinal (GI) parasitic threats in grazing sheep. To this end, level of Zinc (Zn), Copper (Cu), Manganese (Mn) and Cobalt (Co) were determined in grazing forages, respective soils and sera of the grazing sheep of Sialkot, Punjab, Pakistan during spring 2015. The correlation of serum trace elements was done with the burden of GI parasites in grazing sheep. The prevalence of GI parasites in grazing sheep was 32.81% with eight species of GI parasites identified. A significant ($P < 0.05$) difference in the level of selected trace elements was determined in a total of eight collected forages. The mean serum concentrations of Zn and Cu were inversely proportional to the mean magnitude of EPG in the grazing sheep. The present study indicated that trace elements (preferably Zn and Cu) rich forages (*Cichorium intybus* and *Cynodon dactylon*) have ability to control or lower the burden of GI parasites in grazing animals. This mitigation approach may eventually develop the resilience against GI parasitic infections particularly in resource-poor countries.

OA50.04 *Opuntia Ficus-Indica* (L.) Mill. Hydroalcoholic Extract Against Nematodes of Sheep

Dr. Marcelo Molento¹, Dr. Carolina Santos¹, Dr. Luciano Campestrini², MSc Douglas Vieira¹, Dr. Izanara Pritsch¹, Dr. Fabio Yamassaki², Dr. Selma Zawadzki-Baggio², Dr. Juliana Maurer²
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Opuntia ficus-indica (L.) Mill. is a xerophyllous plant originated in tropical America. The aim of this study was to determine the efficacy *O. ficus-indica* hydroalcoholic extract (OFIEOH) against gastrointestinal parasites of sheep. Contents: Initially, the hydroalcoholic extract from cladode peels of *O. ficus-indica* was produced by maceration.

Liquid chromatography coupled to mass spectrometry/electron spray ionization (LC-MS/ESI) was used to characterize the polyphenolic profile of the OFIEOH extract. Fifteen compounds were identified in the OFIEOH extract. Tri-glycosylated methyl quercetin derivatives were the main compounds identified. In vitro egg hatch (EHT) and larval migration tests (LMT) were used in a range of concentrations of OFIEOH: 12.5 to 100 mg/mL for EHT and 12.5 to 200 mg/mL for LMT. In addition, the LMT was used to test ivermectin (IVM), associated with the inhibitory concentration of 50% (IC₅₀) for OFIEOH. The combination of OFIEOH (12.5 to 200 mg/mL) plus the IC₅₀ of IVM was also tested. OFIEOH got up to 90% efficacy in the EHT, and 77% in the LMT, showing a concentration-dependence inhibitory effect. We found a drug-extract antagonistic neutralizing effect when doses of IVM were added to OFIEOH (maximum efficacy of 73.78%), while a positive additive effect was observed when OFIEOH was added to the IC₅₀ of IVM (IC₅₀ of 82.79 for OFIEOH alone against an IC₅₀ of 55.08 of OFIEOH + IVM). Conclusions: OFIEOH alone may be considered as a suitable ecofriendly product to control gastrointestinal parasites of sheep, offering a more holistic approach to improve animal farming and welfare. The drug-extract interaction is also a promising therapeutic alternative, reducing the final dose to the host, with an optimum combination effect.

OA50.05 In Vitro Anthelmintic Efficacy of *Citrullus Colocynthis* on *Haemonchus*

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The appearance of anthelmintic resistance, drug residues that potentially enter the food chain, high costs, and the lack of accessibility to anthelmintic drugs in distant rural areas have motivated investigations into novel alternatives such as medicinal plants. The present project was designed to evaluate the anthelmintic efficacy of *Citrullus* (C.) *colocynthis* against *Haemonchus*. In vitro anthelmintic effects of aqueous-methanol

and ethyl acetate extracts of the fruit of *C. colocynthis* against *Haemonchus* were determined through egg hatch and adult motility assays. The effects of four serial dilutions of 25 mg/ml of each extract compared to levamisole (0.55 mg/ml) and oxfendazole (three serial dilutions of 25 µg/ml) were studied. In the egg hatch assay, means of 83.67% and 80.67% of *Haemonchus* eggs failed to hatch at the same 25 mg/ml doses of ethyl acetate and aqueous-methanol extracts. The ethyl acetate extract exhibited a slightly higher effectiveness than the aqueous-methanol extract, but statistically, there was no significant difference ($P=0.138$). In the adult motility assay, the dose of 25 mg/ml of ethyl acetate extract paralyzed all the worms within only 4h after the start of the experiment, and the aqueous-methanolic extract of the plant at the maximum concentration tested (25 mg/ml) paralyzed all worms by 8h post-exposure ($P=0.001$). Results of the present study strongly suggest that fruit extracts of *C. colocynthis* may present promising alternatives to synthetic drugs for treating *Haemonchus* infections. Further in vivo studies to assess bio-availability of active ingredients from the plant extracts and minimum non-lethal concentrations for the safety of livestock treatment are suggested as needed future studies.

OA50.06 Discovery of Novel Amazonian Stinging Ant Venom Peptides with Activity Against the Sheep Parasites *Haemonchus Contortus* and *Lucilia Cuprina*

Ms. Samantha Nixon^{1,2}, Dr. Akello Agwa¹, Dr. Samuel Robinson¹, Dr. Andrew Walker¹, Dr. Axel Touchard³, Dr. Christina Schroeder¹, Prof. Irina Vetter¹, Dr. Volker Herzig¹, Dr. Andrew C. Kotze², Prof. Glenn F. King¹

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Parasites remain a serious challenge for veterinary medicine and livestock production worldwide. The Australian sheep industry is severely threatened by gastrointestinal nematodes, which cost in excess of \$430 million AUD annually. Blowflies exacerbate

this, costing a further \$222 million. Widespread drug resistance urgently necessitates the discovery of new therapies to control these infections. Venoms have evolved over millions of years to become cocktails of selective and potent bioactive molecules, but their potential as sources of novel antiparasitic compounds has been underexplored. We screened over 250 crude venoms from a diverse panel of spiders, scorpions, assassin bugs, caterpillars, marine snails, ants and wasps for anthelmintic activity against the blood-feeding small ruminant nematode *Haemonchus contortus* in a larval development assay. At 0.2 mg/ml crude venom the hit rate for the screen was 20.6%, with hits dominated by arthropod venoms, particularly tarantulas and ants. Candidate venoms were characterised using bioassay guided fractionation to identify the active compounds. Five novel small linear peptides were identified from Amazonian stinging ant venoms. Peptides were synthesised and found to have low micromolar activity against *H. contortus* larvae (2 – 30 µM). The peptides were also injected into adult *Lucilia cuprina* sheep blowflies, resulting in paralysis within 1 hour of injection at moderate doses ($PD_{50} = 0.5 - 38$ nmol/g) and lethality at high doses. The peptides were counter-screened for cytotoxicity, haemolysis and pain in vitro using sensory neurons soaked in calcium-fluorescent dye. One peptide showed well over 100-fold selectivity for *H. contortus* over mammalian cell lines and no sensory neuronal activation. We subsequently generated mutant peptides to improve potency and selectivity against *H. contortus* and *L. cuprina*, but found that these were closely correlated. Overall, these findings indicate that arthropod venoms may be a useful source for antiparasitic drug discovery.

OA50.07 Development and Evaluation of In Vitro Anthelmintic Activity of Herbal Extracts on Benzimidazole Resistant *Haemonchus Contortus*

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The problem of anthelmintic resistance lead the path to make-in-use the herbal extracts (HE) for the treatment of parasitic diseases. The current study was conducted to evaluate the efficacy of medicinal plants against the benzimidazoles (BZ) resistant strains of *Haemonchus contortus* in small ruminants. Total adult worms (n=1500) that were morphologically identified as *H. contortus* were frequently collected from different small ruminant abattoirs covering two geographic regions of Punjab Province (Pakistan). These worms were confirmed using PCR and then subjected for DNA gene sequencing to isolate the resistant strains. The DNA sequences of the resistant strains were deposited into NCBI GENBANK database with Accession No. MF043121-28. Worms were treated in vitro with combined aqueous extract of *Nigella sativa* and *Tachyspermum ammi*. Anthelmintic activity of the HE has been evaluated by using the Egg Hatch Test (EHT) and Adult Mortality Assay (AMA). Dose-and-time dependent responses of the HE against the resistant strains were recorded. The results obtained from AMA were statistically evaluated by one-way ANOVA. Mortality of worms was comparable with the reference drug levamisole at the maximum dose rate of the HE (200mg/ml). Data from EHT had been evaluated by Probit model test and lethal concentrations were calculated. HE affects the hatching of eggs in dose dependent manner. The LC50 values of the experiments were distinctly different. The P values for the experiments for efficacy of HE concentrations and oxfendazole were recorded as 0.263 and 0.192, respectively. In conclusion, the HE efficacy is comparable with the efficacy of the synthetic anthelmintics. It can be used being field friendly, environmently safe, socially efficient, cheap and easily available alternatives. Moreover use of HE in treating the parasitic problems will help to reduce the dilemma of anthelmintic resistance.

A group of medical professionals, including nurses in blue scrubs and doctors in white coats, are huddled together in a team huddle. They are all wearing yellow gloves and have their hands clasped in the center. The background is a plain, light-colored wall.

POSTER SESSIONS

Poster Sessions

Poster Session 01

July 8, 2019, 10:00 – 16:30

Exhibit Hall A, Level 1

PS01.01 Preliminary Safety Study of Miltefosine in Healthy Cats Treated During 14 Days

Dr. Caroline Bouchez¹, Dr. Emmanuel Briant¹, Dr. Magali Dolon¹, Dr. Christelle Navarro¹, **Dr. Vanessa Chala¹**

¹Virbac, France

Feline leishmaniosis is a chronic disease due to *Leishmania infantum* with clinical signs and laboratory abnormalities similar to those found in dogs. Miltefosine (Milteforan[®], Virbac) has got veterinary marketing authorizations in Europe and Brazil to control canine leishmaniasis. The objective of this non-controlled study was to evaluate the clinical tolerance of miltefosine on healthy cats.

After a 7-day acclimation period, three cats (two males, one female, weighing 3.7 to 6.1 kg) received Milteforan[®] once a day on their food ration at the dose-level of 2 mg miltefosine/kg for 14 consecutive days (D0 to D13) followed by a 14-day recovery period. Animals were observed daily from D-7 to D28. Product consumption was recorded 30 minutes to 1 hour after each treatment and food consumption daily. Clinical follow-up was performed before each treatment and clinical examination including rectal temperature, body weight measurement performed weekly. Blood samples were taken at D-6, D14, D28 for hematology and blood biochemistry, at D58 for hematology.

All animals ingested spontaneously the expected dose-volume of product for 14 consecutive days, showing good palatability. One out of three cats vomited 4 times during the treatment period, without impact on health status. No other clinical signs possibly related to treatment was reported. Rectal temperature, body weight, food consumption remained unaffected. Heinz bodies increased

in 2 out of 3 cats after 14 days of treatment and were still observed in little amounts at D28 for both cats and at D58 for one cat. Other hematology and blood biochemistry parameters remained unaffected.

Miltefosine administered orally at the dose-level of 2 mg/kg for 14 consecutive days induced sporadic vomiting in one out of three cats. At hematology, the number of Heinz bodies increased in two out of three cats with no consequence on other hematology parameters.

PS01.02 Effect of a Long-Acting Moxidectin on the Control of Gastrointestinal Nematodes and the Productivity of Suckling Calves

Fernando Borges¹, Mario Conde¹, Mariana Freitas¹, Matheus Nakatani¹, Vinicius Rodrigues¹, Guilherme Reckziegel¹, Fernando Aono², **Elio Moro²**, Frederico Moreira³

¹Ufms, Campo Grande, Brazil, ²Zoetis Animal Health, Brazil, ³Zoetis Animal Health, United States

The impact of gastrointestinal nematodes (GINs) on different categories of beef cattle in tropical production systems requires studies and little is known about the advantages of anthelmintic treatment in pre-weaned calves. The objective of this study was to evaluate the effect of a long-acting formulation containing 10% moxidectin on the control of GINs and the productivity of calves before weaning. A first study was designed to evaluate the effect of the treatment of calves between 3 and 5 months of age, and later ten other clinical studies were carried out in different Brazilian states to evaluate the repeatability of these results. In the first study, 202 animals were distributed in two groups (moxidectin 500µg/kg or Saline) in a randomized block design, considering the weight, fecal egg counts (FECs), breed, and dam's parity. Other ten field studies were carried out in commercial farms from four Brazilian geographic regions, where 692 calves were randomly assigned to receive either 10% moxidectin or saline

treatments. Calves treated with moxidectin in the first study had an increase of 9.4 kg on the body weight (BW) ($P < 0.05$), a reduction in mean OPG ($P < 0.05$) and a higher percentage of negative FECs (56.8% vs. 36%; $P < 0.05$). In the other 10 field studies, calves treated with moxidectin had an increase of 4.8 kg ($p < 0.02$) and a greater average daily BWG (0.704 vs. 0.653 kg/d; $p < 0.001$). The FECs decreased 68.7% from the treatment to the weaning in the treated calves, whereas in the placebo group, there was an increase of 23.2% in this interval. Therefore, the treatment of suckling calves with long acting moxidectin reduced FEC and increased weaning weight.

PS01.03 Validation of a Semi-Quantitative Real-Time PCR Assay for the Diagnosis and the Monitoring of Giardia Intestinalis Infection in Canine Faeces Samples According to the French Standard NFU47-600-2

Florence Va¹, Myriam Thomas², Sylvain Bellier^{1,3}, Claire Cancia⁴, Valeriia Klubkova⁴, Claire Pelletier⁵, Eric Sellal^{4,5}, **Bruno Polack**^{1,3}
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Infection of young dogs with *Giardia intestinalis* is very common in France and is a major clinical problem, especially in breeding facilities.

In order to obtain a reliable, sensitive and semi-quantitative diagnosis that can easily be carried out in a routine laboratory, we have developed a complete tool combining firstly a ready-to-use quantitative duplex real-time PCR kit (qPCR Premium® *Giardia intestinalis*; BioDev) to detect all *Giardia intestinalis* assemblies A to F (beta-giardin target) by FAM labelling and an exogenous internal positive control (IPC) by Cy5 labelling, and secondly a method of preparation samples and extraction-purification of nucleic acids to obtain an optimized yield (BioPrep® *Giardia* and BioExtract® column, BioSellal).

This tool has been validated according to the recommendations of the French standard NFU47-600-2.

Thus, for the PCR part, the following characteristics were determined: LODPCR (5 copies / PCR), Efficiency (99%) and Linearity Domain (between 10 copies corresponding to the LOQPCR and 1.106 copies / PCR).

Various modes of faecal preparation were compared in terms of the extraction yield of known faeces positives for *Giardia*: either mechanical lysis, or thermal and chemical lysis, or mechanical lysis followed by thermal and chemical lysis; or no pretreatment at all before nucleic acids extraction-purification. It is the association of mechanical and thermal-chemical lysis that reproducibly gives the best extraction yield.

Finally, the complete method part was characterized by the determination of the diagnostic sensitivity and specificity. We analyzed a hundred dog faeces with or without an evocative clinic in order to determine an infection threshold of clinical interest making it possible to distinguish between a chronic carriage and a clinical infection.

Because of its wide range of inclusivity (7 recognized assemblies and exogenous IPC), the qPCR Premium® *Giardia intestinalis* kit can also be used on other animal species such as cats, ruminants or even environmental samples.

PS01.04 Development of a Diagnostic Assay for the Detection and Differentiation of Theileria Spp. In White-Tailed Deer

Mr. Alec Thompson¹, Dr. Michael Yabsley^{1,2}
¹Southeastern Cooperative Wildlife Disease Study, Department of Population Health, College of Veterinary Medicine, University of Georgia, United States, ²Warnell School of Forestry and Natural Resources, University of Georgia, United States

The spread of non-native parasites with the movement of animals is a major concern for disease emergence and native species health/conservation. *Haemaphysalis longicornis* (Asian longhorned tick) is native to eastern

Asia, but has become invasive in several countries including Japan, Australia, New Zealand, and now the United States. Within the established range, *H. longicornis* is a vector of the protozoan parasite, *Theileria orientalis* subtype Ikeda, which until recently was not known to occur in the United States. In 2017, clinical theileriosis caused by *T. orientalis* Ikeda was reported in a cattle herd in Virginia which was also infested with *H. longicornis*, but it is not known if *H. longicornis* transmitted *T. orientalis* to the cattle. Within the United States, white-tailed deer (WTD, *Odocoileus virginianus*) are infected with several genotypes of a *Theileria* sp. (often called *T. cervi*) that is distinct from *T. orientalis*. It is currently unknown if deer are susceptible to *T. orientalis*, a pathogen of agricultural concern. In this study, we developed a restriction fragment length polymorphism (RFLP) assay that can distinguish between *Babesia* spp., selected exotic *Theileria* spp., and the WTD *Theileria* sp. (although not the different genotypes [F, G1, and G2]). Using this assay, we tested cervid blood samples from the eastern United States and found 222 of 264 (84%) positive for the WTD *Theileria* sp., 23 (9%) positive for *Babesia* spp., and 2 (<1%) for exotic *Theileria* spp. Seven of the 264 samples had insufficient quantities of DNA to visualize RFLP results. Sequencing of 20 selected samples confirmed RFLP results. Sequences of the two 'exotic' *Theileria* amplicons were >99% similar to *T. ovis*, a nonpathogenic ovine parasite native to Asia. Our data indicate that WTD *Theileria* sp. infections are common and that WTD can possibly maintain a higher diversity of *Theileria* spp. than recognized.

PS01.05 Report of *Lernaea Cyprinacea* in Axolotl Serrano (*Ambystoma Velasci*) in the Municipality of Atlangatepec in Tlaxcala, Mexico

Prof. Maria Cristina Guerrero-Molina¹, Prof. Ángel García-Hernández¹, Prof. José Manuel Cobo-González¹, Lidia Boleaga-Rivera¹, Itzel Hernández-Aranda¹, Natalia Vieyra-Gómez¹
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Lernaea cyprinacea is a parasite of worldwide

distribution, common in freshwater fish. The life cycle takes place between 15-21°C in 3-4 weeks. In Mexico there are 7 endemic species of axolotl. However, the presence of *L. cyprinacea* has only been reported in *Ambystoma mexicanum*. In the Atlangatepec Aquaculture Center, in the state of Tlaxcala, Mexico, European carps (*Cyprinus carpio*) are raised in ponds for commercial purposes. A group of 8 axolotls (*Ambystoma velasci*) also lived in the aquaculture center and were housed as reproductive couples in separate tanks. The axolotls went to an exhibition outside the center for two months and their tanks were filled with water from the carp's ponds, a species in which *Lernaea cyprinacea* is commonly found. The temperature of the water in the fish tanks housing the axolotls had a range of 11-15 °C. The parasites were collected of all axolotls with the help of tweezers and placed in plastic tubes with 70% alcohol. A total of 22 individual parasites were obtained and identified at the Parasitology Laboratory, UNAM. The frequency of the location of the parasites was: 40.90% (9/22) in the gills, 18.18% (4/22) in the base of the neck, 13.63% (3/22) in the groin, 9.09% (2/22) in the eyes, 4.54% (1/22) in the right shoulder and 4.54% (1/22) in the base of the cloacal glands. In addition, fibrous nodules were observed in axolotls where *Lernaea cyprinacea* was removed from the skin and gills. It was concluded that the water from the ponds where the carps were raised was the source of contamination for the axolotls. In addition, it was speculated that the parasites adapted well to the low temperature of the axolotls' tanks and thus, they survived.

PS01.06 Susceptibility of Selected Strains of Cat Fleas, House Flies and Darkling Beetles Obtained From Selected Laboratory Colonies to Deltamethrin, Fipronil and Imidacloprid

Bill Donahue¹

¹*Sierra Research Laboratories, Inc., United States*

Veterinary pests such as cat fleas, house flies, and litter beetles are routinely reared in entomology laboratories around the world. Bioassays are conducted in the laboratory to determine the efficacy of new compounds

and formulations which may be used for product registration or to conduct product comparisons for marketing claims. Concerns regarding insect health, vigor, behavior and genetic diversity should be addressed and characterized to better understand the results obtained during testing. Whereas standardization of colonies may be impractical and in many cases undesirable, characterization of colonies should be documented.

For example, insecticide resistance from field populations can be quite variable within laboratory reared colonies, often being lost if not periodically challenged or infused with new field collected stock. Baseline data from new insect collections should be established as soon as colonies are reproductively viable and evaluated periodically to note changes in susceptibility to selected classes of insecticides.

Results from dose response bioassays will be presented for several strains of cat fleas, house flies and litter beetles from selected laboratory colonies used for product development and regulatory activities. Technical deltamethrin, fipronil and imidacloprid were serially diluted in acetone and applied to various substrates. Adult insects were confined to the treated substrates and continuously confined to the treated surface. Knockdown and mortality were assessed at predetermined time intervals through 48 hours. Probit analysis (Polo Plus ver 2.0) was used to determine lethal dose values at selected times after exposure to the treated substrate.

PS01.07 Preliminary Study to Localize Adult *Brugia Malayi* and *Brugia Pahangi* Within Mongolian Gerbils (*Meriones Unguiculatus*) Following Subcutaneous Injection of Infective Third-Stage Larvae

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The site of subcutaneous infection with infective stage larvae of *Brugia malayi* and *B. pahangi* has been utilized to limit the

location of adult worms within dogs, cats, and multimammate rats. In the present study, an attempt was made to restrict the location of adult *Brugia* spp. within Mongolian gerbils. Two groups of 5 male gerbils each were infected subcutaneously in the dorsal neck with either 75 infective third-stage larvae of *B. malayi* or *B. pahangi*. The animals were maintained for 90-92 days before necropsy. Just prior to necropsy, blood was collected from each animal for determination of patency. A complete necropsy was done on each animal with examination of lymphatics, testes, hearts and lungs. All dissected tissues and carcasses were soaked overnight and the soakings were examined for worms. Four gerbils infected with *B. malayi* had worms. Recoveries ranged from 2-12 worms (5.5 worms per animal, average) with all but 1 female worm being recovered from the heart and lungs. This single female worm was recovered from the left testicle of the gerbil that had 12 worms. All 5 gerbils infected with *B. pahangi* had worms and all of the worms were recovered from the heart and lungs. Recoveries of *B. pahangi* ranged from 1-19 (5.4 worms per animal, average). Microfilariae were found circulating in the blood of only 1 gerbil infected with *B. malayi*. This gerbil had 12 worms (10 females, 2 males). None of the animals infected with *B. pahangi* were patent. This initial success at limiting the location of worms to the heart and lungs of gerbils may lead to the validation of a rodent model for canine heartworm infection.

PS01.08 Effects of Protein Diet Level and *Haemonchus Contortus* Infection on Lamb Performance

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The aim of this study was to verify the effects of diet protein content and *Haemonchus contortus* infection in sheep performance. Thirty Santa Inês lambs (16 females and 14 castrated males), six months old; initial body

weight (BW) 24.9 ± 4.03 kg (mean $\pm$ SD) were randomly distributed in a factorial arrangement 2x2, with high (HP: 17%) and low (LP: 10%) protein levels, and infected (i) and non-infected (c, control) with *H. contortus*, coming up to four treatments: HPc (n = 6), HPi (n = 10), LPc (n = 6) and LPi (n = 10). On infected groups each lamb received a single dose of 10,000 *H. contortus* L3 larvae. Fortnightly, the animals were weighted (BW). During 62 days, daily dry matter intake (DMI) was recorded for feed intake (FI) determination in g DMI/kg BW^{0.75}. No differences were observed on worm burdens between infected groups ($P > 0.05$). The male lambs (30.1 kg) were heavier than females (26.5 kg) ($P < 0.05$), but there was no interaction between sex and treatments ($P > 0.05$). There was protein and infection interaction on FI and BW ($P < 0.05$). The HPi group (66.3 g DMI/kg BW^{0.75}) had a lower FI ($P < 0.0001$) than HPc (69.1 g DMI/kg BW^{0.75}), LPc (69.3 g DMI/kg BW^{0.75}) and LPi (69.5 g DMI/kg BW^{0.75}). Lambs from HPc presented a significant ($P < 0.001$) higher BW (30.0 kg) in comparison to HPi (26.8 kg), while the other groups showed intermediate values LPc (28.4 kg) and LPi (28.3 kg). In conclusion growing lambs when fed with higher protein presented better performance, but they were more affected on FI and BW by *H. contortus* infection, than those with lower protein availability in the diet.

PS01.09 Highly Effective Selection for Resistance to *Haemonchus Contortus* Experimental Infections May Lead to Reduced Fat Deposition in Growing Sheep

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As anthelmintic resistance is now widespread in gastro-intestinal nematodes (GIN), genetic selection of sheep for a better resistance to GIN infection is increasingly considered as an alternative strategy. One possibility for this is to select breeding animals based on

their responses to experimental infection. To analyze the effectiveness of this strategy and its potential effects on growth traits, we divergently selected sheep of the Romane meat breed based on their response to *Haemonchus contortus* experimental infections. A total of 91 naïve female lambs of the second generation (51 from the resistant line and 40 from the susceptible line) were successively challenged with 3,500 larvae and 10,000 larvae of *H. contortus* at 3 to 5 months of age. In all lambs, we measured fecal egg count (FEC), hematocrit, weight, back fat and muscle thickness at the beginning and at the end of the second infection, and twice a week during the second infection for the 42 most divergent lambs. Lambs from the S line excreted on average ten times more eggs than those from the R line during the second infection. Although growth in body mass did not differ between lines, growth in fat thickness was 20% higher in the S line than in the R line. Overall, selection for resistance to *H. contortus* based on experimental infestation apparently resulted in highly effective immune response in growing female lambs but may compromise fat deposition, suggesting a potential energetic cost of immunity against GIN infection.

PS01.10 Resistance of Helminths to Closantel 10%, Albendazole 5% and Praziquantel 7.5% in Hampshire Down Sheep from the Department of Misiones, Paraguay

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Gastrointestinal nematodes is a serious problem in sheep production, causing a decrease in body condition, weight loss, reduction of productivity, and in severe cases death of the animal. The objective of this research was to evaluate the efficacy of closantel 10%, albendazole 5% (Microtel, Laboratorio Microsules, Uruguay. S. A) and praziquantel 7.5% (Oviquantel, Vetanco, Argentina) in 32, 7 months to 6 years Hampshire down sheep from both sex from a

farm of the Department of Misiones, Paraguay. The anthelmintic resistance was determined by McMaster technique (described by Coles et al., 1992), before and 14 days after treatment. The helminthes species identification was carried out by coproculture and identification of stage 3 larvae (L3), (Fiel et al., 2011). High resistance to anthelmintic treatment was observed since only 6/32 sheep showed reduction in eggs counts $\geq 90\%$. Sheep with less than 1 year showed at the coproculture and identification of L3: *Ostertagia* sp 6%, *Cooperia* sp 38%, *Haemonchus* sp 43% and *Trichostrongylus* 5%. Adult sheep: *Ostertagia* sp 6%, *Cooperia* sp 19%, *Haemonchus* sp 67% and *Nematodirus* sp 8%. This research shows the high resistance to helminths in sheep from the Department of Misiones to the three molecules tested and that should be implemented prophylactic measures that will reduce the problem along the production such as feeding supplementary, pasture rotation and adequate use of anthelmintics in sheep.

PS01.11 Infestation with Larvae of the Fly *Philornis* Sp. Of Harris'Hawk (*Parabuteo unicinctus*) Young Birds in Morelos, Mexico

Prof. Maria Cristina Guerrero-Molina¹, Prof. Cintli Martínez Ortiz de Montellano¹, Prof. Yazmin Alcala-Canto¹, Prof. Juan Carlos Morales Luna¹

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The fly of the genus *Philornis* (Diptera, Muscidae) has 51 species and a distribution is mainly Neotropical. Their larvae are parasites of only subcutaneous location and feed on blood from a wide range of birds. The development of the larva is rapid between 4 and 6 days and forms boils that extend through the dermal openings of its hosts. The black-and-white Hawk (*Parabuteo unicinctus*) is distributed from the southwestern United States of North America, in Mexico, central Argentina and southern Chile. This bird is used frequently in falconry, which is why it is sometimes raised and marketed in a particular way. The objective of this work was to communicate the presence of larvae of flies of the genus *Philornis* sp, in different regions

of the body of Harris'Hawk (*Parabuteo unicinctus*) young birds from Morelos, in Mexico. Sixty-eight fly larvae of forunculus lesions were collected from the head, chest region, thighs, legs and phalanges of two one-month-old Harris'Hawk (*Parabuteo unicinctus*), from the state of Morelos in Mexico. In the Parasitology Laboratory of the Faculty of Veterinary Medicine and Animal Husbandry of the UNAM, the larvae were placed between the holder and covered objects with Hoyer's liquid. The posterior respiratory stigmas of the larvae were observed under a stereoscopic microscope. After observing the morphological characteristics of the larvae, it was determined that they were larvae of the genus *Philornis* sp. The importance of this communication is that the injuries caused by the larvae of *Philornis* sp, in the muscular and cartilaginous tissues can produce irreversible deformations that affect the movement of the birds.

PS01.12 A Retrospective Study on the Occurrence of Parasitic Diseases of Wild Animals in Western Ghats of Karnataka, India

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Wildlife is our national treasure and the wild animals are susceptible to wide variety of parasitic diseases which often go unnoticed. Among factors that threaten the existence of wild animals, infectious diseases in particular those arising from parasites play an important role. The present study reports the occurrence of parasitic diseases in wild animals, both captive and free range received for necropsy in Western Ghats of south-central Karnataka from 2009 to 2018. The necropsy of tigers showed *Taenia* sp., *Ancylostoma* sp., *Paragonimus* sp. and *Toxocara* sp. Leopards harboured *Spirometra* sp., *Taenia* sp. and *Physaloptera* sp. The necropsy of elephants showed *Amphistome* sp., *Cubboldia elephantis* bots and *Murshidia* sp. of strongyles. The necropsy of Pythons showed infections with *Ophidascaris* sp. and *Ascaridia galli* whereas; Checkered Keelback snake had *Ascaridia galli*. The post-mortem

examination of peacock showed *Ascaridia galli* and *Heterachis gallinarum* species, Ibis bird had *Echinostoma* sp. and *Ascaridia galli* whereas, Red Jungle fowls showed *Heterachis gallinarum* in the caecum. The intestinal contents collected during necropsy were examined by qualitative methods for detection of parasitic ova. The intestinal contents of tiger showed the eggs of above said parasites along with *Isosporan* oocyst. Similarly, in species like leopard, python, Checkered Keelback and Ibis, the eggs observed corresponded to the parasites mentioned above respectively. In elephants, along with ova of the above said parasites, there were also eggs of *Fasciola* sp. and *Schistosoma* sp. Whereas, in Peacock and Red Jungle fowls, the ova of *Ascaridia galli* and *Heterachis gallinarum* were observed along with coccidian oocysts. The present study reports the existence of wide variety of parasitic diseases among wild animals which is infectious with a capacity to spread, probably also with zoonotic potential.

PS01.13 Functional Characterization of a Salivary Thrombin Inhibitor from the Flea *Xenopsylla cheopis*

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The salivary glands of hematophagous animals contain a complex cocktail that interferes with the host hemostasis and inflammation pathways, thus increasing feeding success. Analysis of the salivary gland transcriptome of the flea *Xenopsylla cheopis*, the vector of human plague, reveals presence of a 59-aa long peptide (named XC-43) containing a signal peptide, and without significant matches to other proteins in public databases. Mature XC-43 (36-aa long) was synthesized and used in coagulation assays with human plasma, where we were able to observe anticoagulant properties, delaying thrombin time (TT), prothrombin time (PT), and partially activated thromboplastin time (aPTT). XC-43 was found to be a specific, fast and tight-binding

inhibitor of thrombin, blocking its coagulation of plasma and inhibiting S-2238 hydrolysis in a competitive manner with an inhibition constant (K_i) of 7.7 pM. Using isothermal titration calorimetry, we observed XC-43 and thrombin binding reaction reaction was enthalpy-driven ($\Delta H = -2.95 \times 10^4$ kcal/mol) with an equilibrium constant (K) of 1.33×10^{-10} M⁻¹. Although XC-43 has a putative thrombin cleavage site (Lys11-Met12), peptide is not cleaved by thrombin. Evaluation of XC-43 anticoagulant properties in vivo is underway. The identification of novel natural anticoagulants and the understanding of their mechanism of action may offer opportunities for designing new antithrombotics disrupting blood clotting. Taking into account the selectivity for thrombin and its kinetic properties, this peptide could be a candidate for clinical use as an antithrombotic.

PS01.14 Mosquito Distribution and Duck Tembusu Virus Infection on Duck Farms in Sing Buri and Ang Thong Provinces, Central Thailand

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Duck Tembusu virus (DTMUV) is an emerging infectious disease in ducks, belonging to the Ntaya virus group of the Flavivirus genus and Flaviviridae family. The emergence of DTMUV has been observed on layer and breeder duck farms in China since 2010 as well as on layer and broiler duck farms in Thailand since 2013. Infected ducks show neurologic signs, including an incapability to stand, ataxia, and paralysis. A significant drop in egg production is usually observed among layer ducks. The transmission of DTUMV involves insect vectors, with mosquitoes also being important vectors for this virus. However, the exact role of mosquitoes in the ecology of DTMUV in Thailand remains unclear. This study was conducted to examine

mosquito distribution and DTMUV infection in mosquitoes on duck farms in central Thailand. Mosquitoes were collected from two duck farms in Sing Buri Province and two duck farms in Ang Thong Province from September 2015 to July 2016. Four CDC-light traps were used for collecting mosquitoes from each duck farm and were operated overnight. A total of 30,841 mosquitoes were collected and identified. Seven mosquito species were found in this study, comprising *Anopheles barbirostris*, *An. stephensi*, *Culex gelidus*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *Mansonia annulifera*, and *Ma. uniformis*. The most collected mosquitoes from each duck farm and each collected time were *Cx. tritaeniorhynchus*. The pools of mosquitoes were then examined for DTMUV infection by RT-PCR using specific primers. A total of 273 mosquito pools were examined, with only one pool of *Cx. tritaeniorhynchus* collected from Sing Buri Province in November 2015 testing positive for DTMUV. Thus, this study indicates that *Cx. tritaeniorhynchus* may play an important role as a vector in the transmission of DTMUV in Thailand. However, additional studies concerning the vector competence of this mosquito for DTMUV are needed.

PS01.15 Pharmacological Characterization of a Homomeric Nicotinic Acetylcholine Receptor Formed by Ancylostoma Caninum ACR-16

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Parasitic infections are treated using anthelmintic drugs, some of which target nicotinic acetylcholine receptors located in different tissues. The limited arsenal of anthelmintic agents and the prevalence of drug resistance implies that future defense against parasitic infections will depend on the discovery of novel targets and therapeutics. Previous studies have shown *Ascaris suum* ACR-16 nicotinic acetylcholine receptors to be a suitable target for the development of antinematodal drugs. In this study we characterized the pharmacology

of *Ancylostoma caninum* ACR-16 as a drug target using two-electrode voltage-clamp electrophysiology. This technique allowed us to explore the effects of several cholinergic agonists and antagonists on the nAChRs expressed in *Xenopus laevis* oocytes. Acn-ACR-16 was not sensitive to many of the existing cholinomimetic anthelmintics (levamisole, oxantel, pyrantel, morantel, bephenium and tribendimidine). 3-Bromocytisine was the most potent agonist (>130% of the control acetylcholine current). Unlike Asu-ACR-16, the *Ancylostoma caninum* nAChR did not produce a response to oxantel. The mean time constants of agonists for desensitization rates ranged between 1.5 and 4.8s for Acn-ACR-16 and were longer than the rates observed in Asu-ACR-16. In contrast to Asu-ACR-16, the *A. caninum* receptor was completely inhibited by Dh β E and moderately inhibited by α -BTX. In conclusion, we have successfully reconstituted a fully functional homomeric nAChR, ACR-16 from *A. caninum*, which is used as a model for human hookworm infections. The pharmacology of the receptor is distinct from the levamisole sensitive nematode receptors. The ACR-16 homologue also displayed some pharmacological differences from Asu-ACR-16. Hence, *A. caninum* ACR-16 may be a valid target site for the development of agents against hookworm infection.

PS01.16 Anthelmintic Effect of Supplementation with Mimosa Caesalpinifolia and Feeding Behavior of Goats Grazing in Tropical Deciduous Forest

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The use of tropical deciduous forests (TDF) is an important resource in goat production, due to the great diversity of fodder plants rich in protein and condensed

tannins (CT), which may be important in the control of gastrointestinal nematodes. The objective of the study was to evaluate the effect of supplementation of an important tanniniferous plant, *Mimosa caesalpinifolia*, against *H. contortus* in goats grazing in TDF. Egg hatching, larval exsheathment, bromatological analysis and chromatographic profile of the sample plant, were performed. Twenty-four goats were infected with a single dose of infective larvae of *H. contortus*, 28 days prior to the start the experiment. The animals were distributed into two groups according to faecal eggs count and body weight. Group I receiving concentrate with *M. caesalpinifolia* (128.7 mg/CT/kg) and Group II:receiving concentrate without *M. caesalpinifolia*. All animals received isoproteic and isoenergetic supplementation and have daily access to TDF. Animals were weighed weekly and faecal egg counts were performed twice per week. The direct observation method was applied in 4 goats to record the species of plants consumed. After 28 days of experimental feeding, all animals were humanly slaughtered and adult worm populations were estimated. A total of 20 species of TDF plants were consumed. The medium values of dry matter and crude protein Intake was significantly different between the groups ($P < 0.05$). The in vitro exsheathment inhibitory effect (IC50) was 0.46mg/mL. The low inhibitory effect was observed in egg hatch test. The reduction of faecal egg counts was 25%. There was no significant effect of the supplementation with *M. caesalpinifolia* on the reduction of adult worm burdens. There is significant potential in the use of *M. caesalpinifolia* in the goat diet for the control of *H. contortus*, but there is a need for other studies to clarify in more detail how the anthelmintic effect of the tannins or other polyphenols present in the plant studied.

PS01.17 The Use of Deep Amplicon ITS-2 rDNA Nemabiome Sequencing to Investigate Anthelmintic Resistance in Ovine Gastro-Intestinal Parasites

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Gastrointestinal nematodes are a major problem for sheep industry worldwide. Nematode species differ in their clinical manifestations, economic impacts, epidemiology and drug sensitivities. Consequently, quantifying the species composition of parasite communities is important to direct drug treatment choice, diagnose drug resistance and inform parasite management strategies. The recently developed next-generation deep amplicon ITS-2 rDNA nemabiome sequencing, using the Illumina Miseq platform, has many applications in this area due to its high accuracy, throughput, sensitivity and increasing affordability. Here we validate deep amplicon ITS-2 rDNA sequencing for ovine gastrointestinal nematodes and show its reliability in the relative quantification of nematode species from either eggs, L1 or L3 larvae harvested from fecal samples. We have applied the validated assay to a large scale study of more than 40 farms across Western Canada over 5 years to investigate parasite species distribution and anthelmintic resistance. Nemabiome sequencing was applied to samples collected by producers before and after drug treatment and mailed to the laboratory. We also visited farms in Alberta and performed Fecal Egg Count Reduction tests over several years to investigate benzimidazole, ivermectin and closantel resistance. Nemabiome sequencing was used to help confirm resistance and determine which parasite species were resistant to which drug. *Haemonchus contortus* was the most prevalent species on most farms and was most commonly found to be resistant to both ivermectin and benzimidazoles followed by *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. Nemabiome sequencing was also instrumental in determining that the low efficacy of closantel in many Fecal Egg Count Reduction tests was not due to anthelmintic resistance but due to the narrow spectrum of the drug. Results show that ITS-2 rDNA nemabiome sequencing is a valuable adjunct to the FECRT in investigating anthelmintic resistance.

PS01.18 Exploring Thermosensory Behaviours and Pathways in Filarial Nematode Parasites

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Dirofilaria immitis is the etiologic agent of canine and feline heartworm disease. The prevention of this disease is achieved through the administration of macrocyclic lactones as a prophylactic, or melarsomine dihydrochloride in dogs with adult infections. Severely limited pharmacologic options make control of this disease difficult, especially in regions of drug resistance or areas with high non-compliance of preventative supplementation. Therefore, in order to decrease the reliance on a single-drug group, other prevention strategies need to be explored. L3 and L4 larval stages of *D. immitis* are the targets for existing chemotherapies, and these stages exhibit thermosensory behaviours in vitro, which are likely used during transmission and infection of the mammalian host. This behaviour could be exploited by chemotherapeutic intervention, but the molecular effectors of thermosensation are unknown in *D. immitis*. To identify putative thermosensory effects in *D. immitis*, we conducted a pan-phylum comparative genomic and phylogenetic analysis informed by thermosensory pathways elucidated in the model nematode *Caenorhabditis elegans*. To validate these putative thermosensory effectors, we developed a reverse genetics approach to knock-down expression of gene targets predicted to function in thermosensation and assayed the modulation of thermosensory phenotypes. Because of the fragmented nature of *D. immitis* gene predictions, we used long-read isoform sequencing of adult males and females to annotate full-length genes, which enabled the design of improved dsRNA triggers. Larval worms were exposed to dsRNA during in vitro culture or by injecting infected mosquitoes, and worms were tested for thermosensory defects using a linear thermal gradient. In addition to RNAi, we also treated larval worms with chemicals that are known to interact with predicted thermosensory effectors and tested for thermosensory defects. We

foresee this approach enabling the continued identification and annotation of *D. immitis* thermosensory effectors, which could be putative targets for the prevention of heartworm infections.

PS01.19 Study of the Role of P-Glycoprotein-2 of the Parasitic Nematode Heligmosomoides Polygyrus in the Cholesterol Uptake

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Recent studies have described the involvement of *Caenorhabditis elegans* p-glycoprotein-2 in lysosome formation and lipid storage. In that respect, we are carefully trying to identify in *Heligmosomoides polygyrus bakeri* the free-living nematode model *Caenorhabditis elegans* p-glycoprotein homologs using degenerate primers. These experiments have allowed the identification of five partial p-glycoprotein sequences. Among those Hba-pgp-2 has particular interest. Since, *C. elegans* PGP-2 is involved in the transport of cholesterol. Cholesterol is needed for molting and growth of the nematodes. In this study, a full-length cDNA sequence corresponding to Hba-pgp-2 was obtained. It presents high homologies with the Cel-pgp-2 gene sequence. Moreover, we showed that Hba-pgp-2 is expressed among the eggs, fourth-stage larvae and adult and their expression could be induced by cholesterol. In order to characterize the Hba-pgp-2 function, the Hba-pgp-2 promoter was analyzed using the nematode model *C. elegans* and complementation of *C. elegans* pgp-2 mutant by Hba-pgp-2 was performed. This opens the way for studying the involvement of Pgps in parasitic biological process that can lead to develop new way to control nematode parasites.

PS01.20 The Giant African Snail *Achatina Fulica* as Carrier of Nematodes of Medical and Veterinary Importance in the Rio De Janeiro State, Brazil

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Invasive land molluscs have generated economic damage to agriculture, environment and problems to animal and human health worldwide, including Brazil. *Achatina fulica* is an intermediate host of nematodes of medical and veterinary importance, such as *Angiostrongylus cantonensis*, *A. costaricensis* and *Aelurostrongylus abstrusus*. *Achatina fulica* was introduced for commercial purposes in Paraná State, South Brazil, in the 1980's and is currently recorded in all Brazilian states, except in Rio Grande do Sul. Our main objective was to evaluate the occurrence of *A. fulica* infected with *Metastrongyloidea* larvae in the 16 municipalities of the Mesoregion Centro Fluminense of Rio de Janeiro state, Southeast Brazil. Molluscs were collected from December 2016 to October 2018. In all, 358 *A. fulica* specimens were collected and examined through the artificial digestion technique. Morphotypes of nematode larvae were obtained: *Angiostrongylus* sp., *Rhabditis* sp., *Caenorhabditis* sp. and *A. abstrusus*. *Metastrongyloidea* larvae are being identified using DNA Barcode (mitochondrial cytochrome c oxidase subunit I). The municipality of Paraíba do Sul presented the highest infection rate (72%), and Macuco (first report of *A. fulica*) presented the lowest infection rate (8%). *Aelurostrongylus abstrusus* larvae, a feline lung parasite, was observed in 44% of the investigated municipalities. We highlight that the high prevalence of this species indicates that the parasite may be underdiagnosed in cats, and that *A. fulica* may be the main vector for its transmission. In four municipalities, no specimens of *A. fulica* were found (Areal, Bom Jardim, Duas Barras e São Sebastião do

Alto), which requires confirmation, since in bordering municipalities this species is found in dense populations, including specimens infected with nematodes. This survey reinforces the importance of *A. fulica* in the transmission of parasitic diseases of medical and veterinary importance, besides providing subsidies to the control and prevention of those parasites.

PS01.21 Expression of Four Nicotinic Acetylcholine Receptor Subunits in *Ascaris suum* Intestine and Muscle Cells

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Ascaris suum is a gastrointestinal parasite of pigs which is of primary health concern and economic importance to the swine industry. In the absence of effective vaccines, anthelmintics are required for prophylaxis and treatment. However, intensive use has led to the development of resistance globally. Drugs such as levamisole and pyrantel target nicotinic acetylcholine receptors (nAChRs) found on the somatic muscle of the parasite. nAChRs are transmembrane proteins comprised of five subunits that surround a central cation-permeable pore and facilitate coordinated muscle movement that is crucial for the parasite's maintenance of position in the intestine. Additionally, different combinations or stoichiometry of nAChR subunits gives rise to a wide diversity of receptor pharmacology. In vertebrates, nAChR function is not restricted to fast-neurotransmission. Given the diversity of nAChR subtypes in nematodes, we hypothesize that nAChR expression (and function) will not be restricted to nervous and muscle tissue in worms. Here we focus on the nematode intestine, which is involved in digestion and absorption of nutrients making it an attractive site for anthelmintic intervention. PCR was used to confirm expression of nAChR subunits: *Asu-unc-38*, *Asu-unc-29*, *Asu-unc-63* and *Asu-acr-8* that constitute the putative levamisole receptor in adult female *A. suum* muscle and intestine.

Expression of these subunits within the intestine was notable and unexpected. We conducted further experiments using RNAscope in situ hybridization to localize expression of the subunits at higher resolution in the intestine and muscle; and qPCR to compare mRNA levels in both tissues. Calcium imaging was also conducted on isolated intestinal tissue where levamisole and acetylcholine elicited intracellular calcium responses. These findings raise questions on the functional roles of nAChRs in the intestine which may not be limited to neuromuscular transmission, but an acetylcholine paracrine function. Further studies on the mechanisms involved in intestinal nAChR signaling is paramount for therapeutic exploitation.

PS01.22 Anthelmintic Effectiveness of Citronellal and Citronellol on Haemonchus Contortus Evaluated In Vitro Larval Development Test

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Research on natural products is expanding due to the promising results found in the sustainable and strategic control against gastrointestinal nematodes. Citronellal and Citronellol are natural acyclic monoterpenes found in essential oils. The objective of this study was to evaluate the activity of these compounds on larvae of *Haemonchus contortus* resistant to multiple drugs through the larval development test and determination of lethal concentrations (LC) using doses ranging from 5 mg/mL to 0.00001 mg/mL. For each concentration, 6 replicates were performed diluted in Tween and negative control with Tween and distilled water. The eggs were placed in 48-well plates and incubated for 24 hours at 27 °C. On the next day, nutrient medium and oils concentrations were added and they were incubated more 5 days, until the larvae reached the third stage. The counting of L3 and undeveloped larvae was evaluated under the inverted microscope. The SAS Probit Program was performed for

calculation of the LC50 with the independent variables (dose) transformed by natural logarithm (log dose). LC50 of citronellol was 1.08 mg/mL demonstrating better efficacy of this compound in inhibiting the development of larvae in comparison to citronellal which LC50 was 3.57 mg/mL. We concluded citronellol showed high anthelmintic efficacy against resistant strains of *Haemonchus contortus*. - FAPESP/2018-02423-0

PS01.23 In Vitro Anthelmintic Effect of Four Extracts Obtained From Caesalpinia Coriaria Foliage Against an Haemonchus Contortus Isolate. 1. Egg Hatch Test

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The failure of anthelmintics to control parasites of small ruminants is a worldwide problem, alternative control and plants secondary metabolites are one of the potential solutions. The present study evaluated the in vitro anthelmintic effect of extracts obtained from *Caesalpinia coriaria* foliage, a legume consumed by sheep and goats in the department of La Guajira-Colombia, and used for medicinal purposes by the Wayúu community. To measure the susceptibility of an *Haemonchus contortus* isolate the in vitro Egg hatch test was used with extracts of different solvents. The yields of the extracts were 30.54, 23.51, 27.7 and 16.6% for acetone-water (ACW), methanol-water (MW), acetone-dichloromethane-water (ADCW) and methanol-dichloromethane-water (MDCW), respectively. The anthelmintic activity of the different concentrations (125, 250, 500, 1000, 2000 and 3000 µg/ml) of *C. coriaria*, were compared with the hatching percentage in Phosphate Buffered Saline (PBS) as a negative control. Death of *H. contortus* was determined within a period of 48 h. *C. coriaria* extract had mean mortality of 46.7, 37.1, 38.8 and 29.8 % at 4000 µg/ml for the ACW, MW, ADCW, and MDCW, respectively; the extracts inhibited egg hatchability of *H. contortus* in a concentration-dependent manner. Effective concentrations 50% (EC50) were calculate by

Probit Analysis and best-fit values were 6763, 8853, 8762 and 12872 $\mu\text{g/ml}$, for ACW, MW, ADCW and MDCW, respectively. The ACW was the most active against *H. contortus* eggs, with the lowest EC₅₀. This study demonstrates that the extracts of *C. coriaria* foliage have anthelmintic activity; therefore, they could have an application in the control of helminths in livestock.

PS01.24 In Vitro Anthelmintic Effect of Four Extracts Obtained From *Caesalpinia Coriaria* Foliage Against An *Haemonchus Contortus* Isolate. 2. Larvae Exsheathment Inhibition Test

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The increase of anthelmintic resistance worldwide, has caused researchers to focus on the use of plants in order to reduce the use of commercial drugs. The present study evaluated the in vitro anthelmintic effect of extracts obtained from *Caesalpinia coriaria*, a legume consumed by small ruminants in the department of La Guajira-Colombia, used by the Wayúu indigenous community for controlling parasites of sheep and goats. The effect was measured against an *Haemonchus contortus* isolate, the larvae exsheathment inhibition test was performed, using L3 larvae (1 to 3 weeks) incubated for 3 hours in four extracts of *C. coriaria*; then washed with Phosphate Buffered Saline (PBS) solution and placed in 24-well plates to be exposed to a hypochlorite solution (NaClO) at 0.05 % (previously standardized). Six repetitions per concentration (125, 250, 500, 1000, 2000 and 3000 $\mu\text{g/ml}$) were used and a negative control in PBS. The extracts were prepared with different solvents: acetone-water (ACW), methanol-water (MW), acetone-dichloromethane-water (ADCW) and methanol-dichloromethane-water (MDCW). Using PBS control, 100% of larvae were exsheathed at 60 min after adding NaClO. Counts of non-exsheathed larvae were analyzed using a negative binomial

generalized linear model with the log link. Extract by exposure time (ET) interaction effect was statistically significant ($P < 0.05$). At an ET of 10 minutes, there were not statistically significant differences between extracts, while at 60 minutes, all pairwise differences were significant except for MW and MDCW, which yielded the lowest means. Thus, larvae exsheathment was affected by extracts, but the extend of the effect depends on exposure time. At an ET of 60 minutes, EC₅₀ values were 5927.4, 9955.4, 2883.4, 9876.3 $\mu\text{g/ml}$, for ACW, MW, ADCW and MDCW, respectively. These results suggest that at this ET, the most effective extract was ADCW.

PS01.25 Anthelmintic Activity of a Synthetic Peptide Bioinspired in the Protein Rc-2S-Alb Towards *Haemonchus Contortus*

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The nematode *Haemonchus contortus* has been developed resistance to the most common drugs commercially available. In the search for alternatives to conventional anthelmintics, peptides have been investigated. Here, a linear synthetic peptide named RcAlb-Pep bioinspired from the antimicrobial protein Rc-2S-Alb was designed, synthesized, and tested against *H. contortus*. Physicochemical properties of the peptide, the 3D structure model, the egg hatch inhibition and larval development inhibition of *H. contortus* were carried out. Additionally, the ultrastructure of the nematode after treatment with the peptide was evaluated by atomic force microscopy. The peptide showed molecular weight, Boman Index, hydrophobic ratio and net charge of 826.05 Da; -1.28; 62%; and +1; respectively. RcAlb-Pep has a small negatively charged region and a larger positively charged region, due the presence of positively charged amino acid lysine

(Lys). The RcAlb-Pep inhibited the larval development of *H. contortus* with an EC₅₀ of 0.15 mg mL⁻¹ and had no effect on egg hatch. Atomic force microscopy reveals the affinity of RcAlb-Pep with the cuticle of *H. contortus* in L2 stage. For the L3 stage, low affinity with RcAlb-Pep was verified, which indicates the low interaction is due to the formation of the sheath at this stage, promoting an extra protection against biomolecules targeting the cuticle. In conclusion, the bioinspired RcAlb-Pep has potential to be used as a new anthelmintic compound to control of gastrointestinal nematode parasites.

PS01.26 Assessment of Biophysical Properties of Haemonchus Contortus From Different Life Cycle Stages With Atomic Force Microscopy

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Haemonchus contortus is the most pathogenic and economically important gastrointestinal nematode species infecting small ruminants. Light, scanning and transmission electron microscopy have been successfully used to assess damage or structural parameters of *H. contortus*. However, some limitations of these techniques are preventing advances in understanding the effect of different compounds on *H. contortus*. Atomic Force Microscopy (AFM) is a high-resolution microscopy technique that provides information about the topography and surface composition of a wide variety of materials ranging from living individual cells through fixed tissue. The present study report, for the first time, assessed the topographic and biomechanical characterization of *H. contortus* eggs, larvae and adult cuticle

by AFM. *H. contortus* eggs, L1, L2, L3 and adults females were obtained from sheep that were experimentally infected. The L3 larvae were unsheathed with 0.02 % sodium hypochlorite solution. For fixation, all stages of *H. contortus* were maintained in 5% formalin. We observed a qualitative reduction in Young's modulus when eggs develop from the morulae to the larvae stage. The AFM analysis of L1 stage showed a series of periodically separated annuli, with remains from larvae hatching on the nematode cuticle at this stage. The images of *H. contortus* in adult, L3 with sheath, L2 and L1 stages make it possible to compare the alterations of the annuli structures during the evolution of the parasite. The artificially unsheathed process made evident the genital primordium of *H. contortus* in the L3 stage. The results revealed an increase in adhesion values on the surface of the nematode in the L3 stage due to sheath removal. Our results provide early insight into the differential biomechanical and ultrastructural properties of our samples, which can explain biological and biochemical steps in the life cycle of these parasites.

PS01.27 Anthelmintic Effect of Extracts from Petiveria Alliacea and Diospyros Anisandra on Haemonchus Placei Larval Development and Egg Hatching

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Haemonchus placei is one of the most prevalent and pathogenic nematodes that affect cattle. Several anthelmintics have been used for its control; however, resistance to benzimidazoles, levamisole and macrocyclic lactones has been reported. Control alternatives include the use of plant extracts with anthelmintic effect. The objective of the present study was to evaluate the in vitro anthelmintic effect of *Petiveria alliacea* and *Diospyros anisandra* extracts on *Haemonchus*

placei larval development and egg hatching. Extracts from *P. alliacea* (stems and leaves) and *D. anisandra* (bark and leaves) collected in dry and rainy season, were evaluated by the egg hatch assay (concentrations: 600µg, 300µg, 150µg, 75µg and 37.5µg per ml). Lethal concentrations at 50% (LC50) were obtained with Polo plus software. The extracts from stems and bark had higher percent of egg hatch inhibition (EHI) than the extracts from leaves. Extracts from *P. alliacea* stem and *D. anisandra* bark, collected in rainy season showed higher percent of EHI (91.9% and 96.4% in 300 µg/ml, respectively) than the extracts collected in dry season. The extracts of *P. alliacea* stem and *D. anisandra* bark collected in rainy season showed the lowest LC50 (78.9 and 71.6 µg/ml, respectively). Additionally, it was demonstrated that these extracts have high ovicidal effect (91.6% and 89.0% in 300 µg/ml, respectively). The results of the present study showed that the extracts of stem from *P. alliacea* and bark from *D. anisandra* collected in rainy season have high anthelmintic effect in vitro on *Haemonchus placei* larval development and egg hatching, representing a possible alternative for the control of these nematodes.

PS01.28 Anthelmintic-like Activity of Four Polyphenolic Combinations and Their Interactions Against Adult Worms *Cooperia punctata*

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Polyphenolic compounds (PCs) from bioactive plant extracts have been identified and assessed for anthelmintic (Ah)-like activity. Previous reports have proposed the use of PCs-combinations (Coumarin:Quercetin and Caffeic-acid:Rutin) against free-living stages of *Cooperia punctata*. However free-living and parasitic stages have biological differences,

which might impair the effectiveness of a treatment. The objective of this study was to assess the in vitro AH-like activity of PCs-combinations (Coumarin:Quercetin, and Caffeic-acid:rutin; 8:2 ratio) against adult motility of the cattle nematode *C. punctata*. Immediately after recovery, 10-13 motile *C. punctata* adult worms were incubated at 37 °C with the PCs-combinations for 2, 4, 6, 12 and 24 hours, with a 5 % CO₂ inclusion. Five increasing concentrations were used for Coumarin:Quercetin (0.043, 0.087, 0.175, 0.35, 0.7 and 1.4 mg / mL⁻¹) and Caffeic-acid:Rutin (0.052, 0.105, 0.21, 0.42, 0.84 and 1.68 mg / mL⁻¹). Ethanol-2.5 % and physiological saline solution were used as negative controls. Four replicates were run for each concentration and control. Individual motility was calculated as: Worm motility%= 100 (motile worms in the concentration / total number of worms in the same concentration), corrected for by control motility percentages. Motility of worms was not affected after 2 and 4 hours using both PCs-combinations, neither for 6 hours post-exposure to Caffeic-acid:Rutin (P>0.05). Estimated EC₅₀ for Coumarin:Quercetin were: 1.621 ± 0.154 mg mL⁻¹, 0.398 ± 0.064 mg mL⁻¹ and 0.073 ± 0.071 mg mL⁻¹ for time lapse of 6, 12 and 24 hours, respectively. Caffeic acid:Rutin EC₅₀ estimation was: 0.192 ± 0.061 mg mL⁻¹ and 0.051 ± 0.164 mg mL⁻¹ for time lapse of 12 and 24 hours, respectively. Results suggest that PCs-combinations assessed interfere nematodes neurophysiology which leads to their paralysis. After addressing their potential toxicity and pharmacokinetics, formulations could be considered for in vivo field trials.

PS01.29 Prevalence of Gastrointestinal Parasites and the Effective Control Trials in Some Dairy Farms in Bahrain

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Livestock represents an important contributive part of the economy, although the livestock population is very small due to the limited land-space in Bahrain. This field study was conducted to evaluate the parasite

status of dairy cattle in Bahrain. Material and Methods: A clinical epidemiological study in dairy cattle was conducted in Bahrain during 2017 - 2018. The study evaluated fresh fecal samples collected from a total of 92 cattle across 11 dairy farms. The study included various animal ages, breeds, lactation stages and sexes to study the significance on parasitic infestivity across animal breeds and sexes. Results: The overall prevalence rate of *Haemonchus contortus* among sampled cattle was 26.07% (24 of 92 samples tested). Most of the positive cases were found as mixed infections with other species of gastrointestinal parasites, *Trichostrongylus* species, *Trichuris*, *Nematodirus* and *Eimeria* species while all these species were considered as less significant due to the level of infection. Eimeriosis caused by various *Eimeria* species was seen in 17 cases (18.5%) causing serious losses among newly born calves. Discussions and Conclusion: The effects of gastrointestinal helminthes among cattle in Bahrain were varied with animal ages, management and nutritional conditions, lactating stages and severity of infestations.

PS01.30 Clinical Efficacy of Exzolt® (1% Fluralaner Aqueous Solution) In the Treatment of Mite Infestations of Flocks In Tropical Countries

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Three clinical studies were conducted in Brazil and the Philippines in flocks affected with different fowl mites. All flocks were treated with Exzolt® at the label dose of 0.5 mg/kg BW in drinking water twice 7 days apart, D0 and D7.

The first study involved 60 Hisex Brown laying hens sourced from a commercial farm in Brazil. All had a Northern Fowl Mite infestation, *Ornithonyssus sylviarum* (score ≥ 2 of 7). They were separated into 2 groups, treated and untreated. The treatment efficacy

was based on the mean score per group at each time point and assessed by the Abbott's formula: 93.6% on D2 and 100% from D8 until D28 ($p < 0.0001$).

The second study was conducted in a commercial laying farm in Brazil. The Isa Brown laying hens were housed in 2 similar buildings of ca.5000 hens and were infested with the Poultry Red Mite (*Dermanyssus gallinae*). One house was treated and the other left untreated. The infestation was evaluated in blind conditions using mite traps (20 per house left 48h on 12 occasions over 4 months). Treatment efficacy was based on the mite reduction in traps (Henderson-Tilton formula). It reached 95.9% on D2 and 100% from D8 till D115. A positive effect was observed on hen mortality and laying rate. The third study was conducted in a commercial broiler breeder farm in the Philippines of ca.11,000 chickens housed in cages. The visual examination of the vent and the feathers of 79 randomly selected birds revealed a multiple infestation with Tropical Fowl Mites (*Ornithonyssus bursa*) and feather mites (*Megninia cubitalis*, *Pterolichus obtusus*). No mites were seen on these birds from D7 till D56.

These 3 studies showed the fast onset and high efficacy of Exzolt® against not only 3 species of blood-sucking poultry mites but also against feather mites.

PS01.31 Evaluation of DynaTrap Flylight (DT-3009) Plug-In Units to Trap Indoor House Flies and Stable Flies

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DynaTrap Flylight traps with 9-watt UV lights were evaluated alone and paired with Gardner GT-200 traps, an industry standard. Gardner traps are larger than DynaTraps with two 40-watt fluorescent tubes. Evaluations were conducted in a windowless room at 23°C with constant illumination. Tests used colony-reared house or stable flies. Flies were anaesthetized and used after a 30-minute recovery period. Trap pairs were placed

parallel to each other, 2 m apart and 1 m high on a test room counter. Single traps were placed on the same counter with other traps removed. Captured flies were recorded at 1, 4, and 24 hours post-release. After the 24-hour count, remaining live flies were killed and glue boards were replaced. Single and paired traps were tested over 6 dates and traps in paired tests were rotated after every replicate. Because the DynaTrap is designed to plug into outlets in walls, tests were performed with a simulated wall. At 24 hours, individual DynaTraps captured about 83% and 33% of the house flies and stable flies, respectively. These results changed slightly with the simulated wall placed behind the units. In both cases, significantly more house flies were trapped than stable flies. In paired tests, Gardner traps captured significantly more house flies (93%) and stable flies (55%) at 24 h than the DynaTraps (house flies = 4%, stable flies = 9%). Gardner traps, tested individually, captured significantly more house flies than stable flies. Single DynaTraps effectively captured house flies indoors, but were less effective against stable flies, which are not usually found indoors. The Gardner GT-200 captured more flies than DynaTraps because of its size and brightness. The comparison between these traps provides an indication of differential capture rates based on brightness. The DynaTrap performed well and removed house flies from the environment at a better-than-expected rate.

PS01.32 Flight Time and Distance Comparison of Field Collected and Colony Reared Horn Fly and Face Fly

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Flies reared in captivity may exhibit a fitness cost, reducing flight time and distance when compared to wild type flies. To measure potential fitness cost, we compared the flight time and distance of horn fly and face fly using flight mill instrumentation. First generation flies emerging from field collected dung pats and from laboratory reared colonies under teneral, 2d, and 5 day old conditions were compared.

PS01.33 First Detection and Molecular Identification of *Neospora Caninum* From Naturally Infected Cattle, Sheep and Goats in North Africa

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Neospora caninum is a coccidian parasite with a wide range of hosts. It is considered as a major cause of reproductive failure in livestock females causing economic losses. The objective of this study was to estimate the infection rate and molecular identification of *N. caninum* in Tunisian ruminants. A total number of 469 meat samples were collected from 150 cows, 198 ewes and 121 goats slaughtered in the regional slaughterhouse of Béja and tested for the presence of *N. caninum* ITS1 gene using PCR followed by sequencing of some PCR products. The three nucleotide sequences are available in the GenBank database under the accession number KY496699 for cattle, KY562727 for sheep and KY486651 for goats. A phylogenetic tree was then constructed. The overall molecular infection prevalence of *N. caninum* was significantly higher in cattle than in goats and sheep (22, 19 and 10.6%, respectively, $p=0.001$). In sheep, the highest prevalence was observed in the northern Béja (31.2 ± 16.1), with the Noire de Thibar breed as the most infected sheep breed ($31.7\pm14.2\%$) ($p<0.001$). In cattle, and goats, there were no differences in the molecular prevalence of *N. caninum* according to breeds and localities. The association between age and *N. caninum* molecular prevalence was statistically significant in the three species; the highest prevalence was observed in cattle between two and eight years of age ($28.8\pm10.9\%$), in goats aged between two and four years ($31.9\pm13.3\%$) and in sheep of more than one year of age ($19.4\pm9.1\%$). Comparison of the partial sequences of the ITS1 gene revealed

96-100% similarity with amplicon deposited in GenBank.

To our knowledge this is the first detection and molecular identification of *N. caninum* in cattle, sheep and goats in North Africa. This information is pertinent in designing control programmes that would reduce economic losses in the livestock industry.

PS01.34 A Pilot Study to Determine the Efficacy of a Metronidazole Suspension on the Reduction of Cyst and Trophozoite Counts in Dogs Infested with Giardia

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The objective of this randomized masked study was to determine the effectiveness of an oral metronidazole suspension (Eradia/Ayradia, Virbac), when used in dogs infested with *Giardia* spp., to reduce *Giardia* cysts and trophozoites.

From the eighteen dogs screened (> 3 month-old, from 5-10 kg), ten were positive for *Giardia* and six dogs with the highest fecal cyst counts (≥ 750 cysts per gram, determined by immunofluorescent assay) were selected. The dogs were evenly and randomly allocated to either a placebo (control) group or a metronidazole treated group (25 mg/kg of body weight BID) for five consecutive days. Fecal samples were collected once daily and submitted for cyst counts. At the end of the study, samples of the entire small intestines were sent to a laboratory for the trophozoite count. The number of trophozoites recorded for each intestinal section for each dog were summed and the resulting number was the total number of trophozoites per dog. Efficacy of treatment was measured as a reduction in the number of both *Giardia* spp. cysts (Henderson-Tilton formula) and trophozoites after treatment (geometric means). No serious adverse events were reported during the study.

The cyst counts were reduced by > 90% from day 3 to 5 (86.8%, 83.7%, 99.97%, 99.97%, 93.5%, from day 1 to day 5 respectively) and the trophozoite counts by 99.9% in the dogs treated compared to placebo group (192106 in placebo vs 183 in treated group).

These results indicate that the metronidazole suspension was effective for the treatment of *Giardia* infection (cysts and trophozoites) in dogs and demonstrate the link between the reduction of *Giardia* cysts in faeces and of the trophozoite forms in the gut in metronidazole treated dogs.

PS01.35 Approaching to Development of Transmission Blocking Vaccine Based on Developmental Stages of Babesia gibsoni

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Babesia gibsoni is a tick-borne hemoprotozoan pathogen and it may cause canine babesiosis. Clinical signs of canine babesiosis include fever, thrombocytopenia, anemia, and sometimes can be fatal, however, effective vaccine or treatment are not available yet. Recently, transmission blocking vaccine that aims to prevent transmission of pathogens from infected to un-infected host has been studied. The purpose of this study is to develop transmission blocking vaccine based on different gene expression level of *B. gibsoni* in blood stage and in tick stage. First, from *B. gibsoni* (Oita strain) blood stage cultured in vitro, ring form and free merozoites were separated using Percoll density gradient centrifuge. The separated parasites were in alive after centrifuge. However, syringe filtering, refrigeration, and sorbitol treatment that are known can be used for developmental stage separation of *Plasmodium* spp. did not applicable on *B. gibsoni*. On the other hand, to separate *B. gibsoni* in tick stage, semi-artificial feeding system which uses mouse skin instead of dog was used. As a vector tick, *Haemaphysalis longicornis* (Okayama strain) which is known vector of *B. gibsoni* was used. Using semi-artificial feeding system, *H. longicornis* was

infected with *B. gibsoni* in midgut and ovary which was confirmed by PCR. Morphology and transcriptome of each developmental stages will be analyzed.

PS01.36 Establishment of a Stable Transfection System for Genetic Manipulation of *Babesia gibsoni*

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Aims: Genetic manipulation techniques, such as transfection, have been previously reported in many protozoan parasites. In *Babesia*, stable transfection systems have only been established for bovine *Babesia* parasites. We recently reported a transient transfection system and the selection of promoter candidates for *Babesia gibsoni*. The establishment of a stable transfection system for *B. gibsoni* is considered to be urgent to improve our understanding of the basic biology of canine *Babesia* parasites for a better control of babesiosis.

Contents: To establish a stable transfection system, we generated a plasmid construct in which the 5'-intergenic (IG) region-B of the *ef-1α* gene (5'-*ef-1α*) drives the *gfp* reporter gene, and the 5'-actin promotes the expression of the selection marker *hdhfr*. The plasmid was designed for integration into the *ef-1α* locus of *B. gibsoni* genome by double cross-over homologous recombination. Linearized plasmid was transfected by 4D Nucleofector™ into in vitro cultured *B. gibsoni* and 10 nM WR99210 was added for drug selection two days after transfection. GFP-expressing parasites were observed by fluorescence microscopy as early as two weeks after drug selection, and consistently expressed GFP for more than 3 months without drug pressure. Genome integration was confirmed by PCR, sequencing and Southern blot analysis.

Conclusions: We present the first successful establishment of a stable transfection system for *B. gibsoni*. This finding will facilitate functional analysis of *Babesia* genomes using genetic manipulation and will serve as a foundation for the development of tick-*Babesia* and host-*Babesia* infection models.

PS01.37 Optimized Excystation Protocol for Ruminant *Eimeria* Spp. Sporulated Oocysts (Apicomplexa, Coccidia)

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An improved method for excystation and collection of infective sporozoites from sporulated oocysts of *E. bovis* and *E. arloingi*, is here described. The protocol uses conditions which mimics the intestinal anaerobic environment of ruminants in vivo. Firstly, sporulated oocysts were washed with 6% sodium hypochlorite solution. Then, *Eimeria* oocysts have been filtered through different sieves sizes to remove debris with a recovery percentage of 95%. Afterwards, the oocysts have been treated for at least 12 h with 0.02M L-cysteine HCl/0.2M NaHCO₃ solution in a 100% CO₂ environment at 37 °C. The last oocyst treatment was performed with a 0.4% trypsin/8% sterile bovine bile excystation solution, which disrupted oocyst wall thereby releasing up to 90% of sporozoites in approximately 2 h of incubation (37 °C) with a 1:2 (oocysts:sporozoites) ratio. Free-released sporozoites were up to 99% viable, highly motile, capable of infect host cells and to form meront stages in vitro. The improved method is much cheaper, faster, and accessible for all labs with minimum equipment, and without requirement of neither expensive solutions nor sophisticated instruments.

PS01.38 Molecular Characterization of Cryptosporidium in Calves From Rural Settlements in the Northwest Region of the State of São Paulo, Brazil

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The study was conducted on 25 properties of the settlements São José I and Salvador, located in the municipalities of Brejo Alegre and Birigui, in the State of São Paulo, Brazil. A record of variables was elaborated and included data such as gender, breed and age of the animals. A total of 231 stool samples were collected from bovines aged one to six months, 128 being females and 103 males, 131 crossbred and 100 Holstein. Among the 231 samples, 17 (7.36%) were positive for *Cryptosporidium* spp. both by malachite green negative staining and by nested-PCR. Of the 17 positive samples, 14 were sequenced in agarose gel. These sequences were detected between 99% and 100% of genetic similarity for the following species. One sequence was similar to *C. parvum* (AB513880.1), one to *C. bovis* (MF074602.1), two to *C. ryanae* (KT922233.1), one to *C. felis* (KM977642.1) and nine were similar for *C. andersoni* reference MF350628. *C. andersoni* was found in animals aged 2–6 months, an age group which is different from those described by several authors. The presence of *C. parvum* indicates that the calves in the studied region should be considered a potential source for zoonotic transmission. For the first time to our knowledge, *C. felis* was identified in cattle in America.

PS01.40 Targeted Overexpression of Cyclic AMP-Dependent Protein Kinase Subunit in Toxoplasma Gondii Promotes Replication and Virulence in Host Cells

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Toxoplasma gondii (*T. gondii*) is one of the most common parasites that can infect almost any warm-blooded animals including humans. The cyclic nucleotide-dependent protein kinase (PKA) regulates a spectrum of intracellular signal pathways in many organisms. Protein kinase catalytic subunit (PKAC) is the core of the whole protein, and plays an important role in the life cycle of *T. gondii*. Here, *T. gondii* PKAC (TgPKAC) overexpression strain (TgPKAC-OE) was constructed. The growth of the TgPKAC-OE, RHΔKu80, and TgPKAC inhibition strains (TgPKAC-H89) were analysed by SYBR-green real-time PCR, and the ultrastructure was observed by transmission electron microscopy. The survival rate in mice was also recorded to analyse the virulence of the parasites. We also investigated the subcellular localization of TgPKAC in Vero cells by laser scanning microscope. We found that TgPKAC-OE strain exhibited obviously increased growth rate in Vero cells in vitro, and infected mice survived for a shorter time compared to wild type strain. Ultrastructural analysis found more autophagosomes-like structures in TgPKAC-H89 parasite compared to RHΔKu80 strain, and the relative expression level of *Toxoplasma gondii* autophagy-related protein (ATG8) in TgPKAC-H89 parasite was higher than wild type parasite. Laser confocal results showed that TgPKAC was mainly expressed in the cytoplasm of Vero cells. In conclusion, we hypothesized that inhibition of TgPKAC could cause autophagy of *Toxoplasma gondii* and then influence the replication of the parasite. TgPKAC plays an important role in parasite virulence in vivo, and the subcellular localization was successfully detected in Vero cells. Our data will provide a basis for further study of TgPKAC function and help screen drug targets of *T. gondii*.

PS01.41 Klossiella Equi: A Cause of Renal Disease in Horses?

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Klossiella equi (Apicomplexa, Adeleorina) is a monoxenous coccidian parasite that infects the kidneys of equids globally. Usually, *K. equi* is an incidental finding during necropsy because clinical signs referable to it are usually absent. However, gross and histological lesions have been reported from infected kidneys. It is unknown if *K. equi* contributes to or causes clinical kidney disease but long-term infection has been documented in immunocompromised hosts. Although sporocysts that are presumed to be immediately infective to other horses are shed in the urine of infected animals, sporocysts are rarely detected by normal urinalysis; worse, sporocysts are difficult to float even when targeted. Shedding periods and duration of the lifecycle remain unknown. With the difficulty of detecting the parasite antemortem, the true prevalence of *K. equi* is unknown locally (Southern Ontario) and globally. With the recent publication of sequences from *K. equi* (partial nuclear 18S rDNA; whole mitochondrial genome), detecting infections with *K. equi* in horses antemortem may be possible using molecular means. To test this, tissue, blood and urine samples are being obtained opportunistically from horses submitted for routine necropsy to the Animal Health Laboratory at the University of Guelph, regardless of the underlying cause of death. For cases with fresh urine available, detection of sporocysts will be attempted microscopically. DNA extracted from kidney tissues will be tested for the presence of *K. equi* using PCR with species-specific primers to establish the prevalence of this coccidium in Southern Ontario. DNA from blood and urine from positive cases will be used to explore the feasibility of developing a PCR assay to detect *K. equi* antemortem in blood or urine samples. Such a test would permit a better understanding of the prevalence of this parasite globally and its role, if any, in clinically relevant changes to the equine kidney.

PS01.42 The Bacterial Microbiome of Rhipicephalus Sanguineus Ticks in the Mnisi Community, South Africa

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Rhipicephalus sanguineus is a three host tick that completes all its life stages on domestic dogs. It is, however, capable of parasitizing most vertebrates, including wildlife, cattle, dogs and humans. It is known to transmit various tick-borne diseases. In Mnisi, a rural community in Bushbuckridge, Mpumalanga, South Africa, *R. sanguineus* is one of the most prevalent ticks found on dogs. The community lies at the wildlife-livestock-human interface where humans are at risk of infection with various tick-borne zoonotic diseases. The aim of this study was to investigate the prevalence and diversity of tick-borne bacterial pathogens in *R. sanguineus* that may impact human and animal health. To achieve this, we analysed the microbiome of ticks sampled from community dogs over a 12-month period to detect bacterial pathogens and symbionts. To date, *R. sanguineus* (n=582), *R. simus* (n=82), *Amblyomma hebraeum* (n=97), as well as 183 unidentified tick have been collected from 51 dogs. Ticks were kept in a humidity and temperature controlled chamber for two days to allow them to digest their blood meal. Three *R. sanguineus* ticks from each dog were surface sterilized, and dissected to remove their midguts and salivary glands and then pooled. Genomic DNA was extracted and PCR amplified using universal 16S rDNA barcoded primers. Sequencing will be done at Washington State University using Pacific Bioscience's circular consensus sequencing strategy. Environmental conditions and other factors that could influence the tick population or tick microbiome were also

analysed. Preliminary data shows that the microbiome is dominated by *Coxiella* spp, including *C. burnettii*, the causative agent of Q-fever. The information gained about the bacterial communities that abound in *R. sanguineus* will undoubtedly aid health care practitioners in the area with the diagnosis of important tick-borne diseases in animals and humans.

PS01.43 Efficacy of a Single Spot-on Administration of Fipronil and Permethrin Against *Haemaphysalis longicornis* in Dogs

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The efficacy of fipronil plus permethrin (Frontline Tri-Act®, Boehringer Ingelheim), administered once topically at the minimum recommended dose, was assessed in dogs experimentally infested with *Haemaphysalis longicornis* ticks.

The study was a blinded, negative controlled clinical efficacy study using a randomized block design. Fourteen purpose bred Mongrel and/or Beagle dogs, 6 females and 8 males, were included. Dogs were randomly allocated either to the negative control group, or to the treated group. Dogs were experimentally infested with approximately 50 viable, adult, unfed female *H. longicornis* ticks (Okayama strain, Japanese origin) on days -2, 7, 14, 21 and 28. On Day 0, dogs in Group 1 were left untreated and dogs in Group 2 were treated with Frontline Tri-Act® (fipronil plus permethrin). Ticks were thumb counted at 24 hours after post-treatment infestations on Days 8, 14, 22 and 29, and were removed and counted at 48 hours after treatment or tick infestations on Days 2, 9, 16, 23 and 30. Frontline Tri-Act® administered once topically at the minimum recommended dose was highly effective against existing tick infestations (curative efficacy of 98.7%, $p=0.0006$). Regarding the re-infestations, the sustained efficacy of Frontline Tri-Act® was 100%, 100% 98.8% and 97.1% on Days 9, 16, 23 and 30, respectively. In addition, 24h in-situ count ranked from 87.2% to 100%.

PS01.44 Simulation Model of Host-Transferring Adult Males Ticks (*Dermacentor Variabilis*) (Acari: Ixodidae) in the Transmission of Equine Piroplasmiasis (*Theileria Equi*)

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Equine piroplasmiasis (*Theileria equi*) is a tick-borne disease of horses and other equines. In 2009, an outbreak occurred in south Texas, where *Dermacentor variabilis* were collected off infected horses. Experimental transmission studies have shown *D. variabilis* to be a vector of *T. equi* by means of intrastadial transmission, specifically by male adult ticks. Male ticks can remain on the hosts for long periods of time and take multiple bloodmeals. Additionally, males may transfer from host-to-host which can allow for further transmission. Male transfer has been well documented in *D. albipictus* with *Anaplasma marginale* but no studies have investigated male transfer with *D. variabilis*. The aim of this study was to model the transmission of *T. equi* while incorporating the transfer of males from one host to another. Simulations were run over a five-year period with four different infection probabilities (1%, 0.5%, 0.25%, and 0.1%) and five transfer probabilities of males (100%, 75%, 50%, 25%, and 1%). The number of horses infected varied with the combination of the two rates. The duration of the infection was influenced by both rates. Results from this study show that transferring adult male *D. variabilis* may play an important role in the transmission of *T. equi*. In addition, this model may serve as a starting point for further investigation of the role of transferring male ticks in other disease systems.

PS01.45 An Insight into Genetic Diversity of Ticks of Bovines Across Different Agro-Ecological Zones of Pakistan

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Introduction: Tick infestation is one of the leading causes of economic losses in bovines across tropical and sub-tropical regions of the world. In Pakistan, bovine population is exposed to tick infestation throughout the year and little is known about biology and genetic diversity of tick population across different agro-ecological (AE) zones of the country.

Objective: To determine the genetic diversity of hard ticks infesting bovine population across different AE zones of Pakistan. **Materials & methods:** Thirty villages were selected from five different AE zones of Punjab and Sindh provinces of Pakistan and the sampling was carried out from September to November 2017. A total of 753 adult ticks and 20 nymphs were collected from cattle (n = 114) and water buffaloes (n = 88) kept by small-holder dairy farmers (<10 animals). All ticks were identified morphologically to species level using dichotomous keys and then representative specimens were genetically characterized using primers targeted at cytochrome c oxidase subunit 1 (COX1), internal transcribed spacer 2 (ITS2) of the nuclear ribosomal DNA and 16S gene. DNA sequences of all three regions were analyzed separately using Bayesian Inference and Neighbor Joining phylogenetic methods. **Results:** Morphological and molecular identification of 753 specimens showed that they belonged to *Hyalomma* (Hy.) *anatolicum* (n = 680), *Hy. hussaini* (n = 3), *Hy. scupense* (n = 1), *Rhipicephalus* (Rh.) *microplus* (n = 66) and *Rh. (Boophilus) annulatus* (n = 1). Phylogenetic analyses of three genetic markers revealed high diversity in all tick species genetically characterized.

Conclusion: This study provides a detailed account of the genetic diversity of hard ticks

infesting bovines across different AE zones of Pakistan. Findings of this study highlights the importance of using morphological and molecular approaches for identification of ticks.

PS01.46 Evaluation of Four Drug Application Strategies for *Rhipicephalus Microplus* Control in Crossbred Naturally Parasitized Cattle in Água Clara Municipality, MS, Brazil

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The study objective was to evaluate four medication application strategies for tick control in naturally parasitized bovines, pointing out the best cost-benefit. It was selected 72 bovines with superior counting to 10 ticks and divided in four experimental groups: G1-Fipronil 1mg/kg (TopLine Pour-on); G2-Fluazuron 2.5 mg/kg (Forbox Pour-on); G3-Moxidectin 1mg/kg (Onix Injectable); G4-chlorpyrifos 30g, cipermethrin 15g and fenthion 15g (Colosso FC30 - spray). Treatments was realized according to manufacturer recommendations. The G4 group presented best reduction percentual, greater 7° Post Treatment Day (PTD) indice (83.23%) and a 35° PTD without effect. The second best strategy was G3 with inverse reduction percentage, presenting a worst indice in first weeks and better results in last treatments (82.85% in 28°PTD and 44.48% in 35° PTD). G1 and G2 had a better reduction percentage in 21° PTD (32.63 and 2.79%, respectively), with insatisfactory percentage in other dates. Group one, two, three and four presented a USD 0.37, USD 0.36, USD 1.61 and USD 0.28 respectively, for each treatment and each animal. G1, 2 and 3 need

animal weighing and could be measured using balance (fast and high cost) or weighing thoracic tape (low cost and slow). For G4, a spray chamber was acquired with a USD 4,818.63 cost, making a onerous strategy but, for herds above 5,000 head, the return for the low cost of the dose per animal can occur in less than a year. In summary, the acaricide contact formulation administered through the spray chamber presented a better reduction percentage, easier application and lower cost per treatment, and could be indicated as a strategy of better cost-benefit for medium and large herds.

PS01.47 Molecular Surveillance of Tick-Borne Diseases in Dogs, 2017–2018

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The frequency of canine tick-borne diseases, which is babesiosis, granulocytic anaplasmosis, monocytic ehrlichiosis and Lyme disease are increasing globally including Korea. The aim of this study was to identify tick-borne pathogens in dogs and provide base line data in controlling of tick-borne diseases that may affect human and animal health in Korea.

Canine blood samples were taken from companion dogs in veterinary clinic and shelter dogs in abandoned animal shelter. DNA purified from blood samples was used for the detection of 6 of tick-borne diseases (*Anaplasma phagocytophilum*, *A. platys*, *Ehrlichia chaffeensis*, *E. canis*, *Borrelia* spp., *Babesia* spp.) using previously reported PCR assays.

A total of 2,215 dog blood samples were collected. Whole bloods' DNAs were extracted from samples using Maxwell 16 whole blood DNA kit according to the manufacturer's instruction. As a result of PCR analyzed 16S rRNA gene, total of 11 samples were positive for *A. phagocytophilum* (0.5%),

3 samples for *Borrelia* spp. (0.14%) and 2 sample for *B. gibsoni* (0.09%).

In this study, molecular surveillance of tick-borne rickettsial and protozoan infectious diseases was conducted to investigate the infectious status in the Republic of Korea. Continuous monitoring of tick-borne infectious diseases is needed to prevent neglected tick-borne zoonoses having potential emerging diseases in the future due to climate change.

PS01.48 Mortality Time of Rhipicephalus Microplus Larvae Immersed in Binary Mixtures of Essential Oils

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Rhipicephalus microplus causes damages to livestock and transmits diseases to its hosts. Essential oils from different plant species are toxic to the larval stage of this parasite. The objective of this work was to evaluate in vitro the mortality time of *R. microplus* larvae immersed in combinations (1:1) of the mixture of ten essential oils (anethole, vanillin, carvacrol, cinnamaldehyde, 1,8-cineole, carvone, eugenol, linalool, thymol and L-limonene) aiming to evaluate possible synergistic effects of these compounds. The test consisted of immersing approximately 100 larvae of *R. microplus* in 200 µl of the binary mixture, putting each one and its repetition in one well of a 48 well plate; then, with the aid of a magnifying glass and a cronometer, we verified the time in which 100% of the larvae were dead. The ten binary mixtures with the shortest mortality time were diluted in soybean oil: 50, 25, 12.5, 6.25, 3.125 and 1.56%. Negative control (soybean oil, S) and two Pour on acaricides were used as positive controls (C1: Flumethrin 1g, and C2: Cypermethrin 5.0 g + Chlorpyrifos 7.0 g + Citronelal 0.5 g). All binary mixtures, doses and the control treatments were tested in triplicates. The larvae mortality time data

were analyzed by the PROC GLM procedure, whose model included the fixed effects of the compound, dose and its interactions, and the means were compared by the tukey test ($p < 0.05$). The most toxic mixture for the larvae was thymol + limonene, because at the dose of 1.56% presented the shortest mortality time (12 min) ($p < 0.05$). Mortality time for the controls were: C1, 66.67 ± 1.53 ; C2, 141.0 ± 1.0 minutes; S, 2400.1 ± 0.1 min. It is concluded that this methodology is easy to do, low cost and able to test in vitro toxic oil substances for larvae.

PS01.49 Efficacy of Oral Formulation of Sarolaner (Simparica™) Against Naturally Infestation Brown Dog Ticks in Dogs Presented as Veterinary Patients in Thailand

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Brown dog ticks are recognized as one of the most prevalent ectoparasites in Thailand. Ticks are vectors for many pathogens that can cause several diseases in dogs. Treatment and control ectoparasites infestations on dogs is an important role for general veterinary practice. The purpose of this study was evaluated the efficacy of oral formulation of sarolaner treatment naturally infestation brown dog ticks in dogs. Sixty dogs were selected into study from overall country. One dog in each household was allowed to be enrolled as the primary patient. This primary patient had to harbor at least 3 attached alive-ticks at enrolment. Dogs received oral formulation of sarolaner follow recommendation dose at Day 0. Tick counts on primary dogs were conducted prior to treatment on Day 0, and on post-treatment at Day 7 and 30. Dogs were thoroughly examined for within 10 minutes by blinded study personnel until all ticks were removed. Efficacy of sarolaner against ticks was 93%, 96.7% on Day 7 and 30 respectively. Sarolaner administered orally at monthly intervals at a recommendation dose was highly effective against naturally infestation brown dog ticks on dogs in Thailand.

PS01.50 Molecular Characterization of Fasciola Hepatica from Indigenous Animals in Cajamarca, Peru in Comparison with That from Livestock

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Fasciola hepatica is widely distributed in the temperate regions including South American countries. It has been speculated that *F. hepatica* was introduced from European countries to South American continent along with movement of animals. However, limited information is available to reveal the actual dispersal route. In this study, we focused on Peruvian *F. hepatica* collected from indigenous animals Cajamarca, Peru.

Fasciola flukes collected from 11 alpacas and 15 guinea pigs in Cajamarca were fixed with 70% ethanol. One or two flukes were analyzed per animals. Species identification was performed by using the DNA fragment patterns of nuclear phosphoenolpyruvate carboxykinase (pepck) and DNA polymerase delta (pold) genes. Again, the DNA sequences of mitochondrial NADH dehydrogenase subunit 1 (nad1) gene were analyzed to reveal the phylogenetic relationship with *F. hepatica* from livestock (cattle, sheep, pigs) collected from the same area. The median-joining network was constructed to compare the nad1 sequences with the reference European countries.

All of the *Fasciola* flukes collected from alpacas and guinea pigs were identified as *F. hepatica* by using nuclear pepck and pold. Again, the mitochondrial nad1 sequences of *F. hepatica* from alpacas and guinea pigs were almost identical to those of *F. hepatica* from livestock which were previously reported. Therefore, it is demonstrated that there is no difference between Peruvian *F. hepatica* populations from indigenous animals and

livestock. Since Peruvian *F. hepatica* population had close relationship with Spanish *F. hepatica* population, it is concluded that *F. hepatica* was introduced from Europe to Peru.

PS01.51 Controlled Efficacy Test of Triclabendazole Against Fasciola Hepatica Derived from Human Infections

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Cajamarca region of Peru is an area highly prevalent of Fascioliasis caused by *Fasciola hepatica*. The parasite affects domestic species including man. Currently, human patients are treated with a human formulation of Triclabendazole (TCBZ) (Egaten®). The objective of the present work was to investigate the efficacy of TCBZ in humans infected with the liver fluke *F. hepatica* using a controlled efficacy test in sheep experimentally infected with metacercariae derived from human isolates of *F. hepatica*. Twenty-six patients were treated with TCBZ, divided in two groups. Patients in group A (n = 13) were treated with TCBZ at 10 mg/kg bodyweight and those in group B (n = 13) with 20 mg/kg. Oral treatment with TCBZ was administered on two consecutive days. Five patients from both groups failed to cure after TCBZ treatment. From those patients that did not cure, *F. hepatica* eggs were collected and the miracidia produced was used to infect *Lymnaea* snails. Sufficient amount of metacercariae was obtained to perform a controlled efficacy test in sheep. Twelve sheep were artificially infected orally, with two hundred metacercariae each, derived from human patients. The infected sheep were randomly divided into two groups: treated group (n = 6) and control group (n = 6). 106 days after infection, animals in the treated group were treated by oral administration of TCBZ (Bilevon®) at the dose of 10 mg/kg live weight as a single dose. Animals in the

control group received no treatment. Fifteen days later, all the sheep were sacrificed and the number of adult *Fasciola* parasites present in the livers was counted. The results of the controlled efficacy test in sheep show a negative TCBZ efficacy (-125.7%). It is concluded that cases in which therapeutic cure did not occur appear to be related to resistance of *F. hepatica* to TCBZ.

PS01.52 Seasonal Re-emergence of Fatal Acute Immature Paramphistomosis in Goats After Flood – A Longitudinal Study in Tamil Nadu, India

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Paramphistomosis causes severe morbidity and mortality in ruminants in India. Outbreak of acute paramphistomosis in goats maintained under semi-intensive at Mottur-Siruvakkam village, Kancheepuram district, Tamil Nadu was carried out from June 2015 to May 2016. The overall mortality and morbidity was 58.82 and 35.95%. Among the seasons, highest mortality was observed during winter (50.67%) followed by summer (13.51%), northeast monsoon (2.67%) and no mortality during south west monsoon. Similarly, highest morbidity was observed only during winter (336.67%) and no morbidity was observed during other seasons. Incubation period was 2 days in goats. Adult paramphistomes observed in rumen were *Cotylophoron cotylophorum* (40.54%), *Gastrothylax crumenifer* (29.19%), *Paramphistomum cervi* (19.19%), *Fischoederius elongatus* (8.38%) and *F. cobboldi* (2.70%). Affected animals showed severe diarrhoea with bad smell, bottle jaw, protrusion of mucous membrane of eyes and died with 2 days after recumbent. On postmortem examination, adult paramphistomes were found in rumen and immature paramphistomes were found mostly in the duodenum and abomasum. Gall bladder and urinary bladder were enlarged and nodules found on the spleen. On histopathology, rumen showed

presence of adult paramphistome and presence of plug of ruminal papillae in the oral cavity of adult paramphistomes. In immature paramphistomosis, stunting of intestinal villi, desquamation of intestinal villi around the worm, necrosis of villi, infiltration of MNC (eosinophils, lymphocytes, neutrophils), glandular hyperplasia were observed. There was presence of immature paramphistomes deeply embedded in the submucosa as well as just above the Brunner's glands. Snails collected from the lake were identified as *Indoplanorbis exustus* (79.54%) followed by least number of *Pila globosa* (12.50%), *Pila virians* (3.41%), *Bellamya b.eburnea* (3.41%) and *Stenothyroides blandfordiana* (1.14%). Amphistome cercariae were recovered from *I. exustus* snails.

PS01.53 Transcriptional Activity as a Proxy for Eimeria Spp. Oocyst Viability: The Key to Optimizing Coccidiosis Vaccine Performance?

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Eimeria spp. (Apicomplexa) infect the digestive tract of a wide range of vertebrates. These parasites are generally of little concern in wildlife, companion animals, and humans. In the commercial poultry industry, where animal stocking densities are high and feed-conversion efficiency is of top concern, they are a major biological threat. Oocysts are shed in feces of infected birds, and sporulate to become infective to poultry. Parasite replication damages enterocytes to produce disease ranging in severity from simple unthriftiness to death. Severity of this self-limiting infection is a function of three major factors: Number of infective oocysts ingested; parasite species; and the host's previous exposure to that species (i.e. immune status). Infection protects the host against future infection by homologous species; several rounds of infection produce sterile immunity. The self-limiting and immunogenic nature of *Eimeria* spp. make live vaccination an ideal strategy for coccidiosis control. Vaccination requires establishment of infection with sufficiently numerous viable oocysts to stimulate development of immunity, but

few enough to avoid disease. Knowledge of actual viability of oocysts is therefore required for determination of optimal dosage. Unfortunately, no rapid and accurate method for determination of oocyst viability exists. Difficulty assessing oocyst viability ultimately impedes successful implementation of vaccination programs. We have demonstrated an in vitro assay that uses transcriptional activity as a proxy for viability of *Eimeria* spp. oocyst viability. We have investigated a range of specific assay targets, with early data showing a strong correlation between abundance of these targets and oocyst viability. Assay optimization of transcript quantification protocols, including assessment of one- and two-step RT-qPCR strategies and the use of digital droplet PCR (ddPCR) aim to increase sensitivity. Other potential assay applications include use as an epidemiological tracking tool or validation of strategies for environmental control of *Eimeria* and related parasites.

PS01.54 Efficacy of Three Avermectins in EPRINEX®, IVOMEC® F and DUOTIN® Formulations Against Dermatobia Hominis Larvae in Cattle Naturally Infested in Brazil

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Avermectins (abamectin, ivermectin and eprinomectin) are veterinary endectocides used on livestock having a broad-spectrum of activity and a safe profile. *Dermatobia hominis* is amongst the main ectoparasites of bovines, being widely distributed in tropical and subtropical regions of Latin America. Economic losses caused by this parasite in Brazilian herd were estimated at US\$ 380 million per year. The aim of the study was to evaluate the efficacy of eprinomectin in EPRINEX®, ivermectin in IVOMEC® F and abamectin in DUOTIN® against *Dermatobia hominis*. 40 bovines naturally infested with a minimum of 10 grubs per animal were used and splitted in four groups with 10 animals each: EPRINEX® Pour On (500

µg of eprinomectin/kg of body weight), IVOMEC® F (200 µg of ivermectin/kg of body weight + 2 mg of clorsulon/kg of body weight, subcutaneous injection), DUOTIN® (200 µg of abamectin/kg of body weight, subcutaneous injection) and an untreated group. The products were administered to the study animals in the treated groups following the manufacturer's instructions. The animals were examined visually and by palpating the back and the sides of each animal for the presence of live subcutaneous grubs before and after treatment to calculate the efficacy. The number of nodules with live larvae was counted 7 and 14 days after treatment to evaluate efficacy. The tested products were effective in controlling grubs from all treated groups. The percentages of efficacy (arithmetic means) on days 7 and 14 respectively for EPRINEX® were 92.25% and 92.18%; for IVOMEC® F were 93.02% and 94.90% and for DUOTIN® were 90.70% and 92.52%. These data showed that EPRINEX®, IVOMEC® F and DUOTIN® were effective in the treatment of *D. hominis* grubs in cattle. No adverse reactions were observed in any of the treated animals.

PS01.55 In Vitro Anthelmintic Activity of Hymenodictyon pachyanta Stem Bark Extract and Fractions Against Haemonchus contortus

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The development of helminth resistance and high cost of synthetic anthelmintics has prompted the need for an in vitro anthelmintic evaluation of crude stem bark extract and fractions of *Hymenodictyon pachyanta* plant as an alternative in the management of endoparasites including *Haemonchus contortus* which is one of the most prevalent parasitic nematodes in small ruminant farming globally. *Hymenodictyon pachyanta* stem bark is used as anthelmintic by indigenous farmers in Nsukka, Enugu State and Gwagwalada, Federal Capital Territory, Nigeria. The stem bark of *H. pachyanta* were collected from the field in Nsukka, Enugu State, air dried, pulverized and extracted with 80% Methanol. The extract and fractions of *H. pachyanta*

were tested on the egg hatch inhibition assay (EHIA) and the larval development inhibitions assay (LDIA) and compared with Albendazole the positive control. The concentrations for the plant extract, fractions and Albendazole used for the study were 0.78, 1.56, 3.125, 6.25 and 12.5mg/ml. The results showed that the crude extracts, fractions and Albendazole at concentration dose of 12.5 mg/ml produced 100% inhibition of egg hatching and larval development of *Haemonchus contortus*. Although, there was no significant difference ($p>0.05$) in the mean percentage egg hatch and larval development inhibition of the crude extract and fractions when compared with Albendazole. However, significant difference ($p<0.05$) was observed with n-butanol fraction which inhibited 96.17% of egg hatchability. The extract, fractions and Albendazole showed ovicidal and larvicidal activity and produced over 50% inhibition of egg hatching and mortality of larvae at concentration ranges of 0.78 - 12.5 mg/ml. The result obtained from this study suggests that *H. pachyanta* possess anthelmintic effects against *H. Contortus* and validates the folkloric use of the plant in the management of *H. Contortus* infections.

PS01.56 Anthelmintic Activity of Icacinia Trichanta Extract and Fractions Against Haemonchus Contortus

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Haemonchus contortus is an important gastrointestinal parasite of ruminants, which causes anaemia, submandibular oedema, progressive weight loss, weakness and even death. This study was aimed at evaluating the in vitro anthelmintic activity of *Icacina trichanta* leaf extract and fractions against *H. Contortus* eggs and larvae. Phytochemical analysis of the crude extract was carried out using standard techniques to detect secondary metabolites contained in the plant.

The in vitro anthelmintic activity of the crude extract and fractions was determined using eggs hatch inhibition assay (EHA) and larval development inhibition assay (LDA), while Albendazole and distilled water were used as positive and negative controls respectively. The extract of *I. trinchanta* leaves inhibited hatching of eggs and larval development of *H. contortus* in a concentration-dependent manner. Concentrations of 0.78, 1.56, 3.12, 6.25 and 12.5 mg/ml of the extracts completely inhibited the hatching of eggs and development of larvae and these were comparable to Albendazole anthelmintic activity. At the concentration of 12.5 mg/ml, the crude extracts, and fractions and Albendazole produced 100% ovicidal and larvicidal activity against egg hatching and larval development inhibition of *H. Contortus*, except for n-butanol and ethyl acetate fractions which inhibited 98% and 99.8% of egg hatching and larval development respectively. However, there was no significant difference ($p>0.05$) in the anthelmintic activity of the crude extracts and fractions when compared to the anthelmintic effects observed with Albendazole activity. The result in this study showed that crude extract and all the fractions tested possess anthelmintic compounds, warranting further in vivo evaluation for its safety and toxicity profiles.

PS01.57 Dose Determination Study of Entomopathogenic Funghi for the Environmental Control of Amblyomma Ticks

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Two sets of four 6 m square parcels were

demarcated on the lawn next to the Biological Control Laboratory of the Advanced Research Center in Campinas, State of São Paulo, Brazil, where a small group of capybaras (*Hydrochoerus hydrochaeris*) maintained a population of *Amblyomma* ticks. Each set was assigned to treatment with either *Beauveria bassiana* IBCB 66 or *Metharizium anisopliae* IBCB 425 from the Oldemar Cardim Abreu Entomopathogenic Funghi Collection of the Biological Institute. Emulsions containing infectious propagules of each strain were sprayed on the respective parcels at 5×10^{11} , 5×10^{12} and 5×10^{13} conidia/ha. Control parcels were sprayed with the same volume of water. Ticks were recovered from the lawn parcels with the aid of dry ice traps after application and then at four consecutive weekly intervals. Six traps were laid concomitantly on each parcel. Because of the season, only nymphs and adults were captured. Ticks were counted, identified and placed in test tubes closed with cotton stoppers, corresponding to each single trap. Tubes were kept in an incubator at 28°C and 80% humidity. Mortality was checked +1, +3 and +10 days after capture. Provided the number of ticks captured in each trap in each occasion was random and eventually zero, the proportion of dead ticks was used to enable comparison of the different treatments. Mortality and treatment were significantly associated in all cases ($p < 0.001$). Correlation coefficients for *Beauveria* and *Metharizium* were -0.674 and -0.466 for nymphs and -0.619 and -0.589 for adults, respectively. The best LC_{50} adjustment was obtained with Cauchy's generalized linear model for binary data. Median lethal doses for *Beauveria* and *Metharizium* were 1.69×10^{12} and 3.82×10^{12} conidia/ha for nymphs and 8.19×10^{12} and 5.93×10^{12} conidia/ha for adults, respectively. The current results will support further research focused on the biological control of ticks on lawns and pastures.

PS01.58 Foldscope as a Diagnostic Tool for Identification of Canine Parasites with Emphasis on Zoonotic Parasites**Dr. Mandeep Singh Bal¹**, Dr. Rashmi Sagar¹, Dr. Paramjit Kaur¹, Dr. Vishal Mahajan¹, Dr. Gursimran Folia¹¹*Guru Angad Dev Veterinary & Animal Sciences University, India*

The study was undertaken to use foldscope (magnification 140x) as a portable diagnostic tool for canine parasitic infections. Foldscope is a paper microscope developed in USA & is attachable with smart phone to visualize and capture pictures of specimens/slides. Faecal samples were collected from 173 dogs (83 pet, 90 stray) from different districts of Central plain zone of Punjab, India. Skin scrapings were also collected from dogs (15) with history of dermatitis and ticks from dogs (10) with tick infestation. Samples were processed as per standard parasitological techniques and examined by foldscope and optical microscope.

Overall 86 faecal samples (49.71%) were found positive for gastrointestinal parasites with predominance of hook worm eggs (46.24%), followed by Taenid eggs (1.73%), coccidian oocysts (1.15%) and Toxocara eggs (0.58%). Ticks collected were identified as *Rhipicephalus sanguineus*. Skin scrapings were found positive (26.67%) for *Demodex* spp. mites. Gastrointestinal parasitic infections were found to be higher in stray dogs (83.33%) than pet dogs (13.25%). Low grade gastrointestinal parasitic infections were not detected by foldscope. Relative efficacy of foldscope in detecting ticks, mites and parasitic eggs/oocysts evaluated as 100%, 80% and 75.6%, respectively as compare to conventional light microscope (assuming efficacy of light microscope as 100%). Examination of parasitic stages in faecal and skin scrapings by foldscope showed problem in focusing of glass slides. Therefore, a new method using paper slides with slits was also used in the study. Presence of parasites viz; Hook worms, *Toxocara* spp. and Taenid eggs in dogs indicate possible risk of zoonotic infections to the people of the region. Foldscope may be used as a novel, low cost tool for quick diagnosis of canine parasitic

diseases. Authors are thankful to Department of Biotechnology, Government of India for providing the financial grant and facilities to carry out the research work.

PS01.59 Efficacy of Oral Afoxolaner (NexGard®) and Afoxolaner Plus Milbemycin (NexGard Spectra®) Against Rhipicephalus Sanguineus and Ctenocephalides Sp. In Naturally Infested Dogs in Brazil.**Andre Cutolo¹**, Luis Pelissoni¹, Debora Sartor¹, Gabriela Vito¹, Cristiano Nascimento², Tassia Vale³, Isabella Souza³, Naylene Silva³, Livio Costa Junior³, Maycon Heidmann⁴, Itallo Carvalho⁴, Marcela Mendes⁴, Taminis Santos⁴, Isabella Chagas⁴, Bruno de Castro⁴¹*Boehringer Ingelheim Animal Health Do Brasil Ltda., Brazil*, ²*Convolution, Brazil*,³*Universidade Federal do Maranhão, Brazil*,⁴*Universidade Federal do Mato Grosso, Brazil*

Rhipicephalus sanguineus ticks and *Ctenocephalides felis* fleas are the most prevalent ectoparasites of dogs in Brazilian urban areas, potentially causing irritation, blood depletion, pruritus, skin lesions and pathogen transmission. Afoxolaner is a molecule from the isoxazoline family with proven ectoparasiticide action against fleas and ticks infesting dogs. Milbemycin Oxime is an antiparasitic endectocide belonging to the group of macrocyclic lactones and is active against several gastrointestinal worms. Afoxolaner (NexGard®) and Afoxolaner + Milbemycin Oxime (NexGard Spectra®) were administered to the study dogs per the label instructions. Different breed owned dogs, heavier than 2kg and older than 8 weeks old, from urban areas of São Luis, Maranhão state, Sinop and Ipiranga do Norte, Mato Grosso state, were visited in their houses and included in the study if harboring more than 6 fleas and/or more than 4 live attached ticks. 136 tick infested dogs and 146 flea infested ones were included. Part of the animals harboured mixed infestations. Dogs from a same house were treated with the same test product. Sixty-seven dogs infested with ticks and 73 dogs infested with fleas received NexGard® and 69 dogs with ticks and 73 dogs with fleas received NexGard Spectra® single oral dose at Day 0. Efficacy was calculated

based on the comparison of the arithmetic mean number of ticks or fleas before treatment versus the mean number at Day 30 (± 4). The mean number of ticks pre-treatment and percentage of efficacy were respectively for the NexGard® and NexGard Spectra® groups 21.3 and 98.5%; 31.1 and 98.3%; and for fleas respectively 46.4 and 99.2%; 50.9 and 99.8%. Both NexGard® and NexGard Spectra® showed excellent efficacy results against *R. sanguineus* ticks and *Ctenocephalides* sp. fleas in housed naturally infested dogs in Brazil.

PS01.60 Efficacy of the Rapid In-Clinic Speed™ Giardia Test for the Detection of Giardia Duodenalis in Dog Faeces Compared to ELISA and Coproscopy

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Giardia infection is a frequent cause of diarrhoea in dogs and giardiasis should be added on the differentials for any dog with acute or chronic diarrhoea. The aim of the study was to evaluate the diagnostic efficacy of the rapid in-clinic immunochromatographic test, Speed™ Giardia (Virbac) and to compare the accuracy of the immunochromatographic test and the zinc sulfate flotation technique (coproscopy) versus the ELISA, ProSpecT™ Giardia EZ Microplate Assay (Thermo Fisher Scientific).

Method: Faecal specimens from 104 dogs were first examined with coproscopy. Then, samples were blindly tested with Speed™ Giardia test and ProSpecT™ Giardia assays which served as the gold standard for this study.

Results: In comparison with the gold standard, Speed™ Giardia demonstrated high sensitivity (92.7%, CI95 of 82.7% to 97.14%), specificity (95.9%, CI95 of 86.3% to 98.9%) and positive and negative predictive values of 96.2% and 92.1% respectively. The results obtained with the two soluble antigen detection methods presented a strong agreement (94.2%). Coproscopy presented a very good specificity (100%) but lower sensitivity (81.8%) when

compared to ELISA. Positive samples with coproscopy confirm the presence of Giardia cysts, however negative results cannot exclude the presence of the parasite. In this study, 10 negative samples with coproscopy presented specific Giardia antigens with the ELISA test.

The combination of both coproscopy and soluble antigen detection increases the probability of correctly identifying negative samples. In comparison with combined coproscopy and ELISA, Speed™ Giardia presented good sensitivity (87.9%) and specificity (95.7%).

Conclusion: This study confirms the strong agreement between the Speed™ Giardia test and ELISA for the detection of Giardia antigens from faecal samples. Speed™ Giardia can be an efficient way for identification of canine Giardia infections in-clinic. A combination of coproscopy with an antigen detection test increases the probability of identifying canine giardiasis.

PS01.61 The AAVP Educators Meeting: Past, Present, and Future

Prof. Dwight Bowman¹, Prof. Heather Walden², Prof. Brian Herrin³, Prof. Byron Blagburn⁴

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The first AAVP Educators Meeting occurred in 2006 in Atlanta, GA, with a \$15,000 donation from the Companion Animal Parasite Council [CAPC] to cover meals and food and AAVP supplying up to \$300 travel funds for one AAVP faculty member from each North American Veterinary School or College. Additional faculty could attend at their own expense. The CAPC donation has continued allowing the meetings to occur biannually. Since 2006, meetings have followed at the CVM Oklahoma State University, Stillwater (2009); USDA-ARS, Beltsville, MD (2011); Loews Hotel, Chicago IL (2013); AVMA headquarters, Schaumburg, IL (2015), the CVM Ohio State University, Columbus (2017), and coming

in 2019 at the CVM University of Florida in Gainesville. The meeting format, including plenary speakers and group exercises for analysis and discussion, has provided interactive peer support to instructors in veterinary parasitology worldwide, provides opportunities for exchange of information on teaching strategies, course content, computer hardware and software platforms, specimens for laboratories, suggestions for examination content and format, and information on the accreditation process. The Symposium aids instructors in establishing core content for NAVLE preparation and has generated the publication: AAVP recommendations for core competency standards relating to parasitological knowledge and skills. Snowden et al., JVME 43:344-8;2016. This meeting is especially important for parasitologists entering the field and for new and veteran faculty who are developing resources for courses and laboratories, for the sharing of ideas, networking, the development of avenues for teaching advice, and sharing of resources (i.e. specimens, photos, case reports, etc.). Participant feedback supports the value and need for continuation of the program and recognizes its value in when questioned as to the importance of the material within curricular discussions within universities. This biannual meeting has become a valuable part of AAVP and our continued efforts to strengthen and expand veterinary parasitology education.

PS01.62 A Model of Success: World Association for the Advancement of Veterinary Parasitology African Foundation (1997-2017)

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Since 1997, the World Association for the Advancement of Veterinary Parasitology (WAAVP) African Foundation (AF) has achieved a “first” by establishing a successful endowment which awards travel scholarships to Next Generation (NG) African veterinary parasitologists to present their research findings at WAAVP Biennial Conferences. To date, 200 NG veterinary parasitologists from 24 African countries submitted applications; 80 from 15 countries were awarded scholarships to present research at WAAVP conferences in Europe, Asia, South America, USA, Canada, Australia and New Zealand.

The WAAVP AF goals are to support deserving awardees ready to present their research to an international audience, to develop global networks, and to bring positive recognition to their institutions, countries and academic fields. The WAAVP AF Committee oversees a successful financial investment and biennially invites approximately 100 global stakeholders to assist with recruitment across Africa.

From 2008-2017, 42 awardees participated in these conferences, and 37 submitted narratives which were published in the WAAVP quarterly online newsletters (<https://www.waavp.org>). These narratives were analyzed using QDA Miner 5.0 (Provalis Research, Montreal, QC, Canada). The coding process started with an initial open-minded reading to learn the data. During the second reading, each narrative was scrutinized for emerging mutually exclusive categories or themes. A third read through was performed after coding to fine-tune the results.

Nine themes emerged from the analyses with the top five being: 84% expressing gratitude to WAAVP AF for their conference opportunity, 76% for positive learning experiences, 60% for exposure to current research, 51% for networking opportunities and 35% for interaction with other scientists. The WAAVP AF promises to continue for the foreseeable future. It attributes 20 years of

success to being financially well invested in South Africa, a dedicated Committee and awardees committed to identify innovative and novel solutions to address parasites that plague animal and human health.

PS01.63 Development of Veterinary Students' Knowledge, Attitudes, and Practices Towards *Dirofilaria immitis* in St. Kitts

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Dirofilaria immitis (heartworm) has a spatial heterogeneous distribution on the Caribbean island of St. Kitts with a documented higher prevalence in the southeastern region. Numerous Ross University School of Veterinary Medicine (RUSVM) students reside in this area, and we hypothesize that this student population will promote a control campaign for heartworm. To investigate, a standardized questionnaire was developed to assess RUSVM students' knowledge, attitudes, and practices towards heartworm. Seventy-three incoming students completed the questionnaire in September 2017, and one year later, 38 (52%) of these students completed the same questionnaire. Majority of respondents were women (~80%) originally from the United States (70 from 31 states). While there were only five students (7%) living in the southeastern region of the island at the time of the first questionnaire, a 48% increase was reported a year later. Student knowledge surrounding the ability to identify the scientific name of the parasite increased by 47% in a year's time. Attitudes toward heartworm prevention did not change over the year (Wilcoxon paired test, $p > 0.05$), except more advised their family or friends who have a cat in the United States to give it preventative against heartworm ($p = 0.01$). Only six incoming students (8%) had a dog on island versus 15 (39%) after a year. All student dog owners used heartworm preventative, yet only six used a way to protect their dogs against mosquitoes. Our results indicate that despite an improvement of the knowledge, attitudes did not change. This highlights the need for veterinary students to understand

the importance of heartworm disease, their future role in prevention of this disease, and the significance of offering education to support prevention awareness. Further community and academic teaching and learning opportunities, which support these educational needs are warranted.

PS01.64 Serological Evidence of Canine Arthropod-Borne Infections in an Ecotone Area of a Natural Reserve at the Pantanal, Brazil

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Arthropod-borne infections are highly dependent on environmental conditions; therefore, anthropomorphic meddling close to natural reserves may disrupt the natural balance that maintains wildlife and onehealth. Since human populations keep domestic animals as pets or working animals, it is common to find dogs roaming in Brazilian natural reserves and also horses or bovines working under humane orders. This close living favors the spillover of bioagents that can affect wildlife, domestic animals and humans. *Dirofilaria immitis*, *Ehrlichia canis*, *Ehrlichia ewingii*, *Anaplasma platys*, *Anaplasma phagocytophilum*, *Borrelia burgdorferi* and *Leishmania* spp. infect unprotected animals without propensity. The present survey was conducted using 84 sera from dogs residing at the border area of the SESC-Pantanal reserve (16°40'51''S; 56°17'45''W) stored at the Laboratório de Protozoologia e Imunomodulação, Instituto Oswaldo Cruz. Samples were tested with SNAP® Canine Leishmania Antibody Test (IDEXX Laboratories) or DPP® canine visceral leishmaniasis test (Bio-Manguinhos) for the presence of *L. infantum* antibodies and with SNAP® 4Dx Plus Test (IDEXX Laboratories) for *D. immitis*, *Ehrlichia* spp., *Anaplasma* spp.

and *B. burgdorferi* seroprevalences. *Ehrlichia* spp. seroprevalence was the most frequent (76.2%) and of these 19 samples were also *Anaplasma* spp. positive and another sample was positive for the three tick-borne parasites. The frequency of *Anaplasma* spp. positive samples was 27.4%. *L. infantum* prevalence was 13.1% and *D. immitis* 7.1%. *D. immitis* prevalence was higher than expected in the area, although lower than the Brazilian known prevalence (23.1%). Tick-borne diseases presented high seroprevalence similar to the expected for the Pantanal (70.9%), suggesting anthropomorphic impact. *L. infantum* prevalence was lower than the known prevalence for the Pantanal area (61.7%) suggesting that the biodiversity of the reserve depletes *Leishmania* circulation enhancing both wildlife and domestic animal welfare.

PS01.65 Distribution and Dynamics of Canine Babesiosis in France: Results of a Questionnaire Based Survey

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Canine Babesiosis (*Babesia canis*) is an important disease in dogs in France, transmitted by *Dermacentor reticulatus*. A national survey was conducted in summer 2017 covering the period 2012-2017. Questionnaires were sent to veterinary clinics, and distribution and evolution of babesiosis was evaluated by using the number of cases diagnosed per year (<1, 1-10, 11-20, 21-50, >50), autochthonous (= acquired within the territory of activity of the veterinary clinic) or not. The results were compared to a similar study conducted in 2005. Distribution was analyzed using administrative territorial areas ("départements" n= 90). The questionnaires from 620 clinics covered 85/90 areas. The average numbers per clinic was: <1 in 12 areas (mainly in South-east and Corsica), 1-10 in 33 (West, North, South-east). Higher average prevalence were detected: 11-20 in 25; 21-50 in 13 (versus 32 in 2005) and > 50 cases in 8 (versus 26 in 2005), mostly distributed in the Southeast, centre and East of the country. In 14 areas the average number of

cases changed from more than 20 in 2005 to less than 10 in this study. Globally the average number of annual cases per clinic was reduced when compared to 2005 (44 areas). These results indicate a regression of the impact of the disease in many parts of France. However, during the recent period (2012-2017) the evolution is mostly considered unchanged with an increase in 8 and decrease in 12 areas. Interestingly in 12 areas, cases initially considered imported are now autochthonous. Four of these zones are located in the Southeast where the vector of *Babesia canis* is absent. This may suggest that in these areas where *Rhipicephalus sanguineus* (s.l.) is common, the likely agent is *Babesia vogeli*, known quite recently described in France.

PS01.66 Asymptomatic Carrier Status and Seroprevalence of *Leptospira* spp. in Unvaccinated Canines Presented for Elective Sterilization to the St. George's University, School of Veterinary Medicine Junior Surgery and Anesthesia Lab

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Dr. Marta Lanza Perea¹, Dr. Tara Paterson¹,
Dr. Christian Leutenegger², Dr. Ramaswamy Chandrashekar²
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The *Leptospira* genus of bacterium are of global concern as pathogenic species cause Leptospirosis – a zoonotic disease most prevalent in tropical and subtropical developing countries. Domestic and wildlife reservoir hosts harbor the bacteria within the renal tubules and intermittently shed the organism in urine. These asymptomatic carriers are of significant animal and public health concern. While the majority of research on the prevalence of Leptospirosis in animals is based on serology, the few studies that have assessed for asymptomatic carriers within canine populations reported prevalence of 0.2 - 22%. There is currently no data regarding the prevalence of canine asymptomatic carriers of *Leptospira* spp. in the Caribbean. The aim of this investigation is to determine the exposure of dogs to

Leptospira bacteria using serology and assess for the presence of asymptomatic carriers using PCR analysis of urine. Using the IDEXX SNAP® Lepto Test, seroprevalence was determined to be 12.65% (21/166) in 166 owned dogs, not previously vaccinated against Leptospira, presenting to the Junior Surgery and Anesthesia Lab at St. George's University School of Veterinary Medicine during fall of 2017. Urine samples from 149 of the 166 dogs were tested using a Leptospira spp. real-time PCR with 6.71% (10/149) positive for Leptospira spp. DNA. Nine of the 10 PCR-positive dogs were followed up one year later. Blood and urine PCR were negative in all cases and 55.56% (5/9) were seropositive on SNAP® Lepto Test. Four of the 9 dogs (44%) were seropositive on MAT, all of whom had antibodies against the *L. icterohaemorrhagiae* serovar, and one individual also had antibodies against *L. autumnalis* and *L. bratislava*. The identification of Leptospira spp. DNA in canine urine samples in this study suggests that there are asymptomatic carriers within the canine population in Grenada at rates similar to that reported in other countries.

PS01.67 Epidemiological Survey of Eimeria Species in Naturally Infected Calves Throughout Colombia

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There is a lack of information on the epidemiology of eimeriosis in naturally infected cattle in Colombia. A cross-sectional study was conducted to evaluate the prevalence, level of infection, and distribution of Eimeria species in 55 randomly distributed cattle farms across 7 States of Colombia. Fecal samples from 1333 calves less than 1-year-old were examined at a single sampling time from August 2016 to December 2016. Oocysts were identified to species level based on morphological and morphometric characteristics. The overall

prevalence of Eimeria was 75.5% (1006/1333), with no difference observed between age categories, although greater number of oocysts were shed in the lower age groups at 6 months of age. Thirteen species of Eimeria were identified, among which *E. bovis*, *E. auburnensis*, and *E. zuernii* were the most predominant species in 33.5%, 12.5%, and 11.9% of the positive samples examined, respectively. These were followed by *E. pellita* (7.4%), *E. ellipsoidalis* (5.4%), *E. canadensis* (5.3%), *E. wyomingensis* (5.3%), *E. bukidnonensis* (5.3%), *E. brasiliensis* (3.4%), *E. alabamensis* (3.1%), *E. subspherica* (2.9%), *E. cylindrical* (1.6%), and *E. illinoensis* (0.8%). There were 358/1333 (26.9%) samples with single species infections and 352/1333 (26.5%) with mixed infections by two or more Eimeria spp. This is the first large scale study reporting on prevalence and diversity of Eimeria species in calves throughout Colombia and it can be assumed that infections are ubiquitous in the country and may be playing an important role as a subclinical disease affecting production parameters in conventional management systems.

PS01.68 A Survey of Gastrointestinal Helminths and Coccidia in Llamas and Alpacas Raised in France

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Despite relentless increase of llama (*Lama glama*) and alpaca (*Vicugna pacos*) rearing in France, information regarding the presence and prevalence of gastrointestinal parasites in these species remains scarce. This study aimed to determine the presence of helminths and coccidia in llamas and alpacas raised in France and to identify epidemiological factors associated to their detection. The study was performed between May and July 2017 and it included faecal samples from 111 alpacas and 150 llamas coming from 22 French farms located all over the country. All samples were analysed by using saturated magnesium sulfate and quantitative McMaster's technique. Epidemiological analysis to identify risk

factors comprised variables such as camelid species, sex, age, geographical origin, farming practices (one-species or mixed-species farms) and parasite control protocols.

Sampling coverage reached 100% among farms raising between 2 and 24 individuals (18 farms), and 4 farms with more than 30 animals sampled between 25% and 37% of their population. Sixteen out of 22 farms (72%) raised their llamas and/or alpacas without contact with other livestock. Twelve farms raised llamas and alpacas together (54%). Age varied from one-year old to more than 10-years old, while 46% and 31% of samples were coming from 3-10 years-old and less than 3-years old animals, respectively. Strongyle-type eggs, Nematodirus sp eggs, and Trichuris sp. eggs were found on 19.9%, 1.9% and 2.7% of animals, respectively. Oocysts of Eimeria macusaniensis and small coccidia (E. punoensis and/or E. alpacae) were found in 8.4% and 75.3% of animals, respectively. While most results show a low parasite-egg counts in most animals, oocyst excretion was associated to age, being animals younger than 12 months the most infected (Mann-Whitney test $p < 0.001$). The results confirmed that parasite burden in France was comparable to other European countries, regardless the heterogeneity of farming practices and the gastrointestinal parasite-control protocols among llama and alpaca herds.

PS01.69 Regional, Biological, and Exposure Risk Factors That Drive Hunting Dog Seropositivity to Tick-Borne Co-infections

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Background: Ticks and tick-borne diseases have increased both in range and rate of

incidence across the United States. Given the shared environment in which people and dogs interact, dogs can be both primary hosts and sentinel animals for tick and tick-borne disease distributions for humans. Therefore, measures taken to better track, prevent, and/or treat tick-borne diseases in dogs can lead to better prevention or control of human exposure to these infections. Our study sought to identify demographic and biological risk factors for canine seropositivity to Anaplasma spp., Babesia spp., Ehrlichia spp., and B. burgdorferi in a cohort of highly exposed hunting dogs in the United States. Results: After controlling for age and Anaplasma spp. seroprevalence, dogs in regions outside of the west were 2.3x more likely (90% CI: 1.1274 – 4.5438, $p = 0.0216$) to test seropositive for B. burgdorferi. Dogs who tested seropositive for Anaplasma spp. were 1.4x more likely (90% CI: 1.0412 – 1.7846, $p = 0.0242$) to test seropositive for Babesia spp.. Analysis of Babesia spp. seroprevalence indicated that dogs seropositive for Babesia spp. were 1.6x more likely (90% CI: 1.2043 – 2.1826, $p = 0.0014$) to also be seropositive for Anaplasma spp.. Dogs from regions outside of the west were 0.0512x more likely (90% CI: 0.0118 – 0.2226, $p = 0.0001$) to test seropositive for Ehrlichia spp.. Hunting dogs seropositive for B. burgdorferi were more likely to also be seropositive for Anaplasma spp. (0.981 – 2.1225, $p = 0.0569$).

Conclusions: Region, due to the ticks that are found there, significantly influenced the likelihood of hunting dog exposure to ticks and tick-borne pathogens. Our results suggest that hunting dogs were more frequently co-infected with tick-borne pathogens. Dogs had significantly higher exposure to Babesia spp. than previously reported, perhaps because it is not regularly diagnosed through rapid tests and is not susceptible to doxycycline treatment.

PS01.70 Oxifendazole Effect on Gene Expression of P-glycoproteins in Two *Haemonchus contortus* Isolates Selected for Resistance Against Ivermectin and Oxifendazole

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Haemonchus contortus is the most prevalent small ruminant gastrointestinal nematode in tropical areas. It is usually controlled using anthelmintic drugs such as benzimidazoles and macrocyclic lactones. Parasite resistance against these compounds is currently considered a global problem. P-glycoproteins (P-gps) are ABC transporters involved with the removal of compounds from the cell interior and are known to be involved with drug resistance. In this study, we evaluated the gene expression of P-glycoproteins 2, 3 and 10 in *H. contortus* isolates selected for ivermectin and oxifendazole resistance before and after oxifendazole dosing (5 mg/kg) in experimentally infected sheep. Experimental animals (three per group) were infected and, once infection was established, euthanized before and after dosing at the approximate peak of oxifendazole presence in the blood stream. Adult male *H. contortus* were collected and total RNA extracted. *Haemonchus contortus* P-gps were identified based *Caenorhabditis elegans* orthologs and primers for reverse transcribed quantitative PCR (RT-qPCR) were designed based on sequences from genome assembly PRJEB506. RT-qPCR results were obtained for the above mentioned P-gps and GAPDH, actin and ribosomal protein L9 as reference genes. There was no difference in expression between both isolates for all tested genes prior to exposure to oxifendazole with the exception of P-gp 2 which was upregulated in the ivermectin selected isolate. Gene expression levels 8 hours after oxifendazole dosing led to a decrease in gene expression

below detection levels for all tested genes with the exception of L9 ribosomal protein, P-gps 2 and 3. Both resistant isolates presented similar low P-gp 3 expression in comparison to untreated controls. P-gp 2 was also downregulated by oxifendazole dosing but the ivermectin resistant isolate still maintained detectable levels of gene expression.

PS01.71 *Haemonchus Contortus* Infection Modulates Lamb Ruminal Microbiome Composition and Function

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Haemonchus contortus (HC) is the most important gastro enteric nematode in sheep in many regions of the world. However, the interaction between helminth and ruminal microbiome remains largely unknown. Six lambs fed exclusively *Cynodon dactylon* sp. hay were used in an experiment, divided in two treatments: artificially infected with 5,000 L3 (HC, n = 3) and control (n = 3) free of infection. Animals were followed for 34 days. Ovine ruminal contents were collected for DNA metagenomic sequencing to investigate the impact of HC infection on the structure and dynamical function of the ruminal microbial community. Our results showed that HC infection increased Archaea and bacteria abundance in the rumen, but with no effect on overall microbial diversity. The composition and functional analysis revealed less abundance ($P < 0.05$) of *Thermomonospora*, *Saccharomonospora*, *Leptospirillum* genus, as well as less abundance ($P < 0.05$) of genes related to glycine, serine, and threonine metabolism and higher abundance ($P < 0.05$) of genes related to methane metabolism and stress response in the HC infected group. Our findings suggest that

the infection by HC may lead to a decrease of specific genera related to plant biomass degradation and may promote a differential abundance of functional traits essential for immunomodulation, microbiota restoration, and the metabolism of essential amino acids for protein synthesis in infected lambs.

PS01.72 Ugt Genes Have Redundant Functions in Modulating Benzimidazole Sensitivity in the Nematode *Caenorhabditis Elegans*

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The benzimidazoles (BZ) are a family of anthelmintics used in livestock and human parasitic nematode control. Drug metabolism is an important modulator of drug efficacy in many systems including insects and mammals. However, there has been much less research in nematodes. Our previous work has shown that the free-living model nematode, *Caenorhabditis elegans* and parasite *Haemonchus contortus* detoxify BZ anthelmintics by conjugation with a glucose residue. This suggests the potential involvement of phase II xenobiotic metabolizing enzymes (XMEs), specifically the UGT enzyme family. The major objective of this project is to identify and functionally characterize nematode UGT enzymes that metabolize ABZ in *C. elegans*. We undertook an RNAi screening of ugt genes and identified that knockdown of ugt-9, ugt-11 and ugt-22 make worms sensitive to ABZ treatment compared to wild type. Further we identified that RNAi of ugt-9 and ugt-11 may target other six ugt genes present in ugt-9 cluster that are tightly linked and are closely phylogenetically related. We demonstrate the redundant function of ugt-9 cluster genes in modulating ABZ potency in vivo by examining the phenotype of seven loss-of-function ugt mutants present in ugt-9 cluster. Specifically, mutations in ugt-9 and ugt-11 make worms

more sensitive to ABZ and ugt-13 and ugt-12 make worms moderately sensitive to ABZ. By using CRISPR-Cas-9 knockout approach we made a ugt-9 cluster (df) strain (~24 kb deletion knocking out the tandem array of seven ugt genes) that showed a greater sensitivity to ABZ compared to each single loss-of-function mutant demonstrating that multiple ugt-9 cluster genes are involved in modulation of sensitivity to ABZ.

PS01.73 Sub-Lethal Effects of Moxidectin on the Dung Beetle *Onthophagus Landolti Harold* (Coleoptera: Scarabaeinae)

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Macrocyclic lactones can have adverse effects in dung beetles exposed to manure containing them. An evaluation was done of survival and fecundity in *Onthophagus landolti* Harold adults fed manure from cows treated with moxidectin, and of imago emergence from the masses built by these adults. Three cows (*Bos indicus* x *B. taurus*) were subcutaneously injected with 1% moxidectin (0.2 mg kg⁻¹ p.v.) and another three were injected with 10% moxidectin (1.0 mg/kg-1 p.v.). Manure was collected from these animals one day prior to moxidectin administration, at five days post-treatment in the 1% and 10% treatments, and at fourteen days in the 10% treatment. Four bioassays were done: a control using manure without moxidectin; 1% moxidectin at five days post-treatment; 10% moxidectin at five days post-treatment; and 10% moxidectin at fourteen days post-treatment. In each replicate, one pair of adult *O. landolti* was daily fed 30 g manure according to the treatments. No lethal effects were observed in any of the four treatments. Sub-lethal effects ($P < 0.05$) were present in the 10% moxidectin treatments at five and fourteen days post-treatment. Fecundity was reduced by 78.2% at five days and 54.9% at fourteen days, and imago emergence was negatively affected at

both times. Current moxidectin application methods may have negative effects on the environmental services provided by dung beetles, and therefore need to be modified to minimize any impacts they might have on these vital members of tropical livestock systems.

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PS01.74 Effect of *Stylosanthes* Species on Parasites of Cattle and Sheep

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Parasites cause losses in animal production. The ectoparasite *Rhipicephalus microplus* and the endoparasite *Haemonchus contortus* exposed to leguminous forages of the genus *Stylosanthes* which have phenolic compounds with known antiparasitic activity. In this study, *S. guianensis* cv Mineirão, *S. gracilis*, *S. bracteata*, *S. capitata*, *S. humilis* and *S. cv Campo Grande* were evaluated in repellent activity on *R. microplus* larvae and in mortality on *H. contortus* larvae. *Brachiaria ruziziensis* and *B. decumbens* were used as controls, respectively. The leguminous were harvested from the agronomic field of Institute of Animal Science. For the repellency evaluation, triplicates of plants was exposed to 4,000 larvae of *R. microplus*. After 72 hours the tip of each triplicate were put inside Falcon® tubes and washed with 50 mL of distilled water. Ticks were count in five aliquots of 200 µL. For anthelmintic evaluation, 1 g of each plant was placed in Petri dishes in triplicates with 15 mL distilled water with 1,000 L3 *H. contortus* and incubated 24 hours/27°C. The plants were washed with distilled water to a final volume of 50 mL and larvae of 10% of this volume were counted as dead or alive. Statistics were performed with PROC GLM (SAS), and the means of the different plants

were compared by Tukey's test ($P < 0.05$).

The counts of ticks for *S. guianensis* and *S. cv Campo Grande* were 0.0 ± 0.0 and 20.0 ± 56.1 and were significantly lower ($P < 0.05$) when compared to control *B. ruziziensis* (2587.0 ± 85.0). Percentage of larval mortality for *S. gracilis* (53.3 ± 7.7) did not differ among other species ($P > 0.05$) but was greater ($P < 0.05$) when compared to control *B. decumbens* (29.7 ± 10.2). More studies are needed in order to use *Stylosanthes* as part of sustainable control of *R. microplus* and *H. contortus* in pastures.

PS01.75 Distribution Patterns of Sodium Channel Mutations Associated With Pyrethroid Resistance and RDL Mutations Associated With Fipronil in *Rhipicephalus Microplus* Populations From Uruguay and Rio Grande Do Sul, Brazil

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Rhipicephalus microplus resistance to pyrethroids is widely dispersed and three nucleotide substitutions in the para-sodium channel gene have been associated. Resistance to fipronil is increasing in some countries and mutations in GABA-Cl gene were described in laboratory and field fipronil-resistant strains. We developed a multiplex allele specific-PCR (AS-PCR) to screen for mutations in both genes of tick populations from Uruguay ($n=31$) and Rio Grande do Sul state, Southern Brazil ($n=14$). Toxicological in vitro bioassays with larvae and adults were used to determine susceptibility to cypermethrin, flumethrin and fipronil. The AS-PCR included the detection of the three nucleotide substitutions the para-sodium channel gene (C190A; G215T and T2134A) and a non-synonymous mutation in the GABA-Cl gene (G858T/C859C). The mutation C190A was present in all pyrethroid-resistant

populations from Uruguay and Brazil, most frequently homozygous (30.9%). The mutations G215T and T2134A were not detected. Of 12 fipronil-resistant populations, 10 presented at least one mutant allele, more frequently in heterozygosis. Nevertheless, in two fipronil-susceptible populations, the mutation G858T/C859C was detected. The present survey shows a high frequency of pyrethroid resistance mutations widely dispersed in the region. The absence of the G215T and T2134A could suggest a geographic separation of C190A. Other mechanisms besides target site insensitivity may be involved in fipronil resistance, since in some populations the mutation was not detected.

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PS01.76 Benzimidazole Resistance Survey in Gastrointestinal Nematodes of Dairy and Beef Cattle in Western France Using Egg Hatch Assay

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There is a general consensus on the widespread anthelmintic resistance (AR) of gastrointestinal nematodes in Europe in small ruminants. There is much less data available regarding AR in cattle, although reports of lack of efficacy of macrocyclic lactones in several European countries have been published recently. Concerning benzimidazoles (BZ), only one survey on 12 herds in Germany and Sweden is available and indicated a full effectiveness of albendazole. Our study aimed at assessing the anthelmintic efficacy of BZ in dairy/beef cattle in western France using the Egg Hatch Assay (EHA). During summer 2018, 17 cattle farms were selected as a convenience sample. Individual faecal samples were collected and stored anaerobically in approximately 10 first or second grazing season animals per farm

which had not been treated in the previous 2 months. Faecal Egg Count was performed with Mini-FLOTAC technique to select the 2-3 faeces with the highest FEC values and to make a bulk faecal sample. In addition to coproculture, Egg Hatch Assay was performed as follows: i) eggs were isolated by sieving, centrifugation and flotation in saturated NaCl solution and collected with a sucrose step gradient centrifugation, ii) eggs were then tested using 7 concentrations of thiabendazole (TBZ) from 0.01 to 0.5 µg/mL to determine EC50 (cut-off: 0.1 µg/mL). In the 17 farms, EC50 values ranged from 0.027 to 0.051 µg TBZ/mL and indicated the susceptibility of the worm populations. Coprocultures results showed a predominance of *Cooperia* infective larvae. The allele frequency of BZ-resistance associated beta-tubulin isotype 1 single-nucleotide-polymorphisms within pooled samples of each of the 17 examined will be documented using a previously established pyrosequencing-based assay. The results of this study will have to be confirmed on a larger scale but suggest, in contrast to small ruminants, that BZ anthelmintics are fully effective in cattle.

PS01.77 Selective Sweep and Phylogenetic Models for the Emergence and Spread of Pyrimethamine Resistance Mutations in Plasmodium Vivax

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Pyrimethamine resistance is a major concern for the control of human haemoprotozoa, especially *Plasmodium* species. Currently, there is little understanding of how pyrimethamine resistance developed in *Plasmodium vivax* in the natural field conditions. Here, we present for the first-time evidence of positive selection pressure on a dihydrofolate reductase locus and its consequences on the emergence and the spread of pyrimethamine resistance in *P. vivax*

in the Punjab province of Pakistan. First, we examined the dihydrofolate reductase locus in 38 *P. vivax* isolates to look for evidence of positive selection pressure in human patients. The S58R (AGA)/S117N (AAC) double mutation was most common, being detected in 10/38 isolates. Single mutation S117N (AAC), I173L (CTT) and S58R (AGA) SNPs were detected in 8/38, 2/38 and 1/38 isolates, respectively. The F57L/I (TTA/ATA) and T61M (ATG) SNPs were not detected in any isolates examined. Although both soft and hard selective sweeps have occurred with striking differences between isolates, there was a predominance of hard sweeps. A single resistance haplotype was present at high frequency in 9/14 isolates, providing a strong evidence for single emergence of resistance by the single mutation, characteristics of hard selective sweeps. In contrast, 5/14 isolates carried multiple resistance haplotypes at high frequencies, providing an evidence of the emergence of resistance by recurrent mutations, characteristics of soft selective sweeps. Our phylogenetic relationship analysis suggests that S58R (AGA)/S117N (AAC) and S117N (AAC) mutations arose multiple times from a single origin and spread to multiple different cities in the Punjab province through gene flow. Interestingly, the I173L (CTT) mutation was present on a single haplotype, suggesting that it arises rarely and has not spread between cities. Our work shows the need for responsible use of existing and new antimicrobial drugs and their combinations, control the movement of infected patients and mosquito control strategies.

PS01.78 Transcriptomic Analysis of ABC-Transporters (P-GP, MRP and HAF) in *Haemonchus Contortus* Isolates With Different Susceptibility to IVM

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Inadequate and intensive use of anthelmintic compounds has led to the emergence of high

levels of parasite resistance in nematodes of sheep. Changes in drug target sites and the up-regulation of detoxification systems seem to be implicated in this phenomenon. Several studies have shown that ATP-binding-cassette (ABC) transporters such as P-glycoprotein (P-gp) play an important role in multidrug resistance in many organisms, including several nematode species. The goals of the current work were: 1) to compare the gene expression of several cellular transporters in both a susceptible (S-IVM) and a highly ivermectin (IVM)-resistant *Haemonchus contortus* isolate (HR-IVM); 2) to assess the effect of IVM on ABC transporters expression patterns in the HR-IVM isolate under in vivo conditions. To this end, the transcriptional levels of ABC transporters in adult HR-IVM *H. contortus* recovered from IVM-treated lambs (2 mg/kg) at 12 and 24 hours post-treatment, were compared to those obtained from the S-IVM and HR-IVM specimens collected from untreated lambs. The phylogenetic tree with the transporter sequences of the reference nematode *Caenorabditis elegans* and *H. contortus* allowed us to found the putative orthologous genes P-gp 1, 2, 3, 9.1, 10, 11, 13, 16 and 17; MRP 3, 4, 7 and 8; and Haf 2, 3, 4, 6 and 9. Next generation sequencing analysis showed that both *H. contortus* isolates express 6 of the 9 P-gps, 3 of the 5 multi-drug resistant proteins (MRPs) and 4 of the 5 Half (HAF) transporter genes. Some of these ABC transporter genes are differentially expressed in the S and HR isolates. IVM treatment induced slight changes in the mRNA levels of MRP-4 and P-gp transporters, but the biological significance of the observed changes may not be enough to explain the high level of IVM resistance displayed by the isolate under study in the current trial.

PS01.79 Sheep, Strongyles and Sequencing: Investigating Ivermectin Resistance in UK Field Populations

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Parasitic gastroenteritis is a major production limiting disease of small ruminants worldwide, and anthelmintic resistance is steadily increasing. The abomasal nematode *Teladorsagia circumcincta* is the primary pathogen on most sheep farms in the UK during the summer months, when lambs are in their primary growth phase. Ivermectin is commonly used to treat infected sheep throughout the year, but resistance is highly prevalent; recent studies in the UK and Ireland have demonstrated rising numbers of farms with detectable ivermectin resistance by faecal egg count reduction test, a concern for sustainable control of parasites in the future. There are many limitations to the faecal egg count reduction test, and such limitations, combined with a highly complicated disease, make understanding the effect of different management practices on the development of anthelmintic resistance difficult. Ivermectin resistance in *T. circumcincta* is poorly understood, and molecular tests are needed. In this study we have looked at two UK farm populations of *T. circumcincta* pre- and post-ivermectin treatment using next generation sequencing techniques (ddRAD-Seq and Pool-Seq) to identify regions of the genome under selection. Despite the fragmented nature of the *T. circumcincta* draft genome assembly, we found a single large locus to be under ivermectin selection, in addition to many smaller loci. Though some selected loci appear farm specific, several of the same contigs are under selection on both farms. Previous candidate ivermectin resistance genes from the literature do not appear to be under selection so are unlikely to contribute to ivermectin resistance in these populations.

PS01.80 Treatment of Canine and Feline Gastrointestinal and Respiratory Helminths Using a Combination of Milbemycin Oxime and Praziquantel (Milpro® Tablets, Virbac) in Greece

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Dogs and cats are the most common pets living in close relationship with humans, offering several benefits. Both animal species can harbour numerous helminths, which may affect their health and wellbeing, while some of them may have zoonotic implications. The aim of this study was to evaluate the effectiveness of the combination of milbemycin oxime and praziquantel (Milpro, Virbac) against gastrointestinal and respiratory helminths in dogs and cats in Greece. Towards this end, faecal samples were collected for coprological examination from 728 dogs and 670 cats from different parts of the country, targeting potentially infected animals. A questionnaire was accompanying the sample with relevant information (sex, age, lifestyle, breed, owner education etc). Overall, 63.2% (460/728) and 41.8% (280/670) sampled dogs and cats, respectively, were detected to excrete at least one parasitic element. Infected animals were treated at D0 with the above combination and a second sample was tested again at D30 to evaluate the effective removal of the parasitic elements (% efficacy). Dogs were found to be infected with *Toxocara canis* (24.9%, mean efficacy 98.7%), hookworms (19.6%, efficacy 100%), *Trichuris vulpis* (12.1%, efficacy 93.7%), *Toxascaris leonina* (8.7%, efficacy 100%), *Dipylidium caninum* (4.9%, efficacy 99.7%) and *Taenia* spp. (0.5%, efficacy 97.7%). Cats were found to be infected with *Toxocara cati* (31%, efficacy 99.5%), hookworms (21.3%, efficacy 100%), lungworms (5.7%, efficacy 92.4%) and *Dipylidium caninum* (1.6%, efficacy 99.3%). In all cases of reduced efficacy, a second treatment was carried out at D30 and

retested at D60 leading to a 100% parasite removal. The most important risk factor was found to be lifestyle and owner education. It was concluded that the combination of milbemycin oxime and praziquantel (Milpro, Virbac) offers an effective solution to control common gastrointestinal and respiratory helminths in dogs and cats in Greece.

PS01.81 Acaricidal Activity of Plant-Derived Essential Oil Components Against *Psoroptes Ovis* In Vitro and In Vivo

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Psoroptes ovis is a major health problem in beef cattle. Treatment is limited to local administration of amitraz or pyrethroids or systemic administration of macrocyclic lactones. Treatment failures with macrocyclic lactones have been reported in recent years. To investigate potential alternative treatments, the acaricidal activity of four plant-derived essential oil components, i.e. geraniol, eugenol, 1,8-cineol and carvacrol against *P. ovis* was assessed in vitro and in vivo. Three components showed a concentration-dependent acaricidal activity in a contact assay, with LC₅₀ of 0.56 %, 0.38 % and 0.26 % at 24 h for geraniol, eugenol, and carvacrol, respectively. In a fumigation bioassay, carvacrol demonstrated the best efficacy as it killed all mites within 50 min after treatment, whereas geraniol, eugenol, and 1,8-cineol needed 90 min, 150 min, and 90 min, respectively. Following a 72 h incubation period in a residual bioassay, eugenol and carvacrol killed all mites after 4 h of exposure to LC₅₀ and LC₉₀, while geraniol killed all mites only after 8 h exposure at LC₅₀. Topical treatment with 2 % carvacrol in Tween-80 of six calves with experimental *P. ovis* infestations, reduced mean mite counts by 98.48±2.36 % at 6 weeks post treatment. In the control group which was treated with Tween-80 only, the mite population increased

with similar kinetics as a typical experimental mite infestation. Topical application of carvacrol on shaved skin caused mild and transient erythema 20 min after treatment. No other side effects were observed. Considering the strong acaricidal activity of carvacrol in vitro and in vivo and the mild and transient local side effects after topical treatment, carvacrol shows potential as an acaricidal agent in the treatment of *P. ovis* in cattle.

PS01.82 Evaluation of Levamisole Pharmacokinetics and Milk Excretion in Dairy Goats

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Introduction: Levamisole (LVM) is a nematocidal compound available for veterinary medical use for over 50 years. Since the widespread development of drug resistance is a serious productive inconvenient, LVM is currently used extralabel as a therapeutic alternative in dairy sheep and goats. Thus, information on drug residual concentrations in milk is needed to assure protect consumer`s safety. The current work aimed to evaluate the concentration profiles of LVM in plasma and its pattern of milk excretion in lactating dairy goats treated at different stages of lactation.

Material and Methods: Twelve (12) female Saanen dairy goats at early-mid stage of lactation (group A) and at mid-late stage of lactation (group B) were orally treated with LVM (7.5 mg/kg) (Ripercol®, Zoetis). Blood and milk samples were collected between 0 and 5 days post-treatment to characterize the plasma and milk disposition kinetics. LVM concentrations in plasma and milk were determined by HPLC with UV detection. Results: LVM parent compound was detected in plasma and milk up to 8 h and 12 h post-treatment, respectively. Plasma concentrations

increased progressively to a maximum concentration (C_{max}) of $0.89 \pm 0.2 \mu\text{g/ml}$ at 0.27 h (group A) and $0.77 \pm 0.3 \mu\text{g/ml}$ at 0.2 h (group B). LVM milk residual concentrations were lower than measured in plasma.

Conclusion: The pharmacokinetic results reported here confirm that LVM is excreted by milk in lactating dairy goats. The residual concentrations in milk ($0.05 \mu\text{g/ml}$) detected up to 12 h, should be considered before issuing any recommendation on the manufacturing of milk from dairy goats under antiparasitic treatment with LVM.

PS01.83 Uptake of Ivermectin from Growing Substrate to Plant Species

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Ivermectin (IVM) is a worldwide-used antiparasitic drug. However, its high level of faecal elimination together with its transfer from dung pats to the underlying soil as well as the common practice of using manure for soil amendment represents a potential risk to plants growing in these substrates. Two trials were conducted to evaluate the uptake of IVM to: 1) a crop of ryegrass (*Lolium multiflorum*) and clover (*Trifolium repens*) growing for 120 days post treatment (dpt) in IVM-spiked soil at 3000 (High group, HG) and 90ng/g (Low group, LG); and 2) a crop of radish (*Raphanus sativus*) and lettuce (*Lactuca sativa*) growing for 60 dpt in a mix of soil and 10% IVM-spiked manure at 3000ng/g. Soil, soil-manure mix and plants were sampled starting at 15 dpt and at the end of each trial. All matrices were analyzed by HPLC to quantify IVM concentration. Trial 1: In HG, IVM concentration in soil decreased

from 2154 ng/g to 225 ng/g; mean IVM concentration in ryegrass ranged between 378.65ng/g and 21,74ng/g. Strikingly, clover development was delayed until 30 dpt and IVM concentration in this specie ranged between 94,09 ng/g and 4,56ng/g. Significant differences were detected between species ($p=0,0374$). In the LG, IVM concentration was between 22,26ng/g and 1,02ng/g in ryegrass, and between 10ng/g and 1,02ng/g in clover, without statistically significant differences between species ($p=0,8301$). Trial 2: IVM was detected in both plant species at significant levels ($p>0,05$) in all the sampling times; mean IVM concentration was between 10ng/g and 5ng/g in radish, and 17,70ng/g and 6,55ng/g in lettuce. IVM concentration in the substrate decreased from 1311ng/g to 116ng/g. In conclusion, IVM concentrations in soil or composted substrate are transferred to plants during growth period, and could be incorporated into the food chain of both livestock and humans.

PS01.84 The Efficacy of a Proprietary Formulation of Imidacloprid 10% + Moxidectin 1% (Advantage Multi®, Advocate®) Spot-On for the Treatment Against Microfilariae of Brugia Pahangi in Naturally Infected Cats

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Lymphatic filariasis (LF) is one of the most debilitating neglected tropical diseases (NTDs). It is caused by parasitic worms transmitted to humans by mosquitoes. Recent evidence has also indicated that *Brugia pahangi*, a filarial nematode that is naturally found in cats, can cause clinical infection in humans, with clinical presentations that are consistent with lymphatic filariasis. Imidacloprid 10% + Moxidectin 1% has more recently been introduced as a topical spot-on, and marketed worldwide as Advantage Multi® or Advocate® by Bayer Animal Health (Leverkusen, Germany). However, the efficacy against infections with *B. pahangi* in cats

has never been tested. The goal of this project was to determine the efficacy of the combination of imidacloprid 10% + moxidectin 1% against microfilariae of *B. pahangi* in naturally infected cats. Twelve cats naturally infected with *B. pahangi* were divided into treatment and control groups. Cats in the treatment group were given a combination of imidacloprid (10 mg/kg bodyweight) and moxidectin (1 mg/kg bodyweight) spot on monthly for 5 months. Microfilariae were counted weekly for a period of 20 weeks for both groups. The mean microfilaria counts decreased significantly in the treatment group after 3 month application. Moreover, all treatment cats were negative for microfilaria count on week 15, 18 and onward after 4 month application. In this study, cats naturally infected with *B. pahangi* were successfully treated using 5 monthly doses of a imidacloprid 10% + moxidectin 1% spot on formulation.

PS01.85 Anthelmintic Activity of A Herbal Complex Against *Heamonchus Contortus*

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The study was carried out to evaluate anthelmintic properties of a herbal complex (Oregano essential oil, Cinnamon essential oil, Rosemary essential oil and Red chilli powder) against *Heamonchus contortus* a common pathogenic nematode effecting the ruminant by sucking blood from the abomasum resulting to anemia and dullness which leads to low productivity and mortality. The herbal complex with concentration (25mg/ml to 0.39mg/ml) was exposed to the *heamonchus* eggs in the lab with 136egg/0.3ml concentration following the standard procedure of egg hatch test. The reference drug used in the study was oxfendazole with the concentration of 0.01415mg/ml to 0.000215mg/ml. After incubation of 48 hours the results recorded for the egg count in which the herbal formulation used with 25mg/ml (96.70%), 12.5mg/ml (91.13%), 6.25mg/ml (71.30%), 3.125mg/ml (44.12%), 1.56mg/ml (41.18%), 0.78mg/ml (33.83%) and

0.39mg/ml (30.89%) reduction in the egg count as compared to the reference dose (Oxfendazole) concentration 0.01415mg/ml (78%), 0.00707mg/ml (68%), 0.003537mg/ml (68.4%), 0.001768 mg/ml (47.06%), 0.00088 mg/ml (30.89%), 0.00043mg/ml (27.81) and 0.000215 mg/ml (30.15%) reduction in the eggs. So the herbal complex showed a graded response as anthelmintic so Herbal complex seems to be promising. The trials on the large scale for the efficacy and safety, however, are recommended before the herbal complex is considered for commercialization.

PS01.86 Morphological and Molecular Characterization of Feline Filarid Nematode and Its Zoonotic Implication

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Dirofilariosis is a potential zoonotic disease caused by filarid nematodes, prevalent in several parts of the world transmitted mainly by mosquito vectors. In the present study, the filarid worms were recovered from the subcutaneous tissues of seven cats during sterilization in mangalore, a coastal region of Karnataka. The worms were collected in PBS and identified as *D. repens* based on the morphometry. Morphologically, adult worms showed longitudinal ridges on the cuticle with anterior muscular and posterior glandular type of oesophagus. The length and width of female worms were in the range of 112 to 130 mm and 4.1 to 5.8 mm in diameter whereas, male worms of 44 to 58 mm length and a diameter of 3.2 to 4.1 mm respectively. The blood samples of cats showed microfilaria

by modified knott's method (MKM) and quantitative buffy coat (QBC). Morphologically, the microfilariae were unsheathed with blunt head and the tail was long, curved with hook like posterior end. The length and width of microfilaria were in the range of $310.9 \pm 9.10 \mu\text{m}$ and $6.51 \pm 0.14 \mu\text{m}$ respectively. Based on morphometry, the microfilariae were belongs to *D. repens*. During the study, a human filarid worm from the said region received for identification and the morphometry showed similar to that of *D. repens*. For confirmation, DNA from both cat and human filarid worms including microfilariae were extracted and subjected to PCR using pan-filarial primer pair of DIDR-F1 and DIDR-R1 targeting ITS2 of the ribosomal DNA of *D. repens*. The PCR yielded amplicons were sequenced and analysed. The adult worms of cats, human and microfilariae of cats from coastal region of Karnataka were confirmed as *D. repens* based on PCR targeting ITS2 genes. The nucleotide sequence analysis obtained showed more similarity with *D. repens* sequences than *D. immitis*.

of *Cytauxzoon felis*. Sixteen cats were dosed with selamectin/sarolaner or a placebo (vehicle control) on Days 0, 28, and 56. In phase 1, each cat was infested with 50 (± 5) unfed adult *A. americanum* on Day 4 and tick counts were conducted on Day 6 (48 hours post-infestation) and Day 7 (72 hours post-infestation). In phase 2, each cat was infested on Day 60 with 50 (± 5) adult *A. americanum* acquisition-fed as nymphs on two *C. felis*-infected donor cats. Tick counts were conducted on Day 62 (48 hours post-infestation) and Day 63 (72 hours post-infestation). Placebo cats were adequately infested on all count days, with least squares (geometric) mean live tick counts ranging from 34.0 (28.8) to 46.1 (46.0). Treatment reduced the least squares (geometric) mean counts compared to placebo by 27.1 (32.1)% and 90.4 (96.8)% on Days 6 and 7, respectively. The corresponding percent reductions were 56.4 (60.6)% and 94.7 (97.3) % on Days 62 and 63, respectively. Least squares mean counts were significantly lower in the treated group compared with the placebo group on all count days ($P \leq 0.0286$). In phase 2, seven cats in the control group and no cats in the selamectin/sarolaner group became infected with *C. felis* ($P = 0.0017$). Topical treatment with selamectin/sarolaner (Revolution® Plus) was >90% effective in reducing *A. americanum* tick counts 72 hours after infestation and prevented the transmission of *C. felis* from infected ticks following administration of three consecutive monthly treatments. Extracted from <https://doi.org/10.1016/j.vetpar.2018.10.018>.

Poster Session 02

July 9, 2019, 10:00 – 16:30
Exhibit Hall A, July 9, 2019, Level 1

PS02.01 Efficacy of a Topical Formulation of Selamectin Plus Sarolaner (Revolution® Plus) Against Induced Infestations of *Amblyomma Americanum* on Cats and Prevention of *Cytauxzoon Felis* Transmission

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Our aims were to determine the efficacy of a selamectin (6.0 mg/kg) plus sarolaner (1.0 mg/kg) combination (Revolution® Plus / Stronghold® Plus, Zoetis) applied topically once a month on cats for three consecutive months against induced infestations of *Amblyomma americanum* adults and to evaluate the effectiveness of the product in preventing the transmission

PS02.02 Roles of Flies and Beetles Associated with Animal Agriculture in Salmonella Maintenance and Transmission

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Salmonellosis affects over 1.2 million Americans annually, with 65 of every 100,000 children under the age of 5 years suffering from the illness. There are many means by which Salmonella infection is acquired, but research is demonstrating that arthropods are frequent Salmonella carriers. Flies, not surprisingly, are commonly contaminated with Salmonella, and their close association with humans and human food makes them likely means of transmission. Their mobility enhances their vectorial significance. A common pest of poultry production, the lesser mealworm (also known as the poultry darkling beetle) not only acquires and transmits Salmonella as both larvae and adults, but is capable of maintaining Salmonella infection through pupation, emerging as Salmonella-infected adult beetles. Chickens can acquire Salmonella by eating infected beetle larvae or adults. Thus, management of Salmonella exposure necessitates suppression of these arthropods involved in maintenance and transmission of the pathogen.

PS02.03 Use of LongRange® (Boehringer Ingelheim Animal Health) Placei and Nematodirus Helvetianus, Oesophagostomum Radiatum and Trichostrongylus Colubriformis Infections in Cattle

Martin Liebstein¹, Steffen Rehbein¹, Bruce Kunkle¹, Jeffrey Shryock¹, Jonatan Bader¹, Dietmar Hamel¹, Martin Visser¹, Stephen Yoon¹, Becky Fankhauser¹

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INTRODUCTION: LongRange® (eprinomectin extended-release injection) provides therapeutic activity against developing, inhibited and adult nematodes as well as prevention from infection with a range of gastrointestinal nematodes and lungworms for up to 150 days. However, data are lacking on its efficacy against developing and adult *Bunostomum phlebotomum*, *Haemonchus contortus* and *Nematodirus helvetianus*, and developing *Haemonchus placei*, *Oesophagostomum radiatum* and *Trichostrongylus colubriformis*. For this reason, nine blinded studies compliant to GCP and VICH were conducted in the US and Germany to assess the efficacy for each species and stage.

METHODOLOGY: 218 young cattle, 180 with induced and 38 with naturally acquired nematode infections were included in seven and two studies, respectively. In each study, cattle were formed randomly into groups of nine or ten animals each, which served either as saline-treated controls or received LongRange® (1 mL per 50 kg bodyweight) when the parasites were fourth-stage developing (L4) or adult nematodes. Efficacy was determined based on geometric means of nematode counts established following necropsy of animals.

RESULTS: Treatment was well accepted and no treatment-related problems were observed in any study. Counts of each species and stage of nematodes were significantly lower in the LongRange®-treated cattle than in the controls. Percent efficacy for each study by species was 100% against L4 and adult *B. phlebotomum*; 69.6% and >99% against L4 and ≥90.8%, >99% and 100% against adult *H. contortus*; 91.4% and 97.4% against L4 *H. placei*, >99% and 100% against L4 and >99%, >99% and 100% against adult *N. helvetianus*; >99% and >99% against L4 *O. radiatum*; and 98.9% and 100% against L4 *T. colubriformis*.

Results of these studies demonstrated that LongRange® is an efficacious treatment against a broad spectrum of developing larval and adult nematode endoparasites. The efficacy demonstrated was similar to what was previously reported against the most widespread nematodes.

PS02.04 Targeted Selective Treatment Against Liver Flukes (*Fasciola Hepatica*) in Dairy Herds in Sweden

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¹*Swedish University of Agricultural Sciences, Uppsala, Sweden*, ²*Veterinary Research Institute, Brno, Czech Republic*

Control of fasciolosis is primarily based on anthelmintic treatment in combination with grazing routines. In dairy cows, flukicide treatment is restricted due to concerns about drug residues in milk. Although the impact of fluke infection on dairy production is under debate, the costs of fasciolosis and benefits of deworming have never been evaluated in Sweden.

The aim of this study was to evaluate the efficacy of a targeted anthelmintic treatment by investigation of the epidemiology of liver fluke infection and its impact on dairy production.

Four fluke-infected dairy herds in southwest Sweden were monitored for *Fasciola hepatica* antibodies in milk and/or faecal coproantigens between 2017 and 2018. In addition, data on milk yield and quality were collected on a monthly basis between 2016 and 2018. The deworming protocol was oral drenching of all non-lactating animals (heifers and dry cows) with albendazole (10 mg/kg bodyweight) on three different visits both during winters 2017 and 2018. On each visit, dewormed animals along with 15 individual milking cows were also sampled for the detection of coproantigens. Furthermore, milk samples were collected quarterly from the whole herd and examined for antibody levels using an indirect ELISA. Total prevalence in dairy herds based on coproantigen varied from 27 to 85%. However, in all four herds, heifers had lower *F. hepatica* prevalence than milking cows. Deworming in 2017 resulted in a significant decrease of coproantigen-positivity but only in the two herds with higher fluke burden in 2018. Nevertheless preliminary data show a significant increase of energy corrected milk only on one of these farms. More detailed statistical modelling is needed before final conclusion about the impact of liver flukes on dairy cattle.

This study was supported by Swedish Research Council (FORMAS) Nr. 2016-00510 and OP VVV Project CZ.02.1.01/0.0/0.0/15_003/0000495 FIT (Pharmacology, Immunotherapy, nanoToxicology).

PS02.05 Trypanosomiasis in Sedentary Cattle at Previously Assumed Trypanosoma-Free Jos Plateau, Nigeria

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Trypanosomiasis in sedentary Cattle at previously assumed Trypanosoma-free Jos plateau, Nigeria

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Abstract

The occurrence of trypanosomiasis was investigated in sedentary Bunaji (*Bos indicus*) cattle grazing at 5 different villages of Barkin Ladi Local Government Area of Plateau State, Nigeria. Two hundred cattle were examined, 76 (38.0%) of which had trypanosome infection. Three species, *Trypanosoma vivax* (78.9%), *T. Congolense* (15.8%), and *T. brucei* (5.3%) were encountered. More males (42.9%) than females (31.8%) were affected while animals of 3-5 years age cohort had the highest infection rate of 46.7%. The existence of trypanosome infections in sedentary cattle populations of the Jos Plateau seems to suggest that the area which was previously assumed to be Trypanosoma-free may have acquired infection status. Suggestions on the cause of this shift are presented in study.

PS02.06 Profitability of Parasiticides for Control of Horn Flies (Diptera: Muscidae) in Stocker Cattle

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Horn flies (*Haematobia irritans*) have been estimated to cause over US \$2 billion per year to the cattle industry. The efficacy of two parasiticides for controlling horn flies, Corathon® and LongRange®, and their effect on profitability of raising stocker cattle were evaluated. The objectives were to: (1) determine whether either parasiticide would control horn flies better than the control group, and (2) determine the profitability a producer of beef stocker animals could expect to achieve by using either of these parasiticides relative to a control group. A total of 302 stocker cattle records from Kansas State University Beef Stocker Unit were analyzed. Horn fly populations were on average 34% lower with application of Corathon® and 50% lower with application of LongRange® compared with the control group. Comparing value of gains with costs, animals receiving the Corathon® treatment gained an average of 10.01 lbs/head (0.1112 lb. x 90 days), or 4.55 kg/head, and additional profit from the application of Corathon® is estimated at \$5.37/head. Animals receiving the LongRange® treatment gained an average of 14.11 lbs/head (0.1568 x 90 days), or 6.41 kg/head, and additional profit from the application of LongRange® was estimated at \$4.52/head. Although use of LongRange® achieved a greater reduction of horn flies, and a greater increase in pounds of gain, its additional profit is similar to that of Corathon® because of the higher cost per animal to apply (Corathon® = \$4.47 per head versus LongRange® = \$10.00 per head). These profits will be discussed in relation to variable price slides in the cattle market.

PS02.07 Risks with Next Generation Sequencing in the Detection of Strongylus Vulgaris

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Strongylus vulgaris is considered as the most pathogenic equine parasite. NGS-technology is just starting to gain traction in the field of clinical veterinary parasitology. In this study, we tested the ability of NGS for mass screening of *S. vulgaris*. Faecal larval cultures from 279 horses were collected in a routine diagnostic laboratory receiving equine samples from all over Sweden. In addition to NGS all samples were screened for *S. vulgaris* both by microscopy and amplification of a 171 bp product of the second internal transcribed spacer (ITS-2) using standard PCR. For NGS the ITS2-region was amplified using nematode universal NC1 and NC2 primers flanked by barcoding tags. The resulting amplicons were prepared and sequenced using PacBio RSII SMRT cells. Reads were mapped against a local database including all nematode rDNA sequences in NCBI. This way the whole strongyle nematode community was characterized in each sample. *Strongylus vulgaris* was identified in 50 (18%) samples by microscopy, 62 (22%) by PCR and 30 (11%) by PacBio. There was substantial agreement between microscopy and PCR results (Cohens Kappa 0.7 ± 0.05), whereas it was moderate between microscopy and NGS (Cohens Kappa 0.6 ± 0.07). The agreement between PCR and NGS was weak (Cohens Kappa 0.5 ± 0.06). The low sensitivity of *S. vulgaris* using Pacbio was common especially in samples with low relative levels of *S. vulgaris* DNA. This indicates a negative bias for *S. vulgaris* by the NGS method. Thus, a low relative abundance of *S. vulgaris* DNA may lead to underestimation of the infection when diagnosed by NGS. Therefore, diagnosis using microscopy or PCR is advised.

PS02.08 Study on Survival of Nonstrangulating Intestinal Infarctions Associated With *Strongylus Vulgaris*: Treatment Outcomes of 39 Horses (2008–2019)

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Strongylus vulgaris is the most pathogenic gastrointestinal parasite in the horse and it is reemerging, in amongst other places, Denmark. If nonstrangulating intestinal infarctions (NSII) caused by *Strongylus vulgaris* are not diagnosed in due time, the horse may die from this condition. This could most likely be prevented with an increased awareness and recognition of the disease and the initiation of the correct treatment.

The objective was to investigate the survival rate in horses with NSII undergoing exploratory laparotomy.

The study is conducted as a retrospective case series.

NSII was diagnosed in 39 horses with a localized intestinal infarction and concurrent signs of *Strongylus vulgaris* migration and no signs of intestinal strangulation or enterocolitis. Data were obtained from medical records in the period from 2008 to 2019.

39 horses were diagnosed with NSII associated with *Strongylus vulgaris* migration. Exploratory laparotomy was undertaken in 26 horses. 12 of these 26 horses were euthanized intraoperatively due to a poor prognosis. Surgical treatment was carried out in 14 of the 26 horses and of these 7 (50 %) survived to discharge. 13 horses were treated medically due to the owners declining surgery; of these 100 % were euthanized due to no sign of improvement with medical treatment.

A small group of subjects is the main limitation.

In conclusion the findings indicate that an exploratory laparotomy seems to be justified

in cases with NSII in order to improve the survival rate.

PS02.09 Pilot Study on the Parasites of Feral Horses on Cumberland Island, Georgia, USA

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Cumberland Island (147.37 km²), a National Park since 1972, off the coast of Georgia has had feral horses for many years, descended from horses brought to the island from the 1700s through the mid-1900s. The current population, estimated to be 120 to 190, is unmanaged and consists of approximately 15 not well define herds. Health of the horses is a major concern with parasites blamed for many deaths; however, no studies on parasite burdens have been performed and the applicability of data from other island feral horses (e.g., Sable Island off of the coast of Canada) is uncertain due to differences in island size, climate and horse breeds. During the summer of 2015, with permission from the National Park Service, fresh fecal samples from 15 horses and 6 herds (approx. 10% of the horses and 40% of the herds), representing various ages, were analyzed using the Cornell-McMaster technique. Body condition (BC) using the Henneke System (scale 1 to 9) for the sampled horses was assessed at a distance. Strongyle (15 of 15 positive), Oxyuris (7/15) and Parascaris (6/15) eggs were identified with mean fecal egg counts per gram of 1317 (225-2525), 86.7 (0-300) and 38.3 (0-300), respectively. No correlation between BC (mean 4.9; 3 to 7) and strongyle egg counts was found. The results of this pilot study do not indicate that parasites are the primary cause of health issues with strongyle counts lower than that seen in other feral horse populations. However, interpretation of the study results must be made with caution given that only a small number of horses were tested and single samples at one time during the year were analyzed. Further studies are required to understand factors that influence parasite burdens in these island feral horse populations and to assist in developing appropriate management plans.

PS02.10 In Vitro Assays to Evaluate Lethal or Repulsive Activity of Chemical Products Against *Sarcoptes Scabiei*

Dr. Raluca Mindru², Charbel Al Khoury³, Dr. Fang Fang⁴, Dr. Charlotte Bernigaud⁵, **Prof. Jacques Guillot¹**

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The life cycle of *Sarcoptes scabiei* does not include an environmental stage per se and transmission may depend on the ability of mites to survive off the host, and to maintain infectivity. We recently developed in vitro tests to assess the activity of chemical products against motile stages of *S. scabiei*. Mites were collected from experimentally infected pigs as described by Mounsey et al. (2010) and further optimized by Bernigaud (2016, 2018). Crusts present in the external ear canal were gently removed and placed in a Petri dish. Adults, nymphs and larvae were picked with a needle and used for the tests. To evaluate lethal activity, exposure to the products was obtained through immersion, fumigation or direct contact to a solid medium (supplemented with a specific concentration of product) according to Brimer et al. (1993, 1995). Mites were inspected under a stereomicroscope after 10 min, 30 min, 1h, 2h, 3h, 4h, 8h and 24h. Persistent immobility, even when stimulated with a needle was considered as death. To evaluate the repulsive activity, each chemical product was incorporated in Columbia agar through a small hole (3 mm in diameter) or a small piece of tissue. The mites were placed in the center of the Petri dish and the migration ability and potential repulsive effect were evaluated according to the following criteria defined by Brimer et al. (1993): (i) the number of mites having demonstrated the ability to migrate, as indicated by footprints and bacterial colonies, and (ii) the length of the migration tracks. Lethal and repulsive activity was evaluated for commercially available insecticide/acaricide products (mainly pyrethroids), repellents (DEET, icaridin, IR3535) but also for several essential oils. Several molecules were proved

to be highly active against *S. scabiei*. A few products also displayed a repulsive activity against the mites.

PS02.11 Anthelmintic Resistance in Gastrointestinal Nematodes of French Angora Goat Flocks

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Anthelmintic resistance (AR) in gastrointestinal nematodes (GIN) is very frequent in dairy goats, especially to benzimidazoles. Considering that the use of anthelmintics can be quite different between dairy and fiber goats, an anthelmintic resistance survey was conducted in 15 French Angora flocks located all over the country. On each farm, goats were randomly allocated into several groups of animals: an untreated control group, a group that was orally administered oxfendazole (10 mg/kg body weight-BW-) and a group that received orally a moxidectin drench (0.4 mg/kg BW). Individual faecal egg counts and pooled larval cultures were done 14 days after anthelmintic treatment. AR was present when the % of Faecal Egg Count Reduction (%FECR) was <95 % and the lower limit of the 95% confidence interval <90 %. Two farms were excluded from the analysis because the control group was absent. For the 12 flocks where oxfendazole was tested, faecal egg count reductions ranged from zero to 83%, meaning that resistance to benzimidazoles was present in all flocks. Post-treatment larval cultures indicated that *Teladorsagia*/*Trichostrongylus* was the predominant larval type. In the 11 flocks where moxidectin was tested, resistance was present in 3 of them with FECR ranging from 69 to 94%, with *Teladorsagia*/*Trichostrongylus* being also the predominant post-treatment culture larval type. This study confirms the extremely high prevalence of resistance to benzimidazoles for GIN in French Angora goats and demonstrates that resistance to moxidectin is likely developing.

PS02.12 Use of a PCR/Sequencing Technique for Identifying Haemonchus Species and Genotypes From Trichostrongyle Egg DNA Isolated From American Plains Bison (Bison Bison) Fecal Samples

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Haemonchus contortus is a common trichostrongyle parasite of sheep and goats, while *H. placei* is more commonly found in cattle. Both species have been identified in Canadian bison, based upon nemabiome metabarcoding of trichostrongyle eggs. The second internal transcribed spacer (ITS2) region of ribosomal DNA has been used to identify genotypes of adult *Haemonchus* spp. isolated from various ruminants. The purpose of this study was to develop a convenient method for species-level identification/genotype documentation of *Haemonchus* spp. based upon mixed populations of trichostrongyle eggs isolated from bison fecal samples. For this method, six PCR primer pairs were evaluated for their ability to consistently amplify a major portion of the *Haemonchus* spp. ITS2 region so that the amplified product could be sequenced. The goal was to identify two primer pairs collectively spanning the entire ITS2 region, with each pair consisting of a genus-specific primer and an ITS2 universal primer. Samples tested included adult and larval *Haemonchus* spp. and mixed trichostrongyle egg samples from sheep, cattle, and bison; the analyses included amplification consistency, quality values (QV) from Sanger sequencing, and sequence fidelity between individual worms and mixed egg samples. A primer pair set composed of a forward universal primer in the 5.8S subunit gene coupled with a genus-specific reverse primer (at the ITS2 3' end, extending into the 28S gene) provided high QV values over the ITS2 region, except for the 3' end. Two other primer sets provided high QV values in the 3' half of ITS2. Egg DNA results were consistent with individual worm results. *Haemonchus placei* and *H. contortus* were found in cattle samples; only *H. contortus* was found in sheep samples. MEGA7 generated phylogenies from bison

samples and *Haemonchus* sequences from Genbank indicated multiple genotypes of *H. contortus* and *H. placei* are present in South Dakota bison.

PS02.13 Anthelmintic and Anti-inflammatory Activities of Selected Southern African Plants Crude Extracts and Isolated Compounds

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Haemonchus contortus is responsible for over 80% of global parasite infestations of livestock and develop in the animal abomasum, causing inflammation, anemia, and death. This study was aimed to investigate the biological activities of eleven southern African plants used traditionally to treat inflammation. *Typha capensis*, *Ficus elastica*, *Carpobrotus edulis*, *Cotyledon orbiculata*, and *Senna italica* crude extracts showed high antioxidant (IC₅₀ range between 0.22 µg/mL ± 1.18 and 7.11 µg/mL ± 1.71), anti-inflammatory using 15-lipoxygenase inhibition (IC₅₀ range between 3.47 µg/mL ± 0.07 and 18.00 µg/mL ± 4.23), and anthelmintics against nematode parasites *H. contortus* and free-living nematodes *Caenorhabditis elegans* with LC₅₀ between 184.94 µg/mL ± 2.62 and 2669.67 µg/mL ± 4.24. Two new compounds were isolated for the first time from *T. capensis* (Isorhamnetin-3-O-β-D-glucoside, and Isorhamnetin 3-O-rutinoside) showed good anti-inflammatory and anthelmintic activities. *Typha capensis* showed good activity not only against *H. contortus* larvae but by inhibition of inflammatory mediators, increasing consequently the healing process on the abomasum of the animals. *Typha capensis* can be used as potential source of new remedies against inflammation and nematodes infections. There was high correlation between antioxidant and anthelmintic activities, suggesting that antioxidant assay can be used as bioassay-guided fractionation for isolation of compounds with anthelmintic activities.

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PS02.14 Effect of Trichostrongylus Colubriformis Infection and Phosphorus Dietary Levels on Lamb's Nitrogen Metabolism

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The aim of the trial was to compare the nitrogen metabolism of growing lambs fed with different phosphorus dietary levels and infected with *T. colubriformis*. Eighteen Santa Ines castrated male lambs (31.8 ± 4.58 kg of body weight), averaging 6 months of age were randomly allocated in a factorial arrangement 2×2 . The factors were 2 diets containing 50% (Low-P diet) or 100% (Adequate-P diet) of their P requirements based on the National Research Council, and two infection status (infected or not). The infected lambs received a single oral dose of 40,000 L3 of *T. colubriformis*. The animals were individually housed in stalls for a 96-days experimental period. The diets were formulated with a forage source (60%) of Tifton 85 bermudagrass hay (*Cynodon dactylon* cv.), and a concentrate source (40%) composed by cassava meal and glutenose, and the adequate-P diets was also supplemented with dicalcium phosphate. This diet composition provided 14.7% of crude protein, 0.38%, and 0.18% of phosphorus for adequate-P and low-P diets, respectively. Thirty-six days after infection, an apparent digestibility trial was performed, where the feed intake, feces and urine were weighed and sampled for nitrogen analyses. The Kjeldahl laboratorial technique was used to determine the sample nitrogen concentration. The N-absorption was calculated based on N-intake minus N-feces, and N-retention was calculated as N-absorbed minus N-urine. All statistics were performed using the R software, and the fixed effects and interactions were compared by Tukey's test ($P < 0.05$). The results indicated that the infection and the P dietary did not affect the N-absorption. However, the N-retention was greater in non-infected (46% of N-intake) compared to infected (29% of N-intake; $P < 0.01$) animals. In conclusion, the

infection could negatively impact the nitrogen metabolism, increasing the nitrogen losses via urine.

PS02.15 Epidemiological and Molecular Updates on Cystic Echinococcosis in Wild Boars from Italy

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Cystic echinococcosis (CE) is one of the most important worldwide parasitic zoonotic disease and it represents an important public health and socio-economic concern. In the last years, the wild boar in Italy is increased and this ungulate could play an important role in the spreading of CE in wildlife.

This survey was carried out to determine prevalence of hydatid cysts and genotypes of *E. granulosus* circulating in the wild boar population from Campania Region, Southern Italy. The carcasses of animals, obtained from different boars hunting areas (BHAs), were examined during two hunting seasons (2016-2017) by 51 veterinary involved in the project Piano Emergenza Cinghiali Campania. Wild boar origin, gender and age were collected per each animal. When cysts were found, their number, morphology and fertility were determined by visual and microscopic examination. Cysts were classified as fertile, sterile, caseous and calcified. Protoscoleces and germinal layers were collected from individual cyst and DNA was extracted. A specific molecular diagnosis was obtained

by sequencing PCR-amplified mitochondrial genes encoding for the NADH dehydrogenase subunit 4 (ND4), ATPase subunit 6 (ATP6), NADH dehydrogenase subunit 2 (ND2) and partial cytochrome c oxidase subunit 1 (COI). Out of a total of 2,108 wild boars examined for CE, 93 (4.4%) were found positive. The total number of cysts collected was 123, of which 118 (95.9%) in the liver, 4 (3.3%) in the lungs and 1 (0.8%) in the spleen. Cysts were 70 (56.9%) fertile and 53 (43.1%) sterile/acephalous. The presence of fertile cysts was detected in 19.4% of positive boars. Out of a total of 28 boars, molecular diagnosis showed 19 (67.9%) of these infected with the pig strain (G7). These results document the prevalence of *E. granulosus* infection and reveal, for the first time, the presence of the pig strain (G7) in wild boars from Italy.

PS02.16 Prevalence and Economic Losses of Cystic Echinococcosis in The Gambia

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ABSTRACT

Cystic Echinococcosis (CE) is globally distributed, is super endemic in East and North Africa and causes great financial losses to the livestock industry in these regions. In West Africa, the disease is suspected to be prevalent but few epidemiological studies have been conducted so far. The aim of this study was to determine the presence of the disease and estimate the prevalence of CE in slaughtered livestock in The Gambia. In five abattoirs located across The Gambia, from July to December 2017, a total number of 1968 slaughtered cattle, sheep and goats were inspected for the presence of CE using ante-mortem and post-mortem examinations. The distribution of the animals examined was 56.1% goats, 28.9% cattle and 15.0% sheep. The average age of the animals slaughtered was 4-6 years old. Of the total number of examined animals, no hydatid cysts were identified 0/1968 (0.0%) and 16/1400 (1.1%) calcified cysts were collected from the total number of sheep and goats. From the result

of this study it can be concluded that CE is not an issue in the Gambian abattoirs and no financial losses are incurred as a result of organ condemnation.

For a better understanding of the CE status in The Gambia, further investigations should be carried out using advanced molecular diagnostic techniques on samples from both the definitive and intermediate hosts to rule out the existence of the parasite in this country.

PS02.17 Molecular Identification and Function Analysis of SUMO and UBC9 Genes of *Taenia Pisiformis*

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SUMOylation is an essential post-translational modification for protein in eukaryotes, which participates in protein-protein interactions, subcellular localization, protein stabilization and diverse cellular process regulation. The SUMOylation pathway has been studied in several model species. However, SUMOylation system and processes in tapeworms remain unknown. In the present work, the full-length cDNA of two core components genes, small ubiquitin-like modifier (TpSUMO) and E2-conjugating enzyme (TpUBC9), in the SUMOylation pathway of *Taenia pisiformis* were obtained through rapid amplification of cDNA ends using the polymerase chain reaction (RACE-PCR) strategy. Bioinformatic analysis showed that the TpSUMO gene contained a 309 bp ORF, encoding 103 amino acids with a predicted weight of 11.4 kDa and the TpUBC9 gene with 516 bp ORF encoding a 172 amino acid protein with a putative mass of 19.6 kDa. The His-TpSUMO (~18 kDa) and His-TpUBC9 (~25 kDa) proteins were expressed in *Escherichia coli* Transetta (DE3), respectively. In addition, using leucine aminopeptidase of *Taenia pisiformis* (TpLAP) as a SUMOylation substrate we further demonstrated the physical interaction of TpLAP-TpUBC9 via one-to-one yeast two-hybrid and co-

immunoprecipitation analyses. The truncated N-terminus (aa 1-185) and C-terminus (aa 186-522) of TpLAP interacted with TpUBC9, respectively, while TpLAP-TpSUMO or TpLAP-TpSUMO Δ GG showed no direct interactions, suggesting that the SUMOylation of TpLAP was mediated by TpUBC9. The findings provide solid evidence that the SUMO modification pathway plays an important part in regulating the protein functions involved in adult worm growth and development.

PS02.18 Field Flea Collections Reveal New Host and Distribution Records for *Stenoponia Americana* (Baker, 1899) and *Amphipsylla Washingtona* (Hubbard, 1954) (Siphonaptera: Ctenophthalmidae, Leptopsyllidae) in the United States

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In the United States, fleas (Siphonaptera) were extensively collected and described in the 20th century, but currently field collections are rare and primarily aim to further understand their role in plague disease ecology. In this study, fleas were collected from small rodents at several locations in the United States during plague disease ecology studies in the summers of 2013 to 2015. Twenty specimens of *Stenoponia americana* (Baker, 1899) (Siphonaptera: Ctenophthalmidae) collected from sagebrush voles (*Lemmyscus curtatus*) and deermice (*Peromyscus maniculatus*) on the Awapa plateau (Wayne County, Utah) are an expansion of the geographical range and host distribution of this large flea species. *Amphipsylla washingtona* also collected from *Lemmyscus curtatus*, Wayne County, Utah is a new record for Wayne County and expands the known distribution for this rare species an additional 600 kilometers from additional records published herein from the BYU flea collection from the National Reactor Testing Station near Idaho Falls, Butte County, Idaho. These observations illustrate that flea distributions are still changing and that trusted handbooks and regional identification keys need temporary updating.

PS02.19 Do *Dirofilaria Immitis* Infections in *Aedes Aegypti* Affect Wing Beat Frequencies?

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Dirofilaria immitis, the causative agent of canine heartworm disease, is transmitted by numerous genera and species of mosquitoes. Principally among them are *Aedes*, *Anopheles*, *Culex*, and *Ochlerotatus* spp. Within the mosquito, ingested microfilariae develop through three developmental stages (L1, L2, L3). A recent study demonstrated that mobile phones are capable of capturing acoustic data from mosquito wingbeats. Since each mosquito species has a different wingbeat frequency by which they attract mates, a brief wingbeat recording (<1/10th of a second) can be analyzed to determine mosquito species and thus its capability to transmit disease. Here, we examine wingbeat signatures and flight duration patterns of *Aedes aegypti* to determine if wingbeat mobile phone recordings can be used to distinguish infected and non-infected mosquitoes. Individual female mosquitoes were placed into a chamber at various time points after feeding on normal blood or blood containing microfilariae and recorded with a mobile phone for 60 seconds. Recordings were processed using an in-house Python script to determine wingbeat frequency and flight duration. One hundred sixty recordings were obtained. The presence and number of heartworm larvae were determined by mosquito dissection. Our findings indicate that L3 (infective stage) infected mosquitoes have significantly lower (mean=429 Hz) wingbeat frequencies than age-matched negative mosquitoes (mean= 577 Hz; $p < 0.0001$). Flight duration based on wingbeat recordings was substantially lower (17.6% of the time) in L3 infected mosquitoes than

mosquitoes fed on negative blood (35.9% of the time). We present data suggesting that wingbeat frequencies may be used to identify mosquitoes infected with pathogens of public health or veterinary concern.

PS02.20 Correlation of Salivary Antibody to Carbohydrate Larval Antigen With Gastrointestinal Nematode Parasitism in Sheep Under Ontario Grazing Conditions

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Gastrointestinal nematodes (GINs) are an important cause of morbidity, mortality and financial loss on Ontario sheep farms. In light of the rising prevalence of anthelmintic resistance, selecting sheep with a superior immune response to GINs is an attractive complementary control strategy. In New Zealand, such sheep are identified by measuring salivary antibody to a carbohydrate larval antigen (CarLA[®], AgResearch Inc.). However, GIN epidemiology under cold continental grazing conditions, such as in Ontario, is different from New Zealand. The purpose of this study was to determine whether salivary CarLA[®] antibody correlates with GIN burden under Ontario grazing conditions. Replacement ewe lambs (n=107) on an Ontario commercial sheep farm were followed for two years, including their first lambing and lactation. GIN fecal egg counts (FECs) were monitored every 6-8 weeks from May-November in 2016 and 2017. Salivary CarLA[®] antibody was measured at the beginning (May), middle (August) and end (November) of each grazing season, and at mid-gestation in late winter (March). Mean CarLA[®] levels increased in 2016, declined over winter, and rapidly increased during the 2017 grazing season. Spearman correlation coefficients between CarLA[®] levels were consistently positive, of weak to moderate strength ($\rho = 0.19-0.58$), and generally significant ($p = 7.3 \times 10^{-11}$ to 4.7×10^{-2}). In a multivariate mixed model, increased salivary CarLA[®] was significantly associated ($p < 1.0 \times 10^{-4}$) with decreased GIN FEC throughout both grazing seasons, regardless of previous

anthelmintic treatment. These results indicate that CarLA[®] is unaffected by anthelmintic treatment, and that levels measured during a lamb's first grazing season are predictive of subsequent levels. Therefore, under Ontario grazing conditions, selection of replacement ewes with elevated CarLA[®] may reduce pasture contamination following lambing and during their second grazing season.

PS02.21 Developmental Activation of Infective Strongyloides Stercoralis Larvae After Percutaneous Migration Through an In Vitro Model

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Strongyloides stercoralis is a global parasitic nematode of humans and canids. While infections are usually self-limiting, hyperinfection syndrome can occur in immunocompromised individuals, in which autoinfective larvae disseminate throughout the body and helminth burden reaches fatal levels. Currently, albendazole and ivermectin are the only drugs deemed effective against the disease, but often result in incomplete eradication due to the nematode's unique autoinfective lifecycle. Consequently, understanding larval developmental pathways within the lifecycle is necessary for progress towards identifying new therapeutic agents. Parallels between *S. stercoralis* infective third-stage larvae (iL3) arrest/reactivation and the dauer entry/recovery mechanisms in *Caenorhabditis elegans* have been identified, including the importance of steroid hormones known as dafachronic acids (DA) in binding to the nuclear hormone receptor DAF-12 to initiate dauer exit. Although the mechanism for DA production is known, which involves a cytochrome P450 enzyme (CYP), the exact cue to begin synthesis of DA is not. To examine this, we utilized in vitro percutaneous larval migration chambers with mouse skin and a culture medium that does not permit spontaneous resumption of feeding upon a

shift to host-like temperature. In cohorts of iL3 that penetrated mouse skin, 51.7% resumed feeding, as reflected in fluorescein ingestion, while only 9.6% of non-penetrating controls did so. This iL3 activation via skin migration was further investigated using LC/MS to determine whether there was a correlation with DA production, however preliminary results did not indicate a peak in synthesis. Considering the possibility that DA levels required for resumption of feeding are below the level of LC/MS detection, we are now assessing the effect of ketoconazole, a CYP inhibitor, to ascertain the requirement of CYP function for DA biosynthesis in penetration-mediated developmental activation of *S. stercoralis* iL3. Moreover, genetic analysis is being performed on skin-penetrated larvae to identify transcripts associated with the dauer recovery pathway.

PS02.22 Viability of *Haemonchus Placei* Parasitism in Experimentally Infected Young Goats

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The present study aimed to evaluate the viability of *Haemonchus placei* parasitism in experimentally infected goats, since there are no such studies regarding this ruminant species. For that, 14 newborn male Saanen kids were placed in one of the four experimental groups: GI - infected with 5000 *H. placei* L3 (n = 4); GII - infected with 5000 *H. placei* L3 (n = 4); GIII - infected with 2500 *H. contortus* L3 + 2500 *H. placei* L3 (n = 4), and GIV - control, inoculated with distilled water (n = 2). Each kid received, orally, the infective dose in a single inoculum. Based on daily fecal egg counts (FEC), the average pre-patent period was determined as 24 days

for *H. contortus*, and 31 days for *H. placei*. 42 days after the artificial infections, the 14 kids were slaughtered, and the *Haemonchus* sp. parasites were harvested, in totum, from the abomasum. The experimental groups GI, GII, and GIII had, respectively, an average of 25.5, 619.5, and 724.75 (120 *H. placei*, and 604.75 *H. contortus*) adult specimens, and no immature forms. Under the conditions of this study, the viability of goat infection by *H. placei* was confirmed, although, with low susceptibility. Nevertheless, the parasitism of this helminth species was more intense when associated with *H. contortus*. This fact indicates that in common grazing between cattle and young goats, when the latter end up ingesting both *Haemonchus* species, especially in a mixed infection, *H. placei* may also parasitize them.

PS02.23 Ovicidal Effect of Citronellal and Citronellol in *Haemonchus Contortus* In Vitro Egg Hatch Test

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Gastrointestinal nematodes are one of the main causes of decrease in the productivity of small ruminants and are commonly treated with anthelmintics. However, the indiscriminate use of these drugs led to the reduction of efficacy through the selection of resistant parasites, so it is essential to preserve the efficiency of the current anthelmintics, exploring new control alternatives such as the use of phytochemical compounds as essential oils. The objective of the present study was to evaluate the activity of citronellal and citronellol on *Haemonchus contortus* resistant strain through the egg hatchability test and to determine lethal concentrations CL50 and CL90 using increasing doses (0.003 mg / mL, 0.007 mg / mL, 0.010 mg / mL, 0.030 mg / mL, 0.060 mg / mL, 0.120 mg / mL, 0.250 mg

/ mL and 0.500 mg / mL). The eggs were placed in 48-well plates and incubated with the described concentrations for 24 hours at 27°C. The hatchability was evaluated under the inverted microscope. The calculation of lethal concentrations CL50 and CL90 were performed through the SAS Probit program, with the independent variables (dose) transformed by natural logarithm (log dose). Citronellol presented the best anthelmintic efficacy when compared to citronellal as LC50 was 0.02 and Citronellol 0.63 mg / mL, and CL90 was 0.63 and 3.26 mg / mL, respectively. It was concluded that the use of essential oils compounds had a positive influence on *H. contortus* eggs, especially citronellol. The low dose of Citronellol to inhibit the hatch of eggs reinforces the potential anthelmintic activity present in essential oil compounds and deserves further scientific investigations. - FAPESP/2018-02423-0

PS02.24 Efficacy Variation of *Mentha Piperita* Essential Oil on *Haemonchus Contortus* Isolates With Differing Benzimidazole Resistance Backgrounds

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The spread of anthelmintic resistance made treatment options against gastrointestinal parasites increasingly scarce so the search for natural compounds as antiparasitic candidates is now an important field. However, because concentrations of active compounds may vary according to their origin and post-harvest processing, there are many obstacles for the establishment of standards hindering reliable results. To date it is not known if a given compound may vary on efficacy between parasite populations. In this work we evaluate the efficacy of *Mentha piperita* essential oil against eggs of two *Haemonchus contortus* isolates with and without history of resistance to benzimidazole. Tests were carried out using the standard egg hatch inhibition test with a 2% Tween 80 as diluent. Nematode

eggs used in the tests were recovered from adult donor sheep. Egg hatch tests (EHT) were performed with serial concentrations from 1.0 mg/mL to 0.004 mg/mL to obtain a dose-response curve and reach the 99% effective concentration. EHT results for the resistant isolate showed 99.5% efficacy at concentrations above 0.5 mg/mL. However, the EHT results for the sensitive isolate were much lower at 32.3% at 0.5 mg/mL and 41.8% efficacy at the maximum concentration tested (1.0 mg/mL). Our results suggest that the components in *Mentha piperita* essential oil probably have a different mode of action in gastrointestinal nematodes as the resistance background to benzimidazole was not associated with EHT results for the studied isolates. Thus, the importance of testing natural compounds on more than one nematode population is evident in order to provide a better overview of the actual efficacy of said compounds.

PS02.25 Encysted Cyathostomin Larval Counts: Mucosal Digestion Revisited

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Cyathostomins are pervasive equine parasites and may cause larval cyathostominosis. Upon ingestion of the third larval stage, the larvae encyst in the mucosal membranes of the cecum, ventral colon, and dorsal colon. Once encysted, they can arrest development and accumulate in high numbers. No published study has investigated spatial variation of encysted larvae within each intestinal compartment and the current mucosal digestion protocol lacks a description of a standardized area from which to take the tissue sample. Therefore, this study sought to evaluate spatial variation in encysted larval counts in defined sections of each large intestinal organ. Following humane euthanasia, ceca, ventral, and dorsal colons were harvested from 8 foals raised in an anthelmintic naïve parasitology research herd. Each organ was weighed and separated into 3 equal sections by length: the orad, intermediate, and aborad portions. From

each of those sections, two-5% weight tissue samples were selected and underwent mucosal digestion to quantify the number of early third stage larvae (EL3) and late third stage larvae/fourth stage larvae (LL3/L4). A mixed model statistical analysis was performed to evaluate for differences of larval counts between the different organs, sections, and the interaction terms between the organs and sections. There were significant differences between organs ($P = <0.0001$), with the cecum having higher counts than the ventral and dorsal colons. However, there were no significant differences between the three defined sections ($P = 0.1076$). Although not significant, the following sections had the highest average counts of encysted larvae: intermediate cecum, orad ventral colon, and aborad dorsal colon. These trends may become significant in larger data sets and should therefore be investigated further in future studies.

PS02.26 Veterinary-Sanitary Evaluation of Mutton with Mixed Invasions

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The aim of the research is to examine veterinary-sanitary quality of mutton with mixed invasions. The mutton carcasses are divided into 3 groups depending on the intensity of co-members consisted in the Helminthic cenosis. The first group included the material from non-infested animals (control group); the second group (first test group) included samples from two-members (dicroceliasis + moniezirosis); the third group (second test group) included materials from three-members (dicroceliasis + moniezirosis + strongylatosis).

The probes of the meat were taken in 24 hours and on the 15th day of storage (0...40°C) after the slaughter of sheep. The meat of the animals belonging to the II and III groups, unlike that of the control group, showed the humidity increase by 5.1 and 7.9 % respectively; proteins decreased by 3.6 and 6.6%; fat - by 27.2 and 30.1%; caloric value - by 18.3 and 21.1%; In 24 hours after the slaughter of helminthic animals, the microbial

population per field of vision on the average was 2.9 ± 0.43 in the control group; 8.5 ± 2.3 in the first test group; 9.4 ± 0.71 in the second test group. On the 15th day of storage, the bacterial count in 1 g of meat in the first test group was $(43 \pm 13.4) \cdot 10^3$; staphylococcus - $(57 \pm 26.2) \cdot 10^3$; streptococcus - $(9.4 \pm 2.7) \cdot 10^3$; in the second test group respectively - $(139 \pm 13.4) \cdot 10^3$, $(75.3 \pm 26.2) \cdot 10^3$, $(11.0 \pm 2.7) \cdot 10^3$; and the control group: $(2.6 \pm 0.4) \cdot 10^3$, $(1.2 \pm 0.8) \cdot 10^3$ and $(1.4 \pm 0.6) \cdot 10^3$.

The mixed invasions affect the sanitary quality and food value of the products received, and makes meat less stable upon storage. The meat is fresh during 24 hours after the slaughter; on the 15th day its bacterial content increases and shows signs of doubtful freshness.

PS02.27 Parasitic Structures Carried by Sarcophagidae Fly in Two Different Biotopes in the Region of Teodoro Sampaio, Sao Paulo, Brazil

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The synanthropic flies of the Sarcophagidae (Diptera, Insecta) family are potential mechanical vectors of etiological agents such as virus, bacteria, protozoan cysts and helminth eggs. Sarcophagidae have been implicated in facultative wound myiasis. Poor sanitation is probably the most important risk factor for human and animal myiasis. The present study aimed to evaluate the presence of parasites in Sarcophagidae flies captured in two different areas: a rural settlement community and in a countryside city of the Brazilian Southeast. For trapping of the flies, five traps were used per bait with attract of cow liver and fish. Capture of flies was carried out at the beginning, middle and in the end of each season (March/2012 to February/2013), by using of traps. Captured flies were washed for evaluating the

exoskeletal, and desiccated for the removal of the intestines. The fluid obtained from the external lavage and intestinal content were separately subjected to the centrifugation-flotation and sedimentation methods for observation of parasitic structures (helminth eggs and protozoan oocysts/cysts). A total of 88 insects were captured, 53 flies in the city and 35 in the rural area. The parasite structures observed were: *Giardia* spp. (2 cysts, 2 in the city), *Entamoeba* spp. (10 cysts, 6 in the city and 4 in the rural area) and non-sporulated oocysts of protozoa (3 oocysts, 1 in the city and 2 in the rural area). There was a significant difference between the number of cysts recovered from the external portion in relation to the intestine ($p < 0.05$ test t). The results show that Sarcophagidae flies can carry parasites potentially pathogenic to humans and animals, both in the rural and countryside city areas.

PS02.28 Amblyomma Sculptum (Acari, Ixodidae) as a Probable Vector of Dermatobia Hominis (Diptera, Cuterebridae) on a Human Cutaneous Infestation in Brazil

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Dermatobia hominis is a conspicuous Neotropical parasite fly and its larva parasitizes different species of vertebrate hosts including man. On April 15th, 2018 the author (AAC) visited a rural property in Itirapina, São Paulo state, Brazil. The site had few heads of cattle heavily parasitized by *D. hominis* and pasture areas with horses keeping a population of *Amblyomma sculptum* in the environment. On the visit day AAC was wearing boots, trousers and long sleeved shirts aiming to be protected from mosquito bites and walked around but with trousers openings not sealed allowing tick's infestation. On the same day an *Amblyomma sculptum* adult tick was found on AAC's arm. Two days later AAC, which is allergic to tick bites, started to scratch a lesion on the external face of his left thigh. On April 28th episodes of pain like stings on the lesion started to occur. On May 3rd an ulcer less than 1 mm wide was identified and movement of the caudal end of a larva was noticed. On

May 7th, 22 days after infestation, 8 hours after blockage of the ulcer with an adhesive tape, a live larva was removed mechanically. The specimen was identified as an L2 stage of *Dermatobia hominis*. Ticks do not fly and have restricted dispersal around the host after they leave it. These facts make ticks poor vectors of *D. hominis* eggs. Since *D. hominis* is reported to perform oviposition on inanimate objects despite of flying diptera, and ticks are hematophagous attaching to the skin of vertebrate hosts, the rare use of ixodidae ticks as successful egg vectors is possible, as previous described by Dunn (1918). An adequate eco-epidemiological situation with high abundance of *A. sculptum* ticks, *D. hominis* and different co-existing vertebrate hosts might have contributed to this exceptional report.

PS02.29 Diversity of Culicoides Species in New England, NSW, Australia: 28 Years of Data

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Culicoides are biting midges approximately 3mm in length with females feeding on blood of a wide range of mammalian and avian hosts. They play an important role as vectors of more than 50 viruses and can cause host annoyance themselves when present in large numbers. In Australia, the distribution of *Culicoides* is monitored by the National Arbovirus Monitoring Program (NAMP). New England is in the north of the state of New South Wales and is an important area of cattle and sheep production. Under the NAMP surveillance program, *Culicoides* were trapped from thirteen different locations in this region during the spring to autumn months over a period of 28 years (1990 – 2018) using light traps set at approximately monthly intervals for a period of 2 nights during the new moon near cattle camps. Samples from 4482 collections (672 in Spring, 2118 in Summer and 1692 in Autumn) were enumerated and speciated. Of these trapping events 85.2%

resulted in successful *Culicoides* catches with a total catch of 156,101, a median catch of 4 and mean log10 catch of 0.87. A total of 25 different species were identified, of which 15 have been reported to feed on humans, 9 on sheep, 8 on Australian marsupials, 7 on horses, 6 on cattle and smaller numbers on birds, dogs, flying foxes and rabbits. For 7 species, no information on the mammalian/bird host is available. The most trapped 6 species in descending order were *C. marksii*, *C. austropalpalis*, *C. victoriae*, *C. dycei*, *C. bundyensis* and *C. brevitarsis*. The highest mean catch counts were from the plains (mean altitude 198 m) followed by the slopes (512 m) and the tablelands (871 m). Counts were lowest in spring, highest in summer and intermediate in autumn. A diverse range of *Culicoides* species is present in the region.

PS02.30 New World Screwworm (*Cochliomyia Hominivorax*) Population Structure Analysis Using Genotyping by Sequencing

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Larvae of the New World screwworm (NWS) fly (*Cochliomyia hominivorax*) feed on living tissue of warm-blooded hosts, including livestock and humans, causing myiasis. While the U.S. was officially declared NWS-free in 1966 there have been sporadic introductions of NWS into the U.S. including one of the most recent outbreaks that occurred in the Florida Keys in September of 2016. To further explore the origin of NWS flies collected during the 2016 outbreak, we applied Genotyping by Sequencing (GBS) to analyze samples collected from Florida, the Caribbean, Central, and South America to identify diagnostic single nucleotide polymorphisms (SNPs) to distinguish population structure. This method yielded genotypes for thousands of loci across the entirety of the genome from

the individual samples that were collected. These NWS markers lay the foundation for a SNP database for this species which can be used to assess the presence of diagnostic SNPs that discriminate by population and can facilitate the identification of the geographic origin of NWS flies associated with future potential outbreaks.

PS02.31 First Report of *Philornis* sp. in a Protected Area in Uruguay

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The genus *Philornis* (Diptera: Muscidae) is composed of about 50 species, which are distributed mainly in the neotropical zone. The adults of *Philornis* are characterized by laying their eggs in bird nests. After that, the larvae penetrates the skin, producing myiasis in the chicks. Due to their subcutaneous location and type of feeding they generate delays in the development of chicks and, in some cases, death.

As part of a study of dipterous of Veterinary importance, carried out in the Lecocq Park Zoo (Montevideo), dipterous samples were taken during 2018.

The Zoo is located inside the Protected Area: St. Lucia Wetlands (86.517 hectares), where conservation plans are carried out for endangered species. Here, over 150 species of vertebrates and invertebrates develop in wildlife. Every month specimens of *Philornis* were collected.

Many of the birds present in this Protected Area carry out two migratory flows, some are winter migrations and others are visitors of the summer months. Of this last group, several reproduce in the Area. In addition, species that come from migratory movements from tropical climates have recently been found in Uruguay, perhaps due to the effects of climate change.

The protection of the species requires international work and commitment. According to the Red List of birds in Uruguay, of the 458 taxa evaluated, representing 453 species, 46 were identified as Threatened, 31 Vulnerable, 12 Endangered and two Critically Endangered.

This communication represents the first report of *Philornis* sp. in a Protected Area in Uruguay, which is a very important alert to the conservation of bird species because they could be affected by the presence of this parasite.

PS02.32 Seasonal Abundance of *Stomoxys Calcitrans* and *Musca Domestica* in the South Region of Uruguay (2018–2019). Preliminary Data

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Musca domestica and *Stomoxys calcitrans* are the main pests in confined animals and domestic farms with a worldwide distribution. In Uruguay, the population dynamics of these flies are unknown, and this information is fundamental to their control.

The study was conducted in a dairy farm in the department of Colonia and in a wildlife reserve, Parque Lecocq, located in the department of Montevideo, from February 2018 to January 2019. Dairy farm has 250 milking cows and the Zoo approximately 400 animals in 10 paddocks. Three models of traps were used to collect these flies: the Nzy, emergency traps and a homemade trap made with yellow plastic bottle with natural bait. The traps were placed in strategic sites, according to the behavior of each gender. In the Lecocq Park Zoo, 14,998 insects were captured. The abundance of *M. domestica* and *S. calcitrans* was very low. Only 70 specimens of *M. domestica* and 322 of *S. calcitrans* were

captured. In contrast, the abundance of both species in the dairy farm was higher. A total of 52,017 insects were captured and 16,444 were identified as *M. domestica* and 3,206 as *S. calcitrans*.

In both locals, the fly fluctuation was similar. An increase in the *M. domestica* population was observed in May 2018. In the case of the stable fly, an increase in the population was observed during spring (November–December), and a little peak in winter (June) was registered. The winter in this year was extremely warm.

In the Zoo, other little peak of *S. calcitrans* population was observed in autumn, during March–April 2018. Apparently, the behavior of *S. calcitrans* in this zone was bimodal. These preliminary results could be focusing the fly control from the beginning of the spring to avoid the peak of autumn.

PS02.33 Orientation of Stable Fly Larvae

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Stable fly larvae can detect odorants within their developmental substrates and orient in response to them. Ammonium is the strongest attractant eliciting a response from 75% of larvae. Most of the esters examined acted as attractants larvae as well. Alcohols, carboxylic acids, ketones, aldehydes, aromatics and sulfides elicit little response. Stable fly larvae appear to use chemical cues different from those used by gravid females for identifying preferred developmental substrates.

PS02.34 Distribution and Dynamics of Canine Generalized Leishmaniosis in France: Results of the 4th National Questionnaire Based Survey with Veterinary Clinics**Patrick Bourdeau**¹, Laetitia Imparato¹, Fanny Alexandre¹, Mailys Hilary¹¹*Veterinary School of Nantes-oniris - France, Nantes, France*

Canine Generalized Leishmaniosis (L. infantum) is widely distributed in Mediterranean countries, including traditionally the South East part of France. Three previous National surveys (Bourdeau et al 1986-2004-2011) have shown a progressive extension of the endemic area. A fourth questionnaire-based survey with veterinary clinics was conducted in summer 2017 covering the period 2012-2017. For each of the 620 participating veterinary clinics the number of annual clinical cases, autochthonous (infection considered acquired within the range of the territory of activity of the clinic) or not, were collected. From the 90 administrative areas (= French départements), clinics with more than 10 annual cases were found in 21 (20 in 2011). In 33 areas, the average number of annual cases per clinic was >1 (26 in 2011). Autochthonous cases were suspected in 13 areas outside of the endemic zone, with kennel or familial foci in 6 and including 4 where the sand fly vector has never been captured and is not supposed to be present. The endemic area covers 29 départements (26 in 2011) with kennel or familial foci detected in 22 of them. As observed for the period 2004-2011 and predicted, the northern progression of the disease continued on the 2012-2017 interval. The estimated progression of surface of the endemic territory was 9%. In France the “wave effect” described in 2011 is maintained in the periphery of endemic territories, whereas a stabilisation or even a decrease of prevalence is suggested in the traditional center of the endemic zone. However, in the endemic zone, diagnosis was made on dogs originating from new places in 22 areas.

PS02.35 Molecular Detection of Eimeria Species Among Chickens in KwaZulu-Natal Province, South Africa**Mr. Abiodun Fatoba**¹, Dr. Oliver Zishiri¹, Prof. Damer Blake³, Dr. Sunday Peters², Dr. Jeffrey Lebepe¹, Prof. Samson Mukaratirwa¹, Dr. Matthew Adeleke¹¹*University Of Kwazulu-natal, Durban, South Africa*, ²*Berry College Mount Berry, Mount Berry, United State*, ³*The Royal Veterinary College, Hawkshead lane, United Kingdom*

Coccidiosis is a renowned intestinal disease of livestock which impair growth and productivity. The impact of this disease has caused drastic production loss in poultry industry. Effective prevention and control of this disease hinges on proper identification. Despite the prevalent of this parasite species in different regions, there is little or no information of these species in South Africa. The objective of this study was to examine the occurrence of Eimeria parasite using molecular markers. To our knowledge, this is first molecular survey of Eimeria parasite in KwaZulu-Natal province. In this study, 300 fecal samples were collected from 12 different farms in KwaZulu-Natal province among which 102 (34%) were positive for Eimeria infection. Four different species of Eimeria vis-a-vis: E. tenella, E. mitis, E. acervulina and E. maxima were detected using species specific primers targeting ITS-1 regions with E. tenella being the most prevalent. Multiple infections with two or more species of Eimeria parasite were also detected in some of the samples with E. tenella and E. maxima being the most common combination. Single infection prevalent with E. tenella was also detected in some of the samples examined. The report of this study shows that Coccidiosis is widespread in chickens and a concerted strategy to prevent and control this disease is imperative in KwaZulu-Natal province.

PS02.36 Molecular Characterization of Trypanosoma Evansi in Seventeen Isolates from Five Natural Infected Hosts and In Vitro Cultivation in Thailand

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Trypanosoma evansi the causative agent for trypanosomiasis in various vertebrate animals worldwide. There are two type of T. evansi including type A and B. T. evansi type A was predominantly niche and regularly reported in West and East Africa, South America and China. T. evansi type B is restricted in Kenyan camel. T. evansi has endemic in various kind of hosts with high mortality in horses, interfered animal health and reduced livestock production in Thailand. However, there is no report that indicate the T. evansi type in Thailand. The aim of this study was to characterize T. evansi type using molecular technique. Seventeen DNA isolates of T. evansi infected blood collected from 1 dog, 1 buffalo, 2 beef cows, 4 dairy cows, 7 horses and 2 from in vitro cultivation were extract and used for characterization. Specific primers including Rotat 1.2 (type A specific), EVA B (type B specific), ITS 2, VSG, Mini (minicircle kDNA) and NC (ITS 5.8-2 region) were used. Phylogenetic analysis was conducted. Results showed that there were 14/17 isolates of T. evansi type A and 0/17 of T. evansi type B. NC primer targeting ITS 5.8-2 region was very sensitive and be able to detect T. evansi DNA in all 17 isolates. VSG and ITS 2 primers were

not be able to detect T. evansi DNA from two in vitro cultivation isolates. There were 3 among 17 isolates that lacked the Rotat 1.2 gene. Here we are the first T. evansi typing report in Thailand.

PS02.37 3D-Holotomographic Analysis of Respiration of Eimeria Bovis Oocyst Sporogony

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Eimeria species are apicomplexan protozoan parasites with high economic impact in livestock. Oocysts of most enteropathogenic Eimeria spp. are shed by defecation into environment as un-sporulated parasitic stages. Exogenous oocyst development, namely from un-sporulated into sporulated infective status (i. e. sporogony) is only possible in aerobic metabolic conditions. The sporogony process can exclusively be observed in alive oocytes, as commonly used standard staining procedures do not penetrate highly resistant oocyst wall, thereby hampering permanent oocyst circumplasm labeling. Furthermore, it is difficult to prepare Eimeria oocysts for transmission electron microscope (TEM) by conventional techniques explaining why TEM ultrastructural analyses have focused on endogenous merogony- and gamogony-stages, but never so far on exogenous sporogony. In this study, we document for the first time in vivo respiration of E. bovis oocyst sporogony using live cell imaging microscopy techniques. For achieving sporogony, E. bovis oocysts (strain H) were re-suspended in potassium bichromate K₂Cr₂O₇ solution (2%, w/v), at room temperature (RT) at 25 °C with constant oxygenation. Daily, oocysts were documented using 3-D holotomographic microscopy (3D Cell Explorer®, Nanolive) to explore instantly live sporogony in 3D dimensions without any labeling or chemical marker staining. 3D-E. bovis oocyst images were digital stained based on the cell's physical refractive index using STEVE® software (Nanolive).

PS02.38 Seroprevalence of Toxoplasma Gondii Infections in Dogs and Cats of Korea, 2017–2018

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Cats are the natural reservoir of *Toxoplasma gondii* and excrete the resistant oocyst to environments, and dogs play a role in the mechanical transmission of the protozoa. The objectives of this study were to assess the prevalence and risk factors of *T. gondii* infection in dogs and cats, which have an important role in public health, by serological diagnostic test.

Serum samples were collected from companion and shelter animals in veterinary clinic and in animal shelter. The presence of *T. gondii* antibodies was analyzed using the commercial kit based on enzyme-linked immunosorbent assay.

A total 2,169 blood samples of dogs and cats from veterinary clinic and animal shelter. *T. gondii* antibodies were detected in 45(2.1%) of the 1,215 positives in cats. The positive rates of shelters(17.0%) was higher than companions(3.3%) in cats. On the other hands, *T. gondii* antibodies were examined as positive in 17(1.1%) of the 1,613 dog blood samples and shelter and companion dogs had 0.7% and 0.1%, respectively. The seroprevalence of *T. gondii* according to district is shown as follows; Gyeonggi(1.2%), Jeolla(0.2%), Jeju(0.2%), Gyeongsang(0.1%) and Chungnam(0.1%).

Serological surveillance of toxoplasmosis in dogs and cats was investigated in this study. Differences are shown in seroprevalence between dogs and cats, shelters and companions by region. Further epidemiological study is required to determine sources of *T. gondii* infection in dogs because information on the infectious status of *T. gondii* in dogs is important for assessing the risk to public health.

PS02.39 Validation of Single-Tube Nested Real-Time PCR and Genetic Sequencing for Detection and Characterization of Cryptosporidium Spp. in Birds

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Molecular detection and characterization of *Cryptosporidium* spp. in fecal samples are usually performed by two steps conventional PCR assays followed by agar gel electrophoresis and genetic sequencing. The objective of this study is to validate a protocol of single-tube nested real-time PCR assay followed by melting curve analysis and genetic sequencing to detect and characterize the species and genotypes of *Cryptosporidium* in birds. The assay was performed to amplify a fragment of the 18S rRNA gene using DNA samples extracted from 249 fecal samples from birds (47 and 202 tested positive and negative, respectively, by nested PCR targeting the 18S rRNA gene), two sets of primers with different annealing temperatures, and the SsoFast EvaGreen Supermix (Bio-Rad) in the CFX96 real-time PCR system (Bio-Rad). Conditions of the assay were: 98 °C for 2 min was first followed by 20 cycles of denaturation at 98 °C for 5 s and annealing/extension at 70 °C for 30s. This was followed by 35 cycles of denaturation at 98°C for 5 s, annealing at 63 °C for 5 s, and extension at 72° C for 30 s. Fluorescence signal acquisition occurred at the second cycling step. Melting curve analysis was performed from 70 °C to 95 °C. Amplified fragments were purified using ExoSAP-IT (Thermo Fisher Scientific) and submitted to bidirectional sequencing for confirmation of *Cryptosporidium* species/genotype. Melting temperatures of the bird *Cryptosporidium* species and genotypes were 79.6°C, 79.6°C, 79.4°C, 80°C, and 80.2°C for *Cryptosporidium baileyi*, *Cryptosporidium meleagridis*, avian genotype I, *Cryptosporidium galli* and avian genotype III, respectively. The frequency of samples positive for *Cryptosporidium* spp. by nested real-time PCR was 80/249 (32.1%). Our results show that single-tube nested real-time PCR could be used as an alternative to conventional nested PCR with the advantages of lower turnaround time and lower risk of carry-over contamination.

PS02.40 Efficacy of a Single Oral Administration of Afoxolaner Alone or in Combination with Milbemycin Oxime Against Ixodes Hexagonus in Dogs

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The efficacy of afoxolaner (NexGard® and NexGard Spectra®, Boehringer-Ingelheim), administered once orally at the minimum recommended dose, was assessed in dogs experimentally infested with Ixodes hexagonus ticks.

The study was a blinded, negative controlled clinical efficacy study using a randomized block design. Twenty-four purpose bred Beagle dogs, 12 females and 12 males were included. Dogs were randomly allocated either to the negative control group, or to one of the two treated groups. Infestations were performed with 50 adult I. hexagonus ticks on days -2, 7 and 28. On day 0, dogs in groups 2 and 3 were treated with NexGard® (afoxolaner) or NexGard Spectra® (afoxolaner + milbemycin oxime), respectively. Live tick counts were conducted 48 hours after treatment (day 2) and 48 hours after each subsequent infestation (days 9 and 30).

In both treated groups, afoxolaner was 100% effective against existing infestations ($p < 0.0001$). Regarding the re-infestations, overall efficacy of afoxolaner was 100% at day 9 and 98.5% at day 30.

NexGard and NexGard Spectra chewable tablets administered once orally at the minimum recommended dose were highly effective against I. hexagonus infestations for the 4 weeks duration of the study.

PS02.41 Nanocarrier Formulations Against Rhipicephalus (Boophilus) Microplus Larvae

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The aim of this study was to evaluate the larvicidal potential of formulations developed from a nanocarrier system based on cypermethrin (CPM) + chlorpyrifos (CPF) and its association with bioactives isolated from plants. The CPM + CPF based formulations were developed from Nanostructured Lipid Carriers (NLC) and associated with substances isolated from plants commercially acquired (Sigma-Aldrich): cytral (Form. A), menthol (Form. B), and limonene (Form. C). The formulations A, B, C and NLC (alone) were then evaluated against R. microplus larvae from 100 to 0.78 $\mu\text{L.mL}^{-1}$ concentrations by the Larval Packet Test (LPT), in triplicates, negative (water) and positive (Colosso® CPM+CPF at 1.25 $\mu\text{L.mL}^{-1}$) control. After 24h of incubation ($\pm 27^\circ\text{C}$; $> 80\% \text{ RH}$) the count of live and dead larvae was performed. The results were analyzed by ANOVA One-way followed by Tukey's test. Formulations A, B and C caused 100% mortality at 1.56, 3.12 and 6.25 $\mu\text{L.mL}^{-1}$, respectively, with a dose-dependent effect, and significant differences ($p \geq 0.05$). The positive control had 100% efficacy, and the formulations A and B caused 81.31 and 76.27% mortality at 0.78 $\mu\text{L.mL}^{-1}$. Moreover, CLN caused mortality $> 40\%$ at 50 $\mu\text{L.mL}^{-1}$. It was demonstrated that the nanocarrier system evaluated was effective, since the active compounds, even reduced, caused mortality rates similar to those of the commercial reference product. In 100 mL, Colosso® and Formulations (A, B and C) contained, respectively: 15.0g and 0.1875g of CPM; 25.0g and 0.3125g of CPF; 1.0g of citronellal and 0.0125g of isolated from plant (cytral, menthol or limonene) in addition to 5.87*10¹⁵ of CLN in the formulations of present study. Thus, the CLN system can be considered a possible option on the development of new acaricides. New studies are necessary to elucidate the function of these associations, and to validate its efficacy against R. microplus.

PS02.42 Diversity and Geographic Distribution of Brown Dog Ticks in the United States

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Brown dog ticks (*Rhipicephalus sanguineus sensu lato*) are now known to exist as a species complex comprised of several distinct lineages. Although surveys are limited, two lineages have been described in the United States to date: temperate and tropical. To more fully characterize the distribution of *R. sanguineus* s.l. throughout the United States, a 12S rRNA gene fragment was amplified and sequenced from brown dog ticks removed from 153 dogs and 3 cats from different locations across 14 of the 50 states from March 2018 through January 2019; a nested PCR assay targeting an *Ehrlichia* spp. 16S rRNA gene fragment was also used to assess prevalence of infection with *Ehrlichia canis* in ticks. Brown dog ticks were submitted in every month of the year except February. A majority (79.5%; 124/156) of brown dog tick populations evaluated were temperate lineage, while only 32/156 (20.5%) were tropical lineage. As expected, most (26/32; 81.3%) tropical lineage *R. sanguineus* s.l. were from areas with an annual average daily temperature > 20°C (68°F), including Arizona, Florida, Hawaii, Nevada, and southern Texas. However, several (n=6) tropical *R. sanguineus* s.l. infestations were found on dogs from unexpected geographic locations in California, Kentucky, Michigan, Minnesota, Oklahoma, and northern Texas; follow up conversations with veterinarians and owners in these cases suggested some of these infestations resulted from recent travel of dogs from tropical areas. *Ehrlichia canis* infection was not detected in any of the *R. sanguineus* s.l. ticks in this study. These data confirm that most brown dog ticks in the United States are temperate lineage and suggests that pet travel occasionally results in translocation of tropical lineage *R. sanguineus* s.l. to new areas.

PS02.43 Candidatus Neoehrlichia Mikurensis in Ixodes Ricinus Ticks in the Czech Republic

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Candidatus Neoehrlichia mikurensis (CNM) is a tick-borne emerging intracellular bacterium recently found in ticks from over 20 Eurasian countries. Small rodents serve as a reservoirs. Despite this bacterium is acknowledged as a human pathogen since 2010, only little is known about its life cycle, transmission, structure or target cells. In this study, distribution and prevalence of CNM in *Ixodes ricinus* using PCR was investigated in the Czech Republic. In addition, transmission electron microscopy (TEM) was used to identify and visualize CNM in ticks.

To identify the possible hotspots of “neoehrlichiosis”, over 13 600 ticks were collected by flagging in surroundings of major Czech cities. Ticks were subsequently classified by stage and sex and pooled into groups of 5 individuals. In total, DNA was isolated from 2 666 groups. CNM detection was done by conventional PCR. *Ca. Neoehrlichia mikurensis* was detected in *I. ricinus* in 150 of 169 localities examined. Furthermore, several sites were identified as hotspots for neoehrlichiosis, mainly in areas with high tourism activity.

The ultrastructure of CNM in the vector host was examined on the ultrathin sections of infected tissues of *I. ricinus* using TEM. Ticks flagged in the locality of high CNM prevalence were allowed to feed in the artificial in vitro feeding system. After dissection, individual organs were prepared for TEM analysis. Since this pathogen has not yet been cultivated and the surface structures remain unknown, the screening was somewhat hindered by unavailability of labeled probes. However, structures resembling the CNM bacteria in mammalian tissues were found. In summary, we detected *Ca. Neoehrlichia mikurensis* in the close proximity of the majority of sizable

Czech cities and described putative *Ca. Neoehrlichia mikurensis* individual bacteria within the salivary glands of the European tick *I. ricinus*.

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PS02.44 Mortality Time of *Rhipicephalus Microplus* Larvae Immersed in Essential Oils

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Rhipicephalus microplus ticks are responsible for damage to the health of its hosts and generate economic losses to the livestock sector. The development of parasitic resistance to most commonly used commercial acaricides has led to the search for alternative control. In this scenario, the present study aimed to evaluate the toxicity of essential oils by the mortality time of *R. microplus* larvae in direct contact with oils. Among 20 terpenes studied, 14 were cyclic (carvacrol, thymol, 1,8-cineole, anethole, cinnamaldehyde, L-limonene, vanillin, carvone, eugenol, menthol, terpinolene, β -pinene, D-limonene and α -terpinene) and six were acyclic chains (citral, citronellal, citronellol, geraniol, linalool and nerolidol). Mortality time was compared with negative controls (soybean oil) and two positive controls (commercial Pour on). The test consisted in immersing approximately 100 *R. microplus* larvae in 100 μ l of each oil in 100% dose in 48 well polyethylene plates and verifies the time when 100% of larvae were dead, with the aid of a timer. The ten oils with the shortest mortality time were diluted in soybean oil to form the doses of 50, 25, 12.5, 6.25, 3.125 and 1.56%. The essential oil with higher larval toxicity was thymol, in which the lowest dose (1.56%) had the shortest mortality time (15 min) compared to the others. A relationship

was found between the molecular structure of the compounds and their activity. Cyclic compounds which containing ten carbon molecules in their chemical structure were more toxic to larvae compared to acyclic ones. It is concluded that the methodology of evaluation by means of larval mortality time was able to determine which essential oil had the highest toxicity in vitro tests.

PS02.45 Mortality Time of *Rhipicephalus Microplus* Larvae Immersed in Different Solvents

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Researchers are currently looking to study and develop new acaricidal products to control *Rhipicephalus microplus* tick. The objective of this work was to evaluate the mortality time of *R. microplus* larvae immersed in liposoluble solvents (glycerol, vaseline, castor oil, xylol) and water soluble solvents (acetic acid, acetone, Tween 80, DMSO, propylene glycol, ethanol, methanol and isopropyl alcohol) in vitro. The solvents were tested pure (100% concentration) and were diluted in water - negative controls (A) or soybean oil (OS) according to their solubility to perform 50, 25, 12.5, 6.25, 3.125 and 1.56% concentrations. The test consisted of immersing approximately 100 larvae of *R. microplus* in 200 μ l of the solvent tested in a 48 well plate. All larvae were observed with a magnifying glass and with a cronometer, we verified the time in which 100% of the larvae were dead. All solvents, concentrations and the control treatments were tested in triplicates. Data were analyzed by PROC GLM procedure of SAS, whose model included the fixed dose effect. Each solvent was analyzed individually. The means between the different doses were compared by the Tukey's test. All solvents and their concentrations differed from controls (A) and (OS) ($p < 0.05$) which had a mortality

time of 4320 and 2400 minutes respectively. It was observed that larval mortality time is dose dependent for most solvents. Among the liposoluble solvents, xylol presented the shortest mortality time of larvae ranging from 0.2 min (100% dose) to 185 min (1.56% dose). Among the water soluble solvents, acetic acid had the shortest mortality time ($p < 0.05$) with variation from 0.6 min (100% dose) to 97 min (1.56% dose). We concluded that the solvents used in acaricidal products can influence the mortality of larvae.

PS02.46 Exotic and Disease Vectoring Ticks on White-Tailed Deer in Southern Texas

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White-tailed deer were scratch inspected for ticks during the 2018 hunting season at the Laguna Atascosa National Wildlife Refuge in Cameron county TX. Only four of the 73 deer examined were negative for infestation by ticks. A total of 3380 ticks were counted and identified to one of seven species. Five of the encountered tick species are precinctive to the neotropics and just enter the United States in the border area of Texas: *Amblyomma inornatum*, *A. mixtum*, *A. tenellum*, *Anocentor nitens* and *Boophilus microplus*. The latter two species, the tropical horse tick and the southern Cattle Fever Tick, both one-host ticks, are important economic pests of livestock and thus, it is of some concern that they use white-tailed deer as a sylvatic reservoir. Notably, although deer have been reported as an incidental host, the very high numbers (2922) including larvae and nymphs as well as adults, suggests that deer are the native host of the "Horse tick" given that horses are not native to the western hemisphere and *A. nitens* does not occur in the Old World. The gulf coast tick, another pest of livestock was common and coexistent with the other *Amblyomma* spp. in this deer herd. Finally, the detection of 21 specimens of *Ixodes scapularis*, the vector of Lyme disease, suggests that this species may not be as rare in south Texas as previous surveys have indicated.

PS02.47 Efficacy of Different Concentrations of *Duddingtonia Flagrans* Chlamydospores against Different Levels of Faecal Egg Counts of Cattle Gastrointestinal Nematodes

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The nematophagous fungus *Duddingtonia flagrans* is a biocontrol agent that reduces the number of infective larvae of gastrointestinal nematodes in animal faeces, thus, lowering pasture infectivity. However, the minimum amount of fungal chlamydospores in faeces required for this effect is largely unknown. The following in vitro study aimed to determine the fungal efficacy of four different chlamydospores concentrations against three different levels of cattle faecal egg counts. The chlamydospores concentrations tested were 11000, 6250, 3000 and 1000 chl/g faeces. Faeces from naturally-infected calves were used to obtain faecal egg counts of 100, 480 and 840 epg. Ten faecal cultures of 10 g faeces each were set up for each chlamydospores concentration/ faecal egg count combination, plus one control group for each faecal egg count level without chlamydospores. All cultures were kept at room temperature (20-26°C) for two weeks and then L3 were recovered from each individual culture by overnight baermannisation, counted and identified. The larval reductions by *D. flagrans*, in decreasing order of chlamydospores concentrations, were: 100% ($P < 0.0001$), 99% ($P < 0.0001$), 92% ($P = 0.0032$) and 77% (ns) compared to the 100 epg control; 100% ($P < 0.0001$), 98% ($P < 0.0001$), 98% ($P = 0.0002$) and 92% (ns) compared to the 480 epg control; and 100% ($P < 0.0001$), 100% ($P < 0.0001$), 99% ($P < 0.0001$) and 96% (ns) compared to the 840 epg control. There were no differences between the three levels of faecal egg counts at any given fungal concentration, which indicates that the numbers of nematode eggs in cattle faeces would not be a determining factor for the efficacy of *D. flagrans*. These

results suggest that the concentration of 1000 chl/g faeces would not be sufficient to achieve a significant fungal efficacy; however, it could be argued that, in practice, larval reductions of 77% to 96% would contribute to control gastrointestinal nematodes.

PS02.48 In Vitro Anthelmintic Activity of Hymenodictyon pachyanta Stem Bark Extract and Fractions Against Haemonchus contortus

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The development of helminth resistance and high cost of synthetic anthelmintics has prompted the need for an in vitro anthelmintic evaluation of crude stem bark extract and fractions of *Hymenodictyon pachyanta* plant as an alternative in the management of endoparasites including *Haemonchus contortus* which is one of the most prevalent parasitic nematodes in small ruminant farming globally. *Hymenodictyon pachyanta* stem bark is used as anthelmintic by indigenous farmers in Nsukka, Enugu State and Gwagwalada, Federal Capital Territory, Nigeria. The stem bark of *H. pachyanta* were collected from the field in Nsukka, Enugu State, air dried, pulverized and extracted with 80% Methanol. The extract and fractions of *H. pachyanta* were tested on the egg hatch inhibition assay (EHIA) and the larval development inhibitions assay (LDIA) and compared with Albendazole the positive control. The concentrations for the plant extract, fractions and Albendazole used for the study were 0.78, 1.56, 3.125, 6.25 and 12.5mg/ml. The results showed that the crude extracts, fractions and Albendazole at concentration dose of 12.5 mg/ml produced 100% inhibition of egg hatching and larval development of *Haemonchus contortus*. Although, there was no significant difference ($p>0.05$) in the mean percentage egg hatch and larval development inhibition of the crude extract and fractions when compared with Albendazole. However, significant difference ($p<0.05$) was observed with n-butanol fraction which inhibited 96.17% of egg hatchability. The extract, fractions and Albendazole showed ovicidal and larvicidal activity and produced over 50% inhibition

of egg hatching and mortality of larvae at concentration ranges of 0.78 - 12.5 mg/ml. The result obtained from this study suggests that *H. pachyanta* possess anthelmintic effects against *H. Contortus* and validates the folkloric use of the plant in the management of *H. Contortus* infections.

PS02.49 Humoral Response of Canaria Sheep Vaccinated With a Recombinant Teladorsagia Circumcincta Prototype Vaccine

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Among the potential alternatives to anthelmintics against gastrointestinal nematodes, vaccines are an environmentally friendly and promising option. An efficacious recombinant sub-unit vaccine against *Teladorsagia circumcincta* has been tested in Texel-cross sheep (Nisbet et al., 2013; 2016; 2019). Here, we evaluated the effect of this prototype vaccine in another sheep breed, the Canaria Sheep (CS). In vaccinates compared to adjuvant-only control sheep, female worms were found to be significantly shorter and had fewer eggs in utero. Although mean worm burden and faecal egg counts were lower in vaccine recipients, the levels were not significantly lower than found in the controls. Significant negative associations between antigen-specific IgG and IgA and parasitological parameters (worm burden/worm fecundity) were found. These data add to our understanding for the further development of this vaccine for sheep. Acknowledgements: European Union's Horizon 2020 Research and Innovation programme under the Grant Agreement No. 635408 (PARAGONE).

PS02.50 A Predation Test to Evaluate the Efficacy of Macrochelidae Mites as Biological Control Agents for Parasitic Nematode Larvae

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Macrochelidae acari are foretropic predators found in soil, decomposing organic matter and feces. A survey conducted in dairy farms at the municipality of Guaratinguetá - SP, Brazil recovered *Allogynaspis flechtmani*, *Glyptholaspis americana*, *Glyptholaspis saprophila*, *Holostapella bifoliata*, *Macrocheles insignitus*, *Macrocheles mammifer*, *Macrocheles merdarius*, *Macrocheles muscaedomesticae*, *Macrocheles robustulus*, *Macrocheles glaber*, *Macrocheles scutatus* and *Macrocheles subbadius* from partially dry cattle dung patches. Mites were fed *Musca domestica* eggs and infective nematode larvae from coprocultures of cattle, horses and sheep. A colony of *Holostapella bifoliata* was established and assigned to a predation test on coprocultures. Feces collected directly from the rectum of seven cattle were blocked according to their respective EPG counts. Each sample was halved and each half sample was mixed with vermiculite for coproculture. One was assigned to the test group and its pair served as control. After two days at 30°C ± 2°C e 70% ± 10% humidity, five female and two male mites were placed in each test culture. After eight days, infective nematode larvae were recovered and counted. Mann-Whitney test was used to compare recovery rates calculated on the base of the initial EPG counts. Larvae counts were significantly

lower in the test cultures ($p=0.048$), but it was unfeasible to recover mites from the substrate. The procedure was repeated with horse rectal sample used to prepare six repetitions with three coprocultures each. Two cultures from each repetition received a single female *Holostapella* and the third served as control. The additional cultures with acari were placed in the Berlese-Tullgren apparatus. Five cultures yielded live mites, and four produced two. Nematode larvae counts were significantly lower in test cultures ($p=0.037$). The current model has yet to be refined, but it offers positive evidence concerning the potential use of these predators as biological control agents for parasitic nematode larvae on pastures.

PS02.51 Evaluation of the Fecal Dx® Antigen Test for the Diagnosis of Nematodes with Carnivorous Samples from a Germany Zoo

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Common nematodes in zoo felids and canids are *Toxocara* sp., *Toxascaris leonina*, *Ancylostoma* sp., *Uncinaria stenocephala* and *Trichuris vulpis*. Apart from various clinical signs of gastrointestinal parasitosis like anorexia, weight loss, diarrhea or bowel obstruction (especially in young animals), *T. canis* and *T. cati* are also important zoonoses. The diagnosis of these diseases is usually dependent on fecal egg flotation, which is time-consuming and not user-friendly. Compared with fecal flotation, the IDEXX Fecal Dx® antigen test (Fecal Dx) is an ELISA that provides detection of infection in the absence of an egg observation. However, few studies have been reported about the application of Fecal Dx for diagnosis of ascarids, hookworms and whipworms in wild carnivores (cheetah, lynx, leopard, tiger, lion, wolf, polar bear, red panda, meerkat, fishing cat and yellow-throated marten). Since Fecal Dx was designed to detect the secreted/excreted proteins

from *Ancylostoma caninum*, *Toxocara canis*, *Toxocara cati* and *Trichuris vulpis* in domestic canines and felines, very little is known about its performance for the diagnosis of *Ascaris*, hookworms and whipworms in the wild animals mentioned above.

In this study, Fecal Dx, with passive fecal egg flotation as a reference method, was evaluated with 51 fecal samples from 11 different carnivores from a German Zoo. For ascarids, 25.4% (13/51) of the samples were positive by egg flotation, compared with 39.2% (20/51) of the samples with Fecal Dx. Cheetahs and leopards were most likely to be infected with ascarids compared to other carnivores tested. In addition, cheetahs were the only species in which hookworm and whipworm infections were found. Contrasting to flotation where no hookworm or whipworm eggs were observed, Fecal Dx showed 5.8% (3/51) and 1.9% (1/51) of the cheetahs tested were hookworm and whipworm positive, respectively.

PS02.52 Comparative Efficiency of the McMaster, Mini-FLOTAC and FAMACHA® Methods for Diagnosing Helminths in Sheep

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The present study aimed to evaluate and compare three methods of diagnosing ovine helminths. For such, 48 matriarchs were selected, with EPG (eggs per gram of feces) counts superior to 400 eggs on a preliminary McMaster screening. Fecal collections and evaluations of ocular conjunctiva were performed in all ewes, for evaluating parasite burdens by the FAMACHA®, Mini-FLOTAC and McMaster methods, every 14 days over an entire year. Results of all three techniques were dichotomized in Treated (FAMACHA®

evaluations 3, 4 and 5, and EPG counts superior to 1000 for McMaster and Mini-FLOTAC) and Untreated. Prior to the trial's beginning, coprocultures were carried out for the generic identification of helminths. There were 1142 comparable evaluations, and *Haemonchus* (67%) was the most prevalent genus in coprocultures. The FAMACHA® method showed 82.66% of the evaluations in degrees 1 and 2. 69.44% and 67.51% of all evaluations presented EPG counts between 0 and 990, for the McMaster and Mini-FLOTAC methods, respectively. The FAMACHA® method presented small correlation with EPG counts, demonstrating that, the higher the egg mean counts, the more marked were the degrees of anemia. Comparison of McMaster and Mini-FLOTAC techniques revealed that 86.25% of evaluations presented differences from 0 to 490, with 133 results being identical, 578 obtaining differences from 10-190 and 274 from 200-490. Treated and Untreated evaluations for each technique resulted in a 50% reduction in treatment frequency when FAMACHA® was applied ($P < 0.05$). Based on obtained results, it is possible to conclude that all three methods presented expressive helminths diagnosis results, with little difference between them. However, when data were transformed into Treated and Untreated animals, the FAMACHA® method stood out, significantly reducing the needed frequency of treatments.

PS02.53 Association Between *Dirofilaria immitis* and *Euculeus aerophilus* in Naturally Infected Dogs, in the Northern Outskirts of Buenos Aires and the Tigre Region

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The aim of this report is to point out the presence of both *Dirofilaria immitis* and *Euculeus aerophilus* in dogs naturally infected in the littoral region and the northern outskirts of Buenos Aires. Both parasites

can produce chronic cough or dogs can be asymptomatic. *Euculeus aerophilus*, live attached to mucous membranes of trachea and bronchi. The eggs (bipolar and asymmetric) can be found in faeces, measure 60-70µ x 35-40µ, and contain a single cell when passed. Pets are infected by ingestion of the egg with an infective larva; eggs require a period of development in the soil to become infectious. Dogs presented to The Veterinary Virreyes Hospital from 1997 to 2018 were included in this paper. Of 35 dogs positive to *Euculeus aerophila* (diagnosed by fecal flotation or endoscopy), 20 were positive to *Dirofilaria immitis*, (diagnosed by Antigen test and Knott test) and 15 negative. Of 410 positive to *Dirofilaria immitis* 20 were positive to *Euculeus aerophilus*. A complete blood count, creatinine, ALT, urinalysis, urine culture, coproparasitological exam, thoracic radiography, electrocardiography were performed to all of them. The medical treatment sequence recommended by the American Heartworm Society was followed. Complications of heartworm disease or other diseases were managed before adulticide therapy was begun. *Euculeus* was treated with fenbendazole 50mg/kg for 3 days before Heartworm treatment was begun. The adulticide treatment was made with melarsomine dihydrochloride, 2,5mg/kg, all dogs were kept at the hospital for a fortnight. From 2002 on, all dogs were treated with the alternate dose regimen. An analgesic protocol was started from one hour previous to the melarsomine injection. From 2009 on, patients have also received doxycycline orally 10 mg /kg once a day to control *Wolbachia*. It is concluded that in the presence of either *Dirofilaria* or *Euculeus* the other should be suspected, in this region.

PS02.54 First Clinical Case of Demodex Gatoi in a Cat in Argentina

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The authors present the first report in Argentina of *Demodex gato* in one cat. *Demodex gato* is a short mite located in the skin stratum corneum. Unlike *D. cati*, it causes itching and a contagious condition in immunocompetent cats. The main clinical signs are associated with moderate to intense pruritus. Diagnosis was based on skin scraping, acetate tape, and fecal flotation copro-parasitological test to detect parasites. Identification is based on the typical morphology of this *Demodex*.

It was a Feline, neutered female, 4 months old, of Bengali breed referred to the clinic, with diarrhea and intense itching that started since her arrival at home. The cat was from a cattery in the city of Buenos Aires. She showed pruritus, which manifested with scratching, excessive licking and bites all over her body. Inflammation of the bowel and erythema of the head and neck were detected at clinical examination. The blood count and blood chemistry showed no changes. FIV / FeLV negative. Fecal studies were performed with fresh samples: modified Ziehl-Neelsen stain, direct exam with physiological solution, and Bembrock technique. *Tritrichomona* foetus, *Giardia lamblia*, *Cryptosporidium* spp. and *Demodex gato* were found. Trichography samples with acetate strips from affected and unaffected areas of the skin were taken as well as skin scrapings. Eggs and adult mites of *Demodex gato* were observed.

The cat was treated specifically against *Demodex gato* with Advocate spot on (Bayer laboratory, Imidacloprid 100 mg, Moxidectin 10mg) repeated at day 15; negative results were obtained at the end of the treatment. Demodicosis is a rare parasitic disease in feline consulting room; this being the first report of *Demodex gato* in Argentina. Interestingly, diagnosis was made using a fecal flotation. This disease should be then taken into account when facing a pruritic cat mainly if the patient comes from feline communities.

PS02.55 Use of a PCR to Identify the Main Gastrointestinal Nematodes Resistant to Anthelmintics in Cattle Farms, in Uruguay**Celeste Serrano¹***¹Instituto Nacional de Investigacion Agropecuaria, Tacuarembó, Uruguay*

The objective of this work was to set up molecular tests to identify the main resistant gastrointestinal nematodes (GIN) identified as resistant by coprocultures when performing the fecal egg count reduction test (FECRT). Third stage larvae (L3) were obtained from the FECRT conducted on 10 extensive cattle farms in the North of Uruguay during 2018. Morphological and molecular identification was done using L3 obtained for drug groups: Ivermectin 1% (IVM), Levamisole (LEV), Ricobendazole (RBZ), Fenbendazole (BZ) and untreated control. A commercial kit NucleoSpin® Soil DNA (Macherey-Nagel) was utilized to extract DNA from a mixed pool of L3 from each FECRT group. Uniplex PCR reactions were conducted with primer pairs described in the literature specific for regions in the Internal Transcribed Spacer 2 (ITS 2) of *Haemonchus* sp., *Cooperia oncophora*, *Ostertagia ostertagi* and *Trichostrongylus* spp. From 38 L3 cultures where *Haemonchus* spp was morphologically classified, 20 gave an amplicon of 226 base pairs (bp) corresponding to the expected size for this parasite. From 34 samples where *Trichostrongylus* spp. was typified, 29 revealed amplicons of 106 bp reported for this genus. From 38 samples where *Cooperia oncophora* was typified, 20 gave amplicons of 192 bp described for the species and out of 21 samples where *Ostertagia ostertagi* was typified, four gave amplicons of 124 bp expected for this species.

The Cohen's kappa statistics showed a fair agreement (k value=0.21, $p<0.01$) between morphological typification and PCR on 192 mixed L3 samples. Discriminating by nematode, kappa statistics indicated fair agreement for *Haemonchus contortus* (k value=0.29; $p<0.01$), low agreement for *Ostertagia ostertagi* (k value=0.12) and *Cooperia oncophora* (k value=0.14, $p=0.10$). The preliminary results indicate that the applied PCR protocols successfully identify

L3 from mixed GIN samples. The study will continue to improve the agreement between both morphological and molecular tests for our working conditions.

PS02.56 Easy, Cheap and Convenient Method for the Detection of Different Species of Babesia

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Babesia is a disease transmitted by ticks. It is present in whole world. It has impact on animal health as it causes severe anemia which leads to loss of meat and milk and has direct impact on human as babesia microti present in human. Recently there are many costly procedures are adopted for its diagnosis. Among all methods Microscopy detection methods are still the cheapest and fastest method to identify Babesia although their sensitivity and specificity are limited. Molecular and immunological methods have developed and they offer faster, sensitive and specific methods. These includes PCR, RT-PCR, DNA Probes, Loop Mediated Isothermal Amplification (LAMP), Indirect Fluorescent Antibody Test (IFAT), Enzyme-Linked Immunosorbent Assay (ELISA), Immunochromatography Test (ICT), Reverse Line Blot Hybridization (RLB). These methods are time taking, costly and needs experts. but we need cheap, convenient, portable and sensitive method for detection of babesia in blood that should be available at farm which should not require any expert and not time taking. My futuristic approach is that we should go with a kit just cassette of nitrocellulose on which different control lines of specific dye labelled antibodies against specific antigenic proteins of babesia species. With the buffer, blood drop, lysing agent and simple chromatographic process blood along antigenic protein and buffer move on the gel and eventually captured by specific antibodies and with the help of fluorescence of dye we can easily detect which specie is present in blood of specific animal and human.

We can produce its vaccine also if we know the antigenic protein of babesia species so it's another preventive point. It can be done simply at farm level and doesn't need expert person to check as color lines can be visible easily by anyone and need few drops of blood.

PS02.57 Heartworm Microfilaria: Banking the Source for Teaching Diagnostic Techniques

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Proper diagnosis of *Dirofilaria immitis* infection is critical to Day 1 veterinarians and technicians. Antibody-antigen blocking, causing false negatives, confounds some testing. Therefore, detection of microfilaria is an important diagnostic skill. However, finding a supply of blood containing microfilaria when scheduled to teach the procedure presents a challenge. To address this issue, we evaluated the use of cryopreserved microfilaria in two standard diagnostic tests, Modified Knott's and carbonate filter tests. We also evaluated the impact of environmental friendly 2% acetic acid in place of 2% formalin as a fixation reagent in the assay. The specific aims included determine if students could 1) detect the cryopreserved *Dirofilaria immitis* microfilaria added to fresh blood and 2) detect morphological differences between the microfilaria in the modified training assays. With this information, teaching laboratories could utilize cryopreserved microfilaria in freshly obtained blood in lieu of freshly collected microfilaria-containing blood. One aliquot of fresh blood was removed, designated as a baseline and immediately evaluated using the two diagnostic techniques. Microfilaria were isolated from whole blood containing JYD or MO microfilaria and cryopreserved using Glycerolyte 57 solution. The microfilaria underwent rapid thaw, were spun down and were added to freshly acquired blood from a healthy donor. Students (n = 120) were then randomly assigned samples and fixatives (cryopreserved microfilaria, non-cryopreserved microfilaria, and either acetic acid or formalin fixation) to analyze. We

report on the students' ability to correctly identify microfilaria in the blood samples and if they noticed morphological changes based on cryopreservation and fixation used.

PS02.58 Parasitological Procedures, Skills, and Areas of Knowledge Used by Equine Practitioners in North America

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The American Association of Veterinary Parasitologists (AAVP) describes the ability to proficiently perform and interpret common diagnostic procedures in parasitology as a core competency. Mastering these skills requires an understanding of the current parasitological procedures, skills, and areas of knowledge used in large-animal practice. To investigate this need, a study was designed by Ross University School of Veterinary Medicine (RUSVM) where a questionnaire involving common procedures was developed and completed by 46 equine practitioners. Respondents provided general information on practice characteristics identifying the main diagnostician, area of their practice, number of years in practice, number of veterinarians and technicians as well as number of patients examined per day. Following, participants reported their procedures and frequency on diagnoses of equine helminths, protozoa and ectoparasites. Descriptive Results indicated that quick methods requiring minimal involvement are more likely performed at the practice. Specific results regarding the diagnosis of equine helminths indicated that practices located in peri-urban areas are less inclined to perform fecal flotation using centrifugation (p= 0.02). Additionally, the veterinarian is more inclined to perform the Baermann test (p= 0.01), where the majority of diagnosticians perform this test on a yearly basis (88%). Moreover, the adhesive tape test was less likely to be performed in practices where less than 5 patients were examined

per day ($p = 0.02$). In practices with 4 or more veterinarians, skin biopsies to detect mites were more likely to be performed ($p = 0.0014$). Though all diagnostic procedures are termed common by the AAVP core competencies, some results suggest a lack of understanding as to what procedures are performed as well as whether or not they are sent out to a diagnostic laboratory. Outcomes of this study will support parasitology teaching opportunities to further enhance veterinary graduates' expected core competencies.

PS02.59 Prevalence of Gastrointestinal Parasites of Dogs and Cats in Greater Dublin Area, Ireland

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This study was undertaken to obtain a snapshot view of the prevalence of canine and feline endoparasites in Dublin. Faecal samples were randomly collected from dogs ($n=627$) and cats ($n=289$) entering a rehoming centre in Dublin. Samples were analysed by two flotation techniques: one with saturated sugar solution ($SG=1.30$) and the other zinc sulphate ($SG=1.18$) solution, a modified Baermann technique and faecal smears stained with a Modified Kinyoun's staining procedure. Surprisingly, 21.7% all dogs and 31.7% of all cats sampled harboured at least one intestinal parasite. The main parasitic agents detected were ascarids (15.52 and 30.26%), Cystoisospora (3.27 and 3.69%), Giardia spp. (6.02 and 1.84%) and lungworms (0.64 and 2.08%) in dogs and cats respectively. The lungworms identified in dogs were *Angiostrongylus vasorum* (0.48%) and *Crenosoma vulpis* (0.16%) while cats were only infected with *Aelurostrongylus abstrusus* (2.08%). Ascarid eggs and *Cystoisospora* spp. oocysts were more often found in young dogs ($p < 0.001$ and $p = 0.008$, respectively) or cats ($p < 0.001$ and $p = 0.013$, respectively) less than 3 months of age.

This surveys reinforce the need to support the parasite control guidelines recommended by the European Scientific Counsel Companion Animal Parasites (ESCCAP) and emphasize the need to examine pets regularly for intestinal parasites. Continued shedding of parasites in faeces can create heavily contaminated environments, increasing the probability of infection and also of zoonotic transmission of some parasites.

PS02.60 A One Health Paradigm: Multiple Bovine Infections in the Human-Wildlife-Livestock Interface of Lambwe Valley, Western Kenya

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Borne by the tsetse fly, trypanosomiasis is a protozoal diseases complex which has been extensively documented in the Lambwe valley since as early as the 20th century. Despite some level of earlier achievements, the valley lacks long term tsetse and trypanosomiasis control plan. At present, there are several complaints by the local community due to its considerable challenge to livestock productivity and impact the livelihoods. A cross-sectional study was undertaken in villages located within 10 km radial distance from Ruma park with the objectives to estimate the prevalence and to identify risk factors contributing to the occurrence of the disease. A total of 682 blood were sampled from local zebu cattle in December 2018 and tested using two tests, buffy coat technique BCT and using high-resolution melting (HRM) analyses. Using the two tests in parallel, the overall trypanosome infection (*Trypanosoma congolense*, *T. brucei*, and *T. vivax*) was 11.6% and 28.3 %, respectively. Performance of the BCT and HRM was assessed in 682 matching blood samples. The degree of agreement was found to be fair (75.07%, $k = 0.234$). *T. congolense* was observed at a rate of 14.8 %, *T. brucei* at 7.9%, *T. vivax* at 7.8% and mixed infection at 2.3%. The putative risk factors homestead-park distance and village were significantly associated with infection. Our analysis showed that 49% of the overall infection occurred at less than 1.5 km from the

park. This is true for *T. congolense* savannah (58%, $p \leq 0.01$) and *T. vivax* (54%, $p \leq 0.01$). In contrast, more than 67% of the *T. brucei* sub group infections occurred at ≥ 3 km from the park. Therefore risk-based vector control interventions should focus on this distance range. Further experiments are underway to determine what implication of the result exist in the potential spread of infection to human.

PS02.61 Retrospective Analysis of *Dicrocoelium dendriticum* (Rudolphi, 1819) Looss, 1899 Cases Diagnosed During 2010-19 at the Animal Health Diagnostic Center, New York State, USA

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Dicrocoelium dendriticum (Lancet fluke) is an endoparasitic distome with a predilection to the bile duct and has a wide host range including species of domestic and wild mammals. The lancet fluke was considered a parasite of economic importance in sheep and cattle of New York State during the early 1950s. In fact, its occurrence was recorded in six counties (Cayuga, Cortland, Madison, Oneida, Onondaga, and Tompkins) in central NY State, the only region considered enzootic in the United States in 1951. By late 1980s this was not considered a serious parasite of domestic animals. However, sporadic cases of dicrocoeliosis has been reported, even in humans, in NY State in the last few decades. This raised our curiosity to understand the current geographical range in the North Eastern United States. In this study, we retrospectively analyzed *D. dendriticum* cases diagnosed at the AHDC, NY State with an aim to understand the geographical spread and the common hosts that it infects. Diagnosis was based on either detection of ova by modified Wisconsin fecal floatation test using sugar (1.33 SPG) or through morphological identification of the flukes. A total of 145 cases (92 sheep, 31 cattle, 17 goats, 3 bison and 2 llamas) of lancet flukes were recorded over the last ten years

(2010-19), either as sporadic incidents or as outbreaks. Occurrence of *D. dendriticum* was documented from 20 counties in NY State (Chenango, Clinton, Columbia, Cortland, Delaware, Dutchess, Franklin, Herkimer, Lewis, Madison, Monroe, Otsego, Queens, St. Lawrence, Saratoga, Schoharie, Schuyler, Tompkins, Ulster, and Wyoming). This includes 17 new counties that were not identified in the 1951 study. Three cases were from counties outside NY State (Grafton-New Hampshire, Hunterdon-Pennsylvania, and Sussex-New Jersey). This study highlights the continued and expanded threat pose by lancet flukes to livestock and humans in NE United States.

PS02.62 Ehrlichia Canis in Dogs of Mexico: Prevalence, Incidence, Co-Infection With Rickettsia Parkeri and Factors Associated

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The prevalence, incidence and factors associated with *Ehrlichia canis* infection, as well as other Rickettsial infections in dogs of rural communities of Yucatán, Mexico, were investigated. A total of 246 dogs were blood sampled and initially screened for pathogens belonging to the Anaplasmataceae family by a quantitative real-time PCR (qPCR) assay, to detect *Ehrlichia canis*, *E. chaffeensis*, *E. ewingii*, *Anaplasma phagocytophilum* and *Rickettsia rickettsii*. Sixty-five dogs were monitored and sampled twice 7-8 months apart. Using the qPCR, 72 positive dogs to *E. canis* were detected (prevalence of 29.26%). These dogs were also tested by nested PCR to detect the same pathogens. None of the studied dogs were positive to *E. chaffeensis*,

E. ewingii nor *A. phagocytophilum* by both PCR assays. The cumulative incidence of *E. canis* infection was 38.46%. Sequencing analysis of the nested PCR products revealed 100% and 98.1% identity of *E. canis* and *R. parkeri*, respectively. We found a dog co-infected with *E. canis* and *R. parkeri*. It is concluded that high prevalence and incidence of *E. canis* in the dog population of Yucatan were detected and age (>3 years old) was the only factor associated with *E. canis* infection in dogs. This study presents the first report of a *R. parkeri* active infection in a domestic dog in the state of Yucatan, Mexico, presented as a co-infection with *E. canis*.

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PS02.63 Transcriptome Analysis of *Haemonchus Contortus* Infecting Goats from Breeds with Differing Resistance Levels

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Haemonchus contortus is the main small ruminant gastrointestinal parasite in tropical areas. Different host breeds present variable levels of overall resistance against these parasites. Moxotó goat breed is locally adapted to the Brazilian semi-arid and it is known for its resistance against these nematodes. Nevertheless, resistant animals still carry gastrointestinal nematodes but in lower numbers than the susceptible counterparts. In this study, parasite-free goats from two breeds (20 Moxotó and 5 Saanen) known to differ in resistance against nematodes (Moxotó > Saanen) were experimentally infected every two weeks with the same *H. contortus* population for

six months. At the end of this period the animals were euthanized, adult *H. contortus* were collected, counted and stored for RNA extraction. Adult parasite counts were consistently lower on Moxotó goats with the exception of 4 animals that had counts similar to Saanen goats. Total RNA was extracted from pools of 20 adult male *H. contortus* from the three most resistant ($\bar{x}=243\pm70$ parasites) and susceptible Moxotó goats ($\bar{x}=1,981\pm445$ parasites) and from three Saanen goats ($\bar{x}=2,511\pm549$ parasites). RNASeq was done on these samples with 718 million paired reads generated (160 bases long) by Illumina sequencing. The paired-end reads were splice-aligned to the *H. contortus* assembled genome (PRJEB506) using HISAT2 and assembled in 32,460 genes by StringTie. Using Ballgown, 220 genes were determined to be differentially expressed among the treatments and were blasted in order to determine their identities. These results are currently under analysis and we expect to shed some light on the mechanisms related to parasite survival in hosts of different resistance levels.

PS02.64 Cytokine Expression in Calves Experimentally Infected with Two *Theileria Parva* Stocks

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Cytokines play a vital role in the immunity and immunopathogenesis of parasitic diseases. However, most of the studies on the role of cytokines in infection by the tick-transmitted protozoan parasite *Theileria parva*, were performed in in vitro cell lines. Furthermore, there are no comparative studies for different stocks of *T. parva* even though cattle theileriosis caused by *T. parva* manifests in different disease syndromes (East Coast fever and Corridor disease), depending on whether the parasite strain is cattle-derived or buffalo-derived. Hence, an in vivo study was performed to investigate the expression

of cytokines in calves experimentally infected with two *T. parva* vaccine stocks (Katete and Chitongo) used for immunization in Zambia. mRNA expression of anti-inflammatory cytokines, IL-10, and IL-4, as well as that of pro-inflammatory cytokines, TNF- α , IL-2 and IFN- γ , were determined using RT-real-time qPCR. Notably, both Chitongo- and Katete-infected calves reacted differently to infection, with some showing clinical signs (CS) and others not (NCS). The anti-inflammatory cytokine IL-10 expression was up-regulated in both CS and NCS animals; especially in the Katete-infected CS group. While the expression of IL-4 mRNA was generally down-regulated, with significant lower expression in the Chitongo-infected CS group. The pro-inflammatory cytokines, TNF- α and IFN- γ , were up-regulated in all groups; however, IFN- γ expression in the Chitongo-infected NCS group was significantly lower compared to other groups. IL-2 was generally down-regulated, especially in Chitongo-infected NCS group. Overall, these findings suggest that *T. parva* infection influences the expression of investigated cytokines. Similar cytokine expression profiles were generally observed between calves infected with the two parasite stocks. However, significant variations were detected in the expression levels between the CS and NCS groups; suggesting that cytokines such as IL-10, IL-4, IFN- γ and IL-2, may have a vital role on the disease outcome.

PS02.65 Toll-Like Receptors 2, 4, and 7, Interferon-Gamma, Interleukin 10 and Programmed Death Ligand 1 Transcripts in Leishmanin Skin Test Positive Reactions of Ibizan Hound Dogs

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The leishmanin skin test (LST) is an in vivo technique commonly used to evaluate the *Leishmania*-specific cellular immune response in dogs. However, information regarding the local immune response in LST positive reactions is scarce. We examined the pattern

of toll like receptor 2 (TLR2), TLR4, TLR7, interleukin (IL)-10, interferon gamma (IFN- γ) and (program death ligand) PD-L1 gene expression in LST positive reactions and paired normal-looking skin of nine infected Ibizan hound dogs. Normal skin from ten seronegative dogs from a non-endemic area was analysed as a negative control. Immune genes expressions were examined by quantitative PCR (qPCR) analysis. LST positive reactions presented significant upregulation of TLR4, IL-10, IFN- γ and PD-L1 and downregulation of TLR7 when compared with normal skin of control dogs. Moreover, a trend for TLR2 upregulation was observed. All transcripts but TLR7 were higher in LST positive reaction than in paired normal-looking skin. The expression profile of immune genes in LST positive reactions was similar to that previously observed in clinically-lesioned skin of mildly diseased dogs with papular dermatitis due to *Leishmania infantum* infection. Our data provide additional support for the important role of TLRs in canine leishmaniosis.

PS02.66 Human Macrophages Cytokine Response to *Dirofilaria Repens* Antigens

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Helminths are known for producing immunomodulatory molecules, which allow them to evade the host immune system. The aim of the study was to investigate the effect of somatic antigens from an adult zoonotic filarial nematode *Dirofilaria repens* on the in vitro production of pro-inflammatory and regulatory cytokines by human macrophages.

THP-1 human monocytes were differentiated into macrophages and stimulated with *Dirofilaria repens* worm extract (DWE) with and without LPS. TNF- α , IL-6, IL-1 α , IL-10 cytokine concentration was measured in culture medium using ELISA commercial kits.

Macrophages stimulated with DWE released significant amounts of the regulatory IL-10 cytokine comparing to unstimulated cells. The treatment had no influence on the secretion of IL-1 α , IL-6 or TNF- α . The production of proinflammatory cytokines was upregulated only in THP-1 macrophages stimulated with LPS. Additional DWE treatment of LPS stimulated macrophages had no effect on the production of pro-inflammatory cytokines.

Dirofilaria repens extract does not induce the synthesis of pro-inflammatory cytokines and promotes the production of IL-10 immunoregulatory molecule. It might be suspected that M2 phenotype is induced in these cells, promoting the anti-inflammatory response.

These findings allow to better understand the reason for the usual asymptomatic course of skin dirofilariosis. The next step would be to perform an analysis of the T cell polarization in response to DWE as well as the analysis of immunomodulatory proprieties of identified single *D. repens* proteins. This may lead to the establishment of the immunomodulation mechanisms used by the parasite in the human host.

PS02.67 Phylogeny of a Novel Coccidian Parasite Found in the Prostate of Antechinus Flavipes (Yellow-Footed Antechinus)

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Morphological traits, especially those of sporulated oocysts, have historically been the basis of coccidian phylogeny. With development of molecular tools for assessing relationships among these parasites and

construction of ever-expanding databases of DNA sequences, it has become clear that parasite morphology is not as accurate a representation of true genetic relationships as once believed and has contributed to erroneous assignments of generic classifications to many parasites. The genus *Eimeria* is polyphyletic, with members grouping in several separate clades alongside members of *Isospora*, *Cyclospora*, *Caryospora*, and others. *Eimeria taggarti*, recently described from the prostate of an *Antechinus flavipes* (yellow-footed antechinus), further contributes to the polyphyly of the genus *Eimeria*. Based on a partial 18S SSU rDNA sequence, *E. taggarti* groups with reptile-infecting *Choleoeimeria* and *Acroeimeria* spp., as well as several *Eimeria* (s.l.) spp. We completed the sequencing of the nuclear 18S SSU rDNA and generated a full mitochondrial genome of *E. taggarti*; these molecular data were used to conduct a phylogenetic analysis to reassess the validity of its taxonomic assignment. A close relationship amongst *E. taggarti*, *Choleoeimeria* spp., and *Acroeimeria* spp. was confirmed. Together, these parasites form a clade basal to the *Eimeria* spp., sensu stricto. Histological observations of infected tissues showed that endogenous stages are limited to the prostate and that sporulation occurs endogenously. Based on these phylogenetic data and morphological observations, we propose that *E. taggarti* does not belong in the genus *Eimeria*, and merits reclassification. Further, we propose a reconsideration of several of the taxonomic designations within this clade to better reflect the true relationships among its constituents.

PS02.68 The First Determination of Eustrongylides Sp. Larvae in the Gulf of Finland and Ladoga Lake

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Abstract: Cases of *Eustrongylides excisus* larvae detection in the zander *Sander lucioperca* caught in the Gulf of Finland and Ladoga Lake are described. The dynamics of extensiveness and intensity of invasion

since 2003 are traced, and analysis of the relationship of these data with fluctuations in the number of commercial zander in the Gulf of Finland is given. Zander is the paratenic host for *E. excisus* and therefore serves as the best indicator of the state of the ecosystem, other food fish (roach, pike, perch etc.) may also be infected with the fourth-stage larvae of this zoonotic helminth. At the same time zander is the most important commercial fish of both the Gulf of Finland and Ladoga Lake and a very popular object of game fishing. Infection of this species with *E. excisus* has a great ecological, economical and medical significance. Despite the time-distant initial discovery by the author (as well as numerous consumers of fish products in the markets) of *E. excisus* larvae in the zander *S. lucioperca* (2003), this presentation is the first official scientific report confirming the presence of this dangerous nematode in the Gulf of Finland and Ladoga Lake.

PS02.69 Alveolar Echinococcosis in a Dog in the Eastern U.S.

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Echinococcus multilocularis is known to be endemic in Canada and the north central U.S. In early November 2018, an 8-year-old 36 kg neutered male Labrador Retriever was presented to a small animal practice in northern Virginia U.S. The owners reported that the dog had recently been lethargic. Physical exam findings were normal. Radiographs of the abdomen showed loss of detail in the cranial abdomen and a possible hepatic mass. An ultrasound performed within a few days of the initial presentation revealed several large hepatic masses and multiple smaller masses involving the pancreas. Fine needle aspirates of the hepatic masses were submitted to Antech Diagnostics. The cytologic findings included inflammation and necrosis with eosinophilic,

membranous oval structures consistent with cestode infection. A centrifugal fecal flotation test did not detect cestode eggs. In late November, further needle specimens of cystic lesions were submitted to Antech Diagnostics. Histopathologic findings included extensive necrosis, inflammation and irregular frequently folded PAS-positive hyaline-like membranous material interpreted as representing the cyst wall of a larval cestode. Additional aspirate material was submitted to the Animal Health Laboratory, University of Guelph for *Echinococcus* and *Taenia* PCR testing. A PCR product was generated using primers specific for *E. multilocularis*. Subsequent sequence data were 100% homologous (344/344 bp) to *E. multilocularis* NADH dehydrogenase subunit I gene sequences listed in GenBank. The parasite masses were considered inoperable and the dog was placed on albendazole (10mg/kg) daily. In early February, 2019 the dog's condition deteriorated and euthanasia was performed. The dog was purchased in Mississippi as a young puppy and brought to Virginia with no history of subsequent travel. This is the first report of alveolar echinococcosis in a dog in the United States and the first report of *E. multilocularis* infection apparently acquired in the Mid-Atlantic region of the U.S.

PS02.70 Experimental Infection of Domestic Dogs with *Borrelia turicatae*: Clinical Pathology and Cross-Reactivity

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Tick-borne relapsing fever spirochetes are closely related to *Borrelia burgdorferi*, the agent of Lyme disease and both pathogens can cause similar clinical signs in dogs. However, the current knowledge of TBRF in dogs is limited to a handful of case reports and so little is known about the clinical pathology during the course of acute infection. Despite their similarity, diagnosis of TBRF and *B. burgdorferi* differs in that

TBRF diagnostics are primarily detection of spirochetes using blood smear or molecular assays, while diagnosis of *B. burgdorferi* primarily relies on serology. However, reports of cross-reactivity in human cases raises the question as to whether dogs seropositive to TBRF spirochetes will cross-react on any of the several commercially-available assays designed for *B. burgdorferi*.

TBRF infection dynamics and cross-reactivity were evaluated in this study by inoculating *B. burgdorferi*-negative dogs with *B. turicatae*, the most commonly identified TBRF spirochete in dogs in the United States. To describe the dynamic changes in select clinical parameters, temperatures were collected daily, platelet counts twice weekly, and complete blood counts weekly, during the first two months of infection. Seroconversion to *B. turicatae* was determined by GlpQ Western blot. Samples were tested with several commercial and veterinary diagnostic laboratory *B. burgdorferi*-based tests.

Several samples cross-reacted with commercial and veterinary diagnostic laboratory tests. Practicing veterinarians should choose screening and diagnostic tests with care if trying to distinguish between relapsing fever borreliosis and Lyme borreliosis. Clinically, the dogs did not develop signs of disease, but platelet counts decreased within the first two weeks post-inoculation and returned to baseline over the next several weeks. Subclinical infections may be present in the population and that possibility combined with cross-reactivity of some tests highlights the need to critically evaluate positive tests in the context of an animal's risk of exposure.

PS02.71 Bacterial Communities Associated with the Gut of Female House Flies Collected From Three Environmental Niches in Kansas

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Adult house flies serve as a bridge between

sources of bacteria (manure, waste, wounds) and healthy or uninfected animals, including humans, and their habitats. Evaluating and characterizing the bacterial communities in flies collected from natural habitats will help in understanding their role in harboring and disseminating bacteria. In this study, we used next generation sequencing of bacterial 16S rRNA gene to characterize the gut bacterial communities of adult female house flies collected from three different environments in Manhattan, KS: agricultural (beef cattle feedlot), urban (downtown dumpsters), and mixed environment (business located near animal agriculture). Bacterial taxa affiliated to the phylum Firmicutes dominated the bacterial communities in flies from the agricultural setting while Proteobacteria was the predominant phylum in flies from the urban and mixed environments. In the lower taxonomic level, genera associated with feces or the vertebrate gut (rumen-associated microbes such as *Blautia*, *Phascolarctobacterium*, *Anaerovibrio*, Clostridiales, Ruminococcaceae, *Escherichia-Shigella*) dominated the agricultural environment. Importantly, potential human pathogens including *Providencia* and *Aeromonas* were dominant in the mixed environment whereas Enterobacteriaceae, *Providencia*, *Enterococcus* and *Phascolarctobacterium* were dominant in urban environment. Principal coordinates analysis showed that the bacterial communities in the house fly gut were similar between flies collected from the same site, but community composition varied significantly across the environments. Furthermore, bacterial species richness was highest in agricultural environment, which differed significantly from mixed and urban environments. Similarly, Shannon index was higher in agricultural environment than the other two environments. These results demonstrate that the house fly gut harbors complex bacterial communities, including potential human and animal pathogens, and that community composition is strongly influenced by the environment.

PS02.72 Use of Barbervax: A Commercial Haemonchus Contortus Vaccine, in Alpacas over Two Grazing Seasons

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Haemonchosis in camelids remains a challenging disease to treat, and prevention has become increasingly problematic due to widespread anthelmintic resistance. Barbervax is an adjuvanted vaccine containing natural H-11, H-gal-GP antigens obtained from *Haemonchus contortus* adults via a proprietary process and solubilized in Quil A. This vaccine is approved for use in Australia after demonstrating its safety and efficacy in sheep and goats. The vaccine utilizes a mixture of the parasite gut mucosal membrane enzymes including H-gal-GP and H11, involved in digesting a blood meal from the host. This study monitored the efficacy of the Barbervax vaccine in a group of alpacas. In the prior grazing season, three alpacas received the vaccine and two remained as negative controls. These five alpacas were then followed during their second grazing season after either re-vaccination with Barbervax or received no treatment. To further challenge the animals during the study, each alpaca was dosed with 50,000 *H. contortus* larvae. Daily observations were made. Weekly fecal egg counts (FEC) were monitored. The re-vaccinated alpacas developed titers to the *H. contortus* antigen as measured by ELISA. None of the FEC values went over 500 EPGs. Using terminal worm counts, the vaccinated alpacas did not demonstrate adult *H. contortus* worms relative to non-vaccinated animals. In conclusion, the Barbervax vaccine demonstrated safety when used during in the second grazing season in a small group of healthy castrated male alpacas. Despite the large challenge dosing, only one non-vaccinated alpaca demonstrated higher EPGs and higher adult *H. contortus* worm burden when compared to the other alpacas in this trial.

PS02.73 Impact of Shearing on Ectoparasites in Holstein Cattle

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In the tropics, ectoparasites cause extensive damage to cattle, including the *Rhipicephalus microplus* tick, the horn fly *Haematobia irritans*, and the larvae of the fly *Dermatobia hominis*. These parasites are difficult to control because of its resistance to chemical products. Control alternatives are required. With the hypothesis that the short hair length would reduce the infestation by the parasites, this work was carried out. The objective was to know the impact of shearing on natural infestation of Holstein cattle, raised on a dairy farm located in Nova Odessa, State of São Paulo, Brazil. Twenty-seven females from four different categories were evaluated: lactating cows; lactating heifers; dry heifers and calves, each category raised in different places. These animals were clipped by a clipper (blade 10) and were evaluated on three occasions, at 20 days intervals, for infestations by *R. microplus*, *H. irritans* and *D. hominis* larvae. Infestation data were transformed into log₁₀ (n + 1) to approximate the normal distribution and were analyzed by means of mixed models using time-repeated measures. In this model, the category effect, evaluation and their interactions were included, and each variable was analyzed individually. The mean infestations for each category were compared by the tukey test (p < 0.05). The results obtained from the analyzes showed that, in some situations, the ectoparasite count was lower in the first evaluation, made post shearing, in relation to the other two subsequent evaluations, whereas in other situations, the reverse occurred. It was not noticed the impact of the shearing in the infestation by ectoparasites, since the results were not repeated in the categories (Sponsor: Fapesp 2016/19938-7).

PS02.74 Rapid Selection of Multiple-Resistance to Eprinomectin and Benzimidazole in a Dairy Goat Farm

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Eprinomectin was commercially launched in the late 90's for lactating cows as a pour-on formulation with a zero milk withdrawal period, and it was recently registered for small ruminants. Given the high prevalence of resistance to benzimidazoles (BZD) in gastrointestinal nematodes in dairy goats and ewes in France, more and more farmers use eprinomectin exclusively to treat their animals. We report here the first case of multiple-resistance to eprinomectin and benzimidazole in a French dairy goat farm. A veterinary practitioner noted a poor response to two types of eprinomectin treatments (a topical application and an injectable formulation) with high mortality rates. We therefore evaluated the efficacy of several anthelmintic drugs (eprinomectin, topical and subcutaneous routes, moxidectin and fenbendazole), using the fecal egg count reduction test according to the WAAVP guidelines. In parallel, nematode species were identified at day 0 and 14 post-treatment after bulk larval cultures, by morphology and real-time PCR. Plasma concentrations of eprinomectin were analysed by HPLC at day 2 and 5 post-treatment in eprinomectin-treated groups.

Egg excretions remained high in animals treated with topical (-16.7% (CI: -237 to 59)) and subcutaneously (21.5% (CI: -126 to 73)) eprinomectin, and with fenbendazole (-5.8% (CI: -205 to 63)). *Haemonchus contortus* was the main species identified by morphology and real-time PCR before and after treatment (97 to 98% of identified larvae), followed by *Trichostrongylus colubriformis*. Plasma concentrations of eprinomectin were above

2 ng/ml in all eprinomectin-treated animals at day 2 post-treatment, indicating that the lack of effect of this drug was not due to low exposure of the worms to eprinomectin. Interestingly, moxidectin treatment was totally effective. This is the first report of multiple-resistance to eprinomectin and benzimidazole of a *Haemonchus contortus* population in a French dairy goat farm, with moxidectin being a relevant alternative in this case.

PS02.75 Effect of In Vitro Ruminal Digestion on Solid Formulations of the Nematophagous Fungus *Duddingtonia Flagrans*

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Pharmacological control of gastrointestinal nematodes using chemical derived compounds represent different problems such as anthelmintic resistance, drug residues in meat/milk products and alteration in pasture ecosystem. Biological control of nematodes using fungus such as *Duddingtonia flagrans* (BGMSABV-Df-Col-H-001-2014) is a promising alternative to overcome these problems in Colombia. The mode of action of the fungus imply the oral ingestion and the transit through the digestive tract, in the faeces the fungus generates traps that reduce nematode population. Oral formulations need to be developed to protect fungal spores (conidia and chlamydospores) from the chemical, physical and microbiological conditions of the gastrointestinal cavities. In vitro methods are needed as a screening method previous to the in vivo assays. The effect of temperature (39°C), pH and mechanical forces on fungus spores were evaluated in order to correlate the isolated factors with the in vitro ruminal digestion assay. In this work two formulation prototypes and a control (dried fungus without any excipient), were submitted to an in vitro assay simulating the ruminal cavity of ruminants consisting of a mixture of artificial saliva and ruminal fluid under agitation at 150 rpm and 39 °C. Samples were taken at 2h, 6h and 12h evaluating the fungal viability, concentration and in vitro nematophagous activity. A gradual reduction of the in vitro

nematophagous activity along the time was observed achieving 69 % for the control, 77 % and 79 % for the formulations developed. These results showed that the aggressive conditions in the ruminal cavity could affect the fungal ability to capture the nematodes and the in vitro method could be employed as a selection tool for formulations.

PS02.76 Ivermectin-Resistant *Cooperia Oncophora* in Feedlot Cattle from Western Canada

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Gastrointestinal nematode resistance against commonly used anthelmintics in cattle is increasingly reported; however, investigations in Canadian beef operations are limited. Therefore, we determined the efficacy of currently used anthelmintic products in feedlot cattle from western Canada. One hundred and fifty-six auction market-derived, weaned, fall-placed steer calves were randomly allocated to two treatment groups: injectable ivermectin; injectable ivermectin combined with oral fenbendazole. Each group contained replicates of 6 pens and 13 animals per pen. Anthelmintics were administered according to individual body weights and manufacturers' recommendations. Fecal egg counts (FEC) were obtained from each calf before and 14 days post-treatment using a modified Wisconsin floatation technique. To determine the anthelmintic efficacy, we calculated the percentage reduction in post-treatment FEC from pre-treatment FEC using the package "eggCounts" in R statistical software. Anthelmintic resistance was determined when the reduction in mean FEC was <95% and the upper and lower 95% confidence intervals were <95 and <90%, respectively. Relative proportions of gastrointestinal nematode species in calves pre- and post-treatment was determined by deep amplicon ITS-2 rDNA nemabiome

sequencing. Calves treated with ivermectin had an 82.5% (95% confidence interval 67.8-90.5) reduction in FEC. The combination treatment of injectable ivermectin and oral fenbendazole had a 100% efficacy in reducing FEC. In the ivermectin group, *Ostertagia ostertagi* was the predominant parasite species before treatment; however, *Cooperia oncophora* was predominant post-ivermectin treatment. There was an increase in the relative proportion of *Haemonchus placei* in calves following ivermectin treatment compared to that of pre-treatment. This study confirmed *Cooperia oncophora* resistance to ivermectin while *Ostertagia ostertagi* was ivermectin sensitive. The increase of *Haemonchus placei* following ivermectin treatment is concerning because it can cause devastating losses in beef cattle operations. In addition, this study identifies regional, accurately phenotyped, ivermectin-resistant parasite populations for future molecular studies.

PS02.77 Resistance of Sheep Trichostrongylids Against Moxidectin in Austria

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Anthelmintic resistance (AR) in sheep nematodes is increasingly reported worldwide. Few studies have been conducted in Austria and while AR against benzimidazoles (BZ) seem to be frequent, AR against macrocyclic lactones (ML) has not been clearly identified yet. As severe treatment failures were increasingly reported from sheep flocks we aimed to monitor the efficacy of BZ and ML by fecal egg count reduction tests (FECRT) in selected farms. In total 11 farms were examined, 3 from Styria, 8 from Tyrol. Overall, 500 animals were included. At the first farm visit feces was taken and examined by Mini-Flotac (detection limit EpG= 5). Animals with an EpG>100 were included. A larval culture was set up from pooled fecal samples before treatment and in case of a positive FEC also after treatment. Animals were treated with Moxidectin (Cydectin®) (MOX, group 1) Fenbendazol

(Panacur®) (BZ, group 2) or left untreated as controls (group 3). All three groups could be tested on one farm. Moxidectin was applied on ten farms, Fenbendazol on three. FECRT indicated a reduced sensitivity of trichostrongylids against Mox on two farms (78%, 79%) and against BZ on two farms (48%, 89%). The predominant nematode genera before treatment were *Haemonchus* spp., followed by *Trichostrongylus* spp. and the *Chabertiinae*. After treatment predominantly *Haemonchus* spp. and to a lesser extent *Trichostrongylus* spp. were found. The apparent lack of efficacy of the ML in sheep flocks with visible clinical consequences indicates the urgency of implementing sustainable control practices in Austria.

PS02.78 Acaricide Resistance Profile of *Rhipicephalus Microplus* From Arauca, Colombia

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Rhipicephalus microplus, commonly known as the southern cattle fever tick (SCFT), prevents raising pure *Bos taurus* cattle breeds in Colombia because it feeds on blood and transmits pathogens. Intense chemical treatments with synthetic acaricides as the only control method selected for SCFT populations that are resistant to multiple classes of acaricides. The objective of this

study was to assess the resistance profile of SCFT infesting livestock at a farm in the Department of Arauca, Colombia, through bioassays and molecular techniques. Results from multiple Adult Immersion Tests (AIT) showed complete lack of deltamethrin efficacy at concentrations of 2 and 4x above therapeutic levels of 50 ppm. The Larval Immersion Test (LIT) with deltamethrin confirmed the high resistance status (RR = 241.6). At label concentrations, the organophosphorus chlorpyrifos (312 ppm) had an intermediate value efficacy of 65-75%, whereas ethion (622 ppm) maintained 100% efficacy. With the (LIT), the LC50 (6.34 ppm) and LC99 (22.79 ppm) for ivermectin were not different to a susceptible SCFT strain, and 5-6 fold lower than values published before showing lack of field efficacy. Due to complete lack of efficacy against SCFT, pyrethroids were no longer used by the producer in this farm. Ethion was the main acaricide employed with alleged good SCFT control. A PCR-RFLP technique to investigate the presence of a carboxylesterase mutation associated with pyrethroid resistance revealed a mixture of homozygous wild-type (n=4), heterozygous (n=13), and homozygous (n=10) mutant genotypes, although SCFT showed a resistant phenotype against deltamethrin. Thus, different resistant mechanisms to pyrethroids may be involved including mutations in the para-sodium channel. These results complement reports from other regions of Colombia where a complete lack of efficacy occurs towards pyrethroids. Best practices need to be developed regarding the use of ethion as the only acaricide affording complete SCFT efficacy across different parts of Colombia.

PS02.79 The Prevalence of Anthelmintic Resistance in Gastrointestinal Nematodes of Beef Cattle in Uruguay

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The aim of this study was to determine the prevalence of anthelmintic resistance (AR) in gastrointestinal nematodes (GIN) in beef cattle farms under extensive production systems in Uruguay. A cross-sectional study was designed and the sample size (n=35-40 farms) estimated under the assumption of 20% prevalence of AR; 5% allowable error and 95% confidence interval. The farms were conveniently selected mainly in the North part of the country, based on willingness to participate. Drug efficacy was evaluated using the in vivo Fecal Egg Reduction Test (FECRT) for the following drugs: Ivermectin (IVM, 200µg/kg bodyweight), Levamisole (LEV, 7.5mg/kg bodyweight), Ricobendazole (RBZ, 4mg/kg bodyweight) and Fenbendazole (BZ, 5mg/kg bodyweight). Additionally, BZ efficacy was evaluated by the in vitro Egg Hatch Test (EHT). At each farm on Day 0, 15 animals were assigned randomly to each of the treatment groups (T) and one group was left untreated (C). For each group, individual fecal samples were collected at baseline and day 14 and analyzed by the Mini-FLOTAC technique. The efficacy was obtained using the formula described by Dash $[1 - ((CO/C14)/(T14/T0))] * 100$ and a threshold of <95% was the criteria to declare AR positive. A pool of samples for each farm was kept under anaerobic conditions for EHT. Coprocultures were done for each treatment group. From 27 farms sampled until present, results from the FECRT indicated a proportion of 100%, 29.6%, 22.2% and 4.7% presenting lack of efficacy for IVM, LEV, RBZ and BZ, respectively. The EHT showed a range EC50 values of 0.01 µg/ml-0.088 µg/ml, indicating no evidence of GIN resistance to Thiabendazole (Threshold >0.10 µg/ml). *Cooperia* spp. was the main GIN genera identified as resistant in all drug groups. The preliminary results of the present study demonstrated the widespread prevalence of AR in GIN of beef cattle in Uruguay as already reported for sheep.

PS02.80 Comparative Efficacy of Doramectin 1% and Closantel 10% + Albendazole 5% in Goats from the City of General Artigas, Canindeyú Department, Paraguay

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Small ruminant like goats are susceptible to helminth infection so generally the control of gastrointestinal nematode relies primarily on antihelminthics administration by oral or subcutaneous route. The objective of this study was to determine the efficacy of doramectin and closantel in 38 Boer and mixed goats from Yvy Kui farm, in General Artigas City located at the Canindeyú Department in Paraguay. Two groups were used. Group 1: Received doramectin (Dectomax®, Zoetis, Argentina), at the recommended dosage of 200 µg/Kg by subcutaneous injection) and Group 2: received closantel 10%, 10 mg/kg plus albendazole 5% 5 mg/kg (Microtel®, Laboratorios Microsules Uruguay, delivery orally). Fecal samples were collected at the time of treatment and 7, 14 and 21 days later. The fecal egg count was performed by the McMaster technique (Coles et al., 1992) and the fecal culture and three stage larvae genus identification (L3) according to Fiel et al., 2011. The fecal culture before the treatment showed the infection by *Cooperia* sp. (45%), *Haemonchus* sp. (32%), *Ostertagia* sp. (18%) and *Nematodirus* sp. (5%). In both groups 5/19 (26,3%) goats showed a reduction of 90% in the fecal egg count at 14 days post treatment and the same percentage was registered at 21 days post treatment in 7/19 (36,8%) goats of both groups. An average of 13/19 (68.4%) goats of the tested animals in both groups did not showed any reduction in their egg counts. This study showed the high resistance to these antihelminths molecules in goats and major studies should be carried out in order to evaluate the real impact of the antihelminth resistance in these animals so appropriate strategies can be developed to address this problem.

**PS02.81 Pomegranate (*Punica Granatum*)
Aqueous Extract for Control of
Gastrointestinal Nematodes in Sheep**

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Helminth infections, and in particular Gastrointestinal nematode (GIN) are amongst the most important production-limiting pathogens of grazing ruminants globally. The negative impact of GIN on livestock farms is further exacerbated by the escalating spread of anthelmintic resistance (AR). Phytomedicine has been used by farmers to treat parasitism and improve performance of livestock, however, scientific evidence on the anti-parasitic efficacy of most plant products is limited. Scientific validation of the anti-parasitic effects is necessary prior to their adoption as a novel method for parasite control. The aim of this study was to evaluate the anthelmintic efficacy and benefits on the milk production of the natural extract rich in tannin present in the peels and seeds of the pomegranate (*Punica granatum*). The research was conducted in sheep naturally infected by GIN. 30 sheep were selected and divided into 2 groups of 15 sheep each: TG, treated orally at single-dose with 50 ml of aqueous extract 21 days before calving; CG, untreated. On Days 7, 14 and 21, individual faecal samples were collected to evaluate the Faecal egg count (FEC) using FLOTAC technique and FEC reduction (FECR) on the different days. The formula used to evaluate was $100 \times (1 - [\text{epgTG}/\text{epgCG}])$. The milk production was measured 8 times every 7 days starting from beginning of lactation. The results of epg mean and FECR (%) were: TG-D0: 460; D7: 264 (55.7%); D14: 298 (53.8%); D21: 392 (46.1%); CG-D0: 450; D7: 596; D14: 646; D21: 728. Regards the milk was observed an increase average over the entire lactation both quantitatively (15.5%) and qualitatively

(protein 5.5%, casein 4.1%, lactose 4.3%, fat 8.3%). Although results showed a discrete anthelmintic efficacy, the benefits derived from not using synthetic drugs and the qualitative increase in milk production indicate the use of the mixture as useful and promising in control strategies against GIN.

PS02.82 Efficacy Evaluation of a Formulation with Ivermectin 1% w/V Plus Clorsulon 10% w/V (Ivomec® F) in Cattle Naturally Infected with *Fasciola Hepatica* in Brazil

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Fasciola hepatica is a trematode that parasitizes the liver of several species including ruminants and is responsible for a decrease in animal welfare and significant economic losses in cattle and sheep. In Brazil, the area with higher prevalence of bovine fasciolosis is the Southern region, however the disease has also been noted in the states of Rio de Janeiro, São Paulo, Espírito Santo, Minas Gerais and Goiás. The treatment of *Fasciola hepatica* infection is mainly done through the use of chemical substances. This study aimed to evaluate the efficacy of a single subcutaneous injection of a commercial formulation with ivermectin 1% plus clorsulon 10% w/w (Ivomec® F) in bovines naturally infected with *Fasciola hepatica*. The study was conducted on the facilities of the Federal Rural University of Rio de Janeiro State and the animals were obtained from Alegre city, state of Espírito Santo. Twenty bovines (age = 9.5 ± 4.1 ; weight = 380.3 ± 59.8) were allocated pre-treatment to blocks based on decreasing arithmetic means EPG counts for *Fasciola hepatica* eggs, performed on Days -7, -5 and -3 and divided in two groups (Control and Treated) of 10 animals each. EPG means pretreatment for control and treated group was 8.7 ± 5.2 years and 8.8 ± 5.5 kg, respectively. Animals from Treated Group received (Day 0) a single subcutaneous injection of Ivomec® F (1mL/50kg bw). On

Days 19 and 20 the animals were euthanized and the livers were removed and sliced into 2cm pieces to recover adult specimens of *Fasciola hepatica* on bile ducts. All flukes recovered were counted. No specimens of *Fasciola hepatica* were recovered from Treated Group animals, while in Control Group mean fluke number observed per animal was 12.60 ± 8.64 . In conclusion, Ivomec® F efficacy was 100% on the treatment of *Fasciola hepatica* in bovine naturally infected.

PS02.83 Comparative Ivermectin Plasma Concentration Profiles After Subcutaneous Administration of Different Long-Acting Formulations to Cattle

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The pharmacokinetic behaviour and systemic availability of ivermectin were compared following subcutaneous application of six different Long-Acting formulations to cattle. Forty-two healthy calves were randomly allocated into six experimental groups (n=7). Animals in each group were subcutaneously treated with one ivermectin formulation at label recommended dose rate (Formulation A= 700 µg/kg; B= 700 µg/kg; C= 630 µg/kg, D= 800 µg/kg; E= 700 µg/kg; F= 630 µg/kg). Blood samples were collected over 120 days post-treatment (14 sampling times). Ivermectin concentrations in plasma were measured by HPLC. Complete pharmacokinetic analysis were performed for all ivermectin preparations. The mean peak plasma concentration (C_{max}) and the area under the concentration vs time curves (AUC, drug exposure) obtained for each formulation were compared following dose rate normalization. The statistically significant differences observed in the kinetic

parameters reflecting the rate and extent of IVM absorption, indicate the existence of some differences among preparations in terms of pharmaceutical behaviour. The relationship between the plasma kinetic profiles of the different formulations and the “theoretical threshold” to obtain an optimal efficacy against ticks was calculated using the period of time during which ivermectin concentrations were above 10 ng/mL. Formulations A, B and D remained above 10 ng/mL for a longer period of time compared to Formulations C, E and F (a highest dose rate of 800 µg/kg was used for formulation D). The tested Long-Acting ivermectin formulations showed slight differences in their absorption patterns, which was reflected in the observed plasma pharmacokinetic behavior. Formulations A and B showed the best performance from the pharmacokinetic point of view, showing initial higher ivermectin concentrations followed by sustained plasma concentrations above 10 ng/mL for more than 40 days that may be relevant to obtain an optimal efficacy against ticks.

PS02.84 Effect of Monepantel on Gastrointestinal Nematodes Infection and the Influence on Gestant Sheep

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One of the main impasses in sheep breeding is the gastrointestinal nematodes (GN) caused by the high morbidity and mortality of the animals. Several anti-helminthic molecules have been used to control GN, especially during the critical period of sheep peripartum. However, the indiscriminate use of drugs favors the development of resistance by helminths. Monepantel belongs to the new class of aminoacetonitrile derivatives and is indicated for the treatment and control of multiresistant GN in sheep. The objective of this study was to evaluate the influence

of the peripartum period on the helminth parasite load, the anthelmintic efficacy of Monepantel during the gestational period of the sheep and whether its use generates any influence on the development of gestation and the health of newborn lambs. Thirty-two pregnant sheep were divided into two groups (G) of 16 animals each, according to egg count per gram of feces (EPG) at the 4th week of gestation (WG). G1 was treated with Monepantel at the 8th and 16th WG; G2 was untreated (control). Stool were collected for EPG at 4th, 8th, 12th, 16th and 20th WG and two weeks after lambing. At birth, all newborns were clinically examined. Monepantel did not present side effects in the gestation of the sheep, and all the offspring were born healthy. It was also verified that the first 10 days after delivery correspond to the period with the highest parasitic infection. In the present study, the treatments with interval of 63 days showed efficient reduction of EPG during peripartum. In addition, Monepantel did not cause clinical and gestational changes in the offspring, the two treatments performed at the 8th and 16th week of gestation had satisfactory efficacy in the control of GN in pregnant sheep.

PS02.85 Combination of Bioactive Phytochemicals and Synthetic Anthelmintics: In Vivo and in Vitro Assessment of the Albendazole-Thymol Association

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The search of novel strategies is an urgent need considering the widespread increasing of anthelmintic resistance in livestock. Bioactive phytochemicals may contribute to improve parasite control by enhancing the effect of existing anthelmintic drugs.

This work evaluated the in-vivo and in-vitro pharmaco-chemical interaction and the in-vivo efficacy of the combination of albendazole (ABZ) with a phenolic natural monoterpene, thymol (TML), in lambs naturally infected with resistant gastrointestinal nematodes. Thirty (30) lambs were allocated into three (3) experimental groups. Each group was treated orally with either ABZ (5 mg/kg), Thymol (150 mg/kg, twice every 24 h) or the co-administration of both compounds. Blood samples were collected between 0 and 51 h post-treatment and TML, ABZ and its metabolites were determined by HPLC. Individual faecal samples were collected at days -1 and 14 post-treatment to perform the faecal eggs count reduction test. Additionally, the effect of TML on the metabolic sulphoreduction and the sulphonation of ABZ sulphoxide was in-vitro assessed using ruminal content and liver microsomes, respectively. No changes on the pharmacokinetic behavior of ABZ sulphoxide were observed in the presence of the natural product (TML). In contrast, the ABZ sulphone Cmax and AUC were lower ($p < 0.05$) in the co-administered animals ($0.16 \pm 0.07 \mu\text{g/mL}$ y $3.63 \pm 1.21 \mu\text{g.h/mL}$) compared with those that received ABZ alone ($0.45 \pm 0.15 \mu\text{g/mL}$ and $9.50 \pm 2.84 \mu\text{g.h/mL}$). TML was detected in the bloodstream between 1 and 51 h post-treatment, which indicates the time of target nematodes exposure to the bioactive monoterpene. However, the in-vivo efficacy of TML was 0% and the presence of TML did not increase the efficacy of ABZ. The presence of TML inhibited significantly ($P < 0.05$) the ruminal sulphoreduction and the hepatic sulphonation of ABZ sulphoxide. In-vivo pharmaco-parasitological studies are relevant to corroborate the adverse kinetic/metabolic interactions and the efficacy of bioactive natural products combined with synthetic anthelmintics.

PS02.86 Evaluation of the Ethnoveterinary Potential of *Picralima Nitida* (Apocynaceae) as an Anthelmintic

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Aim of the study: The study evaluated the potentials of *P. nitida* as an anthelmintic. Materials and methods: Up and down method of acute toxicity was used to determine the acute toxicity of the ethanol seed extract of *Picralima nitida* (ESEPN) up to 2,000 mg/kg body weight given orally. The anthelmintic efficacy of ESEPN was investigated in vitro using the egg hatch assay and in vivo using thirty adult male albino mice randomly divided into 6 groups (A–F) of 5 mice each and experimentally infected with *Heligmosomoides bakeri* (with the exclusion of mice in Group A which was the negative control group). Graded doses of ESEPN (250mg/kg, 500mg/kg and 1,000mg/kg) and Albendazole (25mg/kg) were administered to mice in groups D – F and C respectively. The body weight, packed cell volume, erythrocyte, total leucocyte and faecal egg counts (FEC) were assessed.

Results: No death or sign of toxicity was observed in the mice following acute toxicity assay. LC50 of 0.120 and -1.557 for ESEPN and Albendazole respectively was obtained following a Probit log regression analysis of the percentage egg hatch inhibition. A pre-patent period of 7±3 days was observed. Anaemia, weight loss and leucocytosis were observed following infection. A dose dependent increase to near pre-infection values of the body weight and haematological parameters was observed following treatment with either albendazole or ESEPN. A drop in FEC was observed till days 9 and 10 post treatment for ESEPN and Albendazole respectively however; none was able to clear the helminth infection.

Conclusion: Despite promising in vitro anthelmintic activity of ESEPN, its in vivo was poor. Thus ESEPN should be exploited for other uses.

PS02.87 Emerging and Reemerging Zoonotic Parasitic Diseases of Companion Animals in India

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There are well recognised zoonotic infectious diseases transmitted from companion animals to human beings across the globe and among the various pathogens responsible for this zoonoses, parasites play an important role. Pet animals play a significant role to the social, physical, mental and emotional development of kids, and are a companion to the elderly people and these pets are almost a family member in most of the houses. Most of the cases or instances, the disease due to such zoonotic parasites goes undiagnosed or unnoticed due to lack of awareness or knowledge. The presence of the gastro intestinal parasites in pet animals leaves a major problem in transmitting the disease to human beings and cause severe morbidity. The transmission is possible to human beings by ingestion of infective stages such as oocysts, cysts, spores, ova, larva or larval stages or through consumption of raw or undercooked meat with infective larval stages in tissues. Companion animals like dogs and cats act as reservoir host for a number of pathogens of parasitic zoonoses, viz., toxoplasmosis, giardiasis, toxocarosis and ancylostomosis. Their roles in transmitting human infections have been recognized worldwide. An integrated multidisciplinary 'One Health' approach is the need of the hour to prevent transmission of zoonotic parasites, necessitating collaboration between veterinary and medical scientists, policy makers and public health officials. The interaction amongst veterinary and human medicine with clinicians, researchers, agencies and governments together will benefit domestic as well as wild animals, human health and the global environment in general.

PS02.88 Plasmodium Knowlesi and Other Malaria Parasites in Long-Tailed Macaques from the Philippines

Mr. Lief Erikson Gamalo¹, Dr. Judeline Dimalibot¹, Ms. Khamisah Abdul Kadir², Dr. Vachel Gay Paller¹, **Prof. Balbir Singh²**

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Macaca fascicularis (long-tailed macaque) is the most common species of macaque in Southeast Asia and the only species of monkey found naturally in the Philippines. The species is the natural host for the zoonotic malaria parasites, *Plasmodium knowlesi* and *P. cynomolgi* and for the potentially zoonotic parasite, *P. inui*. Other *Plasmodium* species such as *P. coatneyi*, *P. simiovale* and *P. fieldi* are also natural parasites of *M. fascicularis*. The aims of this study were to identify and determine the prevalence of *Plasmodium* species infecting wild and captive long-tailed macaques from the Philippines. A total of 95 blood samples from long-tailed macaques were collected from three locations; 30 at the National Wildlife Rescue and Rehabilitation Center (NWRRC) in Luzon, 25 at the Palawan Wildlife Rescue and Conservation Center (PWRCC) in Palawan and 40 from Puerto Princesa Subterranean River National Park (PPSRNP) in Palawan. The *Plasmodium* spp. infecting the macaques were identified using species-specific nested PCR assays on DNA extracted from these blood samples. All 40 of the wild macaques from PPSRNP and 5 of 25 captive macaques from PWRCC were *Plasmodium*-positive, while none of the 30 captive macaques from the NWRRC had any malaria parasites. Overall, *P. inui* was the most prevalent malaria parasite (44.2%), followed by *P. fieldi* (41.1%), *P. cynomolgi* (23.2%), *P. coatneyi* (21.1%), and *P. knowlesi* (19%). Mixed species infections were also observed in 39 of the 45 *Plasmodium*-positive macaques. Wild long-tailed macaques from the island of Palawan, the Philippines are infected with *P. knowlesi*, *P. inui*, *P. coatneyi*, *P. fieldi* and *P. cynomolgi*. The presence of these simian *Plasmodium* parasites, especially *P. knowlesi* and *P. cynomolgi* in the long-tailed macaques in Palawan presents risks for zoonotic transmission in the area.

Poster Session 03

July 10, 2019, 10:00 – 16:30

Exhibit Hall A, Level 1

PS03.01 Treatment of *Tritrichomonas Foetus* in a Cat Colony with Pre-existing Neurological Disorders

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Aim of the study was to eliminate the diarrheal agent *Tritrichomonas foetus* from a cat colony with co-existing morbidity. Seventeen Maine-Coon cats were confiscated for animal welfare reasons and kept in an animal shelter. Six cats showed neurological disorders before treatment. Liquid diarrhea was observed in three cats, soft faeces in four. Fourteen cats were tested positive for *T. foetus* by PCR. All cats were treated with ronidazol (Ridzol®, 30 mg/kg PO q24h) for 14 days. Two weeks after treatment *T. foetus* could not be detected by PCR anymore; however, three to five weeks later three cats were again positive for *T. foetus*. The success rate for elimination of the pathogen within the observation period of 8 weeks post treatment was 79%. Liquid diarrhea could be controlled in all three cats. During treatment nine cats showed neurological disorders at least once. In five of cats that showed neurological disorders before treatment a minor impairment of clinical signs (coordination problems, head tilt) was observed. As neurological signs occurring during treatment were similar to the clinical signs observed before treatment it could not be determined if these were side effects of the ronidazol therapy. Three cats showed neurological disorders for the first time during treatment (slight head tremor and slight ataxia). In conclusion, due to possible side effects of ronidazol in cats a treatment of feline

tritrichomonosis should always be decided on a risk-benefit assessment. Nonetheless, systemically ill animals should not generally be excluded from treatment, especially when they are living in animal shelters where a good health status is a prerequisite for adoption. Because the affected cats were all closely related, we hypothesize that a high coefficient of genetic relation might be a significant risk factor for the development of tritrichomonosis in cats.

PS03.02 Efficacy of a New 0.5% Eprinomectin, 5% Diflubenzuron-based Pour-On Formulation against Gastro-Intestinal Nematodes in Cattle Population under Field Conditions in Brazil

Dr. Fabio Barbour Scott², Dr. Gabriela Ferreira de Oliveira², Dr. Barbara Rauta de Avelar², Márcia Cristina Alves Teixeira³, Dr. Marcio Dentello Lustoza³, Dr. José Manuel Moreno Montroy⁴, Dr. Emmanuel Briant¹, Dr. Christelle Navarro¹, **Dr. Vanessa Chala¹**

¹Virbac, Carros, France, ²Universidade Federal Rural do Rio de Janeiro, Seropédica, Brasil,

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Gastro-intestinal nematodes (Trichostrongylidae) are the most frequently observed parasites in grazing cattle worldwide causing digestive disorders, anemia, delays in growth and production. The objective of this study was to test the efficacy of a new 0.5% Eprinomectin, 5% Diflubenzuron-based pour-on formulation on the elimination of a natural gastro-intestinal nematodes infestation in a Brazilian cattle population.

For this study, 20 male or female cross-breed animals *B. indicus* x *B. taurus*, 8 to 24 months old, 100-260 kg live weight, were selected from the experimental herd of Universidade Federal Rural do Rio de Janeiro. Animals were kept on pastures, without parasiticide treatment and were naturally infested by gastro-intestinal nematodes. On D0, these animals were randomized to the control group (C, n=10) or the treatment group (T, n=10) according to the average EPG counts performed on D-7, D-2. On D0, 1 mL/10kg/

Bw of this new formulation containing 0.5% Eprinomectin, 5% Diflubenzuron (Virbac) were poured on the back of group T animals, from the base of their horns to the base of their tails. EPG counts and coprocultures were performed on days D-7, D-2, D+7, D+14, D+21, D+28 and D+ 35.

On D-7, D-2 the average EPG counts in arithmetic mean were 695 and 790 in groups C and T, respectively. Coprocultures at D-7 included *Cooperia* spp (38%), *Haemonchus* spp (32%), *Oesophagostomum* spp (27%), *Trichostrongylus* spp (3%), respectively. In group T, EPG counts decreased significantly ($p < 0.05$) in comparison with group C for all time points from D+7 until D+35, and, in arithmetic means by 91.2%, 95.60%, 89.5%, 89.5 and 74.5%, respectively.

In this study, the efficacy of this new pour-on formulation was demonstrated by the reduction of EPG counts greater than 80 % until 28 days after treatment in Brazilian cattle naturally infested by gastro-intestinal nematodes.

PS03.03 Toxoplasma Gondii and Besnoitia Besnoiti Infections Differentially Modulate Host Cell Cycle Progression in Primary Endothelial Host Cells

Dr. Zahady Velasquez¹, Dr. Ivan Conejeros¹, Dr. Katharina Kerner², **Prof. Carlos Hermosilla¹**, Prof. Anja Taubert¹

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²Institute of Hygiene and Infectious Diseases of Animals, Justus Liebig University Giessen, Giessen, Germany

Background: *Toxoplasma gondii* was described to modulate the host cellular cell cycle and to dampen host cell replication, while no respective data are available on *Besnoitia besnoiti*. However, *T. gondii*-related data are inconsistent since some authors detected a *T. gondii*-induced cell cycle arrest in the G0/G1 phase whilst others report on a G2/M-based cell cycle arrest in infected cells. Objective: We here analyzed effects of *T. gondii* and *B. besnoiti* infections on host cell cycle progression in primary endothelial cells.

Methods: Proliferation of infected and non-infected bovine umbilical vein endothelial cells was estimated microscopically. Cell cycle phases (G0/G1-, G2/M-, S-phase) were analyzed by a FACS analysis of cellular DNA content. Cell cycle-specific key regulatory proteins (cyclin A2, cyclin B1) and histone H3 S10 as a marker of mitosis progression were analyzed via Immunoblotting.

Results: *T. gondii*-infections induced enhancement of host cellular proliferation when compared to non-infected controls. In contrast, *B. besnoiti* infections had no effect on host cell division. Referring to their impact on cell cycle phases, *T. gondii* induced a G2/M phase arrest in infected cells with impairment of host cellular cytokinesis while *B. besnoiti* seemed to arrest cell cycle progression in G0/G1 phase. *B. besnoiti* infections caused a decrease of cyclin A2 and of cyclin B1 phosphorylation whilst in *T. gondii*-infected BUVEC no respective alterations were found. In contrast to *B. besnoiti*, confocal microscopic analyses of the different mitosis phases revealed that chromosome segregation in *T. gondii*-infected cells was significantly impaired in infected cells, which occasionally presented more than two poles of cell division. Thus, *T. gondii* impedes correct mitosis process and subsequent host cell division.

Conclusion: The current data show that *T. gondii* and *B. besnoiti* differentially interfere with cell cycle machinery of primary endothelial host cells and thus use different strategies of host cell modulation.

PS03.04 Bovine Neutrophil Extracellular Traps Induce Damage of *Besnoitia besnoiti*-Infected Host Endothelial Cells but Fail to Affect Total Parasite Proliferation

Dr. Ivan Conejeros¹, Dr. Zahady D. Velasquez, Dr. Ershun Zhou, Prof. Anja Taubert, **Prof. Carlos Hermosilla**

¹*Justus Liebig University Giessen, Germany*

Abstract: *Besnoitia besnoiti* tachyzoites infect and develop in bovine endothelial cells in vivo and triggers the release of neutrophil extracellular traps (NETs) from

bovine polymorphonuclear neutrophils (PMN). The purpose of this study was to analyze if pure *B. besnoiti* tachyzoite- or calcium ionophore (A23187)-triggered NETs would damage endothelial host cells and subsequently influence intracellular development and proliferation of *B. besnoiti* tachyzoites in primary bovine endothelial cells. Thus, we here evaluated endothelial host cell damage triggered by histone 2A (H2A) and *B. besnoitia* tachyzoite-induced NET preparations and furthermore estimated the effects of PMN floating over *B. besnoitia*-infected endothelium under physiological flow conditions on endothelial host cell viability. Overall, all treatments (H2A, *B. besnoiti*-triggered NETs and floating PMN) significantly induced endothelial cell death of *B. besnoiti*-infected host cells. However, thought host cell damage led to significantly altered intracellular parasite development with respect to meront diameter and numbers, the total proliferation of the parasite over time was not significantly affected by these treatments thereby denying any direct effect of NETs on intracellular *B. besnoiti* replication.

PS03.05 Strategic Control of Gastrointestinal Nematodes in Beef Cattle in Brazil

Fernando Borges¹, Dyego Borges¹, Mariana Freitas¹, Flávia Fávero¹, Elvis Coelho Filho¹, Jonis Genro², Welton Cabral³, **Elio Moro³**, Frederico Moreira⁴

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The aim of this study was to compare two protocols for strategic control of gastrointestinal nematodes in beef cattle in Brazil. Sixty Nelore weaned calves were distributed in three groups, based on the number of fecal egg counts (FECs) and live weight: T1 Placebo, T2 May – moxidectin 500 µg/Kg, November – doramectin 700 µg/Kg, and T3 May – doramectin 700 µg/Kg, August – moxidectin 200 µg/Kg, November – doramectin 700 µg/Kg. Animals were kept in exclusive paddocks with an area repetition. Individual weighing and faecal collections were performed every 28 days from d0

(May/2017) to d313 (April/2018) after a 12 h fastening. At the end of the study, calves from T2 and T3 were 11.3 and 17.3 kg heavier than those of T1 ($P < 0.05$) respectively, but there was no difference between T2 and T3. Two peaks of FECs were observed in untreated animals: the first in May and June and the second from October to December, with a predominance of *Haemonchus* sp. and *Cooperia* sp. The treatment with long-acting moxidectin resulted in a significant reduction of FEC in June (d 28) and maintained FEC reduced until September (d 112), whereas this pattern was not observed in T3. The treatment with a long acting formulation of moxidectin in T2 (May) had the same effect on performance than using the first two formulations in T3 (May and August). Both T2 and T3 protocols can be used depending on the level of resistance and the availability of handling the animals two or three times.

PS03.06 Development of a Natural Host Model for *Tritrichomonas Foetus*

Dr. Katy Martin¹, Dr. Jeba R J Jesudoss Chelladurai¹, Dr. Matt Brewer¹
¹*Iowa State University, Ames, United States*

Tritrichomonas foetus is a sexually-transmitted protozoan parasite and the cause of bovine trichomoniasis. Infection with *T. foetus* results primarily in early embryonic death, which in turn causes significant economic losses for producers. There are currently no approved treatments for bovine trichomoniasis in the United States. Due to the economic impact of the disease, infected animals are generally culled to prevent further transmission of the disease.

Typically, only adult bulls maintain infection with *T. foetus*. This has contributed to significant challenges in the study of bovine trichomoniasis, due to the cost and potential safety concerns of maintaining bulls in a research setting. We hypothesize that developing a calf infection model for the study of bovine trichomoniasis will allow for advancements in diagnostic tests, treatment options, and prevention strategies for the disease.

In the present study, we describe experimental infection in bull and heifer calves with *T. foetus* trophozoites. We discuss culture and PCR findings following infection and describe the distribution of parasites in host tissues. Our results provide a framework for future studies regarding diagnostic and treatment options for bovine trichomoniasis.

PS03.07 Mini-FLOTAC Automated System

Alessandra Amadesi¹, MP Maurelli¹, Antonio Bosco¹, Davide Ianniello¹, ME Morgoglione¹, Mirella Santaniello¹, **Laura Rinaldi**¹, Giuseppe Cringoli¹

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In an era of technological revolutions in the diagnostic industries, also diagnostic methods for parasitic helminth infections are evolving, for instance through the use of Mini-FLOTAC techniques, the use of pooled samples, point-of-care diagnosis and automation of faecal egg count (FEC) and FEC reduction (FECR) tests for assessing helminth infections and anthelmintic resistance.

The aim of this study was to design and test a prototype of the Mini-FLOTAC automated system that allows a rapid laboratory workup and can be used directly on livestock farms for automated FEC/FECR including image software analysis.

The scanning device for the Mini-FLOTAC was designed as an equivalent of a XYZ motorized microscopy stage that is automatically moved step by step to scan the two entire flotation chambers by taking partially overlapping pictures. The movement of the stage is controlled via software by a main processing unit, which can be either a PC, a Tablet or a Smartphone. The development of an image-analysis software is able to identify and count helminth eggs in order to reduce the time required for the analysis and the human errors. Furthermore, the development of an automated software permits to transmit collected data via internet to expert diagnostic centers.

The use on farm of this simple, automated system will allow a rapid assessment of FEC/FECRT in large and small ruminants to assist the new generation of veterinarians and farmers.

PS03.08 Palatability and Safety of a Soft Chewable Tablet Containing Ivermectin, Praziquantel and Pyrantel Pamoate in Client-Owned Adult Dogs

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Palatability and safety of heartworm preventative treatments are crucial to ensure good compliance.

An open-label field study was conducted at five veterinary clinics in Texas to determine the palatability and safety of a new soft chewable tablet (Iverhart Max soft chew, Virbac) under conditions of field use. There were 132 dogs enrolled in the study (including 14 dogs from breeds known to bear the MDR1 mutation allele), who received each 3 treatments at monthly intervals. The number of doses taken voluntarily (offered by hand or in a bowl with or without food) was evaluated to determine the palatability of the bacon-flavored chews. The reported adverse events were analysed to establish the safety profile of the product. The incidence of vomiting, choking, obstruction and other gastrointestinal incidents were closely evaluated to address any safety concerns regarding dosing with the chew.

Three hundred and eighty-nine dosing events occurred during the study: 336 doses were accepted as a treat (by hand or in empty bowl) (86.3%), 39 in a bowl with food (10%) and 11 had to be pill and placed in the mouth (3%). Three dogs (0.7%) could not be successfully dosed with this dosage form. The adverse events reported by the owner were diverse (vomiting (2.3%), diarrhea or loose stool (1.2%), increased salivation (0.7%)), generally required little treatment or follow-up and were self-limiting. Overall the incidence of potential gastrointestinal

side effects was 5%. Obstruction and choking were not observed or reported during this study. Two serious adverse events (blindness and renal failure) were reported during this study and were most likely due to factors other than treatment administration (genetic predisposition and long term exposure to non-steroidal anti-inflammatory drugs).

The product was found to be well accepted (96.3% voluntary acceptance) and safe (low incidence of self-limiting side effects reported) during this trial.

PS03.09 Quantitative Proteomics Analysis of Angiostrongylus Vasorum-Induced Alterations in Dog Serum

Dr. Lucienne Tritten¹, Dr. Tobias Kockmann Kockmann², Dr. med. vet. Nina Gillis-Germitsch¹, Prof. Manuela Schnyder¹

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Blood contains hundreds of proteins, reflecting ongoing cellular processes and immune reactions. Infection with *Angiostrongylus vasorum* in dogs is associated with a perturbed blood protein profile, but existing reports lack the necessary depth of analysis to resolve the observed pathologies in *A. vasorum* infections, including bleeding disorders.

Serum proteins from eight experimentally-infected dogs (i) before inoculation with *A. vasorum*, (ii) 34 days post-inoculation (p.i.; immature infection), and (iii) 75 days p.i. (mature patent infection), were analyzed using liquid chromatography and tandem mass spectrometry (LC-MS/MS). Sera from two dogs were additionally examined at days 104 and 230 p.i.. A data-independent acquisition workflow was employed in order to generate quantitative data. Computational analysis revealed 139 up- and down-regulated proteins following infection (log2 ratio cutoff ≥ 1.0 ; q-value ≤ 0.05).

Differences in serum profiles were most pronounced at day 75 p.i. when compared to before inoculation. Among up-regulated

proteins, chitinase 3, several saposin-like proteins and heat shock proteins were greatly increased ($\log_2$ fold-changes ≥ 5). Levels of pulmonary surfactant protein B were already elevated at day 34 p.i. in the prepatent phase. Pathway enrichment analyses revealed that complement (especially the lectin pathway) and coagulation cascades as significantly affected upon analysis of down-regulated proteins. Among them there were mannan-binding lectin serine peptidases, ficolin, and coagulation factors.

These results reflect the ongoing immune response and stress imposed to the lungs by the parasite. In addition, they add new elements towards understanding the coagulopathies observed in *A. vasorum*-infected dogs.

PS03.10 Prevalence of Intestinal Parasites and Heartworm in Northeastern Oklahoma Shelter Dogs

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Intestinal parasites, including several zoonotic agents, and heartworm (*Dirofilaria immitis*) are commonly detected in shelter dogs due to inconsistent treatment and frequent exposure. To determine the prevalence of intestinal parasites in fecal samples and evaluate interactions between age, sex, and infection status, semi-quantitative centrifugal flotation in sugar solution (SG 1.25) was performed on samples (4g) from 218 dogs with results evaluated by chi-square ($P < 0.05$). Flotation revealed parasites in 173/218 (79.4%, 95% CI 73.5–84.2%) samples, including *Ancylostoma caninum* (118; 54.1%), *Trichuris vulpis* (73; 33.5%), *Toxocara canis* (21; 9.6%), *Cystoisospora* spp. (43; 19.7%), *Giardia* sp. (41; 18.8%), *Cryptosporidium* sp. (12; 5.5%), and *Sarcocystis* sp. (4; 1.8%); 1 sample each (0.5%) contained *Demodex* sp., *Dipylidium caninum*, or *Taenia* sp. Dogs < 1 year of age were more likely to be shedding *Giardia* sp. ($P < 0.01$) and *Cystoisospora* spp. ($P = 0.03$)

than adult dogs, while adult dogs were more likely infected with *T. vulpis* ($P < 0.0001$); egg/cyst/oocyst per gram estimates for positive samples were not significantly different by age class ($P > 0.05$). Infection with *T. vulpis* was significantly more common ($P < 0.0001$) in dogs also infected with *A. caninum*. Antigen testing for *D. immitis* was performed on 157 dogs (≥ 5 months), 99 with fecal flotation, and modified Knott's test to detect microfilariae in blood. Heartworm antigen was detected in 13 (8.3%) before and 16 (10.2%) after heat-treatment; microfilariae were detected in 9 (4.5%), including 7 dogs with *D. immitis* and 2 with *Acanthocheilonema reconditum*. Two dogs with microfilariae of *D. immitis* were antigen negative before but positive after heat treatment of serum. Heartworm infection was not found to be significantly associated with age or detection of any parasites by fecal flotation ($P > 0.05$). Intestinal parasites and heartworm remain common in shelter dogs, with younger dogs particularly likely to be shedding protozoa and adult dogs more likely infected with *T. vulpis*.

PS03.11 Surveillance of Parascaris Equorum Infections and the Efficacy of Fenbendazole in Horses Across the United States

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Parascaris equorum is the most significant internal parasite pathogen of foals, presenting as respiratory disease as the juvenile ascarids migrate through the lung, and as gastrointestinal disease as the adult parasites remove nutrients or large populations cause impaction colic or intussusception. The current study enrolled 410 horses on 27 farms (197 weanlings; 213 horses > 1 year old) from across the United States, to determine the ascarid burdens by fecal egg count (FEC), as well as the efficacy of fenbendazole as judged by fecal egg count reduction test (FECRT) from horses with any *P. equorum* eggs on the first FEC. Horse owners were also asked to fill out a survey on their normal

deworming and management strategies. All horses under 1 year old received one dose of fenbendazole (10 mg/kg), and the horses > 1 year of age received 5 consecutive days of treatment of fenbendazole at 10 mg/kg. Of the 130 horses that had an ascarid-positive fecal, 107 of them were weanlings (p-value < 0.00001), and average ascarid FEC for those horses was 174 EPG (Range: 1 – 6,768). The overall efficacy of fenbendazole against *P. equorum* was very high with a FECRT of 98.3% (Range: 83.0 – 100.0%). Because of the high efficacy, there were no significant differences in egg reductions between age of animal, deworming strategy, or even between farms. Additionally, there were no management practices that significantly changed the pre-treatment FEC or reduction of ascarids egg shedding. Infections with *P. equorum* continue to be a significant problem for horses under the age of one, but fenbendazole remains a highly effective dewormer for controlling those infections. Overall, this study reiterates the need for a tailored deworming strategy based on the horse's age and other risk factors, incorporating fecal egg counts into the program for assessing the deworming efficacy.

PS03.12 Coprological Prevalence and Intensity of Helminth Infections In Polo and Work Horses (*Equus caballus*) In Abuja, Federal Capital Territory (FCT), Nigeria

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This study aimed to estimate the prevalence and intensity of helminth infection of horses (*Equus caballus*) in the Federal Capital Territory (FCT), Abuja, Nigeria and investigate the associations between infections and horses age, sex and owner-reported use of anthelmintics. In a cross-sectional survey, fresh faecal samples were obtained from 103 horses in nine randomly selected horse stables in three Area Councils of the FCT, viz: Abuja Municipal Area Council (AMAC), Gwagwalada and Kuje, and worm egg counts performed at the parasitology laboratory, University of Abuja using the direct faecal smear, floatation method, sedimentation

method and the Modified McMaster technique. Details of anthelmintic use were collected using a standardized face-to-face owner/horse-keeper questionnaire. The Student's T-test of independence was used to investigate the association between exposure variables and infection status/intensity. The following helminthes were identified in the study: *Parascaris* spp.(51.97%), *Dictyocaulus* spp.(17.32%), *Strongylus* spp.(14.96%), *Oxyuris* spp.(7.8%), *Strongyloides* spp.(2.36%), *Gastrodiscus* spp.(3.15%), *Anoplocephala* spp.(0.79%), *Habronema* spp.(0.79%), and *Trichonema* spp.(0.79%). Of the 103 samples examined, 87 (84.47%) were positive for helminthes ova while 16 (15.53%) were negative. *Parascaris equorum* was the most prevalent helminth among the sampled horses, followed by *Dictyocaulus arnfieldi* and then *Strongylus* spp. The class Nematoda has the highest percentage of infection (94.18%), Trematoda (1.16%), Cestoda (1.16%) and mixed infections (3.48%). Eggs of *Strongylides* spp were not seen. Intensities of helminth infection was found to decrease with horses age and use of proprietary equine anthelmintic products. There was no significant variation in the mean intensities of infection with respect to the location of stables as well as the sex of the animals sampled. In conclusion, strongyle infection is endemic in the FCT but equine anthelmintics assists in managing the infection. Recommendations made include the need to institute wholistic parasite control measures involving strategic deworming programme for stabled horses.

PS03.13 Coproscopical, Antibody-Based and Molecular Survey on Equine Helminth Infections on Horse Farms in Berlin/ Brandenburg, Germany

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Knowledge concerning the current prevalence

of helminth infections in horses is sparse, while at the same time this information is urgently needed to guide new worm control approaches with less routine anthelmintic treatments. This study aimed at providing representative data on the occurrence of the major groups of intestinal helminths on horse farms in Berlin and Brandenburg. From a total of 484 horses on 48 farms faecal, saliva and serum samples were collected from May 2017 to January 2018. Faecal samples were analysed using mini-FLOTAC technique and combined sedimentation-flotation. Additionally, PCR on DNA from strongyle larvae was performed to investigate the potential presence of *Strongylus* spp. The latter was also approached by testing serum samples for antibodies against *Strongylus vulgaris* (rSvSXP-Antigen). Serum (ADB IgG(T) serologic ELISA) and saliva (EquiSal® Tapeworm test) were examined for antibodies against *Anoplocephala*. In 66.9% of the faecal samples, eggs of strongyles were found (64.2% in mini-FLOTAC technique, 57.6% in combined sedimentation-flotation). Additionally, 1.2%, 0.6% and 0.4% of faecal samples were positive for *Oxyuris equi*, *Anoplocephala* spp. and *Parascaris* spp. eggs, respectively. Out of the 365 horses examined using the *Anoplocephala* saliva test, 29.6% were positive on 28 out of 37 (75.7%) farms, while of 481 serum samples 16.2% were positive on 25 out of 48 farms (52.1%). Regarding *S. vulgaris*, seropositivity was observed for 21.2% of 481 samples corresponding to 83.3% farm prevalence, while in 13 of the 484 examined faecal samples (2.7%) from 6 farms (12.5%) *Strongylus* spp. DNA was detected. The unexpectedly high (sero-) prevalence data encountered for both tapeworm and large strongyle infections point at an urgent need for routine parasite surveillance. Due to its high pathogenicity the exceptionally high seroprevalence of *S. vulgaris* is alarming and requires further attention, particularly in the context of 'targeted selective treatment'.

PS03.14 Anticoccidial Efficacy of Naringenin and a Grapefruit Peel Extract in Naturally-Infected Growing Lambs with *Eimeria* Spp.

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The current study aimed to determine the anti-*Eimeria* efficacy of an extract of grapefruit peels (GF) and commercial naringenin (NAR) in naturally-infected lambs, as well as the influence of these flavonoids on the oxidative status. Pharmacokinetic profiles were also determined. Extracts were administered per os to *Eimeria* naturally infected growing lambs during 90 consecutive days. The commercial anticoccidial drug toltrazuril (TTZ) was included in this trial as a standard. Twenty-four lambs were divided into four groups: NAR, lambs given a daily dose of 5 mg of a commercial naringenin extract of 98% higher purity per kg body weight; GF, lambs that received a daily dose of 5 mg of ethanolic extract of grapefruit peels per kg body weight; TTZ, lambs treated with 20 mg of toltrazuril/kg body weight on days 0 and 15 of the experiment; and CTRL, untreated lambs that received daily dose of 30 ml of water. Daily doses of GF and NAR were dissolved in 30 ml of water. The CTRL group received 30 ml of water; as well as the TTZ group for the period after the single dose administration. Fecal and serum samples were collected from all lambs. Anticoccidial efficacy was estimated by coprological techniques. Generation of nitric oxide levels and the antioxidant capacity of the experimental compounds were determined by the Griess and ABTS assays, respectively. On day 30 post-ingestion, anticoccidial efficacy was 91.76% (NAR) and 89.65% (GF); whereas 99.63% of efficacy was achieved with TTZ 15 days after treatment. NAR, GF and TTZ significantly reduced oxidative stress in infected animals. Following the oral administration of NAR and GF, values in plasma approached maximum concentrations within 2.1 to 2.5 h. In conclusion, the administration of NAR and the GF extract reduced *Eimeria* oocyst output and oxidative stress in infected lambs.

PS03.15 Mechanism and the Effects of Urea on the Development and Hatching of *Trichostrongylus Colubriformis* Eggs

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Interrupting the parasite lifecycle outside of the host may provide novel methods of control that complement the use of anthelmintic drugs. Topical application of Urea can interrupt nematode parasite egg hatching, although the mechanisms are still to be determined and are the objective of this study. Firstly, the role of osmolality was investigated using egg hatch assays with either Urea, NaCl, Glucose or CaCl₂ solutions at an osmolality ranging between 0 and 1500 mOsmoles per l. All solutions reduced percentage egg hatch from in excess of 85% in water to less than 3% at 1500 mOsmoles. However, there was a solution x concentration interaction whereby the lethal concentration (LC) 50 was 792 ± 32.1 , 486 ± 41.2 , 498 ± 36.5 and 299 ± 36.8 mOsmoles per l for Urea, NaCl, glucose and CaCl₂, respectively, being least ($P < 0.05$) in CaCl₂, not different ($P > 0.05$) between NaCl and Glucose ($P > 0.05$) and greatest in Urea ($P < 0.05$). As the osmolality of solutions increased the egg hatching percentage decreased and differences in egg hatching percentage between solutions at the same osmolality indicates that this effect is not solely due to osmolality. The second study examined the penetration of Urea and NaCl when applied to faeces. Following topical application, faeces were left for 24h before samples measuring 1cm x 1cm were sectioned throughout the faecal pat and placed in mini-bearman apparatus. The number of larvae recovered from either NaCl or Urea treated faeces were reduced relative to water controls in the first 1 cm by 64% and by 34% in the second 1 cm cube from the surface only. Results indicate the mechanism of Urea interrupting egg hatching is not solely due to osmolality and when Urea is topically applied to faeces interrupts the egg development in the first 2cm of faeces nearest to the surface.

PS03.16 Anthelmintic Efficacy and Cytotoxicity of Diospyros Whyteana Compounds Derived Using Antifungal Activity

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Gastrointestinal (GI) nematode infections are one of the major health challenges affecting performance of small stock. Furthermore, animals have developed resistance against the use of clinical drugs, and therefore, this led to the utilisation of alternative products. Hence, the aim of this study was to investigate if antifungal assays can be used as a model to isolate anthelmintic plant compounds, based on potential correlations in the two activities. Anthelmintic egg hatch assay (EHA) and the larval development assay methods were used. Bioactivity of the fractions as well as cytotoxicity (IC₅₀, tested using four human cells in the colorimetric MTT assay) was assessed during fractionation, which resulted in isolation of three active compounds. The *Diospyros whyteana* acetone leaf extract had good activity with EC₅₀ values of 4.43 and 5.58 µg/ml respectively. The extract was active against *Candida albicans* with MIC of 40 µg/ml, and this organism was used as a model to isolate antifungal compounds from *D. whyteana*. EC₅₀ values of the compounds ranged from 0.11 to 3.30 in the EHA and 1.5 to 4.35 in the LDA, with promising selectivity index (SI) values above 28. The three compounds had the same MIC of 160 µg/ml against *C. albicans*. Conclusions: Results showed good correlation between the EHA and LDA as well as good antifungal activity, indicating that the use of antifungal assay assisted with the isolation of anthelmintic compounds. Consequently, α-amyrin and β-amyrin were isolated and structures elucidation were also identified.

PS03.17 Identification and Molecular Characterization of Exosome-Like Vesicles Derived from *Taenia Asiatica* Adult Worms

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T. asiatica is one of the most important food-borne parasites and poses a great threat to human beings. So far little is known about the specific infection and immune escape mechanisms of *T. asiatica*. Recently, exosome-like vesicles have been emerging as a regulator in the interactions between parasites and hosts, providing a new direction for research on infection of *T. asiatica*. Here, exosome-like vesicles were harvested from the excretory/secretory products of cultured *T. asiatica* and isolated by differential centrifugation. The purified vesicles, ranging from 30 to 150 nm in size, were identified as exosome-like vesicles by transmission electron microscope and nanoparticle tracking analysis. Proteomic analysis showed that a total of 464 proteins were identified in the exosome-like vesicles (455 derived from *T. asiatica* and 9 derived from the human). Of these proteins, enzymes involved in metabolic processes, such as glyceraldehyde 3 phosphate dehydrogenase, fructose 1, 6 biphosphate aldolase, cytosolic malate dehydrogenase, enolase and so on, were enriched. The abundant proteins from proteomic analysis, 14-3-3 and enolase, were present in the exosome-like vesicles as shown by immunogold labeling. In addition, we found 20 known miRNAs present in *T. asiatica* sRNA libraries. MiR-71 was the most abundant, followed by let-7 and tas-miR-4989. Also, some other common miRNAs, such as miR-1, miR-9 and miR-10 were also found in the vesicles. We further validated the expression of *T. asiatica* miRNAs, including 6 known miRNAs (tas-miR-71, tas-miR-1, tas-miR-7, tas-miR-9, tas-miR-10, tas-let-7) and 3 novel miRNAs (tas-m0022-3p, tas-m0816-3p, tas-m0082-5p). The nine miRNA abundance were consistent with high-throughput sequencing results. Additionally, we demonstrated that the exosome-like vesicles experimentally labeled with PKH67 were internalized by

LoVo cells in vitro. These findings provide new insights into the interaction between tapeworms and host mediated by exosome-like vesicles.

PS03.18 Efficacy of a Permethrin and Fipronil Combination (Effitix® Spot-On), Against Flea Infestations in Dogs: A Randomized, Controlled Study

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Flea infestations can seriously impair dog's health through parasites they can transmit as *Dipylidium caninum*, or allergic conditions they can arouse (as Flea Bite Allergy)(1) or may worsen (as Atopy)(2). Thus an adapted flea control remains mandatory.

This study evaluates the efficacy of a Permethrin (44.88%) plus Fipronil (6.01%) based spot-on (Effitix®, Virbac, Brasil) against fleas infestations, compared to a reference product (Frontline® top spot, fipronil 10 %, Boehringer, Brasil) in adult dogs. Eighteen healthy Beagle dogs were equally randomly allocated to group 1 (Frontline®), group2 (Effitix®) or control group (no treatment). Treatment was applied once on D0, following manufacturer recommendations (at least 60mg/kg of permethrin and 6.7mg/kg for Fipronil). Dogs were infested with +/- 100 fleas on D-2, D5, D12, D19, D26, D33, D40, D47, and D54. Flea counts were performed accordingly on D2 and then 48 hours following other flea challenges.

The mean geometrical efficacies against a preexisting infestation were very high as soon as D2 (99.02% in group 1 vs 100 % in group 2), as well as the prevention of new infestations (100 % on D7, D21 and D28 for both groups, 99.76% vs 100% on D14, 98.45% vs 98.33%

on D35 and 95.42% vs 96.96% on D42 for group 1 and 2 respectively, with no statistical differences ($p < 0.05$).

This trial allows to confirm the rapid onset of action of the permethrin-fipronil combination, regarding a preexisting infestation, with no living fleas as soon as 2 days after product application. This spot-on offers beside a very good protection against fleas over 6 weeks.

PS03.19 Using Electropetography (EPG) to Surf the Waves of Host Choice in *Aedes Aegypti*

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Electropetography (EPG) was originally developed for use on Hemipteran phloem-feeding insects to study feeding activities, including points of pathogen acquisition and transmission. Because mosquitoes feed in a similar manner, it is possible that EPG could be used to study mosquito probing activities. Host choice is often studied in mosquitoes by observing behavior when exposed to a specific host characteristic. Using EPG, host choice could be studied via direct measurements of mosquito probing activities while taking a blood meal from a given host. EPG recordings were taken of *Aedes aegypti* females exposed to three different hosts. The hosts included one typical male, one typical female, and one atypical male. The typical hosts have a past of feeding mosquito colonies and experiencing no issues with regular feedings, including *Aedes aegypti*, and report average mosquito feeding in natural environments. The atypical host reported below average mosquito feeding in natural environments and negative impact when feeding mosquito colonies: the mosquitoes died shortly after the second feed. EPG recordings from the three hosts were collected using a completely randomized design. Present results show irregular feeding patterns in *A. aegypti* when exposed to the atypical host, such as prolonged probing time lengths and disrupted probing and digestion. The results of this study demonstrate that EPG provides quantifiable recordings of

mosquito feeding activities, as well as variation of mosquito feeding between hosts. Patterns of mosquito feeding such as points of insertion, probing, and digestion were also discernible from the recordings. From this study, EPG could likely be implemented in future research to investigate the feeding patterns of other mosquito species and pathogen acquisition and transmission.

PS03.20 Can Cell Phones Detect Mosquitoes Infected with Canine Heartworm?

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Dirofilaria immitis is a filarial nematode and the causative agent of canine heartworm. *D. immitis* can be transmitted by mosquitoes of the genera *Aedes*, *Anopheles*, *Culex*, and *Ochlerotatus*. Within the mosquito, the nematode parasite matures through three developmental larval stages (L1, L2, L3). A recent study has shown that mobile phones are capable of capturing acoustic data from mosquito wingbeats. Since each mosquito species has a different wingbeat frequency by which they attract mates, with just a brief recording ($< 1/10$ th of a second) these acoustic signatures can be analyzed to quickly determine if mosquitoes belong to a species that is known to transmit different pathogens. We examined wingbeat signatures and flight duration patterns of *D. immitis* infected and non-infected *Aedes aegypti* to determine if wingbeat mobile phone recordings can be used to distinguish infected mosquitoes from non-infected ones. Female mosquitoes were recorded prior to and at various time points after feeding on infected or non-infected dog blood by placing individual mosquitoes into a chamber and recording for 60 seconds using a standard mobile phone. To uniformly analyze audio data, recordings were processed using an in-house Python script

to determine wingbeat frequency and flight duration. One hundred sixty recordings were gathered, and mosquitoes were dissected to confirm the presence and number of heartworm larvae. Our findings indicate that L3 (infective stage) infected mosquitoes have significantly lower (mean=429 Hz) wingbeat frequencies than age-matched negative mosquitoes (mean= 577 Hz; $p<0.0001$). Flight duration based on wingbeat recordings was substantially lower (17.6% of the time) in L3 infected mosquitoes than non-blood fed mosquitoes (35.9% of the time). We present data suggesting that wingbeat frequencies may be used to identify mosquitoes infected with *D. immitis*, a pathogen of veterinary and public health concern.

PS03.21 Mining the Excretory-Secretory System of Filarial Nematodes for New Drug Targets

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Filarial nematode parasites significantly impact human (e.g. *Brugia malayi*), veterinary (e.g. *Dirofilaria immitis*), and wildlife health; but the fundamental mechanisms underlying parasitism are not fully understood. The excretory-secretory (ES) system of filarial nematodes is likely the primary conduit for the release of products essential to the establishment and maintenance of infection. There has been significant progress in cataloging the complex makeup of filarial ES products released at the host-parasite interface, and there is continued interest in resolving the role of these products (proteins, vesicles, and nucleic acids) in modulating host responses to infection. In contrast, comparatively little attention has been paid to the structure and function of the underlying ES system in filarial nematode species. We have utilized low-input and spatial transcriptomic approaches to identify gene transcripts enriched in ES-associated cells and tissue. We have used these spatial data to identify ES-enriched cell-surface receptors and to highlight transcripts with spatial expression profiles that cluster with

known drug targets and antigens. Lastly, we've performed comparative analyses of the transcriptomic state of the filarial ES system with the model nematode *Caenorhabditis elegans* to identify conserved transcripts and to elucidate differences between free-living and parasitic nematodes. We hypothesize that cell-surface targets prioritized by this effort are promising targets for the development of new anthelmintics.

PS03.22 Effects of Gastrointestinal Nematode Parasitism on Growth and Reproductive Performance in Ewe Lambs in Ontario, Canada

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Infection with gastrointestinal nematodes (GINs) is an important cause of loss of productivity on sheep farms in Ontario. Efforts to quantify the effect of GIN infection on growth have demonstrated mixed results; investigation of its impact on reproductive performance has been limited. This study evaluated the effect of GIN parasitism on growth and reproductive performance of ewe lambs under Ontario grazing conditions. Replacement Rideau cross ewe lambs ($n = 140$) on a farm in central Ontario were followed for two years (2016-2017) from nursing through their first lambing and lactation event. The lambs grazed from May to November of each year and were sampled every 6-8 weeks during both grazing seasons and once at mid-gestation in March 2017. At each sampling the ewe lambs were weighed, body condition scores assigned, serum proteins and hematocrit measured, and fecal egg counts (FECs) performed. Rainfall levels and numbers of infective larvae on pasture were unusually low during the first grazing season, but were more typical of Ontario conditions in the second grazing season. The three most common GIN species were *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus* spp. Multivariate mixed models were generated for weight change over time and litter size at lambing. Gastrointestinal nematode FEC was

not a significant predictor of weight change in replacement ewe lambs during their first two grazing seasons. However, higher FECs at lambing were associated with larger litter sizes ($p = 0.05$), likely reflecting increased periparturient egg rise in ewes with larger litters. GIN-associated morbidity was not observed during the study, indicating that infection levels may have been insufficient to negatively impact productivity. Therefore, subclinical GIN infection appears to have minimal impact on growth and reproductive performance in Ontario sheep.

PS03.23 Anthelmintic Efficacy of Nutritionally Balanced Cranberry Vine Pellet Against Strongyle Infection in Dorset Lambs

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Plants containing secondary compounds such as proanthocyanidins have been shown to suppress gastrointestinal nematode (GIN) infections, providing promise for alternative methods of GIN control in small ruminants. To date, in vivo studies from our laboratory have demonstrated a decrease in fecal egg count (FEC) after six weeks of feeding cranberry vine (CV), however palatability and nutritional quality limited consumption of the CV. The purpose of this study was to test the anthelmintic efficacy of feeding varying amounts of CV incorporated into and nutritionally equivalent to a 16% sheep pellet on lambs naturally and experimentally infected with GIN. Cranberry vine prunings were incorporated into a feed pellet containing 500g CV in 1kg. Five-month-old parasite naive lambs were turned onto pasture lightly infected with GIN. An experimental infection (5000 *H. contortus* L3) was superimposed on the natural infection after one week on pasture to ensure adequate infection. After the infection matured, lambs

were stratified by FEC, then balanced for sex, weight, and genetic variation and fed one of three levels of dietary CV ($n=7$ per group): 0 (control), 250, or 500g CV/day for 10 weeks. The lambs were fed 1kg daily using a combination of CV pellets and commercially produced sheep pellets to achieve the targeted CV consumption. Weekly weight, FEC, and packed cell volume (PCV) were determined. The FEC of lambs fed 500g CV were significantly lower than those of control lambs at week 1 ($p = 0.02$, 864 ± 205 vs 1807 ± 387 ; mean $\pm$ SEM) and week 2 ($p = 0.03$, 800 ± 273 vs 1671 ± 289). The FEC decreased in each group over the duration of the trial. There was no effect on PCV or weight gain. Further research is needed to establish the optimal time to feed CV for maximum anthelmintic effect against GIN.

PS03.24 Chromosome-Scale Assembly of *Haemonchus Contortus*, a Model Gastrointestinal Parasite

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Haemonchus contortus is an economically important gastrointestinal parasite of small ruminants and a model for anthelmintic research. Building upon the draft genome published in 2013, we have completed a chromosomal-scale assembly for the MHco3(ISE) isolate, which now represents the largest, most contiguous assembly of any parasitic nematode to date. We describe the distribution of genetic diversity throughout the genome from within and between globally distributed parasite populations, and together with a significantly improved transcriptome annotation aided by full-length cDNA sequencing, we explore coordinated gene expression throughout the life cycle.

The assembly and annotation is available on WormBase Parasite (https://parasite.wormbase.org/Haemonchus_contortus_prjeb506/Info/Index/), providing a significant genomic resource for a broad group of Clade V nematodes, which include parasitic species of major veterinary and medical importance. This removes some of the dependency on using *C. elegans* as a genome reference for which parasite-specific traits are not relevant. This chromosome-scale assembly now allows insight into chromosome evolution among these species, and offers a robust scaffold for genome-wide analyses of important parasite traits such as anthelmintic resistance.

PS03.25 Tissue-Specific Heterologous Expression of Filarial Parasite G Protein-Coupled Receptors (GPCRs) in *Caenorhabditis Elegans*

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Parasite G protein-coupled receptors (GPCRs) remain unexploited as anthelmintic targets despite their involvement in critical sensory, neuromuscular, and physiological processes. One significant bottleneck in exploring the pharmacology of parasite GPCRs results from difficulties in consistently establishing heterologous expression in single-cell systems. Yeast and mammalian cell culture systems have paved the way for deorphanization of helminth GPCRs, but not all receptors express or behave properly in cell types derived from distant phylogenetic lineages. The combinations of accessory proteins, molecular chaperones, G proteins, and membrane determinants required for the successful folding, cell-surface expression, and signaling of parasite receptors in surrogate systems have not been comprehensively identified. To avoid some of these complications, we've established new endpoints for parasite GPCR expression in the model nematode *C. elegans*. Building upon the work of others, we have expressed *Brugia malayi* aminergic receptors in the body wall muscle, pharynx, and sensory neurons of the

C. elegans N2 strain. We show that activation of parasite receptors in body wall and pharyngeal tissue can be measured in simple plate-based assays and through the use of electropharyngeogram (EPG) recording, and that activation of parasite receptors in the ASH sensory neuron can be measured through microfluidic trapping and single-neuron calcium recordings. The suitability of these approaches for a given parasite GPCR will depend on the complement of related receptors and endogenous ligands that signal in targeted tissues. We show that transgenic strains can be created in various genetic knockout backgrounds to help mitigate these concerns. While expression in scalable single-cell systems will remain an important objective for high-throughput screening (HTS) against GPCR targets, functional parasite receptor assays in a more native nematode cell and physiological environment can provide important baseline pharmacological data. Further, it is possible that these transgenic whole-organism assays can be ultimately adapted for medium or high-throughput screening.

PS03.26 A One Health Approach: Translating Livestock Parasite Research to Scan for Benzimidazole Resistance Mutations in Human Soil Transmitted Helminths

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Soil Transmitted Helminth (STH) infections are a major health concern among developing nations. Mass Drug Administration (MDA) programs that aim to reduce the morbidity associated with STH infections involve the administration of benzimidazole drugs to school age children considered to be at risk of infection in endemic regions. The increasing use of these drugs leads to a greater risk of the emergence of drug resistance which would potentially confound the aims of the MDA programs. However, the detection

of resistance in STH the field is extremely challenging and unreliable. Benzimidazole resistance is common and well characterized in parasitic nematodes of livestock and several Single Nucleotide Polymorphisms (SNPs) in the β -tubulin genes encoding the drug targets are known to be associated with resistance. We have developed a number of deep amplicon sequencing assays, using the Illumina Miseq platform, to undertake large scale scanning for these benzimidazole resistance associated SNPs in gastro-intestinal parasites of ruminants. We are now applying the same approach to investigate the potential emergence of benzimidazole resistance in the major human soil transmitted helminths (STH); *Necator americanus*, *Ancylostoma duodenale*, *Trichuris trichiuria* and *Ascaris lumbricoides*. We have combined phylogenetic and RNAseq analysis of the complete β -tubulin gene families in each of the major STH species to prioritize the genes for targeting. Primer sets have been designed to amplify fragments encompassing the relevant codons - 167, 198 and 200 - of our prioritized genes and the assays are deep amplicon sequencing assays currently being optimized. Once the assays are optimized and validated, we they will be used to screen STH populations from endemic regions, including Tanzania and Ethiopia, to detect and quantify the presence of benzimidazole resistance associated SNPs.

PS03.27 Evaluation of Biochemical Assays for the In Vitro Testing of Drug Response in the Canine Heartworm *Dirofilaria Immitis* Microfilaria

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Dirofilariosis caused by *Dirofilaria immitis* is a source of great suffering in dogs and cats in endemic regions in the United States. Resistance development to the prophylactic macrocyclic lactones (MLs) is emerging in these regions and is a cause for concern. Although bioassays and molecular markers have been developed, there are

no reliable in vitro biochemical assays to study pharmacological responses in the L3 or microfilarial stages. The currently used resistance validation methods include experimental infection establishment in dogs and microsatellite analyses, both of which are expensive and time-consuming. We report the evaluation of in vitro biochemical assays to test responses to drugs in validated resistant and susceptible isolates of *Dirofilaria immitis* microfilariae. These assays test response parameters of microfilaria such as cell membrane integrity, cellular metabolism, apoptosis, and efflux proteins to dilutions of FDA approved MLs. We discuss how the presented data can supplement the currently used resistance validation methods to identify isolates of drug resistant heartworm.

PS03.28 Effects of Alpha-Terpinene, Citronellal, Citronellol and Limonene in *Caenorhabditis Elegans* Motility Test

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Caenorhabditis elegans is a small nematode that can be maintained at low cost and handled using standard in vitro techniques. Unlike other in vitro tests, *C. elegans* motility assays provide data from a whole animal with intact and metabolically active systems. The model has the potential to inform pharmacological doses, as well as in vivo studies and also to predict active doses. Plant and our derivatives including essential oils are globally acclaimed for their medicinal and therapeutic values, especially anthelmintic effect. The present study was designed to explore the effect of different concentrations (5; 2.5; 1.25; 0.6 mg/ml) of alpha-terpinene, citronellal, citronellol and limonene on *C. elegans* motility test. For each concentration, 6 replicates were performed diluted in Tween and negative control with Tween and distilled water. Data

from the motility were analyzed by the PROC GLM procedure of SAS, which model included fixed effects of oil, concentration and interactions. The means between different oils and doses were compared by the Tukey's test ($P < 0.05$). The means of the highest concentrations (2.5 and 5 mg/mL) differed from the control. Citronellol at the lowest concentration had 100% mortality and must be evaluated in lower doses to calculate LC50. The LC50 followed by their confidence limits for citronellal were 2.48 mg/mL (2.18 ± 285), 4.16 mg/mL (3.44 ± 5.50) for limonene and 5.11 mg/mL (3.20 ± 16.39) for the alpha-terpinene. Results suggest that the anthelmintic effect of the oils on nematode *C. elegans* and the potential to be investigated on other nematodes. - FAPESP 2017/23540-1.

PS03.29 New Insights in the Biology of *Troglostrongylus Brevior*

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While feline lungworms share a similar life-cycle with terrestrial gastropods as intermediate hosts, other aspects of their biology may differ. First contributions to the biological characteristics of *Troglostrongylus brevior* were reported by Gerichter in 1949 who verified the infectivity of *T. brevior* third-stage larvae, after developing in snails, for the domestic cat. More recent research focused on the understanding of the biology of the larvae within the intermediate host, potential pathways of transmission and risk factors for transmission. However, several questions concerning the development of *T. brevior* in the final host remain open, including the duration of larval shedding and their variability in individual definitive hosts.

In preparation for a clinical study, six domestic short hair cats (8 to 9 months old, 2 male, 4 female) were inoculated orally with 12 to 53

third-stage larvae isolated by peptic digestion of infected *Cornu aspersum* snails (5 cats) or with tissues of an infected snail (1 cat). Snails had been inoculated with *T. brevior* isolated from a cat from Sicily. Starting 19 days post inoculation (dpi) fecal samples of 10 grams each were collected daily and subjected to the Baermann technique. At 38 dpi, two cats were necropsied for lungworm recovery and count.

None of the cats showed any respiratory signs during the observation period. Cats started shedding larvae at 21 dpi and all cats shed larvae from 23 dpi onwards. Arithmetic mean fecal larva counts peaked at approximately 40 dpi (831 larvae per gram feces). Necropsy of two cats revealed 16 (cat inoculated with 16 larvae) or 112 (cat inoculated with snail tissue) adult *T. brevior*. Larval shedding from the four cats followed up ceased 107 dpi, 141 dpi, 141 dpi or 227 dpi for the cats inoculated with 53, 12, 29 and 13 third-stage *T. brevior* larvae, respectively.

PS03.30 Development of an Automated Motility Assay Using Infrared Tracking on Exsheathed Third- Stage Larvae of *Haemonchus Contortus* for New Anthelmintics Discovery

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Haemonchus contortus is one the most prevalent nematodes in gastro-intestinal parasite infections of small ruminants and responsible of major economic losses in livestock production. Several anthelmintic drugs are available in the market to control this helminthiasis, but development of resistance against all major classes, has been reported. Therefore, the development of novel anthelmintic compounds in veterinary medicine is essential. Availability of parasitic stages for in vitro screening relies on artificial infections and adult stages require ruminant necropsy. Contrary, exsheathed L3 (xL3) parasitic stage, can be easily obtained starting from eggs in feces. In this work we show the

set-up of a whole-organism automated motility assay for new anthelmintics discovery on xL3 stage of *H. contortus* (Kirby anthelmintic-susceptible McMaster isolate). xL3s were cultured in multiwell plates according to Preston et al 2015, with minor modifications and the larval motility was measured for 30 min at 37°C, 72 hs post-incubation using an infrared tracking device with temperature control (WMicrotracker Arena, PhylumTech). This system uses video recording of infrared microbeams illumination through multiwell plates and the analog signal is mathematically processed to detect the passage of the larvae in the sensing area. This assay was calibrated using albendazole, monepantel and ivermectin as standards, with different mechanism of action, and the half-maximum inhibitory concentration (IC50) was determined for each anthelmintic from four independent assays with six replicates of each concentration evaluated, being 0.81 ± 0.23 , 0.10 ± 0.04 and 0.16 ± 0.04 μM (average $\pm$ standard deviation) respectively. Also controls of culture medium and culture medium with DMSO were also carried out. Due to its fully automated characteristics for reading this is a rapid method for the screening of new compounds with potential anthelmintic activity using a easily obtained parasitic stage of *H. contortus*.

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References: Preston, S. et al. Int. J. Parasitol., 2015, 45, 333-343.

PS03.31 Can We Combat Anthelmintic Resistance in Ruminants?

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Helminth parasitic pathogens cause severe disease and are amongst the most important production-limiting diseases of grazing ruminants. Frequent anthelmintic use to control these infections has resulted in the selection of drug resistant helminth populations. Anthelmintic resistance (AR) is today found in numerous major helminth species across the EU and globally. COMBAR (COMBatting Anthelmintic Resistance in Ruminants) is a COST Action (launched in 2017) which aims to advance research on the prevention of AR in helminth parasites of ruminants and disseminate current knowledge among all relevant stakeholders. COMBAR aims to integrate, evaluate and assess the economic trade-off of the novel developments in the field mainly by networking and has already attracted scientists from 31 countries. The Network has been organised around three Working Groups (WG): WG1 "Improving Diagnosis" which aims to prioritise, evaluate and implement cost-effective methods for the diagnosis of helminth infections and AR; WG2 "Understanding the socio-economic aspects" which aims to develop, disseminate and apply methods to study the economics and human behaviour in the field of helminth control in ruminants and WG3 "Innovative, sustainable control methods" which aims to develop practical and sustainable helminth control strategies that integrate current insights from diagnostics, Targeted (Selective) Treatment approaches, epidemiology, anti-parasitic forages, vaccinology, farm economics and human behaviour.

PS03.32 A Putative Cryptic Freeze-resistant Species of *Trichinella* Discovered in Wolverine

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Trichinellosis is caused by nematodes of the genus *Trichinella*, which has two major clades. The encapsulated clade includes six species and three currently unnamed genotypes, whereas the non-encapsulated clade includes three species. In North America including Canada, five species of *Trichinella* (*T. spiralis* (T1), *T. nativa* (T2), *T. pseudospiralis* (T4), *T. murrelli* (T5) and *Trichinella* genotype T6) have been found in homeothermic vertebrate hosts. Here we discuss the discovery, phylogeny, geography and host range of a previously unrecognized, encapsulated, cryptic species of *Trichinella* designated as the T13 genotype. This novel genotype was discovered while sequencing the mitochondrial genome of an isolate mistakenly determined to be *T. nativa* based on multiplex PCR. Subsequent phylogenetic analysis showed that the new genotype is at the base of the subclade containing *T. patagoniensis* (T12), *T. nativa*, *T. britovi* (T3), *T. murrelli* (T5), and genotypes T6, T8, and T9 which is sister to the lineage containing *T. spiralis* (T1) and *T. nelsoni* (T7). Of 95 animals from Canada that tested positive for *T. nativa* based on multiplex PCR, 14 wolverines (*Gulo gulo*) were infected with the novel genotype. These occurred mostly as single infections (11/14), but occasionally as mixed infections with *T. nativa* and *Trichinella* T6. We recommend use of a newly developed PCR-RFLP or DNA sequencing to confirm identification of the isolates of *T. nativa* or any species of *Trichinella*, respectively. Our results indicate that T13 is not geographically widespread in Canada, and may be limited to

wolverine in northwestern Canada, especially Yukon, suggesting a possible historical link to Beringia and the Palearctic. Exploration of Alaskan and Siberian isolates may contribute to further resolution of a geographically complex history for *Trichinella* and other parasites across the Western Hemisphere, Beringia and Eurasia.

PS03.33 The New Rat Lungworms: The Occurrence of *Physaloptera hispidus* and a *Mastophorus* Sp. In Pulmonary Vessels of the Hispid Cotton Rat (*Sigmodon hispidus*) From Georgia, U.S.A.

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During 2017-2018, a survey for the rat lungworm, *Angiostrongylus cantonensis* (Nematoda: Metastrongyloidea) in rodents from Piedmont and Lower Coastal Plains physiographic regions of Georgia, U.S.A. was conducted. On four occasions, a single worm was recovered from the pulmonary vessels of a single cotton rat (*Sigmodon hispidus*). One of these worms was identified as a *Physaloptera* sp. and the remaining three as a *Mastophorus* sp. by morphology. *Physaloptera* (Nematoda: Physalopteroidea) and *Mastophorus* species (Nematoda: Spiruroidea) are stomach parasites of many wild and domestic animals. This is the first report of these species in the pulmonary vessels of a definitive host. To better characterize these parasites, representative specimens were collected from cotton rat stomachs and identified morphologically and molecularly. Based on partial cytochrome c oxidase subunit 1 (COI) gene sequences, *Physaloptera hispidus* from stomachs were identical to the *Physaloptera* sp. from the pulmonary vessels. The COI sequences from the *Mastophorus* sp. from the stomach exhibited a higher degree of variability but confirmed that the pulmonary worms were the same *Mastophorus* species. Furthermore, sequences

of *Mastophorus* from a coastal site clustered separately from a clade of *Mastophorus* sequences from cotton rats from a Piedmont site. Collectively, our data show that adult worms recovered from pulmonary vessels of cotton rats could be either *Physaloptera* or *Mastophorus* sp., indicating that these parasitic worms are not always restricted to the stomach and that worms from pulmonary vessels must be carefully examined to obtain a definitive diagnosis of *A. cantonensis* infection.

PS03.34 Nematode Exsheathment 1: Heat Shock and *Haemonchus contortus*

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Understanding the processes involved in the transition from the free living to the parasitic life stage of ruminant parasitic nematodes may possibly identify new targets amenable to future intervention. The transition to parasitism is initiated by exsheathment and is triggered by the sudden change in environment after ingestion of the infective larva by the host. Two major changes in environment are the increases in temperature and carbon dioxide (CO₂) levels. For CO₂ a role in exsheathment has been described previously, but the exact role of temperature was unclear. This study investigated the importance of temperature in triggering exsheathment of *Haemonchus contortus*. Carbon dioxide induced exsheathment in *H. contortus* proved to be temperature dependent, as no exsheathment was observed at room temperatures. The temperature requirement to trigger exsheathment was quite specific, with a rapid change in temperature (heat shock) very efficiently inducing high levels of exsheathment. In contrast, when the larvae were exposed to a slow increase in temperature the exsheathment response was smaller and delayed. Further investigation revealed that timing of the heat shock in relation to the CO₂ administration was crucial, as well as the final temperature and magnitude of the heat shock.

In conclusion, these data indicate that heat

shock rather than temperature itself is a crucial aspect in triggering the biological exsheathment cascade, and thus infection process, of *H. contortus*.

PS03.35 Conserved and Divergent Aspects of Chemosensory Signaling in Filarial Parasitic Nematodes

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Chemosensation - the process of receiving exogenous chemical cues, initiating a signaling pathway, and precipitating an evolved behavioral response - is a fundamental life process that is shared from animals to bacteria. The model nematode *Caenorhabditis elegans* has been shown to display positive and negative chemotaxis to soluble and volatile compounds, and much is known regarding the neuronal networks and molecular pathways underpinning these behaviors. Some of these pathways have been recently explored in parasitic nematodes, particularly soil-transmitted helminths. In contrast, there is very little known about chemosensory behaviors in vector-borne filarial parasites such as *Brugia malayi* and *Dirofilaria immitis*, the causative agents of the neglected tropical disease lymphatic filariasis and dog heartworm, respectively. We hypothesized that chemosensory signaling is integral to the migration and transmission of filarial parasitic nematodes, and that sensory pathways could present new targets for vector or chemotherapeutic methods of filariasis control, and we have taken the first steps toward elucidating the molecular mediators of chemotaxis in filarial nematode species. Pan-phylum analyses of the nematode chemoreceptor GPCR family, as well as TRP and CNG channels that are hypothesized to function downstream of chemoreceptor activation in the amphid sensory neurons, revealed that filarial nematodes have a reduced and divergent subset of chemoreceptors, while they retain one-to-one homologs to the canonical TRP and CNG families annotated in *C. elegans*. We

show enrichment of expected chemosensory proteins in the parasite anterior and explore stage-specific and temperature-dependent aspects of chemosensory receptor expression. Using plate-based chemotaxis assays, we show that in vitro chemotaxis can be dysregulated by pharmacological and reverse genetic approaches targeting chemosensory proteins. Lastly, using long-read isoform sequencing, we have cloned *B. malayi* *osm-9* and *tax-4* and are currently testing transgenic rescue of chemosensory function in corresponding *C. elegans* knockout strains.

PS03.36 Clinico- Histopathological Observations of Camel Sarcopticosis: A Case Report

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Cutaneous ectoparasitoses is one of the important skin diseases of camels and ranks second commonest infestation after trypanosomosis. Sarcopticosis affects productivity but mostly cause debilitation leading to heavy economic loss. Camel sarcopticosis also known as sarcoptic mange is common at all ages in camels. It is usually a chronic condition with high morbidity and low mortality. In this study, a skin sample was collected from camel (*Camelus dromedarius*) suspected for cutaneous lesions, was processed mechanically for paraffin embedding by acetone and benzene technique for histopathological examination. The blood sample was also collected from suspected case for analysis of various haemato-biochemical parameters. Sarcopticosis was confirmed after the mites were identified as *Sarcoptes scabiei* var *cameli* from the skin scrapings. The affected areas in head and neck showed papules and eruptions. Microscopically, minute cavities in the epidermal layer extending into dermis were present. These cavities were filled with tissue debris and infiltrated mononuclears. The epidermis showed hyperkeratosis and acanthosis and there was proliferation of fibrous connective tissue. Haematological observations revealed decrease in total

erythrocyte count (TEC), haemoglobin and packed cell volume (PCV) and an increase in total leucocyte count (TLC) and eosinophilia. The biochemical studies revealed hypoalbuminemia and hyperglobulinemia.

PS03.37 Metazoan Parasitic Infections for *Clarias Gariepinus* (Burchell, 1822) in Native and Extralimital Distribution Ranges Within Southern Africa

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As one of the most widespread freshwater fishes and an economically important fish in Africa, few studies investigate the parasitic communities of the African sharp-tooth catfish, *Clarias gariepinus* (Burchell, 1822) in southern African freshwater systems. More specifically, studies investigating the parasitic communities and what determines the composition of these parasitic communities in both the native and extralimital distribution range are limited. A recent review emphasises the potential for *C. gariepinus* to be a suitable host for various parasite taxa, with ca. 106 parasitic species across seven major metazoan taxa recorded from *C. gariepinus* in Africa. Currently only ~24 parasite species are known from *C. gariepinus* in South Africa. In addition, in recent years only five descriptions of new species from South Africa highlighting the paucity of knowledge on the parasitic communities of *C. gariepinus* in southern Africa. The present study aims to investigate the metazoan parasitic communities of *C. gariepinus* in native and extralimital distribution ranges in southern Africa. Furthermore, it aims to determine which invasion mechanisms (i.e. parasite spillback or enemy release) are at play and how the diversity and abundance of these

parasite communities change throughout the distribution range, taking into account the age of the fish, metal concentrations and nutrient levels in the environment as well as the method of translocation to the extalimital regions.

PS03.38 *Thelazia Callipaeda* Eyeworm and Its Vector *Phortica Variegata*: Strategies to Survive the Winter

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Thelazia callipaeda (Spirurida, Thelaziidae) is a vector-borne nematode that infects the conjunctival sacs and associated orbital tissues of many mammals, including humans. The nematode was once commonly referred to as the 'Oriental eyeworm', but after more than 20 years, it has spread throughout Europe and many studies have elucidated the role of a bizarre fruit fly (i.e., *Phortica variegata*) as the intermediate host and vector of this zoonotic nematode, through its lacriphagous behaviour. Though domestic and wild carnivores are the main definitive hosts for this parasite, more than 1000 and 10 human cases have been reported, respectively, in Asia and in Europe. The presence of adult nematodes and their serrated cuticle cause a variety of clinical signs in the infected animals which range from mild conjunctivitis, follicular hypertrophy of the conjunctiva, foreign body sensation, epiphora, itchiness, congestion, swelling and even blindness. The close relationship among *T. callipaeda*, their definitive hosts and their vector *P. variegata* remains in many instances enigmatic and, unlike many other arthropod vectors, flies feeding around the eyes of definitive hosts (therefore transmitting the third infective larval stages) are almost exclusively males. Indeed, the development of the nematode larval stages (L1-3) closely associated with the biology of the male. In this poster the authors will describe recent field and laboratory

studies to assess male/female ratio of flies collected around the eyes of human baits, the *T. callipaeda* natural infection rate of flies and the potential overwintering of *T. callipaeda* in diapausing *P. variegata*.

PS03.39 Detection of *Theileria Orientalis* Genotypes and Identification of Potential Vectors in Central Queensland

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Theileria orientalis is a tick-borne protozoan parasite of bovine red blood cells inducing persistent infection. Clinical theileriosis, caused by *T. orientalis* has become a cattle disease of economic importance in Australia since 2006 with the emergence of pathogenic genotypes of the parasite. There is a scarcity of information on the identification of these genotypes and their epidemiology in central Queensland. We used recently developed molecular methods to differentiate and quantify pathogenic and non-pathogenic *T. orientalis* genotypes in cattle and ticks on a property near Capella, Central Queensland where clinical theileriosis is believed to be absent. Thirty blood samples were collected from cattle and ticks were collected off pasture and off animals fortnightly between February and June 2018. Real-time quantitative PCR (qPCR) analysis of the blood samples revealed a generic *T. orientalis* prevalence of 100% with prevalence of the different genotypes being 100% for Buffeli (non-pathogenic), 80% for Chitose (possibly pathogenic) and 0% for the pathogenic Ikeda genotype. Absolute quantification revealed similar parasite burdens of Buffeli and Chitose with no association between the two. It also revealed significantly higher *Theileria* burdens in sampled cows than heifers. On pasture, two tick types were detected, *Amblyomma* sp. (95.8%) and *Haemaphysalis bancrofti* (4.2%). The ticks collected from cattle were very different being 98.6% *Rhipicephalus microplus* and 1.4% *Amblyomma triguttatum*. Quantitative PCR of pooled tick head parts of

the three tick species revealed high levels of genotype Buffeli in *H. bancrofti* and moderate levels in *R. microplus*. No other *Theileria* genotypes were detected. Based on these and other findings the most likely vector of *T. orientalis* in this area is *Haemaphysalis bancrofti* despite the lack of detection on cattle. Further research is required on the role of *Rhipicephalus microplus*. *T. orientalis* was endemic on this property but the most pathogenic genotype, Ikeda was absent.

PS03.40 *Eimeria* Spp. And Its Association With Diarrhea in Dairy Calves in Uruguay

Licenciado en Laboratorio Anderson

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Bovine eimeriosis is a disease of worldwide distribution that cause significant economic losses. It is produced by *Eimeria* spp. and affects young animals, mainly. Many species have been described but only few are pathogenic. Usually, it is a self-limiting disease.

In Uruguay, only two cases of eimeriosis have been reported, one by *Eimeria zuernii* (1918) and, recently, other by *Eimeria bovis* (2015). Although several suspects were reported, little is known about the different species that could affect cattle and which are more relevant in clinical cases. The objectives of this study were to identify the *Eimeria* species in feces of 757 calves (<10-month-old) from ten different dairy farms with diarrhea in calves and to determine their responsibility as a cause of diarrhea.

The oocyst count per gram (OPG) was determined by McMaster method and for identification species, fecal sample cultures to sporulation were used. Six species were identified: *E. bovis*, *E. zuernii*, *Eimeria ellipsoidalis*, *Eimeria auburnensis*, *Eimeria canadensis* and *Eimeria alabamensis*, with a high prevalence of *E. bovis* and *E. zuernii* being

these ones the cause of diarrhea in all clinical cases. A significant association ($p = 0.007$) among calves with diarrhea and high OPG was found. There was a positive association between number of oocysts (≥ 4000 OPG) and diarrhea. On the other hand, the risk of diarrhea increases (Relative Risk = 2.37) when the number of oocysts eliminated increases too. The presence of *E. bovis* and *E. zuernii* in cases of diarrhea shows that this disease is being underestimated in calf rearing. It is recommended to maximize hygienic-sanitary measures in these dairy farms. The high incidence of calves' infections suggests that fecal monitoring is a necessary tool for the control and prevention of this parasite disease.

PS03.41 Mitochondrial DNA of *Babesia Odocoilei* as a Means of Diagnosis for Babesiosis in Cervids

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Babesia odocoilei is a piroplasm (Apicomplexa) infecting cervids that is transmitted through blood-feeding by its tick definitive host, *Ixodes scapularis*. In dead-end cervid hosts (e.g. reindeer and elk), *B. odocoilei* can cause hemolytic anemia and death. Although frequently used for species identifications, nuclear 18S ribosomal DNA (rDNA) sequences may lack sufficient species-level divergence to provide reliable identifications. Consequently, diagnostic tests based on this locus may lack the ability to differentiate among closely related piroplasms. In contrast, piroplasm mitochondrial (mt) DNA exhibits extensive species-level diversity that can be exploited for diagnostics. Complete mt genome sequences of related piroplasms (e.g. *Babesia gibsoni*, *Babesia bigemina*, *Babesia bovis*, and *Babesia caballi*) are available but not for *B. odocoilei*. We used polymerase chain reaction (PCR) and amplicon sequencing to generate a substantial majority (>5.3 kbp) of the mt genome of *B. odocoilei*. DNA was obtained from the spleen of a reindeer confirmed to have died from a *B. odocoilei* infection. Standard PCR amplifications were performed

using primers targeting conserved regions of apicomplexan mt genomes. PCR amplicons were purified and Sanger sequenced. The mt genome has the same components and arrangement as other related piroplasms including three protein-coding regions (CDS; i.e. cytochrome c oxidase I [COI], cytochrome c oxidase III [COIII], and cytochrome B [CytB]) and numerous fragmented rDNA. Unlike the nuclear 18S rDNA sequences, the mt CDS regions demonstrated considerable genetic divergence among these related piroplasms. For example, pairwise sequence identities of partial COI sequences ranged from 76 to 82% among the related *Babesia* species listed above. This newly generated *B. odocoilei* mt genome sequence will provide a target for more reliable species delimitation, potentially including species specific PCR, than the existing nuclear 18S rDNA sequences and will support the detection of babesiosis in wild and farmed cervids.

PS03.42 Metabolic Budget of Ticks in The Field in S.W. England

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The spread of tick-borne diseases in many parts of the world represents a significant health challenge for livestock and companion animals despite many advancements in the understanding of tick biology. Of crucial importance to the success of a tick species to reproduce and thrive in its specific environmental niche is the ability to efficiently obtain, store and metabolise the key bio-nutrients from their blood meal. Neither the metabolic requirements of developing ticks nor the allocation of resources to development have been studied extensively. Therefore, many aspects of tick physiology remain unclear. In the present work, a range of biochemical assays were used to estimate the seasonal patterns of free sugar (glucose), glycogen, lipid and protein accumulation through the life cycle of ticks in s.w. England. A total of 1,303 nymphs, males and females were analysed over the course of a year. The metabolic patterns observed provide a direct insight into the feeding history and life-history partitioning of resource during tick development.

PS03.43 Repellent Effects of Encapsulated Carvacrol on the Southern Cattle Tick, *Rhipicephalus (Boophilus) Microplus* (Acari: Ixodidae)

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Rhipicephalus (Boophilus) microplus (Canestrini) is a problem for livestock production systems, and its control has become challenging due to the selection of tick populations resistant to synthetic chemical acaricides. The use of repellent compounds prevents contact between the arthropod parasite and the host and can thus contribute to increases in the efficacy of these acaricides. Carvacrol monoterpene phenol is a possible alternative method for controlling *R. (B.) microplus*; however, this compound is highly volatile, and its volatilization can be decreased through microencapsulation, which results in the timed release of the compound. The cell wall of *Saccharomyces cerevisiae* can be utilized for the protection of volatile molecules. The aim of this study was to evaluate the in vitro repellent effect of yeast cell wall-encapsulated carvacrol on susceptible *R. (B.) microplus* larvae. Specifically, the vertical filter paper bioassay was employed to analyze the repellent activity of encapsulated carvacrol, nonencapsulated carvacrol and DEET (N,N-diethyl-methyltoluamide) at concentrations ranging from 0.75 to 0.001 mg/cm², and the repellent activities were evaluated. Both carvacrol and encapsulated carvacrol exhibited repellent effects on *R. (B.) microplus* larvae, and the encapsulated compound showed the highest repellent activities at the lowest concentrations. Carvacrol encapsulated exhibited a low repellent concentration in all times (≤ 0.05 mg/cm²), while the carvacrol nonencapsulated ranged CR50 from 0.13 to 0.27 mg/cm² at 1 to 6 h posttreatment. The present paper provides the first description of the use of a microencapsulation technique for achieving the highest repellent effect of carvacrol and indicates that this technique might be used to obtain new delivery systems for volatile and hydrophobic compounds.

PS03.44 Distribution of Ticks and Molecular Detection of Tick-Borne Diseases From Dogs in the Republic of Korea, 2017–2018

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Ticks are vectors of a number of pathogens that are important to humans and also veterinary practice. Many of the pathogenic agents transmitted by ticks, including Ehrlichia spp., Anaplasma spp., Borrelia spp., and Babesia spp., are known to be humans and animal pathogens worldwide. In this study, we are proposed to detect and characterize tick-borne diseases in ticks from Korea using molecular techniques.

Ticks were removed from shelter and companion dogs, also collected near the animal shelter by dragging and flagging. Based on microscopic examination, collected ticks were identified to species and developmental stage characterized. DNA purified from ticks was used for the detection of 6 of tick-borne diseases (Anaplasma phagocytophilum, A. platys, Ehrlichia chaffeensis, E. canis, Borrelia spp., Babesia spp.) using previously described PCR assays.

A total of 2,294 ticks from two genera and three species (2,261 of Haemaphysalis longicornis, 10 of H. flava, 22 of Ixodes nipponensis) was collected from northern area (1,461 ticks), central area (718 ticks) and southern area (115 ticks) of ROK. 24 of 1,111 tick pools (2.2%) were PCR-positive for A. phagocytophilum, and 4 of 1,111 tick pools (0.9%) PCR-positive for Borrelia spp.

In this study, tick identification and molecular detection were conducted to monitor of ticks and tick-borne diseases in the Republic of Korea. It is important to continue the efforts to identify additional tick-borne pathogens in ticks because of public health significance of these agents.

PS03.45 The Use of Computed X-ray Microtomography (microCT) for Non-invasive Imaging of Ixodes Ricinus Ticks

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Candidatus Neoehrlichia mikurensis is an emerging bacteria transmitted by Ixodes ricinus in Europe. C. N. mikurensis can infect several mammals, including humans. However, the impact of C. N. mikurensis on internal morphology of I. ricinus is not known. Internal and external structure of ticks is commonly visualized using light, electron microscopy or histology techniques. Recently, computed X-ray tomography (microCT) has been used as a novel tool for investigation of morphologic features in insect. In comparison to electron microscopy and histology, microCT enables fast and easy sample processing, examination of several specimen at the same time. While final resolution of images from microCT scans is sufficient (2.5 to 6 µm), major challenge of X-ray scanning consists in low contrast of internal structures. Aim of the study was to develop and optimize protocol for microCT scanning of both unfed and fully fed ticks of I. ricinus. Unfed ticks were dehydrated in a graded 70-100% ethanol, then incubated in 2% osmium tetroxide and dried using hexamethyldisilazane. Another group of unfed ticks were dried by letting them placed at room temperature for several weeks. All ticks were scanned on Bruker Skyscan 1276 with no filter, resolution 4 µm and 4K binning. Scanned data were reconstructed using N-recon, further modified and analyzed using CTan software. 3-D rendered volumes of ticks were visualized in CTvox. Contrast and quality of internal structure did not differ between naturally dried and ethanol dehydrated ticks contrasted with osmium. In group of fed ticks, artificial in vitro blood feeding of the ticks with addition metal-based contrast agents will be carried out as next step. In conclusion, our study shows that microCT is a novel efficient tool for imaging of tick internal structure.

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PS03.46 Ticks and Piroplasms of Equines in Nigeria: Prevalence, Risk Factors and Health Implications.

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The aim of the study was to conduct an epidemiological investigation of ticks and equine piroplasms by determining the species diversity of ticks, detection of piroplasms, risk factors associated with prevalence and the possible effects of these pathogens on the health of equines in Nigeria. Ticks and blood samples were collected and counted from 463 equids (horses and donkeys) in four (4) states, namely, Kano (n =169), Jigawa (n=139), Katsina (n = 108) and Kaduna (n = 47). Epidemiological questionnaire was also administered to the animal owners to ascertain risk factors. A total of 445 ticks representing four species were collected. The overall prevalence of tick infested horses was 24.6% while that of donkeys was 0.5%. *Rhipicephalus evertsi evertsi* 85.84% was the most abundant, followed by *Hyalomma truncatum* 8.76%, *Hyalomma dromedarii* 3.59% while *Rhipicephalus decoloratus* 1.79% was the least abundant. The average tick burden was 6.5 with a range of 1-14. The highest collection of ticks was from Kano state 32.35% while Kaduna state 15.06% was the least.

Analysis of the risk factors associated with tick infestation reveals that the Arewa breed of horses has the greatest risk 15.9% of been infested with ticks. Other breeds include Talon 4.71% and Sudanese 3.99%. Male horses had higher tick infestations 16.5% than females 8.69%. Furthermore, younger horses (<12 years) 21.38% had higher infestations than older horses (>12 years) 3.62%.

Microscopic and molecular analysis revealed the presence of piroplasms such as *Babesia caballi* and *Theileria equi*. The overall prevalence of equine piroplasmosis using

microscopic and molecular technique was 9.29% and 19.0% respectively. The infection was higher in males 6.05% than females 3.24%. Younger equids (<12 years) were more infected 6.91% than older ones (>12 years) 2.38%. The Arewa 4.7% and 6.42% Idabari breeds of horses and donkeys respectively were the most infected.

PS03.47 Cross Border Transhumance, a Dissemination Way of Ticks and Tick-Borne Pathogens in West Africa

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The extensive and low-input livestock system occurring in West Africa lay on a cross-border transhumance which represents an important animal production strategy. Meanwhile, cattle tick infestations constitute one of the main constraints to the development of livestock production in this region. Pathogens involved can be spread by live animals or vectors movements. This study aims to evaluate the involvement of transhumance in the spread of ticks and associated pathogens between the eastern Burkina Faso (BF) and northern

Benin (BN), where the sanitary conditions of cattle, considering ticks and tick-borne diseases, are less known. The first step has been a cross sectional survey, which consisted in an investigation on ticks and tick-borne pathogens in cattle in the Eastern BF (490 cattle) and the Northern BN (456 cattle). The second step was a longitudinal survey including 311 cattle from BF, monitored during one season of transhumance. These two surveys were carried out with cattle ticks and peripheral blood collection from December 2016 to March 2017. According to the cross sectional survey, the most abundant tick species recorded during this period were *Amblyomma variegatum*, *Hyalomma truncatum* and *Rhipicephalus (Boophilus) microplus*. This latter species was evidenced in some areas in Northern BN where it wasn't reported yet, and only in a herd of BF coming from transhumance in BN. The microscopic examination of blood smears revealed a high prevalence of *Anaplasma* sp up to 86,12% and 85,75% respectively in BF and BN. The genus *Theileria* was solely diagnosed in BN within 8,11% of cattle. Fisher exact test showed a prevalence of *Babesia* significantly higher ($p < 0.05$) in BN (36.84%) than in Burkina Faso (5.31%). Such pattern of ticks and tick-borne pathogens distribution suggest a south-north expansion gradient which will be more investigated using longitudinal survey data and by Reverse Line Blot hybridization essay.

PS03.48 Threat of Ticks and Tick Borne Diseases in the Scenario of Changing Climate

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Zoonotic tick-borne diseases have become increasingly prominent and the incidence of these diseases have risen dramatically over the last decade. Climatic suitability for a tick population is the fitness of a set of climatic conditions for the existence of that population in a given region. All the tick species are important vectors of diseases

and an increasing incidence of these diseases is the most significant potential outcome of climate changes that affect the ticks directly or indirectly. Transmission of infection occurs when there is an overlap of activities between reservoir, vectors and humans, and differs according to the pathogens and location. Climate change may impact all of these stages and their interactions. The changes in climate and the length of different seasons directly affect tick survival, activity and development because of the concept that rising temperature will result in greater abundance of ticks. Additionally, climatic change will also have indirect effect on tick borne pathogen transmission by affecting the survival and abundance of tick maintenance hosts. The magnitude of the effects of climate change in an endemic area depends on local conditions, ecosystems and biodiversity, migration patterns of birds and immunity in the population. There may be difficulties in determining the future scenario, however, it can be overcome by conducting long-term studies regarding disease incidence, tick biology, tick distribution and tick abundance, host abundance and distribution and relevant vegetation biology, specifically in relation to climate change.

PS03.49 Development and Evaluation of an Innovative Product Formulated With Essential Oils for *Rhipicephalus Microplus* Tick Control

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Rhipicephalus microplus tick causes a lot of damage to susceptible cattle. Its control is increasingly difficult due to the resistance to acaricides. Some plants naturally contain active principles to protect them from insect attack, among them are the essential oils. The Institute of Animal Science, in partnership with HYG Systems, has developed an essential oils based product (HYGIZ) that has been tested in vitro and in vivo. In the in vitro tests, performed in the Laboratory of Parasitology of the Institute of Animal Science in Nova

Odessa, SP, the product presented excellent results: 100% efficacy in adult immersion test; 100% repellency in 72 hours; 100% mortality in larvae immersion test and odor test. Two in vivo tests were performed. In the first one, animals treated with the HYGIZ had a drastic drop (more than 80%) in the tick count made in the first week of the experiment already and in the follow ones; unlike the group which received a commercial Pour on product that only dropped tick counts in the following counts (+14 and +21 days). In the other in vivo test, teleogines were counted throughout the body of 10 animals on days -2, -1, 0 in order to distribute the animals homogeneously in two groups: treated or not, and evaluated for 22 consecutive days. After application, with a hand pump in the most infested regions (neck, barb, arms, belly, groin, perineum, scrotal sac and inside and outside the ears), HYGIZ controled all tick stages, and the daily effectiveness of the product ranged from 73.6 to 98.5%, with an overall effectiveness of 91%. HYGIZ did not alter parameters related to hepatic and renal functions of treated animals.

PS03.50 Acaricidal Effect by Odors of Eucalyptus Globulus, Corymbia Citriodora, 1,8-Cineole and Citronellal Essential Oils Against Rhipicephalus Microplus

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Essential oils have been investigated as an alternative for parasite control, aiming to delay the development of tick genetic resistance, a consequence of the extensive use of chemicals for its control. The present study aimed to evaluate the acaricidal effect of the odor of *Corymbia citriodora* and *Eucalyptus globulus* essential oils and its main constituents, citronellal and 1,8-cineole, at the doses of 10, 20, 40, 60, 80 and 100 mg.g⁻¹ and in binary mixtures (1:1) on *Rhipicephalus microplus* larvae and engorged females. Commercial acaricides (amitraz 12.5%, cypermethrin 15% and cypermethrin 30% + chlorpyrifos 30% + fenthion 15%) were used

as positive controls and, as negative controls, water and alcohol/acetone (1:1). In order to verify the acaricide odor effect on ticks, it was created an open experimental unit composed by two Falcon tubes connected and interleaved with filter paper to prevent the direct contact of the ticks with the disc impregnated with the oils that stayed in the inferior tube. An opening was made in the plastic cover of the upper tube that was shut with filter paper to allow only the odor to escape to the environment. In each treatment, larvae or engorged females were exposed to the odor for 72 hours. In the bioassays with engorged females, the oviposition inhibition, percentage of hatched larvae, and product efficacy were determined. Efficacies ranged 80-100% for almost all essential oils and its combinations in certain doses. Citronellal, 1,8-cineole and its combinations caused larvae mortality > 90%. In general, the odor of all essential oils and their combinations had acaricide effect on both larvae and engorged females. The odor of commercial acaricides had no effect on larvae and teleogines. (Sponsor: Hyg Systems).

PS03.51 Estimates of Correlations and Repeatability Between Infection Level of Tick Fever and Tick Infestation in Holstein Calves Raised in Paddock

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The present study aimed to estimate the repeatability and correlation between the infection levels of *Babesia bovis*, *B. bigemina* and *Anaplasma marginale* and natural infestations by *R. microplus* in calves reared individual with shelter in paddock. Twenty newborns calves were monitored biweekly in the period between 08/21/2018

and 10/30/2018 (5 evaluations), wherein *R. microplus* adults females were counted and blood samples were collected for DNA extraction. The infections levels for each hemoparasite specie were estimated by qPCR. The qPCR assays set primers for *B. bovis* and *B. bigemina* were located in a region of cytochrome B (mt-cyt B) gene, whereas for *A. marginale* were located in surface major protein 1b (msp1b) gene. The qPCR assays were performed using HOT FIREPol EvaGreen® qPCR Supermix (Solis Biodyne) and Rotor Gene Q thermocycler (Qiagen). The hemoparasites loads and tick counts were transformed into $\log_{10}(n + 1)$ for normal distribution approximation and were analyzed by the SAS Mixed procedure, in which the model included the fixed effects of evaluation, sex, hemoparasite species and interactions. Animal was included in the model as a random effect. The estimated correlation coefficients between tick counts and hemoparasites loads were low or close to zero, as well as the correlations between *A. marginale* and *Babesia* spp loads. However, the correlation between the two species of babesias levels was 0.49. The repeatability estimates of *B. bigemina*, *B. bovis* and *A. marginale* were 0.74, 0.77 and 0.70, respectively. Based on these results, under conditions of the present study, we suggest that it is possible to identify animals presenting a most resistant phenotype against infection by hemoparasites. Furthermore, the high correlation found between both babesias indicates that the factors that determine an increase or decrease in parasitemia for one species cause the same variation in the other species.

PS03.52 Studies on the Insecticides Against Hard Ticks and Poultry Mites

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Ticks and poultry mites are very fecund and resistant to environment, so that we can easily see them in pasture of cattle and poultry house. They are bloodsucking and cruel to animals, and transmit many kinds of pathogens to host animals, such as

Babesia spp and *Theileria* spp in cattle. They kill humans and animals, and have many economic losses of cattle and poultry farms. Human tried to control the ticks and poultry mites very long times but it was very difficult. In these trials, we tried to evaluate some commercial insecticides and select some good effective drugs. Six anti-insecticide drugs, Cyper-Killer (A), CyperKill Power (B), New-Goldenpex EC (C), New-Goldenpex Powder (D), Green Ticks(E), and Natural herb extracts(F) were evaluated the efficacy against bovine ticks and poultry red mites in the experimental laboratory, field poultry farms and Korean cattle farms.

In the anti-insecticides effect of insecticides against bovine ticks in vitro culture, drugs A, B, C and D were good effective against ticks in vitro culture. In the anti-insecticides effect of insecticides against poultry red mites in vitro culture, drugs A and C were good effective against poultry mites in vitro culture. In the anti-insecticides effect of insecticides against bovine ticks in Korean cattle farms, drugs A, B and D were good effective against ticks in field trials. In the anti-insecticides effect of insecticides against poultry red mites in the poultry farms, drugs C and F were best effective against poultry red mites. And drugs B, D and E were good effective against them.

So that drugs A, B and D were recommended to be used in the bovine ticks and C and F were recommended to be used in the poultry red mites.

PS03.53 A High Throughput Deep Amplicon Sequencing Method to Show the Emergence and Spread of *Calicophoron Daubneyi* in United Kingdom Cattle Herds

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The prevalence of *C. daubneyi* infection in the United Kingdom has increased, but despite the potential for rumen flukes to cause production loss in ruminant livestock, understanding of their emergence and spread is poor. Here we describe the development

of a method to explore the multiplicity of *C. daubneyi* infection and patterns of the parasite's emergence and spread, based on Illumina MiSeq deep sequencing of meta barcoded amplicons of a fragment of the mt-COX-1 locus. Our results show high levels of genetic diversity per infection and between populations of 10 to 47 of adult *C. daubneyi*, each from a total of 32 finished prime cattle consigned to slaughter from northern United Kingdom; with 18 unique mt-COX-1 haplotypes. This has implications for the adaptability of environmental and intermediate host stages of the parasite to changing climatic and animal management conditions, or of parasitic stages to exposure to anthelmintic drugs; potentially allowing for greater pathogenicity, or the development of anthelmintic resistance, respectively. Our results illustrate the impact of high levels of animal movements in the United Kingdom, whereby multiple common mt-COX-1 haplotypes were identified in 26 populations in the absence of geographical clustering of clades.

PS03.54 Can *Fasciola hepatica* Metacercariae Survive Within Grass Silage And Retain Viability?

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Fasciola hepatica, the common liver fluke, is an important cause of morbidity and mortality in ruminant livestock worldwide. It is recognised that metacercariae can overwinter on pasture and infect grazing livestock the following spring. However, the infection risk posed by feeding silage is not well described. Existing studies date back to 1961 and pre-date the modern advantages of applying molecular techniques to detect *F. hepatica* DNA persistence within silage. The impact of aerobic spoilage on survival of ensiled metacercariae and the ability of juvenile parasites to excyst must be understood, to establish how silage provision impacts *F. hepatica* transmission.

F. hepatica DNA was detected in silage samples inoculated with as few as 6 metacercariae using ITS-2 PCR. Metacercariae from laboratory-maintained *G. truncatula* specimens were shed onto grass samples of 20%, 30% and 40% dry matter (DM) content. Ensiling vessels were either sealed with an anaerobic fermentation lock or perforated plastic wrap and ensiled for 2, 6 and 10 weeks. Following ensiling, PCR and in vitro assays comparing excystment rates of ensiled and un-ensiled control metacercariae were conducted.

F. hepatica DNA persisted at 2, 6 and 10-week timepoints within silage originating from grass of all DM contents, under both anaerobic and aerobic conditions. In terms of excystment rates; following 2 weeks anaerobic ensiling no excystment occurred irrespective of initial grass DM. However, under aerobic (spoiled) ensiling conditions, ~28% excystment occurred after 6 weeks (30% DM content) and unhatched juvenile parasites developed following 10 weeks (20% DM content).

In conclusion, whilst detection of *F. hepatica* DNA in silage is useful, excysting metacercariae post-ensiling is a more useful indicator of viability. Anaerobic conditions are vital to prevent survival of metacercariae, highlighting the importance of forage sealing. These investigations will lead to informed husbandry advice on reducing fasciolosis prevalence through improved silage management.

PS03.55 Efficacy of Oral Afoxolaner Plus Milbemycin (NexGard Spectra®) on the Reduction of Eggs per Gram (EPG) of Feces of Dogs Naturally Infected With *Ancylostoma* Sp., *Toxocara* Sp. And *Trichuris Vulpis* in Brazil

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Ancylostoma spp., Toxocara spp. and Trichuris vulpis are the most prevalent nematodes infecting dogs in Brazil. Infection by these worms may be asymptomatic but also lead to different lesions and blood/nutrient depletion in the gut depending on the level of infestation. Ancylostoma spp. and Toxocara spp. are also important as zoonotic agents since L3 larvae may infect humans as larva migrans. NexGard Spectra® is a combination of Afoxolaner and Milbemycin Oxime promoting a broad efficacy action versus canine ecto and endoparasites. Different breed owned dogs, weighing greater than 2kg and more than 8 weeks old, from urban areas of São Luis, Maranhão state, Sinop and Ipiranga do Norte, Mato Grosso state, were included in the study if harboring quantifiable eggs of any of these worm species on a McMaster EPG evaluation. Eighty-four Ancylostoma spp., 31 Toxocara spp. and 21 Trichuris vulpis. infected dogs were included in the study and treated once orally with a single tablet delivering 2.50 to 5.2 mg/kg of afoxolaner + 0.50 to 1.07 mg/kg of milbemycin oxime per dog. Few included animals harboured mixed infections. The individual EPG were transformed to Log (x + 1) and the efficacy was calculated based on the comparison of the geometric mean number of EPG before treatment versus the mean number at Day 14 (± 4). The geometric means were calculated by averaging the EPG log-counts, taking the anti-logarithm, and then subtracting 1. The mean number of EPG pre-treatment and percentage of efficacy were 1031 and 98.5% for Ancylostoma spp., 665 and 96.8% for Toxocara spp., and 400 and 96.8% for Trichuris vulpis.. NexGard Spectra® showed excellent efficacy results in the field against Ancylostoma spp., Toxocara spp. and Trichuris vulpis. in housed naturally infected dogs in Brazil.

PS03.56 In Vitro Hatchability of Eggs of Haemonchus Contortus and Trichostrongylus Colubriformis With Powdered Bark of Acacia Mearnsii

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Gastrointestinal helminths in small ruminants are one of the major health problems affecting the production of these animals worldwide. The resistance of these parasites to commercial anthelmintics entails great economic losses. Therefore, the search for effective alternatives for the control and eradication of these helminths has been the target of researches, among which the use of phytotherapeutic compounds. Among these compounds, plants with high contents of condensed tannins, such as Acacia mearnsii, have been presented as a promising alternative in the control of these parasites. The objective of this study was to evaluate the effect of Acacia mearnsii powdered bark (AM) on egg hatch assay (EHA) to Haemonchus contortus and Trichostrongylus colubriformis. Decreasing concentrations of the AM (50; 25; 12.5; 6.25; 3.125; 1.56; 0.78; 0.39; 0.19; 0.09; 0.04; 0.02; 0.01; 0.006; 0.003; 0.001 mg/mL) was solubilized with Tween80 and distilled water, were added in 48 well plates, each well containing approximately 100 eggs of H. contortus and the same conditions for T. colubriformis and incubated for 24 hours at 27°C. The data were analyzed with the software SAS® using the PROBIT procedure to estimate the LD50 (lethal dose enough to inhibit hatchability of 50%). The LD50 for T. colubriformis eggs was 1.28 mg/mL, and for H. contortus eggs was 13.55 mg/mL. Thus, we can conclude that AM may be a potential alternative for the control of gastrointestinal parasites in small ruminants, due to inhibitory effect showed on egg hatchability, being more effective in T. colubriformis eggs, once it presented a lower LD50.

PS03.57 Effect of Different Faecal Concentrations of Anthelmintics on Mycelial Growth of the Biological Control Agent *Duddingtonia Flagrans*

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Duddingtonia flagrans acts as biocontrol agent by preying on pre-parasitic nematode larvae in animal faeces. This fungus could be exposed to anthelmintic drugs eliminated in faeces, but little is known as to whether these drugs could alter the fungal development and its efficacy. Thus, the aim of this study was to determine the in vitro effect of certain anthelmintics on the growth of this fungus. In two assays, active ingredients diluted in methanol of the five anthelmintic drugs most commonly used in Argentina were used in concentrations reported as found in bovine faeces: levamisole, 1 ppm; albendazole, 0.027, 0.054 and 1 ppm; fenbendazole, 0.027, 0.054 and 1 ppm; ricobendazole, 1 and 2.77 ppm; ivermectin, 1, 2 and 10 ppm. Each of these drug concentrations were added to corn meal agar (CMA) 2% and then poured on Petri dishes (n=12/concentration). Plates with CMA and containing only methanol and only *D. flagrans* were used as control. All plates were inoculated with 1 cm² of fresh *D. flagrans* mycelia growing in CMA, and incubated at 27°C for 7 (assay 1) or 12 (assay 2) days. The fungal growth rate was determined every 24 h by measuring the radial growth. Similar fungal growth was obtained from the control plates containing only *D. flagrans* (6.79 to 8.12 mm/d), only methanol (6.5 to 7.17 mm/d), levamisole (6.49 to 6.99 mm/d) and both concentrations of ricobendazole (6.51 and 7.53 mm/d). Ivermectin slowed significantly (P=0.0181 – P=0.0351) the fungal growth in all tested concentrations (6.14, 5.67 and 6.29

mm/d). Albendazole reduced the mycelial growth only at 1 ppm (0.89 mm/d, P<0.0001), while fenbendazole affected negatively the fungal growth in all concentrations (0.46 to 2.19 mm/d, P<0.0001). These results represent a first step on elucidating whether anthelmintics could be used in very specific situations while biological control is applied.

PS03.58 Evaluation of Attractiveness and Specificity of New Plastic Blue Screens for Monitoring and Control of *Stomoxys Calcitrans* Populations

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Stomoxys calcitrans is considered as a major pest of livestock worldwide. Insecticides have been extensively used to control this pest but resistance to these chemical compounds is now reported in many countries. Therefore, a more sustainable and efficient control is needed. A lot of different traps have been tested to catch stable flies such as Vavoua and Nzi traps or Alsynite sticky traps. However, low numbers of stable flies are caught per day and per trap with these devices (usually less than two hundreds individuals per day and per trap), which is sufficient for establishing a population's dynamics but not for control. The objective of this study was to evaluate the attractiveness and the specificity of seven different new types of blue screens (one polyester fabric blue screen and six plastic blue screens) for stable flies. These screens, showing slight differences in their reflectance around 460 nm, were tested during summer 2016 in southwestern France. Height of the screen and its east or west orientation were also considered. High levels of *S. calcitrans* captures were recorded during this study (from 141 to 7301 individuals per blue screen and per day) whereas the numbers of

Tabanids and pollinator insects remained very low (less than 10 individual per screen and per day). No significant difference in attractiveness has been shown between the different types of blue screens. The lower half of the blue screens caught significantly more stable flies (70%) than the higher half (30%). The “east” side of the screen was a little more attractive (60% of stable flies) than the west side. These results are highlighting the interest of these blue screens not only to monitor populations but also to control stable flies in cattle farms as some insecticides could be incorporated in the plastic material.

PS03.59 Alternative Treatments for Southern Cattle Fever Tick, *Rhipicephalus Microplus* (Acari: Ixodidae) Infested White-Tailed Deer Hides

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Southern cattle fever tick (SCFT), *Rhipicephalus microplus*, is the most economically important invasive species of ticks in the United States. SCFT parasitizes cattle and also wild ungulates as the white-tailed deer (WTD), *Odocoileus virginianus*, which can spread tick populations posing a threat to the cattle fever tick eradication program (CFTEP). Hunter killed WTD and other game hides are inspected for ticks and treated with coumaphos before they leave tick quarantined areas of Texas. Concerns over human intoxication have led the CFTEP to consider alternative treatment methods. Essentria® IC3 (EIC3), a plant essential oils-based product and entomopathogenic nematodes (EPN) (*Steinernema carpocapse*)

are known to be lethal to ticks. Simple Green® (SG), a household cleaning product, has anecdotal information of insecticidal activity. The present study aimed to determine efficacy of EIC3, EPN and SG against adult ticks on deer hides. In vitro bioassays with adult and larval stages of SCFT were carried out to establish doses of EIC3 and SG to be used in the hides treatment. For the deer hide test, each hide (10 replicates) was inoculated with ten engorged female ticks and treated with one liter of EIC3 (6.25%), EPN (50,000 infective juveniles/ml), SG (100%) or water (control). After treatment the hides were rolled and placed into plastic bags and held at 80°C for 12 hours. After 12 hours, ticks were incubated to allow for egg laying and larval hatch determination. SG treatment resulted in low mortality rates both in vitro (10%) and in the hides (27%). EN resulted in even lower mortality (<15%). EIC3 was the most effective treatment tested against SCFT resulting in 100% mortality of larvae, 94% reduction of fertility of adults and 98% mortality of fully engorged females in treated hides. EIC3 can be used as an alternative to treat SCFT infested deer hides.

PS03.60 Dog's Parasite Control Practices by Veterinarians in Brazil

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The aim of the present study was assess dog's parasite control practices by Vets from different regions of Brazil. The on-line survey were performed with 403 Vets. From all professional surveyed 41% (n=168) routinely ask stool test being 21% every six month, 16% yearly and 98% performed prophylactic treatment to helminth. The most commonly antihelminth (AH) used

was the association of Benzimidazoles+Pyrimidines+Pirazinoisoquinolone (33%) and Benzimidazoles+Macrocyclic lactones+Pyrimidines+Pirazinoisoquinolone (22%). The AH group are change by 57% of the Vets and 26% already suspected the inefficiency, being the Benzimidazoles the group most cited (23%). The data showed that 47% of the vets change routinely the drugs against ectoparasites and 92% associate with environmental control. A high percentage of surveyed (58%) reported a dog hypersensitivity to collar for fleas and ticks control. The Isoxazoline was the most indicated group (40%). The Vets report inefficiency of different chemicals groups to tick control. The most report was Pirazol (63%), followed by Pyrethroids (8.2%) and Isoxazoline (5%). These results show the indiscriminate use of antiparasitic compounds and the reports of resistance accelerates the need for guidelines for treatment and control of dogs parasites in the veterinary clinic.

PS03.61 A Teladorsagia Circumcincta Recombinant Vaccine Prototype Is Able to Protect Lambs of Canaria Hair Breed Sheep

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Gastrointestinal nematodes (GIN) are a major constraint on production in the small ruminant sector worldwide. As anthelmintic resistance is now widespread, alternative non-chemical control strategies have become a priority. Recently, a recombinant vaccine prototype against *Teladorsagia circumcincta* has been shown to be protective in Texel-cross sheep. The level of immunity stimulated by the vaccine varies between individuals and with age. Previous studies suggest that Canaria Hair Breed (CHB) sheep have an element of 'natural resistance' to GIN infection, including in young lambs. Here, we tested the effect of the *T. circumcincta* recombinant vaccine in three-month-old CHB lambs subjected to a trickle challenge with this parasite.

Vaccinated animals responded favorably to immunization; over the sampling period, vaccinates shed 66% fewer eggs (when measured as mean cumulative faecal egg counts) than challenge control lambs. At postmortem, vaccine recipients had a 70% mean reduction in mean total worm burden compared to the control group. These results indicate that, in young lambs of this breed, the subunit vaccine is capable of inducing vaccine -protective responses at an early age. Acknowledgements: European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No. 635408 (PARAGONE).

PS03.62 Anthelmintic Effect of Banana Plantation Residues in Sheep

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Acetone extracts of leaves, pseudostems and bunch peduncles of the "Nanica" banana cultivar, a triploid clone (AAA) of the Cavendish subgroup, significantly inhibited *Haemonchus contortus* eggs from hatching in vitro, even when condensed tannins were suppressed with polyvinylpyrrolidone ($p < 0,0001$). A further extraction was performed with n-butanol. Thin layer chromatography (TLC) was conducted with chloroform: methanol: water (70: 30: 4) as solvent system and vanillin – phosphoric acid as reagent. A retention factor corresponding to syringin was obtained. Syringin is a phenylpropanoid,

a group of phenolic compounds related to banana plant resistance against phytonematodes. Total phenolic contents of leaves, pseudostems and bunch peduncles were 66.2 ± 0.4 ; 30.9 ± 0.4 and 47.1 ± 0.7 mg of gallic acid equivalents per 100 g of sample, respectively. No alkaloids were detected by TLC. Samples were negative for saponins, producing neither persistent foam nor hemolysis. Wet basis moisture contents were 89.01%; 93.00% and 94.28%, respectively. Crude protein was 17.47%; 12.47% and 8.68%, respectively. In spite of lower protein contents, bunch peduncles are easily available at the packing houses of banana plantations, so they were chosen for in vivo trials. Twenty four crossbred sheep artificially infected with *Haemonchus* were blocked into four groups according to their fecal egg counts (FEC). Each group was assigned to treatment with fresh chopped bunch peduncles fed at 0, 10, 20 and 30% dry matter during 14 consecutive days. Diet was complemented with *Brachiaria* hay ad libitum and mineral salt. Sheep developed a normocytic anemia at the end of the trial. FAMACHA scores did not correlate to FEC under trial conditions. Chopped peduncles have significantly reduced FEC on day +13 ($p=0.0286$) compared to those fed 0%. Although long term studies and further phytochemical investigation are required, the current results offer evidence for use of these plantation residues as an anthelmintic.

PS03.63 *Giardia* Spp.: Diagnostic Evaluation of the Immunochromatographic Strip Test, the Parasitological Method of Flotation, the Enzyme Immunoassay and the Polymerase Chain Reaction

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Introduction: The protozoa of the genus *Giardia* remain a common cause of diarrhea in dogs and constitute a public health concern. The aim of this study was to evaluate the diagnostic performance of the immunochromatographic strip test Speed™ *Giardia* (Virbac) compared to the parasitological method of flotation, the enzyme immunoassay (ProSpecT™ *Giardia* Microplate) and the polymerase chain reaction (PCR) for canine giardiasis.

Materials and methods: Initially, fecal samples from dogs with the typical clinical signs of giardiasis were tested with the strip test Speed™ *Giardia* and two groups of 50 dogs emerged, namely group A and group B with positive and negative samples, respectively. Thereafter, all samples were examined by zinc sulfate 33.2% flotation, the ProSpecT™ *Giardia* Microplate assay and PCR. The combination of the last two methods was considered as a gold standard during statistical analysis.

Results: *Giardia* cysts were not detected with microscopy in 16 out of the 50 samples (32%) of group A, neither in samples of group B. Eight out of 50 samples of group B (16%) were tested positive both with the ProSpecT™ *Giardia* Microplate assay and PCR. Fecal examination with the strip test Speed™ *Giardia* was significantly more sensitive (86.2%, CI95 of 75.1% to 92.8%) than the parasitological method (58.6%, CI95 of 45.8% to 70.3%) and gave results comparable to those of the ProSpecT™ *Giardia* microplate assay. The specificity was excellent (100%, CI95 of 91.6% to 100%) for both Speed™ *Giardia* and parasitological method.

Conclusions: The immunochromatographic strip test Speed™ *Giardia* (Virbac) is a quick, easy to perform and efficient method. However, in case of a negative result in dogs with compatible clinical signs it is advised to confirm by an enzyme immunoassay and PCR.

PS03.64 Cost-Benefit Analysis of Four Helminth Control Strategies in Naturally Infected Sheep on a Low Technification Property

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This research evaluated cost-benefits of four helminth control strategies in ovines. Forty-eight matriarchs, naturally parasitized by gastrointestinal helminths, were distributed in four groups: GI - animals with egg per gram of feces (EPG) counts ≥ 1000 by Mini-FLOTAC evaluations were treated; GII - similar to GI, but using McMaster analyzes; GIII - all animals treated every 56 days; GIV - deworming of animals with FAMACHA® degrees 3, 4 or 5. Coprocultures were performed on fecal samples, to identify helminths genres, and parasitological necropsies were performed in five animals that died. For cost-benefit calculations, sums of expenses with treatments, laboratorial exams and materials for sample collection were calculated. According to results of coprocultures and necropsies, *Haemonchus* and *Trichostrongylus* were the most prevalent amongst all 10 diagnosed genres, representing 94.49% of total parasitic burdens. Statistical differences in EPG counts between groups were observed in only five dates, with GIV showing higher averages in three of those. Regarding treatments, only GIII and GIV were similar ($P > 0.05$), receiving a smaller number of doses at the end of one year. Total treatment costs were USD 2.74, USD 3.57, USD 4.65 and USD 3.82 for groups IV, III, I and II, respectively. Strategies I, II and IV required extra expenses of USD 1,462.11, USD 1,391.71 and USD 11.89, respectively. In spite of these extras, the strategy adopted in group III presents disadvantages in relation to presence of drug

residues in meat, and pressure on helminth populations for selection of resistance. Therefore, it can be inferred that application of the FAMACHA® method significantly reduced costs, in comparison to other techniques, making it possible to indicate it as a strategy with the best cost-benefit ratio.

PS03.65 A Case of Chorioptic Mange in a Dairy Farm in the Municipality of Descalvado, SP, Brazil

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Fourteen lactating cows in a dairy herd of 300 Holstein Frisian cattle presented bilateral crusty focal lesions on the base of the tail. Skin scrapings were collected for direct examination after clarification with 10% potassium chloride. Tissue samples from the affected areas and from the adjacent healthy skin were collected for histopathological examination with the aid of disposable dermatological punches. Skin samples were immediately placed in cassettes for fixation with 10% buffered formaldehyde. At the laboratory they were dehydrated with increasing ethyl alcohol concentrations and then treated with xylol for later paraffin inclusion and block assembly. Samples were cut in a microtome (3 micrometers) and assembled on slides to be stained with hematoxylin and eosin. Direct examination revealed *Chorioptes bovis* in all of the samples. Histopathological examination revealed intense hyperkeratosis and acanthosis of the epidermal strata; crusts with acari and polymorphonuclear cells; intense multifocal eosinophilic, neutrophilic

and lymphoplasmacytic infiltrate in the epidermis and dermis; sudoriparous glands with dilated ducts; accentuated edema and congestion of the epidermis and dermis; moderate eosinophilic and lymphoplasmacytic perivascular cuffing in the reticular dermis, consistent with an eosinophilic epidermidis caused by parasitic mites. Infected animals were treated with 0.5% eprinomectin pour on (Eprinex Pour-on, Boehringer Ingelheim). Except for one cow, all skin scrapings performed thirty days after treatment were negative. However, on this occasion, another two animals with clinical signs were identified. These findings are an evidence of the usual mild nature of this parasitic infection. Skin lesions were restricted to the base of the tail and didn't result in apparent pruritus or decreased productivity noticeable by the farmer. The recurrence after a single treatment with eprinomectin and the occurrence of another two cases in the period exposes the difficulty of eradicating chorioptic mange, provided it seems to go unnoticed amidst the intense dairy farming routines.

PS03.66 A Novel Application of Enzyme-Linked Fluorescent Assay for Detection of Veterinary Toxoplasma Gondii Infections in Multiple Hosts

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Toxoplasma gondii is a global zoonotic protozoan parasite of endothermic animals. In addition to causing clinical disease in domestic and companion animals, this parasite threatens captive exotic and wild animals. Veterinary diagnostics rely primarily on serologic methods, most requiring species-specific conjugates. Our laboratory has adapted the modified agglutination test (MAT) for simultaneous IgG and IgM detection in warm-blooded animals, testing more than 18,000 samples over 30 years. The commercially produced MAT is ideal in veterinary testing due to the nonspecific reagents suitable for testing multiple host species. However, the federally regulated reagents require special import permits and the necessary tachyzoite

propagation remains challenging. To expand the diagnostic capabilities of our lab, we have validated the novel application of an automated platform and assay developed for human diagnostics. Using 312 previously MAT IgG antibody tested frozen serum samples (Negative=154; Positive=158), we evaluated the miniVIDAS[®] and TOXO Competition (TXC) assay (bioMerieux) for comparison. Representative animal groups included our most common clinical and research submissions: canine (n=62), feline (n=63), macropod (n=62), primate (n=61), and aquatic mammal (n=64). Nonparametric analyses evaluated interrater agreement (K), homogeneity (χ^2), and performance (ROC AUC). Overall, TXC was an excellent test compared to the MAT for combined animal groups. Stratified analyses indicated that each canine, feline, macropod, and primate animal group had near perfect or perfect agreement with excellent performance. However, among aquatic mammals, TXC had a poor to fair performance depending on the species. In this study, we determined that the novel use of the TXC assay has excellent overall performance for IgG detection, particularly in canines, felines, macropods and primates. However, additional work is required to validate this technique for aquatic mammals. This assay represents a valuable tool for detection of *T. gondii* in companion animals, captive exotics, and wildlife.

PS03.67 Seroprevalence, Biogeographic Distribution and Risk Factors for Aelurostrongylus Abstrusus Infections in Cats

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Aelurostrongylus abstrusus is a worldwide occurring feline lungworm. The spectrum of clinical signs in infected cats ranges from mild to severe respiratory distress. We performed a seroepidemiological study to define prevalence and risk factors for *A. abstrusus* infections in Swiss cats and to

assess the biogeographic distribution of this parasite. Sera of 4067 domestic cats were tested for the presence of antibodies against *A. abstrusus* by a novel ELISA and the results correlated with biogeographic aspects. A subsample of 1000 datasets was used for risk factor analyses. Overall, 10.7% (434/4067, 95% confidence intervals [CI]: 9.7-11.7%) of the cats were tested positive, with variations from 0.0% to 20.0% among ten different biogeographic regions. Differences were significant between the Western (13.9%, CI: 11.4-16.7%) and the Eastern (9.2%, CI: 8.0-10.5%) Swiss Plateau, possibly attributable to the suitability of the areas for intermediate hosts. In total 90.3% (392/434) of the seropositive cats originated from regions lower than 700 m above sea level. Correspondingly, 98.9% (429/434) of positive samples were obtained from regions with a mean temperature higher than -2 °C in January, suggesting altitude and temperature being limiting factors for *A. abstrusus* infections. Concerning individual risk factors, prevalence was higher in intact (15.5%, CI: 9.5-23.4%) than in neutered cats (5.8%, CI: 7.9-10.4%). Young adult cats (aged 11-22 months) were significantly more often seropositive (10/76, 13.2%, CI: 6.5-22.9%) than kittens aged 1-10 months (1/34, 2.9%, CI: 0.1-15.3%) or adult and senior cats > 22 months (58/889, 6.5%, CI: 5-8.4%). We here confirm that the use of a serological test can contribute to improve the identification of infected animals, through evaluation of risk factors on a population level and for a better management on an individual level, overcoming the challenges represented by faecal examinations and the correlated underestimation of the occurrence of *A. abstrusus* in cats. Further seroepidemiological studies are ongoing.

PS03.68 *Morishitium Polonicum* in Black Birds (*Turdus Merula*) in Baden-Wuerttemberg, Germany

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The family Cyclocoelidae comprises numerous flattened digenetic trematode species parasitizing in diverse organ systems and the thoraco-abdominal cavity of birds. Most of the cyclocoelid trematode species develop in wet land mollusks except *Morishitium polonicum*, which is transmitted by terrestrial snails of genus *Helicella*. In 2018, 11 black birds (*Turdus merula*) from Northern Baden-Wuerttemberg were routinely examined for parasites by section after being found dead or severely injured and euthanized due to human endpoints for health burden. Trematodes were found in 4 animals, being mainly located in the thoraco-abdominal cavity above posterior air sacs, scattered from caudal part of lungs until caudal end of thoraco-abdominal cavity with a parasite burden range from 12 to 51 worms per bird. No trematodes could be found in visceral organs (air sacs, lungs, liver or intestine). According to morphological criteria (size and localization of testes, ovary, suckers, oesophagus etc.) and sequencing of 672 bp of 18S rRNA gene, the parasites were identified as *M. polonicum* (Cyclocoelidae). This species has been described in several European countries including Poland (Baltic seacoast), Czech Republic, Spain (Mallorca) and Italy, but no reports on occurrence of *M. polonicum* are available from Southern Germany and Northern Baden-Wuerttemberg. It can be assumed that *M. polonicum* was introduced into this region by migratory birds. The spreading of the trematode parasite might have been increased by Usutu virus, which had caused massive *Turdus merula* die-off circulating in Southern Germany since 2011-2012, influencing the population dynamics of black birds. The reduction of the black bird population in Germany could have led to additional immigration of *M. polonicum* infected black birds from neighboring countries. To our knowledge, this is the first detection of *M. polonicum* in this region of Germany.

PS03.69 Canine Cardio-Vascular Helminthoses in Dogs in France: Distribution and Dynamics of Heartworm (*Dirofilaria Immitis*) and French-Heartworm (*Angiostrongylus Vasorum*) in France: Results From a National Survey With Veterinary Clinics

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A national survey on canine Angiostrongylosis and Dirofilariosis was conducted in summer 2017 with questionnaires sent to veterinary clinics, to evaluate the distribution and prevalence of these diseases covering the period 2008-2017. Information obtained from 620 questionnaires was compared to previous studies (2000 and/or 2008). More than one case of angiostrongylosis and dirofilariosis was detected in clinics from respectively 50 and 28 administrative territories (départements n = 90). Cases were considered autochthonous (= suspected to be acquired within the territory of activity of the veterinary clinic) in 41 for Angiostrongylus and 9 for Dirofilaria (6 in 2008). The two parasites seem to overlap in only 6 areas, mainly located in the Mediterranean zone. Up to 10 annual cases of Angiostrongylosis were diagnosed in clinics from 8 areas. For Dirofilaria, clinics diagnosing 2-4 annual cases were found in 3 areas (up to 10 in one). Angiostrongylus was widely distributed but more frequently in 3 regions: Ile de France (around Paris), Rhône Alpes (mid east) and the historical region of South-west. Multiple cases in kennels were diagnosed in 19 areas. The distribution of Dirofilariosis is much dispersed with more cases in the perimediterranean zone and Corsica. The dynamics was evaluated. In 19 areas Angiostrongylosis was considered increasing, stable in 29. For Dirofilaria the diagnosed cases (mainly considered imported) were considered increasing in 24. Interestingly Angiostrongylosis was diagnosed in 19 areas not previously identified (2000 and 2008) suggesting a possible extension. In return Dirofilariosis was newly mentioned only on area. Canine Angiostrongylosis is an active and widely distributed canine helminthosis

in France and Dirofilaria remains apparently located to the southern part of the country, also possibly slowly extensive. However the global increase of diagnosis of cases of Dirofilariosis suggest a wide distribution of dogs as sources to mosquitoes vectors with potential changes in the future.

PS03.70 Companion Animal Parasite Council: One Health in Practice

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Over the past few decades, companion animals have become an integral part of many family households in the United States and around the world. Not only do pets share a home with their human counterparts, they often accompany the family away from the home, particularly dogs. Consequently, companion animals and humans share in risks of exposure to a variety of vectors and pathogens. The Companion Animal Parasite Council (CAPC) recognizes this shared risk and aims to conduct scientifically rigorous studies on the relationship of infectious disease between humans and animals. CAPC currently maintains a repository of testing data from dogs and cats covering vector-borne pathogens (*Borrelia burgdorferi*, *Anaplasma* spp., *Ehrlichia* spp., and *Dirofilaria immitis*), gastrointestinal parasites (*Toxocara* spp., *Ancylostoma* spp., *Trichuris* spp., and *Giardia* spp.), and viral pathogens (Feline Leukemia Virus, Feline Immunodeficiency Virus). These data are used to maintain surveillance of these pathogens within the United States through maps available at <https://capcvet.org/maps> which are updated monthly. Additionally, annual forecasts are provided in the early spring each year predicting the seroprevalence of *B. burgdorferi*, *Anaplasma* spp., *Ehrlichia* spp., and *D. immitis* in dogs. A recent publication describes the association between the canine *B. burgdorferi* data and human incidence of Lyme disease. This further substantiates the

use of dogs as sentinels for zoonotic vector-borne disease. CAPC aims to use canine data to inform on human risk of vector-borne disease with the ultimate goal of providing a tool for health care providers and public health officials to use in the prevention and control of vector-borne disease. The data is available on a monthly and county-level scale, eliminating much of the missing data, underreporting, and reporting lags found in human case reporting. With these, CAPC can produce maps regularly that estimate the level of risk of exposure to various vector-borne pathogens.

PS03.71 The Prevalence and Intensity factors of Otodectic Mange In Cat In Two Districts of Pakistan (Lahore and Sheikhpura).

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The study was conducted to update the status of Otodectic mange in commensurate with age, sex, breed, housing and intensity. To this end 450 samples were collected from the ear wax of cats brought on pets' clinic from district Sheikhpura and Lahore. Skin scraping and ear wax were collected from all the Cats selected for sampling and observed under the Microscope. By statistical analysis the prevalence observed was 38.03% and 32.40% in Lahore and Sheikhpura. The ubiquity in cats with the age of less than 12 months and more than 12 months was 39.67% and 29.8% respectively. Prevalence rate was noted higher in male cats (36.25%) than female (34.39%). No specific breed associations have been found. Outdoor cats have more prevalence (46.85%) than those that lived indoor (12.29%). So more the grubby environment more will be the prospects of ear mites. Besides this Cotton bud soaked in liquid paraffin is construct to be the easiest and the affordable method for the detection of ear mites. Clean environment for housing and regular ear cleaning is the best way to control its infestation.

PS03.72 A Rapid, Parasite-Dependent Cellular Response to *Dirofilaria Immitis* in the Jird (*Meriones Unguiculatus*)

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The Mongolian jird (*Meriones unguiculatus*) is long-recognized as a permissive host for the filarial parasite *Brugia malayi*. This is believed to result from the immunological characteristics of the species, as particular immunodeficient mouse models exhibit this same permissivity. At the same time, the jird is nonpermissive to another filarial parasite, canine heartworm (*Dirofilaria immitis*), and so by elucidating differences in early response to infection, we hope to identify mechanisms involved in the species-specific clearance of these parasites. We hypothesized that the early clearance of *D. immitis* in intraperitoneal infection of the jird is immune-mediated and parasite species-dependent.

When *D. immitis* third-stage larvae (L3) were injected intraperitoneally into jirds (200 each, $n = 3$), only 20% of parasites were recovered at 1h (range: 0 – 74), with 85% exhibiting host cell attachment. We then assessed the species-dependency of this cell attachment in vitro. Cultures were prepared as three groups: *D. immitis* L3, *B. malayi* L3, and co-culture of both parasites. Each group was cultured with peritoneal exudate cells (PECs) from naïve jirds and paired with a media-only control (20 L3 per well, $n = 3$ for 3 experiments). We then assessed host cell attachment and parasite survival microscopically after 20h incubation.

In all conditions, cell attachment to *D. immitis* was 100%, while *B. malayi* was much lower (mean = 5.56%), suggesting a strongly species-dependent response from which *B. malayi* could not confer immediate protection in co-culture. When we replicated these experiments with PECs derived from jirds subcutaneously infected with *B. malayi*, results were similar (99.4% and 4.72% of *D. immitis* and *B. malayi*, respectively, exhibited cell attachment). Applying Wright's stain, the cells responsible for this L3 attachment were morphologically most consistent with lymphocytes, and the specific nature of this attachment is the subject of ongoing study.

PS03.73 Diving Deeper into Fecal Counts: Exploring New Technologies to Identify and Quantify Eimeria in Mixed-Species Samples

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Coccidiosis, caused by a number of protozoan parasites in the genus *Eimeria*, is a common and costly intestinal disease affecting the poultry industry globally. *Eimeria* species are ubiquitous in commercial poultry facilities, but presence does not equate with disease due to the biological characteristics of the parasites' self-limiting life cycles. Multiple *Eimeria* species can infect a chicken simultaneously with each parasite species varying in its pathogenicity and perceived commercial importance. Therefore, identification of individual *Eimeria* species in mixed samples is valuable for understanding this disease complex.

Molecular assays can provide a more reliable means of identifying these parasites based on DNA targets present in a sample. Conventional PCR, both direct and nested, has been used successfully for *Eimeria* species identification but standard PCR cannot provide quantification data. Newer technologies may be able to provide the necessary data for understanding relative abundance of *Eimeria* species in sample. Digital droplet PCR (ddPCR) is a sensitive quantitative method that could be applied to the identification and quantification of *Eimeria* species. A ddPCR-based method was developed that exploited mitochondrial genome targets that were genus-specific or species-specific. DNA and purified oocyst samples of known identities were tested in combinations to assess the sensitivity and repeatability of this experimental assay. Early results have been promising using samples of three *Eimeria* species considered to be the most important to industry with good assay sensitivity. Following additional validation studies, samples from commercial facilities with unknown contents will be tested. Obtaining relative species abundance

and correlating it with oocyst per gram (OPG) counts will create data needed by veterinarians, researchers, technical representatives, nutritionists and producers. Converting uninformative OPG counts into precise measures of parasite diversity and relative abundance is prerequisite for unraveling this disease complex and thereby improve control of coccidiosis in commercial poultry industries.

PS03.74 MarkerDB: A Pipeline to Build Bespoke Databases of Marker Gene Sequences for Identifying Taxa in Samples

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Progress in next-generation sequencing technologies is revolutionizing our ability to use molecular approaches to identify and quantify the presence of different parasite and other pathogen species in fecal, environmental and other samples. However, there are a variety of challenges to the accurate identification of taxa, particularly to the species level, including marker selection, bioinformatic pipeline choice and parameterization, reference sequence availability and database selection and curation. High quality databases are essential for accurately identifying taxa in samples using marker genes such as the ribosomal small subunit (SSU) and the internal transcribed spacer (ITS). Although large publicly available databases exist for both the large and small ribosomal subunits, such as SILVA, there are few options for other marker regions such as ITS-2 rDNA that may be more suitable for particular projects. Furthermore, large databases often have long delays between release cycles. markerDB is a pipeline to build databases of any chosen marker gene sequence for a given taxonomy built in R and snakemake. Currently it supports ITS-2 and 18S rDNA sequences but can be easily expanded to be use for any

gene. The pipeline retrieves sequences from NCBI annotated with the provided marker, identifies the correct region using a hidden markov model (using barnap, <https://github.com/tseemann/barnap>) or covariance model (using infernal) and formats them for common pipelines like RDP, dada2 and mothur. This pipeline provides speed and flexibility beyond what is available in public databases. markerDB is available at <https://github.com/ucvm/markerDB>.

PS03.75 Evaluation of the Preventive Efficacy of a Permethrin-Fipronil Based Spot-On (Effitix® Spot-On) for Canine Leishmaniasis and Dirofilariosis in a Highly Endemic Area in Greece: An Open Field Trial

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Canine dirofilariosis (*Dirofilaria immitis*) and leishmaniasis (*Leishmania* genus) are two vector borne diseases, occurring worldwide including now in northern countries(1)(2). Therefore affordable and efficient options are needed to struggle against reservoirs carriage, to protect both animals and humans health. Thirty privately owned dogs, living outdoor, in a highly endemic area of northern Greece (Serres), were recruited. In clinic diagnostic tests (Speed LeishK /Diro, Virbac BVT, La Seyne sur Mer, FRANCE) were used to screen and exclude positive carriers (detection of anti leishmania's kinesin antibodies and antigens from adult filaria) before enrollment. Each dog was treated with Effitix® (Permethrin 44.88% - Fipronil 6.01%) following manufacturer recommendations, at inclusion time, then on a monthly base, over 18 months (from June 2017 to November 2018). Dogs were monthly blood sampled to follow their status.

Among the 30 dogs, one accidentally died, two were found to be dirofilariosis positive (in August and September respectively: which implied a contamination prior to enrollment), three additional leishmaniasis infected dogs were removed from the study; all were excluded accordingly from the

analysis regarding Dirofilariosis. At the end of the study 22 out of 24 dogs remained clinically healthy with negative testing, giving a protection of 91.7% against Dirofilariosis. Regarding leishmaniasis, the 4 dirofilariosis infected dogs as well as the car accident were removed from the calculations; at the end of the study, 22 of them remained negative, giving an 88% protection against leishmaniasis.

Those encouraging results show Effitix® spot-on can be one efficient option to prevent Dirofilariosis and Leishmaniasis transmissions; results would need to be confirmed on larger scale with a control population.

PS03.76 Evolution of the Efficacy of a Combined Moxidectin-Levamisole Treatment Against Resistant Gastrointestinal Nematodes in Lambs

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Nematocidal combinations can be a valid strategy to achieve effective nematode control in the presence of drug resistance. The aim of the current trial was to evaluate the pharmaco-parasitological outcome after the continuous use of moxidectin (MXD) and levamisole (LEV) as a combined treatment in lambs naturally parasitized with ivermectin-resistant gastrointestinal nematodes. Forty (40) lambs were divided into four groups (n=10): untreated control and subcutaneously treated with either MXD (0.2 mg/kg), LEV (8 mg/kg) or with MXD+LEV (0.2 and 8 mg/kg, respectively). Blood samples were collected at different times up to 1 (LEV) or 14 (MXD) days post-treatment. LEV and MXD plasma concentrations were measured by HPLC. Faecal samples were collected on days 0, 7, and 14 post-treatment to perform the

faecal egg count reduction tests (FECRT). No significant pharmacokinetic (PK) adverse changes were observed for either MXD or LEV after their co-administration in sheep. The clinical efficacy of the MXD+LEV combination was evaluated after its continuous use (3 treatments/year) over five (5) years at the same farm. The initial anthelmintic efficacies (1st year) were 99% (MXD), 85% (LEV) and 100% (MXD+LEV). Following repeated annual treatments over five years, the clinical response for the combined treatment reached 87% efficacy. The combination reached efficacies of 100% (1st year) and 98.5% (5th year) against *Haemonchus contortus*. *Teladorsagia* spp. and *Trichostrongylus* spp. were the main nematode genera surviving the individual and combined treatments. The co-administration of MXD+LEV during five years resulted in a significant higher anthelmintic effect compared to MXD or LEV given alone. Even when MXD and LEV individual efficacies were reduced during the five-year period, the combined treatment maintains acceptable efficacy levels against *H. contortus*.

PS03.77 Polymorphisms in the Acetylcholinesterase 3 Gene in Cattle Fever Ticks (*Rhipicephalus Microplus*), Isolates from Uruguay and Southern Brazil

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The objective of this study was to identify mutations in acetylcholinesterase 3 gene (BmAChE3) of cattle fever ticks (CFT - *Rhipicephalus microplus*) previously associated with resistance to organophosphates (OP) in field isolates of CFT obtained in Uruguay and Rio Grande do Sul state, Southern Brazil. CFT populations (n=24) were submitted to the larval packet test with ethion in order to characterize phenotypic resistance. To identify nucleotide polymorphisms in BmAChE3, we amplified and sequenced a segment of 308 bp where three mutations (I48L, I54V and R86Q)

were found in OP-resistant ticks. In total, the genomic DNA of 134 individuals from susceptible and resistant populations were analyzed. The I54V mutation was found in 133 individuals, the R86Q in 131 and the I48L in 44 individuals. In all ticks that survived ethion exposure, mutations I54V and R86Q were detected. The I54V was found in only 20% of ethion-treated survivors. Both resistant and susceptible ticks presented any of these three mutations, including ticks from a susceptible reference strain (100% with I54V/R86Q). The results obtained in the present study disagree with previous published data associating these mutations with OP resistance in CFT. Mutations in other acetylcholinesterase genes (BmAChE1 and 2) and metabolic detoxification may also contribute with OP resistance in this tick species.

PS03.78 Anthelmintic Resistance and Common Worm Control Practices in Sheep Farms in Belgium

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In contrast to many other European countries, no data were available on the presence of anthelmintic resistance in gastrointestinal nematodes in sheep in Belgium. A faecal egg count reduction test was performed in 26 sheep farms (29 flocks) in Flanders, Northern Belgium. Results indicated widespread resistance against benzimidazoles (albendazole, fenbendazole and mebendazole), with treatment failure (FECR < 95 %) in all 8 flocks investigated. *Haemonchus contortus* and *Teladorsagia circumcincta* were the predominant species after treatment failure. Amino acid substitutions associated with benzimidazole resistance were detected at the codon positions 167 (8%) and 200 (92%) of the isotype-1 beta tubulin gene in *H. contortus*, codon positions 198 (47%) and 200 (43%) in *T. circumcincta* and position 200 (100%) in *T. colubriformis*. Resistance against

macrocyclic lactones (ivermectin, doramectin and moxidectin) (FECR < 95%) was recorded on 7 out of 20 flocks, mainly in *H. contortus* and *T. circumcincta*. Treatment failure was also observed for closantel (in combination with mebendazole) (FECR 53%) and for monepantel (FECR 44%), on one farm each. *Trichostrongylus* spp. and *Cooperia curticei* were implicated with resistance against monepantel.

A questionnaire survey on farm management and worm control measures indicated that worm control was often not sustainable. Ewes and lambs were treated frequently (on average 2.6 and 3.2 times per year), mostly without weighing. Only few sheep farmers (9%) regularly used faecal egg counts to monitor worm infections. Despite the FECRT showing treatment failure, most of the farmers perceived the efficacy of anthelmintics as good (54%) to very good (29%). Only 12% and 4% evaluated the anthelmintic efficacy as mediocre or insufficient, respectively. In conclusion, anthelmintic resistance is widespread in Belgian sheep flocks. There is an urgent need to efficiently promote sustainable worm control practices to sheep farmers and veterinarians.

PS03.79 Storage Stability Study of Solid Based Formulations of the Nematophagous Fungus *Duddingtonia Flagrans* for Biological Control of Gastrointestinal Nematodes in Sheep

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The inappropriate use of chemical antiparasitic agents in ruminants has generated anthelmintic resistance in gastrointestinal nematodes. Biological control using the nematophagous fungus is a promising alternative due to its capacity to develop traps that capture the nematodes in free living phase reducing the use of chemical drugs. A native species of the fungus *Duddingtonia flagrans* (BGMSABV-Df-Col-H-001-2014) was isolated in Agrosavia Colombia and a fermentation medium was developed for its production. Solid- based

formulations for oral administration in sheep were developed, employing a fluidized bed coating process adding different excipients in order to protect the fungus during its transit through the gastrointestinal tract. The aim of this work was to assess the storage stability of the fungal formulations developed. Two formulation prototypes (E3, E16) and a control, consisting of the dried fungus substrate, were stored in glass vials at three temperatures ($20 \pm 2^\circ\text{C}$, $25 \pm 1^\circ\text{C}$, $30 \pm 3^\circ\text{C}$) for 90 days. The response variables evaluated were the in vitro nematophagous activity, fungal viability, fungal concentration and moisture content. The results showed that the viability and concentration did not have significant variation ($P < 0.05$) and remain higher than 1×10^6 UFC/g and 1×10^7 UFC/g respectively, the moisture content also remain under 5 %. The ability of the fungus to trap the nematodes was higher than 80 % during the storage period which is an important characteristic to continue with the product development. Agrosavia will test the developed formulations in sheep at different climatic conditions.

PS03.80 Canine Heartworm Treatment Using a Combination of ProHeart® SR 12 (Injectable Moxidectin) and Oral Doxycycline

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Dogs infected with *Dirofilaria immitis* may develop a life-threatening disease affecting the heart and lungs. Treatment should focus on the elimination of the adult worms as soon as possible after diagnosis of infection, as this will minimize side effects from killing of the worms. ProHeart® SR-12 (SR 12) is approved in Australia, Japan and some Latin American countries for the prevention of heartworm disease for 12 months via a single subcutaneous injection. In this study, conducted in Brazil, after obtaining owner

consent 16 naturally infected dogs were confirmed positive for microfilariae and adult heartworm antigen (SNAP 4Dx Plus). Dogs were then administered SR-12 (0.5 mg/kg of injectable sustained release moxidectin) at the same time as they started daily administration of doxycycline (10 mg/kg BID) for 30 days (Day 0). All dogs received the same dosing schedule of both drugs every 6 months (modified from the approved 12 month dosing interval for SR-12 for heartworm prevention) until two negative adult heartworm antigen tests (SNAP 4Dx Plus) were obtained 6 months apart. Dogs had chest x-rays, echodopplercardiograms, Knott's tests and antigen testing done every 6 months. Microfilariae counts were performed on Days 0, 30 and 150. On Day 150, all dogs were amicrofilaremic. Eleven dogs became antigen negative at 6 months after the first dose and 5 dogs at 12 months of treatment. At 12 months of treatment 7 dogs were considered free of the infection and by July 2019, as the study is ongoing, 4 additional dogs will be tested to confirm their infection status at 12 months and the 5 remaining dogs at 18 months of treatment. All dogs tested 6 months after the first negative antigen test remained negative 6 months later. Preliminary disease evaluation suggests that lung and heart lesions tended to remain unchanged throughout the treatment.

PS03.81 Combined Use of Ivermectin and Levamisole to Control Resistant Nematodes in Cattle: Assessment of Pharmacokinetic Interactions and Therapeutic Responses

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Combination of anthelmintics with different mechanisms of action has been suggested as an effective strategy to delay the development of resistance. In this context, the current study evaluated the efficacy and the potential pharmacokinetic (PK) interactions occurring after the subcutaneous administration of ivermectin (IVM) and levamisole (LEV)

given both separately and co-administered to calves in two commercial farms (A and B). Sixty(60) male calves naturally infected with gastrointestinal nematodes were randomly allocated into four groups(n= 15): Control: animals did not receive anthelmintic treatment; IVM: treated with IVM (0.2 mg/kg); LEV: treated with LEV (8 mg/kg); IVM+LEV: simultaneously treated with IVM and LEV (at the same dose rates). Seven (7) animals from each treated group (Farm A) were randomly selected to perform the PK study. Drug concentrations were measured by HPLC. The efficacy was determined at 14 days after treatment by the FECRT. The IVM area under the concentration vs time curve (AUC) obtained after administration of IVM alone (274 ± 65 ng.d/mL) was similar to that obtained after IVM co-administered with LEV (295 ± 111 ng.d/mL). Likewise, LEV AUC values were similar after LEV administration alone (8.90 ± 2.69 µg.h/mL) or combined with IVM (9.11 ± 1.82 µg.h/mL). No adverse PK interactions were observed after the combined treatment, with similar PK parameters ($P > 0.05$) obtained between the single-drug and combination-based strategy. In Farm A, the overall efficacies were 54%(IVM), 99%(LEV) and 100%(IVM+LEV). While *Cooperia* spp. survived IVM treatment, *Ostertagia* spp. survived LEV treatment. In fact, the efficacy against *Cooperia* spp. was 41%(IVM), 100%(LEV) and 100%(IVM+LEV), and the efficacy against *Ostertagia* spp. was 91%(LEV), 100%(IVM) and 100%(IVM+LEV). Similarly, in Farm B, total efficacies were 55%(IVM), 99%(LEV) and 100%(IVM+LEV). Although LEV alone achieved high efficacy in both farms, the combination was the only treatment that achieved 100% efficacy against all genera (*Cooperia*, *Ostertagia* and *Haemonchus*). Further work is required to understand the advantages of nematocidal combinations in different commercial cattle farms.

PS03.82 Impact Evaluation of a Regular Shampoo Use, on the Efficacy of a Permethrin and Fipronil Combination (Effitix® Spot-On), Against Flea Infestations in Dogs: A Randomized, Controlled Study

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Regular bathing can be required in dogs, especially in subjects with skin conditions impairment, which can discourage from spot-on use, especially when ingredients have no or few systemic activity.

This study evaluates the impact of a weekly neutral shampoo (Shampoo Pet Bril® Neutro) application on the efficacy of a Permethrin (44.88%) plus Fipronil (6.01%) based spot-on (Effitix®, Virbac, Brasil), regarding fleas infestations, in adult dogs. Eighteen healthy Beagle dogs were equally randomly allocated to group 1 (Effitix® without shampoo), group 2 (Effitix® with weekly shampoo) or control group (no treatment). Treatment was applied once on D0, following manufacturer recommendations (at least 60mg/kg of permethrin and 6.7mg/kg for Fipronil). Dogs were infested with +/-100 fleas on D0 (after treatment), D5, D12, D19, D26, D33, D40, D47, and D54. Flea counts were performed regularly 48 hours after each flea challenges. Dogs were subsequently bathed with 50 ml of shampoo and dried with a fluffy towel on D0 (before treatment application for group 1 and 2) and on days 8, 15, 22, 29, 36, 43 and 50.

The mean geometrical efficacies against fleas' infestation (prevention) were equal to 100% for both treated groups as soon as D2, until D21. Subsequent assessments on D28, D35, D42 allowed the calculation of a high efficacy (100% vs 99.2%, 98.92 vs 97.97%, and 98.06 vs 94.74% for group 1 and 2 respectively).

This trial allows to confirm the persisting killing effect of the permethrin-fipronil combination, even when applied just after shampooing, over 35 days together with a weekly bathing of the dog. Those very encouraging results would need to be confirmed on a larger scale.

PS03.83 Efficacy of Topical 0.5% w/v Eprinomectin (EPRINEX®) Administered at 1 Mg per Kg Body Weight Against Larval Oestrus Ovis Infestation in Sheep and Goats

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INTRODUCTION: Oestrus ovis larvae mainly parasitize sheep and goats, but humans are also vulnerable to infestation. Currently, there are no products authorized in Europe for the control of oestrosis for use in lactating dairy animals. Topical 0.5% w/v eprinomectin, administered at 1 mL per 5 kg body weight to sheep and goats, has been recently authorized in Europe as EPRINEX® Multi as broad-spectrum anthelmintic with zero hours milk withdrawal and was thus evaluated against O. ovis larval infestation.

METHODOLOGY: Three blinded clinical studies compliant with GCP and WAAVP guidelines were conducted in Bulgaria (sheep) and Greece (sheep and goats). At each location, recovery of O. ovis larvae from five of six or six animals necropsied pre-study supported the inclusion of animals into the studies (34 adult female sheep or goats per study). Animals were ranked based on pre-treatment bodyweight and allocated at random to remain untreated (control) or to be treated with EPRINEX® topically. Three weeks after treatment, animals were necropsied and O. ovis larval counts were established.

RESULTS: Treatment was well accepted and no health problems were observed throughout the studies. Live *O. ovis* larvae were recovered from 13 of 17 and 16 of 17 control animals in the sheep studies and from all 17 controls in the goat study comprising first, second and third instars in each study. In each study, EPRINEX®-treated animals had significantly fewer live *O. ovis* larvae than the controls ($p < 0.001$). Efficacy of EPRINEX® treatment, based on *O. ovis* larval counts of treated vs. untreated animals, was 100% and 91.3% against infestation with all three *O. ovis* larval stages in sheep and in goats, respectively.

In conclusion, results of the studies demonstrated EPRINEX® to be an efficacious treatment against ovine and caprine oestrosis and thus extend the efficacy profile of this product beyond nematode endoparasites.

PS03.84 Can Combination Deworming Conquer Double-Drug Resistance? Moxidectin and Oxibendazole Combination Treatments Against Double-Drug Resistant Cyathostomins Naïve to Macrocyclic Lactones

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Equine cyathostomins are well-known contributors to the anthelmintic resistance crisis with wide-spread resistance to the benzimidazole drug class, and reports of resistance to the pyrantel (PYR) and macrocyclic lactone drug classes. Combination deworming is suggested as an alternative control regimen for drug-resistant parasites and is commonly implemented in the small ruminant community. Some products exist for horses despite the lack of supporting evidence. Previously, we found that the combination of oxibendazole (OBZ) and PYR did not effectively control double-drug resistant cyathostomins. This population remains naïve to macrocyclic lactone drugs and offers a unique opportunity for research.

The goal of this study was to determine the efficacy of the first moxidectin (MOX) treatment and MOX-OBZ combination treatments against this cyathostomin population, and observe changes in OBZ efficacy. Treatments were given when ten ponies exceeded 100 eggs per gram. Two OBZ treatments (initial and final), one MOX single-active, two combination treatments were given over the course of two-and-a-half years. Fecal egg counts were performed every two weeks, and efficacies were evaluated using the fecal egg count reduction test. The absolute efficacy of MOX single-active also provided 100% efficacy for both combination treatments. The egg reappearance period for MOX was 16 weeks, and the combination treatments were 12 and 18 weeks. There was no significant difference between the first and last OBZ treatment efficacies. There was no evidence of cross-resistance to MOX from the previous OBZ/PYR resistance status. Combining OBZ with MOX did not affect the efficacy as MOX single-active was already 100% efficacious. The lack of improvement in OBZ efficacy suggests that combination treatments using an active where resistance is heavily established may not provide a beneficial alternative regimen for cyathostomin control. Longer term studies and increasing the refugia may affect the OBZ efficacy and warrants future investigation in this population.

PS03.85 In Vitro Characterization of the Effects of Polyhexamethylene Biguanide (PHMB) Alone or in Complex With TLR4 or TLR9 Agonists in Leishmania Infantum Infected Canine Macrophages

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The dog is the main reservoir for *Leishmania infantum* infection which can manifest as subclinical, self-limiting or a severe and fatal

disease. Limited drug treatment options are available. New drugs as well as new immunomodulators for treatment of canine and human leishmaniosis are actively pursued. Polyhexamethylene biguanide (PHMB) is a polymer that has broad antimicrobial spectrum and is used as a disinfectant and antiseptic. However, very limited studies are available regarding PHMB's activity against protozoan parasites. Here, we examined how PHMB alone or in complex with Toll like receptor agonists (TLRa) selectively kills *L. infantum* parasites. DH82 cells were infected at a 10:1 parasite:host cell ratio with *L. infantum* promastigotes. After 3 hours, the cells were washed and treated with 1 µg/mL of PHMB alone or combined with TLR4a (Monophosphoryl Lipid A) or TLR9a (CpG ODNs) agonists. At 24h, cells were fixed with methanol and stained with diff quick to determine the rate of intracellular infection. Furthermore, the supernatants were collected for measurement of IL-6 and TNF-α by ELISA. Results indicated significantly lower infection when cells were treated with PHMB alone ($p=0.043$), PHMB+TLR4a ($p=0.0031$) and PHMB+TLR9a ($p=0.0338$) compared with infected but not treated cells. Additionally, higher production of TNF-α was observed after treatment with PHMB alone or in combination with TLR4a when compared with uninfected and infected untreated-macrophages ($p=0.043$), but not with TLR9a. Moreover, PHMB+TLR4a induced significantly higher production of TNF-α when compared with PHMB+TLR9a ($p=0.043$). No differences were found in IL-6 concentrations in all conditions studied. In conclusion, PHMB combined with TLR4 agonists is a potent antiparasitic drug combination and also induces a proinflammatory response, as demonstrated by decreased infection and increased TNF-α production.



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