

WAAVP



4-8 Sept, 2017



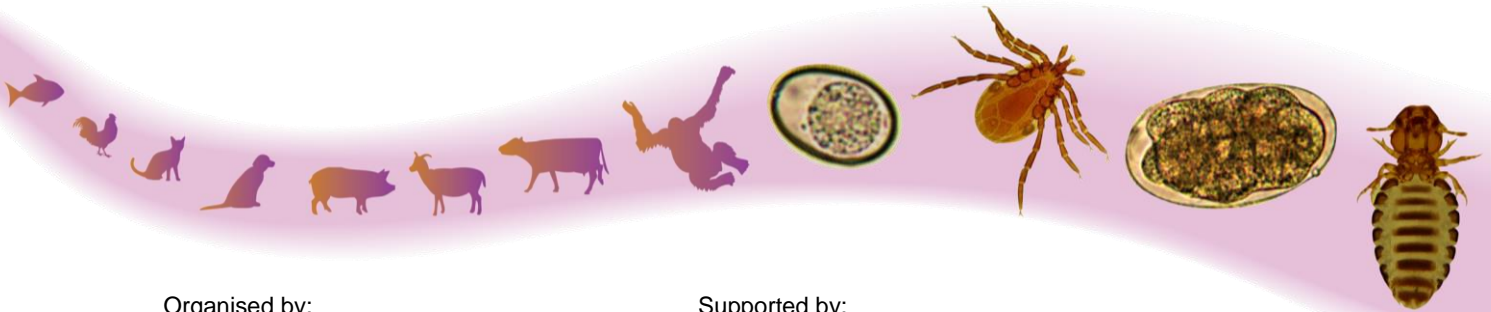
26th International Conference of the World Association for the Advancement of Veterinary Parasitology

In conjunction with 53rd MSPTM Annual Conference

Conference Theme

Combating Zoonoses: Strength in East-West Partnerships

ABSTRACT BOOK



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Keynote Session



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One Health, Zoonosis and the Ivermectin story

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Abstract Content

The imminent elimination of two of the world's most devastating and disfiguring of diseases will be testament to the power of human partnerships to conquer even the most intractable of health problems. Diverse and novel partnerships which have encompassed individuals, the Private and Public sectors, NGOs, affected communities, Scientists and Donors, among others. The fight against the ancient scourges of River Blindness and Elephantiasis, which have plagued poor communities throughout the tropics for centuries, is being won. At the very heart of this success is the Discovery, Development, Donation and Delivery of ivermectin by WHO/TDR, MERCK and various NGOs. A single drug, originating from Japanese soil, that has been recognised as one of the greatest global public health interventions of the last 50 years. Pivotal to the advent of ivermectin was the chemical discovery expertise that exists in Japan, together with the Vision, Endeavour and Commitment of a group of individual multidisciplinary scientists who created and drove forward the collaboration that resulted in what has been widely referred to as a 'Wonder Drug'. The Kitasato Institute, with a long history of ground-breaking drug discovery, was central to the origins of the ivermectin story. And one of its world-leading alumni, Satoshi Omura, was instrumental in establishing the international research collaboration that witnessed the creation of the drug. His fundamental approach and personal philosophy with regard to collaborative research, plus his fortuitous meeting and long-standing friendship with Prof Max Tishler, and Professor William Campbell both of Merck's Research laboratories, was the catalyst for the eventual appearance of the avermectins, a complete novel class of chemicals, and the subsequent incomparably successful derivative, ivermectin. This presentation will briefly cover the major innovative steps, science, key partners and serendipity involved in the various stages of the ivermectin story, along with how the drug was used to help move two socioeconomically destructive and highly-stigmatising diseases to the brink of elimination. The Two Eminent Scientists- the Discoverers of the Drug- Professor Toshi Omura from Japan and Professor William Campbell from the United States were awarded the Nobel Prize for Medicine in December 2015 - The award ceremony in Stockholm which I was privileged to attend.

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Plenary Sessions



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Abstract No: 4956

5 Sept 2017, 1100 – 1130

The expanding parasite genome universe – giving ‘big data’ meaning

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Abstract Content

Diseases caused by parasitic worms have a devastating, long-term impact on hundreds of millions of people and animals worldwide. No vaccines are available against most of these parasites, and control relies heavily on the use of a relatively small panel of anti-parasitic drugs. The excessive and widespread use of such drugs, particularly in livestock animals, has led to serious drug resistance problems around the world, such that there is an ongoing need for the discovery of new interventions, preferably built on sound knowledge and understanding of the molecular biology, physiology and biochemistry of parasites. However, very little is known about these aspects for most parasitic worms. Various research groups have been using advanced nucleic acid sequencing and informatic technologies to decode and annotate the genomes and transcriptomes of socioeconomically important parasites, providing first ‘global’ glimpses of their molecular landscapes. Thus, much progress has been made and major resources established, providing exciting opportunities to underpin fundamental molecular genetic and biochemical investigations as well as applied research endeavors such as discovering new anti-parasite interventions (drugs and vaccines). However, ‘dark matter’ in parasite genomes is vast, and there are still decades of work ahead of us, to make sense of the big data sets emanating from these sequencing efforts using advanced, tailored *in silico* tools. The present talk will give a perspective on the expanding parasite genome universe and informatic challenges for ‘big data’ analyses as well as the need for laboratory- and field-based investigations to complement ‘omic research of parasites.

Keywords: Parasite; worm; genomics; transcriptomics; big data

Abstract No: 3272

5 Sept 2017, 1130 – 1200

Anthelmintic resistance: 50 years and counting...

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Abstract Content

Fifty years after anthelmintic resistance was first reported, it is now timely to reflect on how the research has unfolded and what we can learn for the future. Important aspects are to look at our understanding of the phenomenon of resistance, how resistance has increased in range and severity, how we measure it and how we have responded to it. We can tally up how many hosts, parasite species, drugs and countries are involved. We can also count the monetary costs to gauge impact. Critical in this exercise and in the success of control is how we estimate prevalence. Yet measuring and diagnosing resistance continues to develop. What do we actually count and how do we derive meaning? Looking forward, how has our knowledge influenced parasite control. One example is how we have applied our knowledge of resistance mechanisms at the physiological and molecular levels. Another is which of the numerous factors that are implicated in the selection for resistance are pivotal and how this knowledge has been used to benefit control. Parasites do not give up their secrets readily and research into resistance has delivered great benefits in the field. Counted amongst those are a major impetus for improving our understanding of parasite biology and host parasite relationships. Finally, what more do we need to learn? Who will do it? and How will we pay for it?

Keywords: Anthelmintic resistance, diagnosis

Arthropod-borne pathogens of dogs and cats: pathways and times of transmission and disease control

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Abstract Content

Vector-borne pathogens have evolved a close relationship with blood feeding arthropod ectoparasites and exploited a huge variety of vector transmission routes. Therefore, the biology of these pathogens result in a long evolved balance with the respective arthropod biology, ecology and blood feeding habits, which is instrumental for the pathogen to the infection of several animal species, including humans. Amongst the many modes of parasite transmission routes, such as ingestion of the arthropod, with its faeces or secretions, blood feeding has a central event to the life of the majority of arthropod vectors. Pathogen transmission times are governed by a large number of biological variables related to the vectors, the pathogens, the host and environment. Scientific data available on arthropod transmission times for each pathogen are discussed relative to their impact for the success of vector-borne disease control strategies. Blocking pathogen transmission, and thus preventing the infection of dogs and cats with vector-borne diseases may be achievable by the use of chemical compounds if they are characterised by a fast onset of killing activity or repellence against arthropods. The fast speed of kill exerted by systemic isoxozaline, as well as the repellent effect of pyrethroids have renewed the interest of the scientific community and pharmaceutical companies towards reducing the burden of vector borne diseases under field conditions. However, endosymbionts and vaccines targeting arthropod or pathogen antigens should be further investigated as control strategies towards the goal of achieving an effective integrated strategy for vector-borne diseases.

Keywords: Transmission; Vectors; Arthropod-borne pathogens; Dog; Cat; Emerging

The molecular architecture of the African trypanosome cell surface

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Abstract Content

African trypanosomes are always extracellular and have evolved intricate surface coats that allow them to obtain nutrients while also protecting them from the immune defenses of either insects or mammals. The mammalian host's innate and adaptive immune system are counteracted by a densely packed protein array on the exterior face of the plasma membrane comprised predominantly of a single member of a large gene family, the variant surface glycoprotein (VSG). There is a mechanism of monoallelic exclusion that ensures only one VSG is expressed at any one time and a switch in identity of the expressed VSG gene results in antigenic variation that underlies a population survival strategy. The VSG coat contains a set of other proteins that interact with host molecules. There is a Complement Factor H receptor that presumably counteracts C3. The acquisition of macromolecular nutrients requires receptors that function within the context of these surface coats. The best understood of these is the haptoglobin-haemoglobin receptor (HpHbR) of *Trypanosoma brucei*, which is used by the mammalian bloodstream form of the parasite for haem acquisition. Structural and functional studies of HpHbR receptors from different trypanosome species have highlighted how the receptor functions without eliciting an adaptive immune response. Taken together, recent work has shown that the interaction between African trypanosomes and their mammalian hosts an excellent example of the sophisticated interactions that occur between host and pathogen.

Keywords: Trypanosoma; immune evasion; antigenic variation; nutrient acquisition; cell surface receptor

The uncertain future of anthelmintic pharmaco-therapy in ruminants

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Abstract Content

The Plenary Lecture to be presented at the 2017 WAAVP Conference will emphasize on how the use of pharmacology-based information may be critical to achieve sustainable parasite control in ruminants. The inadequate use of anthelmintics has led to therapeutic failures and to the dramatic widespread development of parasite resistance, which has been spreading in prevalence and severity. The accumulated scientific knowledge on the pharmacology of anthelmintics has been relevant to design strategies for parasite control in livestock. The assessment of the drug disposition in the host and the comprehension of the mechanisms of drug influx/efflux/detoxification in different target helminths, has signified a relevant progress on the understanding/optimization of drug activity. Different approaches to enhance parasite exposure and the use of combination of drugs from different chemical families have been proposed as valid strategies to delay the development of anthelmintic resistance. However, further research is required to identify the advantages/disadvantages of the use of combined drug preparations. There is urgent need for new drugs which will not share mechanisms of resistance with existing molecules. The emergence of novel anthelmintic molecules into the veterinary pharmaceutical market reinforces the need for deeper understanding of their pharmacological properties to prolong their lifespan. The progress made on the knowledge of the pharmacological basis of drug activity has not been sufficient to delay the widespread development of resistance. There is a need to strengthen the pharmaco-parasitological research linkage in Veterinary Medicine, which seems to be a key issue for the future of chemically-based parasite control.

Keywords: Anthelmintic Therapy, Resistance, Drug Activity, Pharmaco-Parasitological research approaches

Anthelmintic Resistance – An Escalating Issue in Malaysia

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Abstract Content

Anthelmintic resistance (AR) monitoring has been ongoing since the 1980's to the present time. Early studies reported severe cases of resistance in small holders as well as commercial farms limiting the small ruminant industry which was plagued by Haemonchosis followed by secondary pasteurellosis and as well as other chronic diseases including myiasis, ticks and brucellosis. Small ruminant farming has evolved over the years, moving from temperate breeds like the Merino crosses to hair breeds such as Barbados Black belly and Dorper which are better able to tolerate the tropics, thereby reducing stress and subsequently reducing disease incidence. However, parasitic infections still pose a significant problem with AR situation in many rural farms escalating. Over the period from 1990 to 2010, almost 80% of over 300 farms all over the country tested for anthelmintic efficacy by FECRT, showed that common drugs such as Benzimidazoles, Levamisoles, Closantel and Ivermectin were ineffective in controlling helminthiasis in goat and sheep farms. Characterization of Benzimidazole Resistance Profile of *Haemonchus contortus* was also conducted further emphasizing the need for alternative control methods. This led to the establishment of counter measures to control helminthiasis by introducing rotational grazing, developing local herbal remedies from agricultural by products as well as ethnoveterinary products, the use of FAMACHA and good nutritional intervention and management in small ruminant farms. The Department of Veterinary Services is now actively engaging small ruminant farmers to educate and encourage farming for food production for the nation and simultaneously promoting drug free

Abstract No: 3868

8 Sept 2017, 1130 – 1200

Investigation on Intra-Erythrocytic Development of Babesia Parasites Using Bioimaging Analysis

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¹Obihiro University of Agricultural and Veterinary Medicine/ National Research Center for Protozoan Diseases/ Japan

Abstract Content

Bovine babesiosis is a tick-borne disease caused by several *Babesia* species which produce acute and fatal disease in cattle and affect livestock industries worldwide. After entering the host erythrocyte, *Babesia* merozoite rapidly escapes from the parasitophorous vacuole (PV) that is formed by the invagination of the cell membrane during invasion. Following establishment of the free parasite within a host erythrocyte, the parasite produces two merozoites by binary fission. After erythrocytic lysis and egress, each merozoite invades a new erythrocyte and successive intra-erythrocytic development occurs. In recent years, genetic manipulation methodologies have advanced in apicomplexan parasites and among which, fluorescence labeled parasite populations are being employed in time-lapse imaging analysis to investigate their behavior within the mammalian hosts. However, this approach had not been applied in *Babesia* parasites which may reveal important mechanisms of the parasites during host invasion. Here, we have observed intra-erythrocytic development of the *Babesia* parasites using time-lapse video microscopy of green fluorescent protein (GFP)-expressing *Babesia bovis* merozoites developed in our previous study. Time-lapse video images delineated the sequential process of parasite-infected erythrocyte rupture, merozoite egress, gliding motility of the merozoites, attachment and invasion of merozoites into new erythrocytes, and finally formation and breakdown of PVs. Currently, we are investigating the role of the thrombospondin-related anonymous/adhesive protein (TRAP) family in the gliding motility of *Babesia* merozoites by taking advantage of the recently developed reverse genetics technologies. In addition, we have also applied this technology in investigating the role of antioxidant enzymes in the intra-erythrocytic development of the parasite.

Keywords: Babesia; time-lapse imaging analysis; transgenic parasites

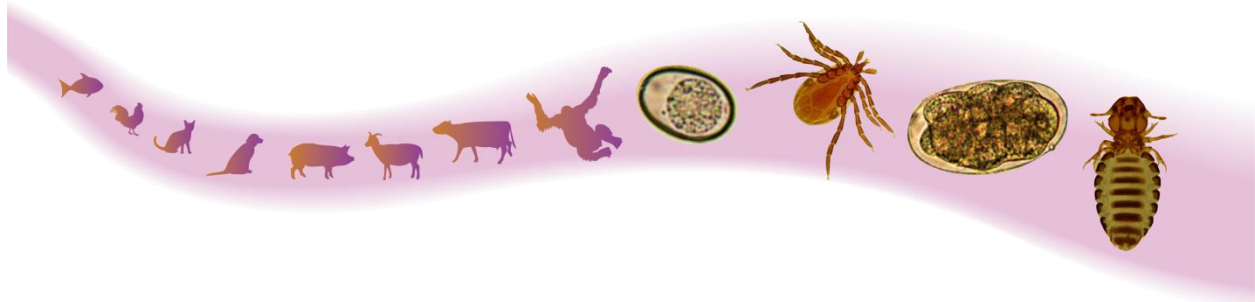
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Bayer Animal Health Symposium: News on Important Parasites for Mankind



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Abstract No: 4966

5 Sept 2017, 1200 – 1300

Varroa: the leading cause of colony mortality in the USA?

Dennis Vanengelsdorp^{*1}

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Abstract Content

Honey bees have been dying at high rates for the last 10 years. Many drivers for these elevated loss rates have been proposed, including Pesticides, Pathogens and parasites, and poor nutrition. There is general consensus that Varroa mites are the leading and most important contributor to elevated losses. Since transferring from its original host, *A. cernea*, the Asian honey bee, to *A. mellifera*, the European Honey bee, these mites have caused significant harm to the apicultural industries in Europe, North and South America, and most recently New Zealand. Several efforts to monitor mite populations in the US show a large proportion of operations have mite levels well in excess of damage threshold just prior to the winter. These large numbers occur even in operations that treated for mites recently. In this talk we will discuss the challenges of mite control, highlighting the need for better beekeeper training, more and diverse mite control methods, and vigilant surveillance. We will also consider how risk factors, such as pesticides and poor nutrition synergize with mite populations to cause colony losses.

Keywords: VARROA; HONEY BEE; CONTROL



Abstract No: 5643

5 Sept 2017, 1200 – 1300

Call in the veterinarians! – Testing an innovative strategy for Guinea worm eradication in Chad, 2016-2017

James Zingesser^{*1}

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Abstract Content

In 1986, there were 20 Guinea worm (GW) endemic countries and approximately 3.5 million human cases annually. By 2016, this painful and debilitating disease affected only 25 persons in four countries. However, Chad had 1,011 laboratory confirmed dog infections with *Dracunculus medinensis* in that year. Large numbers of dog infections is a new phenomenon, limited to Chad, first reported in 2012. In response, scientists from The Carter Center (TCC) and the U.S. Centers for Disease Control and Prevention (CDC) began exploring innovative strategies to stop transmission, including chemoprophylaxis for dogs. Based on recent research, moxidectin was considered a possible GW preventive, and TCC purchased Advocate® (Bayer Animal Health), topical imidacloprid/moxidectin, for mass treatment of domestic dogs near Chad's southern border. The campaign began in October 2016 as an adjunct to ongoing GW eradication activities. From January-May 2017, an average of 5,843 dogs were treated monthly without documented adverse events. Comparing treatment villages from January through June 2016 and the same period in 2017, dog infections reduced 54% (from 208 to 95) and emerging GWs reduced 66% (from 452 to 155). Comparable villages elsewhere had no change in incidence of dog infections, and extracted worms decreased 16%. The data here represent operations research evaluating an ongoing program, not a controlled clinical trial, and therefore should be interpreted with caution. However, this intervention demonstrates that mass treatment of domestic dogs with Advocate is feasible and safe in remote villages. The epidemiologic results are preliminary, yet highly encouraging. Further studies are planned.

Keywords: Dracunculiasis, disease eradication, one health, anthelmintic



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Teaching and Learning Veterinary Parasitology Symposium



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Veterinary Parasitology teaching in Australia: challenges and opportunities

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Abstract Content

At the beginning of the 21st Century, Professor Johannes Eckert highlighted the future vision, challenges and directions for teaching veterinary parasitology in increasingly internationalised institutions as well as in a digital era. Since this time, the higher education sector has undergone major changes in the delivery and management of course content delivery, and digital technologies have revolutionised our day-to-day life, offering unprecedented opportunities for the teaching and learning of veterinary parasitology. Currently, there are several approaches to teaching parasitology, including the disciplinary-based and problem-oriented approaches as well as a combination of both. The main aims of this paper are to (i) present the structure of veterinary parasitology courses taught at the seven veterinary school across Australia, (ii) highlight current challenges (institutional and others) faced by the parasitology discipline, (iii) discuss parasitology core competencies for veterinarians and (iii) present opportunities offered by digital technologies which might be used for effective and engaging parasitology teaching and learning.

Keywords: Teaching, Parasitology, Australia, Digital technologies

Abstract No: 3899

6 Sept 2017, 0915 – 0930

Unsticking from Time to Create a Parasitologic Amalgamation

Dwight Bowman^{*1}

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Abstract Content

Parasitology is a relevant and integral part of veterinary medicine, and the WAAVP membership has skills ranging from morphological diagnostics and routine parasite control recommendations through the cutting edges of many disciplines, e.g., pharmacology, immunology, molecular biology, and genomics. We regularly face a majority of students who want only the practical information useful the moment they enter the clinics. However, we are preparing them for careers lasting 30-50 years. Thus, we also must help them prepare for their futures. There is a constant squeeze on parasitology in the curricular footprint accompanying a mandatory need to cover the licensure basics. The basic material has stood the test of time, and until the agents are eradicated or the hosts extinct, they have value. But, a critical need is the interweaving of the marvels of modern science into the parasitology regularly presented. Often this has been done with boxes highlights, or examples within classes or texts, but asides are mentally treated as such. Also, many of those teaching parasitology are unfamiliar with many of the concepts and details of this material, but these same folks remain a grand part of the profession. Also, it is hard to sneak this apparently unwarranted material past the clinically oriented veterinary student. Somehow, WAAVP needs to work with its membership to develop and assist faculty in the presentation of a curriculum that can meld the old and the avant guard into a fusion of tastes and flavors palatable to today's veterinary student and tomorrow's practitioner.

Keywords: Education, Curriiculum, Training, Future, Past

Simple, but not easy: Opportunities and Challenges from a Teacher's and Student's Perspective in 21st Century Veterinary Parasitology Teaching

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Abstract Content

One of the main goals in academia is and has been high quality education of students to provide theoretical and practical knowledge essential for professional life. Achieving this goal is highly dependent on teaching procedures and consequently on constant adaptation of teaching styles to technical advancements as well as to current leading topics. Technical advancement strongly influences teaching and learning in the complex subject area of veterinary parasitology. Students today are provided with extensive digital lecture notes and e-learning offers including virtual microscope technology to independently obtain intensified theoretical knowledge. As veterinary parasitology is also highly reliant on proficient practical skills, lectures with integrated diagnostic exercises are mandatory. Nowadays, these practical skills, e.g. faecal examination techniques, can be individually strengthened by having access to clinical skills labs. Advances such as digital lecture notes, e-learning and virtual microscopes do not only provide new innovative opportunities, but also comprise challenges. Availability of excess study material or provision of sufficient relevant material, respectively, with the consequence of reduced responsibilities for autonomous gathering of additional information may influence learning processes. Beside technical advances, zeitgeist changes shape teaching contents, which are progressively expanding as zoonoses are increasingly put into focus. Towards the one-health concept, students today are more and more expected to not only bear responsibilities for animals, but also for their owners and, moreover, public health. This presentation will cast light on different aspects of opportunities and challenges in modern veterinary parasitology teaching from a teacher's and student's perspective.

Keywords: Veterinary education; Teacher; Student; Digital learning; One health

Established and novel approaches in veterinary parasitology education in Berlin

Peter-Henning Clausen^{*1} ; Sandra Stelzer² ; Ard Nijhof¹ ; Jürgen Krücken¹ ; Georg Von Samson-Himmelstjerna¹

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Abstract Content

The teaching of veterinary parasitology to the usually high number of students at the Freie Universität Berlin is largely limited to conventional face-to-face lecture series supplemented by practical courses. Extensive parasite descriptions and diagnostic techniques are the core aspects of the practical courses that are also intended to provide emphasis on key biological and veterinary aspects highlighted in the lectures. Further in-depth and specific learning is achieved within a detailed framework of elective courses with defined learning outcomes for small groups of students focusing on themes such as 'diagnosis and treatment of ectoparasites in companion animals' or 'zoonotic parasites'. Additionally, structured excursions are designed to offer experience in collaborative international studies. Organ-based approaches are also an integral part of our veterinary parasitology teaching and is done in collaboration with the clinical and para-clinical departments, either by face-to-face interactions or online. Wide ranging themes, such as 'causes of colic in horses' or 'atopic dermatitis in dogs' are covered. A new platform to provide online lectures for students, termed VET Talks, was launched in public in 2015 by the International Veterinary Student's Association (IVSA) and acts as a non-commercial educational support for students. Provided free to veterinary students throughout the world, the platform offers the opportunity to access great lectures on interesting topics by outstanding speakers who are nominated by their students. Finally, continuing education (CE) opportunities are provided through specific Masters courses (Master of Equine Medicine, Master of Small Animal Sciences), classical seminars and recent webinars.

Keywords: veterinary parasitology; education; established and novel approaches

Balancing active knowledge and basic principles in veterinary parasitology: competences for future Danish veterinary graduates

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Abstract Content

Veterinary parasitology has always been considered interesting but difficult by the Danish veterinary students. They have to acquaint with a lot of new, small creatures with complicated life cycles and with intricate Latin names that are difficult to pronounce and only few have Danish names. In our veterinary curriculum, zoology has disappeared as discipline, and parasitology has gradually moved from the third year to the beginning of the second year which implies that e.g. pathology or pharmacology are unknown fields. The number of hours has been gradually cut to 24 lectures (of 35 minutes) and practical exercises (18 hours), including 6 hours coprology. It is taught and examined jointly with bacteriology and virology in a 9 weeks course. With the comprehensive course it has become increasingly difficult to get enough time to obtain both an active knowledge of the most common parasites and an understanding of the basic principles in e.g. transmission and control. Despite much information are readily accessible through e.g. online resources, we still believe that a competent clinician knows a range of parasites by heart as an active resource for their work. The dilemma has been tackled (partly) by introducing a veterinary paraclinics brush-up course of 18 hours (half practicals and lectures) in the fourth study year. The focus here is host(herd)-oriented clinical and diagnostic parasitology. The students can also now select a One-health track for half a year in which zoonotic parasites is an obvious relevant topic.

Keywords: teaching; veterinary curriculum; competences; active knowledge; basic principles

Veterinary parasitology teaching: ten years of experience with the Vetsuisse-Curriculum

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Abstract Content

The European Bologna System was introduced at the Vetsuisse Faculty (Universities Zurich and Berne) in Switzerland in 2007. Parasitology is initiated with selected examples in the context of ecology in the first academic year. The second and third years of the Bachelor program comprise non-organ-centred (NOC) and integrated organ-centred (OC) course modules. In the NOC modules, parasitology is taught in consecutive courses focussing on topics including occurrence, biology, pathogenesis, clinical manifestations, diagnostics and the strategic principles of therapeutic and prophylactic interventions against major veterinary or zoonotic parasites. This syllabus is complemented with live demonstrations as well as practical laboratory exercises. A course script with defined learning objectives is based on the book "Parasitology in Veterinary Medicine". Furthermore, students review relevant parasitoses in the diagnostic context of OC case presentations. In another module, immunological aspects of parasitic diseases are deepened in group works. The two-year Master program is divided into a core syllabus for all students and an elective subject chosen from six areas of specialization. Within the clinically focused specializations, interactive teaching on control strategies against parasitoses of companion and farm animals is in focus. Students specializing in "Pathobiology" experience a deep immersion in parasitology. Learning objectives are verified in different test formats. E-learning tools, including a learning management on-line platform, allow interactive student training in coproscopy and arachno-entomology and provide case-oriented teaching. The fragmentation of teaching in veterinary parasitology, the reduction of the number of diagnostic exercises and clinically oriented day-1-skills in control of parasitoses will be critically commented.

Keywords: Parasitology; teaching; Switzerland

Parasitology Summer Course (ParSCo) in Southern Italy: a bench-to-field approach

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Abstract Content

Parasitology Summer Course (ParSCo) is a one-week intensive residency course organized by the European Veterinary Parasitology College and the Department of Veterinary Medicine of Bari (Italy). ParSCo aims at providing parasitologists and post-graduate students with theoretical and hands-on knowledge of arthropod vectors, transmitted pathogens and many other parasites in the heart of the Mediterranean area. It takes place in Basilicata (Southern Italy), a region which has received considerable attention from researchers, for its outstanding animal and parasite species richness and because it represents a model for other countries of the Mediterranean basin. ParSCo is divided into oral lectures (35.3%) and practical activities (64.7%) led by an international team of scientists working in veterinary parasitology. Oral lectures cover various aspects of vectors (e.g., ticks and sand flies) and their transmitted pathogens (e.g. *Leishmania infantum*, tick-borne pathogens, *Thelazia callipaeda*, and filarioid species), lungworms of carnivores and many other parasites of dogs, cats, wildlife as well as livestock. The main focus of the course is on practical activities including collection of arthropods, necropsies, taxonomic identification of collected parasites and execution of various diagnostic techniques. Until 2017, in the previous five editions, 63 participants from every corner of the world took part in this summer course allowing new research partnerships, which resulted in more than 23 scientific articles published under the ParSCo direct or indirect influence.

Acknowledgments: ParSCo is sponsored by Boehringer Ingelheim and Bayer HealthCare - Animal Health, with the participation of Italian Society for Parasitology and of Parasites & Vectors.

Keywords: *teaching, education, field, vectors, Mediterranean*

Abstract No: 5682

6 Sept 2017, 1115 – 1130

“Now I feel like a true parasitologist” - Concept-based training for Early Career Scientists

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Abstract Content

One of the allures of parasitology is its breadth of aspects spanning everything from molecules to ecosystems. Very few institutions have the capability to cover this breadth in educating parasitologists. As the national professional body, the Australian Society for Parasitology has developed a training course that aims to fill this gap. The course offers a comprehensive overview over the field, highlights the current research foci and introduces key methods. The program equips participants with an appreciation of parasites and with strategies to deal with the complexity of parasitological systems. The course provides an innovative model for training parasitological key concepts with a focus on professional development for early career researchers.

Veterinary Parasitology Teaching at London – Meeting the Needs of Our New Graduates

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Abstract Content

Over the past four decades, there has been an exponential increase in veterinary parasitology knowledge coinciding with the advent of molecular biology in research. It is therefore unrealistic for teachers to expect students to graduate with an encyclopaedic knowledge of the subject. As a result, a new curriculum was introduced in 2007 designed to meet the needs of our new graduates, i.e. RCVS Day One Skills. The aims of this curriculum were, *inter alia*, to ensure that new graduates had an up-to-date body of core knowledge and were able to use such knowledge and newly-acquired information in scientific and clinical problem-solving. Veterinary parasitology is taught primarily in Year 2, following a brief introduction in Year 1; clinical aspects are covered in Year 3 and original research projects undertaken in Years 4 and 5. Parasitology is taught in parallel with other subjects enabling both horizontal and vertical integration. Core material is provided in lectures supplemented by directed learning (DL) in small groups and interactive, clinical scenario-based practical classes. Student learning is supported by Moodle 3.2 (VLE, RVC Learn) which provides access to an on-line study guide (annotated using Adobe Reader), PowerPoint presentations with synchronized lecturer commentary (Echo ALP), detailed feedback for DL and practical classes, parasite potcasts and CAL packages, and a Clinical Skills Centre. A parasitology textbook has also been published recently to support courses taught at the College. Assessment of student learning is achieved using a variety of written formats (essay, PSQ, MCQ, EMQ), integrated oral examinations and OSCEs.

Keywords: Assessment; Curriculum; London; Problem-solving; Teaching

Abstract No: 4314

6 Sept 2017, 1145 – 1200

Pitfalls and opportunities of teaching veterinary parasitology within an integrated curriculum

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Abstract Content

In 2006 an integrated curriculum was implemented at the Faculty of Veterinary Medicine, Utrecht University. This change was made because of the European change to a Bachelor Master system. Coming from a curriculum that was divided in a pre-clinical and clinical phase and based on separate disciplines, these changes led to a drastic revision. Veterinary parasitology needed to adjust their pedagogical approach towards this new curriculum. An integrated study design focusing on improved problem solving skills and being organ-oriented was deemed warranted. This change offered the possibility to recurrently teach students basic principles of veterinary parasitology in successive organ-oriented courses. Though there is no doubt about the benefits of this approach, it led to the loss of clearly identifiable veterinary parasitology courses with associated separate exams. Short term visions of calculative students aiming mainly at passing the exam make it possible to pass a course in which parasitology is taught without studying this topic thoroughly. This seems to apply less for other disciplines as students often indicate they find parasitology in particular difficult because of the diversity in parasite species and lifecycles. Now, more than 10 years after the integrated curriculum started, it is still a huge challenge to get bachelor students to understand the reason why they need to learn about these basic principles. Finding a balance between blending in and still remaining recognisable as a specific discipline has not yet been completely sorted out.

Keywords: Veterinary Parasitology; teaching; Bachelor Master; integrated curriculum

Abstract No: 3854

6 Sept 2017, 1200 – 1215

Overcome monotony thanks to diversity: for efficient and motivating veterinary parasitology practicals

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Abstract Content

The aim of this study is to initiate a reflexive practice for Veterinary Parasitology practicals. An important deficit in motivation was observed during these practicals where students must observe about fifteen preparation after an introductory slideshow presentation. In order to overcome the monotony, teaching activities were diversified (flipped classroom, individual and collective work, formative test, authentic situation, peer review). We studied how students perceived new teaching activities during practicals and their impact on learning process. Objective and subjective data were collected to verify their use, their perception and achievement towards these novel activities. This study showed that students used and enjoyed the new provided pedagogical activities and particularly: learning platform (practical preparation), collective work and formative quiz (memorization, fun). Clinical medicine aspects were plebiscited as taking them closer to their future job (motivation). Thus, some ways to improve were highlighted.

Keywords: Veterinary Parasitology Practicals; Motivation; Formative test; Flipped classroom

Veterinary parasitology teaching in China in the 21st Century: Challenges, opportunities and perspectives

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Abstract Content

China has made tremendous achievements in social-economic development in the last three decades, and the numbers of farming animals and companion animals are increasing swiftly. Significant advances have also been made in the prevention and control of animal parasitic diseases, and some of the soil-transmitted animal parasites have been well under control. However, parasites of animals (including food-producing animals and companion animals) are still of major animal health and socio-economic significance in China, and quite a number of animal parasites are also zoonotic and can cause death and severe diseases in humans. In particular, Food-Borne Parasitic Zoonoses (FBPZ) and Companion Vector-Borne Diseases (CVBD) are becoming significant threat to public health and animal production. Also, the application of new technologies such as the “Omics” technologies has advanced veterinary parasitology into the “post-genome era”. Given these dramatic changes of circumstances, the teaching of veterinary parasitology in Chinese universities has undergone significant reforms in the past ten years, both in the range of parasites and contents being covered, and the ways of the course being delivered. In this article, we describe the current status of veterinary parasitology teaching for undergraduate students and postgraduate students in Chinese universities, summarise the progress and advances in the reform and improvement of veterinary parasitology teaching quality, and discuss the challenges, opportunities and perspectives of veterinary parasitology teaching in the 21st Century, including the use of cutting-edge teaching technologies and the integration of the “One Health” concept into the course.

Abstract No: 3901

6 Sept 2017, 1230 – 1245

Teaching Veterinary Parasitology in South Africa – A Look at the Past, a Vision for the Future

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Abstract Content

Finding a healthy balance between classical parasitology and clinical veterinary medicine remains a challenge. Veterinary Parasitology, of vital interest in sub-Saharan Africa, has always featured prominently at the University of Pretoria. The Faculty of Veterinary Science (founded 1920) was initially an integral part of the Onderstepoort Veterinary Institute (OVI). Parasitology was taught by specialist researchers from OVI; a cult of total coverage prevailed. Presenting three separate courses – ectoparasitology, helminthology and protozoology – continued for many decades. From 1995: “refresher” in parasitology for final-year students (during their clinic rotation): diagnostic parasite identification; problem-solving group discussions (prepared and led by students). Student contact time (including practicals and assessments), initially 80 hours/discipline/year, was gradually reduced. Species-based approach (introduced 2005) had a major impact: introductory course in general parasitology followed by fragmented lectures in subsequent 2 years on specific parasitic diseases in the species-based subjects. Degree reverted from 7 to 6 years in 2013; curriculum reverted to original approach, i.e. all aspects of parasitology covered during one academic year. The 3 sub-disciplines are included in a 2-semester course, with total contact time of 100 hours. Vision: erasing artificial division between “pre-clinical” and “clinical” in minds of academics and students; enhancing analytical thought and problem-solving capacity of undergraduates.

Keywords: Curriculum development; Problem-solving; South Africa; Teaching; Undergraduate

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Estimates of the global and regional burden of foodborne parasites as determined by WHO

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Abstract Content

Parasitic diseases may result in high disease burdens, particularly in low and middle income countries, and are frequently transmitted to humans via contaminated food. Comparable information on the population health impact of foodborne parasites is crucial to inform and prioritize health policies and research funding, both at national and international levels. Recently, the World Health Organization launched the first-ever estimates of the global and regional burden of foodborne disease, including that of four protozoa (*Cryptosporidium* spp., *Entamoeba histolytica*, *Giardia* spp., and *Toxoplasma gondii*) and ten helminths (including two nematodes: *Ascaris* spp., *Trichinella* spp.; three cestodes: *Echinococcus granulosus*, *Echinococcus multilocularis*, *Taenia solium*; and five trematodes: *Clonorchis sinensis*, *Fasciola* spp., intestinal flukes, *Opisthorchis* spp., *Paragonimus* spp.). Data were abstracted from systematic reviews, disease databases, and reports from national surveillance systems; and used to estimate the number of infections, sequelae, deaths, and Disability-Adjusted Life Years (DALYs), by age and region for 2010. A Bayesian random effects model was used to impute data gaps, while expert elicitation was used to attribute disease burden to different exposure routes and food items. Together, the considered parasitic diseases caused more than 400 million illnesses, resulting in nearly 100 000 deaths and 12 million DALYs. Intestinal protozoa were responsible for nearly 90% of all illnesses, while helminths were responsible for the majority (60%) of all deaths and DALYs. Across all parasites, 22% of all illnesses, 55% of all deaths and 61% of all DALYs were estimated to be due to foodborne transmission. The highest numbers of foodborne deaths were due to *Taenia solium*, *Echinococcus multilocularis*, and *Clonorchis sinensis*; while the highest numbers of foodborne DALYs were due to *Taenia solium*, *Paragonimus* spp., and *Toxoplasma gondii*. The largest burden of foodborne parasitic disease occurred in the Western Pacific and African regions. These estimates represent an important step forward in understanding the true impact of foodborne diseases globally and regionally. Further efforts should focus on addressing key data gaps and on unraveling the burden of foodborne parasites not considered in the WHO study.

Molecular epidemiology of waterborne protozoan parasites of humans

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Abstract Content

Molecular diagnostic tools have played an important role in improving our understandings of the transmission of waterborne protozoan parasites in humans. They are commonly used in the characterization of *Cryptosporidium* spp. and *Giardia duodenalis*, which are two most important waterborne parasites in industrialized nations. For these organisms, genotyping tools are frequently used in the identification of host-adapted *Cryptosporidium* species and *G. duodenalis* assemblages, allowing the assessment of infection sources in humans and public health potential of parasites found in animals and environment. In contrast, subtyping tools are more often used in case linkages, advanced tracking of infections sources, and assessment of disease burdens attributable to anthroponotic and zoonotic transmission. More recently, multilocus sequence typing (MLST) tools have been developed for human-pathogenic *Cryptosporidium* species and *G. duodenalis* genotypes. They offer higher resolution, thus are increasingly used in population genetic characterizations of transmission dynamics and delineation of mechanisms for the emergence of virulent subtypes. With recent development in next generation sequencing techniques, whole genome sequencing and comparative genomic analysis are increasingly used in typing *Cryptosporidium* spp. and *G. duodenalis*. The use of these genotyping and subtyping tools in epidemiologic studies has identified significant differences in the transmission of *Cryptosporidium* spp. in humans between developing countries and industrialized nations, especially the role of zoonotic transmission in human infection. Geographic differences are also present in the proportion of giardiasis caused by *G. duodenalis* assemblages A and B, the two genotypes infecting humans. In contrast, there is little evidence for widespread zoonotic transmission of giardiasis in both developing and industrialized countries. Differences in virulence have been identified among *Cryptosporidium* species and subtypes, and possibly between *G. duodenalis* assemblages A and B, and genetic recombination has been identified as one mechanism for the emergence of virulent *C. hominis* subtypes. These recent advances are providing insight into the epidemiology of waterborne protozoan parasites in both developing and developed countries.

Foodborne Trematode Infections in Asia

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Abstract Content

Foodborne trematodes are diverse and can be classified largely into liver flukes, lung flukes, and intestinal flukes. Geographical distribution of these parasites is wide, and Asia is the most important endemic area among all continents. Zoonotic liver flukes in Asia include at least 6 species; *Clonorchis sinensis*, *Opisthorchis viverrini*, *Metorchis orientalis*, *Fasciola hepatica*, *F. gigantica*, and *Dicrocoelium dendriticum*. Zoonotic lung flukes in Asia include 4 species; *Paragonimus westermani*, *P. heterotremus*, *P. skrjabini skrjabini*, and *P. skrjabini miyazakii*. As to intestinal flukes, up to 46 zoonotic species are known. The largest group is heterophyid flukes (more than 17 species); the major genera are *Haplorchis*, *Heterophyes*, *Metagonimus*, *Pygidiopsis*, and *Stellantchasmus*. The next is echinostomes (more than 16 species); the major genera are *Echinostoma*, *Echinochasmus*, *Isthmiophora*, *Hypoderaeum*, *Artyfechinostomum*, *Acanthoparyphium*, and *Echinoparyphium*. Several other genera infecting humans include *Gymnophalloides*, *Neodiplostomum*, *Plagiorchis*, *Phaneropsolus*, and *Prosthodendrium*. The foodborne sources of human infection include fish, snail (including oyster), amphibia, reptile, and insect, and reservoir hosts are mammals or birds. The pathogenicity and clinical aspects of each parasite species and host defense mechanisms are poorly understood. The diagnosis of liver and intestinal flukes can be done by fecal examinations, whereas the diagnosis of lung flukes can be done by recovery of eggs in the sputum or serologically by ELISA. Praziquantel is an effective anthelmintic for use against these parasitic infections. Epidemiological surveys and detection of human cases should be continued for better understanding of these parasites and to provide control strategies.

Abstract No: 4770

6 Sept 2017, 1100 – 1130

Waterborne parasite transmission among the Association of Southeast Asian Nations (ASEAN): An overview

Yvonne AL Lim^{*1}

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Abstract Content

Most of the global outbreaks of waterborne parasitic protozoa have been reported in regions with established surveillance and reporting systems such as North America, Europe, Australia and New Zealand. Given that only an estimated 1% of these outbreaks have occurred in Asia, it is evident that there is a paucity of information from this region where organised mechanisms of documentation of parasitic infections or waterborne outbreaks are lacking. This presentation attempts to provide an overview of the available epidemiological data and studies on waterborne occurrences among the Association of Southeast Asian Nations (ASEAN) which comprises of the ten member states (i.e., Brunei Darussalam, Cambodia, Indonesia, Lao People's Democratic Republic (PDR), Malaysia, Myanmar, the Philippines, Singapore, Thailand, and Vietnam) with the aim of identifying some directions on how to progress.

Abstract No: 4771

6 Sept 2017, 1130 – 1200

Risk-based management of drinking water safety in Australia: implementation of health based targets to determine water treatment requirements and identification of pathogen surrogates for validation of conventional filtration

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Abstract Content

In Australia, the safety of drinking water is ensured using a risk management framework embedded within the Australian Drinking Water Guidelines (ADWG). This framework includes elements for hazard identification, risk assessment, risk mitigation, verification of barrier performance and monitoring for any changes to the hazards that influence source water quality. The next revision of the ADWG will incorporate Health Based Targets (HBTs) for achieving microbiologically safe drinking water. This incorporates Quantitative and Microbial Risk Assessment and the metric of Disability Adjusted Life Year (DALY) to define safety, with a target of 1 microDALY set as the maximum tolerable disease burden from drinking water, which in the case of *Cryptosporidium* is $<1.3 \times 10^{-5}$ oocysts / L. The resulting product water specification, in combination with knowledge of pathogen challenges in source waters, allows the determination of the treatment requirements to ensure public safety. The HBT manual provides default removal values for *Cryptosporidium* for particular treatment processes, such as conventional coagulation and dual media filtration. However, these values are based on assumptions regarding treatment plant design, operation and water quality. To properly manage risk and demonstrate compliance with the guidelines, water utilities need to be able to validate treatment performance for *Cryptosporidium* removal. A particular limitation is the absence of *Cryptosporidium* surrogates for full-scale filter validation. This presentation will provide an overview of risk-based management of drinking water safety in Australia, the development of health-based targets for microbial pathogens and the evaluation of *Cryptosporidium* surrogates conventional coagulation and dual media filtration.

Two new OIE Collaborating Centres in Europe and Asia to improve food safety and reduce the burden of food-borne zoonotic parasites

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Abstract Content

An increase of meat and sea food products consumption is expected over the next 20 years (OECD-FAO), particularly in developing countries where new food habits combined with a lack of knowledge of food-borne parasites and the related public health risks. The designation of two OIE-CC for Food-borne Zoonotic Parasites in June, 2014 proposed by Jilin University (PR China) and ANSES (France) for Asia-Pacific Region and European Region, respectively to address these emerging risks. These laboratories have the independent capabilities and skills to perform diagnostic tests for several food-borne parasites as prescribed by the OIE Manual of Diagnostic Tests. The two new OIE-CC benefit from over 20 years of robust cooperation between their respective institutes. These laboratories follow the recommendations described by FAO: Multicriteria-based ranking for risk management of neglected parasites transmitted by food (2012). The main goals of the OIE-CC include: 1) To provide scientific and technical expertise for detection of food-borne parasites in livestock, wildlife or animal products; 2) To implement direct or indirect diagnostic methods; 3) To participate in international standardization particularly in OIE, ISO, OECD, and the International Commission on Trichinellosis; 4) To establish reference material banks and propose ring trials; 5) To organize training within OIE Member Countries and participate in twinning programs to build new local competences; and, 6) To organize information for consumers through various media. The OIE-CC should also perform research in the field. The aim is to improve control strategies for food-borne parasites by understanding their interactions with the host and exploring their infectious potential. Within food-borne parasites, the OIE-CC focus research activities on those responsible for major zoonotic diseases such as *Trichinella spp.*, *Toxoplasma gondii*, *Cryptosporidium spp.* and *Giardia duodenalis*. These parasites have important impact on public health, and control strategies for most of them include specific regulations in many countries. Research at the OIE-CC is based on two strategic axes: 1) the study of host-parasite interactions aiming to either protect parasites' hosts or define new therapeutic approaches; and, 2) develop innovative tools for the evaluation of parasite virulence or their detection in animals/environment for evaluation of risk factors in animal populations. The results of such research should facilitate new protocols for meat curing and harmonization of diagnostic methods. The two OIE-CC also have a collaborative link with the first OIE-CC which is located at the Canadian Food Inspection Agency in Canada, and a new project in Africa is in progress to overlap with other OIE Regions. Food-borne zoonotic parasites are at the heart of the "One Health" concept as they are closely related to both animal health and food safety. Their management requires a global approach where the OIE-CC can be a leading actor.

Abstract No: 4354

6 Sept 2017, 1230 – 1245

Rapid detection and characterization of *Giardia* and *Cryptosporidium* in food and water using a portable lab-on-a-chip platform

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¹*Food Directorate/ Health Canada/ Canada*

Abstract Content

Giardia duodenalis cysts and *Cryptosporidium* spp. oocysts are commonly found in raw water samples worldwide, and have also been associated with fresh produce in many surveillance studies and illness outbreaks. Current detection methods are labour-intensive and require a high level of technical expertise. The objective of the present study was to design and develop a portable, semi-automated lab-on-a-chip platform for the rapid detection and characterization of these parasites. Separate microfluidic chips were designed, manufactured, and tested for their ability to specifically concentrate *Giardia* cysts and *Cryptosporidium* oocysts from large elution volumes, lyse the (oo)cysts by bead-beating and extract DNA, perform a multiplex PCR, and identify species and genotypes using a cloth-based hybridization array system (CHAS) using custom-designed probes. A linear inertial focusing chip was very effective in concentrating (oo)cysts from large volumes, with the volume being reduced by 33% during each circulation cycle. DNA was extracted from lysed (oo)cysts for the amplification of *Giardia* and *Cryptosporidium* gene fragments by PCR, which were then detected by colour reaction following CHAS. Preliminary results suggest that the system will provide rapid and specific detection and characterization of *Giardia* and *Cryptosporidium* in food samples and water concentrates. As the system is portable and semi-automated, it will be well suited to in-the-field use, including farm level, processing or retail. It is anticipated that this novel technology will allow for the generation of rapid and reliable results in support of outbreak investigations and parasite surveillance studies.

Keywords: Giardia; Cryptosporidium, food, water, detection

DNA aptamers for the detection of *Cryptosporidium* spp. oocysts on fresh produce

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Abstract Content

In recent years, several foodborne illness outbreaks due to infections with *Cryptosporidium* spp. have been reported in developed countries. Detection of *Cryptosporidium* spp. oocysts in food using standard PCR is difficult due to the low concentrations of parasites, the difficulty in eluting from foods, the lack of enrichment methods, and the presence of inhibitors. Aptamers are single-stranded oligonucleotides that fold into specific three-dimensional shapes and are capable of binding strongly and selectively to target cells or molecules. DNA sequences from large libraries were screened using an *in vitro* process known as systematic evolution of ligands by exponential enrichment (SELEX) for their ability to bind strongly and specifically with the oocyst wall of *C. parvum*. SELEX involves repetitive rounds of two processes: (i) partitioning of aptamers from non-aptamers using an affinity method, and (ii) amplification of aptamers by PCR. In the present study, a total of ten rounds of selection led to a number of promising aptamers with high affinity for *C. parvum* oocysts. Using flow cytometry, these aptamers were found to bind to *C. parvum* oocysts with an affinity in the low nanomolar range. The sensitivity and specificity of aptamers and the aptasensor platform showed rapid detection and identification of *C. parvum* oocysts on spiked fresh fruits, as compared to conventional methods such as microscopy and PCR-based methods. This is the first study of PCR amplification of random DNA libraries used in *C. parvum* aptamer selection.

Keywords: Cryptosporidium, Foodborne, Waterborne, Diagnosis, Aptamer

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Abstract No: 5599

7 Sept 2017, 1400 – 1430

Blastocystis sp. - Recent advances, Current status and future perspectives

Suresh Govind¹; Tan TC¹; Chandramathi S¹ ; Gaythri T¹ ; Rajamanickam A ¹ ; Girish S¹

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Abstract Content

Blastocystis sp., is one of the most commonly found microorganism in stools and has been implicated to cause bloating stomach, diarrhea and other non-specific gastrointestinal symptoms. Various subtypes have been previously reported with certain ones predominantly shown to exert pathogenicity. The presentation will summarize key findings that have been made from our laboratory in the past two decades especially with regards to aspects of the parasite which includes biology, pathogenesis, immunology, molecular and prevalence. The parasite's role in contributing to colorectal cancer and irritable bowel syndrome will also be discussed.

Resistance development in *Aedes aegypti* (Linnaeus) against metofluthrin in mosquito coil

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Abstract Content

This study was conducted to determine the resistance development rate in *Ae. aegypti* to metofluthrin in mosquito coil in the presence of selection pressure. Adult mosquitoes were exposed to coil burnt up to 2.41 minutes in the glass chamber. The burning time of the coil would induce 50% mortality based on earlier test. The surviving mosquitoes were reared to produce next generation. Each generation has undergone similar selection pressure continuously for 10 generations. To measure the resistance development rate, each generation of mosquitoes after selection pressure were exposed to 0.5 gram of coil according to standard test protocol of SIRIM. The knockdown rates were recorded every minute up to 20 minutes. The mosquitoes showed different level of susceptibility to metofluthrin when compared to reference strain. Bioassay results revealed that KT_{50} values of metofluthrin-selected *Ae. aegypti* ranged between 1.75 – 2.35 minutes for 10 consecutive generations. Complete knockdown of reference and selected strains were achieved within 20 minutes of exposure period. However, metofluthrin-selected *Ae. aegypti* exhibited lower mortality (92.45%) compared to reference strain (96.61%) at 24 hours post treatment. This study revealed the potential resistance development in *Ae. aegypti* after 10 generations of selection pressure by metofluthrin in mosquito coil.

Keywords: Aedes aegypti; metofluthrin, mosquito coil, selection pressure, Malaysia

Abstract No: 4935

7 Sept 2017, 1445 – 1500

Validation and comparison of the PrioCHECK *Trichinella* AAD Kit for the detection of larvae in pork, horse meat and wildlife tissue

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Abstract Content

A new artificial digestion assay was recently developed and commercialized for the detection of *Trichinella* larvae in the muscle of infected animals. The PrioCHECK™ *Trichinella* AAD Kit uses an alternative enzyme, serine protease, and no hazardous substances such as HCl or pepsin. Activation of the enzyme requires an elevated digestion temperature of 60°C which kills the parasite and reduces the risk of contaminating the environment with *Trichinella*. Compared to the pepsin/HCl method, digestion using the PrioCHECK *Trichinella* AAD Kit is significantly faster. To assess the Kit's suitability for *Trichinella* testing, and to validate its performance relative to the conventional pepsin/HCl digestion method, several comparative studies were conducted using meat from domestic food animals and wildlife species. Multiple muscle samples were collected from diaphragm, tongue, masseter, loin or foreleg of adult pigs, horses, wild boars, bears and wolves. Samples were naturally infected or spiked with 3, 4, 5, or 25 *Trichinella spiralis* larvae. A total of 638 100 g meat samples were used to validate and compare the diagnostic proficiency of the Kit with the pepsin/HCl digestion method. Analysis of the data produced from these studies showed that both methods are capable of consistently detecting *Trichinella* in 100 g samples which contained as few as 3 larvae. Overall, the PrioCHECK *Trichinella* AAD Kit performed satisfactorily according to various international guidelines for the detection of *Trichinella* infection in all of the various types of meat samples tested.

Keywords: Trichinella, diagnostic kit, pork, horse meat, wildlife

Intestinal Parasite Diagnostics - Advances in Coproantigen Detection

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Abstract Content

Three independent antigen detection assays have been developed for the detection of coproantigens for *Ancylostoma caninum*, *Toxocara canis*, and *Trichuris vulpis*. The antigen assays detect excreted or secreted proteins from the young adult to adult stages. The protein markers are not associated with reproduction or with eggs and thus provide a different marker for nematode infection. Experimental infection studies demonstrate earlier detection by antigen ranging from 7 days for *T. canis*, 14 days for *A. caninum*, and 46 days for *T. vulpis*. These assays were applied to a 1000-member field population to assess performance compared to egg flotation results. The majority of samples agreed with either the egg positive or egg negative flotation result. Discrepant results were observed for each nematode. These discrepant results were investigated in detail for the *T. canis* samples. Four of the five *T. canis* egg positive, ELISA negative samples most likely were spurious eggs. One of the five was confirmed to be *T. canis* egg positive and the ELISA signal was elevated but below cut-off. Seven samples were egg negative, ELISA positive. ELISA signal was confirmed to be specific to *Toxocara* antigen. Flotation and ELISA data was collected for over 54 thousand tests. The overall pattern of detection was similar to that seen in the smaller 1000-member population. Together the data supports that the combination of the traditional flotation assay and the new antigen detection technology will allow identification of more infected pets.

Estimates of the global and regional burden of foodborne parasites as determined by WHO

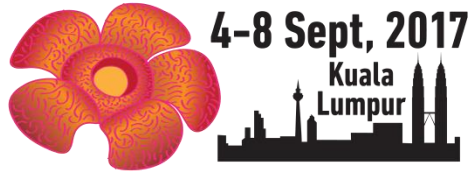
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Abstract Content

Parasitic diseases may result in high disease burdens, particularly in low and middle income countries, and are frequently transmitted to humans via contaminated food. Comparable information on the population health impact of foodborne parasites is crucial to inform and prioritize health policies and research funding, both at national and international levels. Recently, the World Health Organization launched the first-ever estimates of the global and regional burden of foodborne disease, including that of four protozoa (*Cryptosporidium* spp., *Entamoeba histolytica*, *Giardia* spp., and *Toxoplasma gondii*) and ten helminths (including two nematodes: *Ascaris* spp., *Trichinella* spp.; three cestodes: *Echinococcus granulosus*, *Echinococcus multilocularis*, *Taenia solium*; and five trematodes: *Clonorchis sinensis*, *Fasciola* spp., intestinal flukes, *Opisthorchis* spp., *Paragonimus* spp.). Data were abstracted from systematic reviews, disease databases, and reports from national surveillance systems; and used to estimate the number of infections, sequelae, deaths, and Disability-Adjusted Life Years (DALYs), by age and region for 2010. A Bayesian random effects model was used to impute data gaps, while expert elicitation was used to attribute disease burden to different exposure routes and food items. Together, the considered parasitic diseases caused more than 400 million illnesses, resulting in nearly 100 000 deaths and 12 million DALYs. Intestinal protozoa were responsible for nearly 90% of all illnesses, while helminths were responsible for the majority (60%) of all deaths and DALYs. Across all parasites, 22% of all illnesses, 55% of all deaths and 61% of all DALYs were estimated to be due to foodborne transmission. The highest numbers of foodborne deaths were due to *Taenia solium*, *Echinococcus multilocularis*, and *Clonorchis sinensis*; while the highest numbers of foodborne DALYs were due to *Taenia solium*, *Paragonimus* spp., and *Toxoplasma gondii*. The largest burden of foodborne parasitic disease occurred in the Western Pacific and African regions. These estimates represent an important step forward in understanding the true impact of foodborne diseases globally and regionally. Further efforts should focus on addressing key data gaps and on unraveling the burden of foodborne parasites not considered in the WHO study.

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Abstract No: 4948

5 Sept 2017, 1400 – 1415

A 16-year retrospective analysis of anthelmintic resistance on small ruminant farms in the United States

Ray Kaplan^{*1}; Sue Howell¹; Bob Storey¹; James Collins¹

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Abstract Content

The DrenchRite[®] larval development assay (LDA) is a diagnostic bioassay that measures and detects resistance to benzimidazoles, levamisole, ivermectin, and moxidectin. From 2000-2016 we performed DrenchRite[®] LDA on 291 small ruminant farms in 40 of 50 USA 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 time period. For analysis, data were grouped by 5-6 year intervals (2000-2005; 2006-2010; 2011-2016). Resistance to both benzimidazoles and ivermectin was highly prevalent; ≥95% of both sheep and goat farms demonstrated resistance to benzimidazoles, and ≥92% and ≥63% of goat and sheep farms, respectively demonstrated resistance to ivermectin at all time intervals. Levamisole resistance was less prevalent and was similar for sheep and goat farms (average prevalence = 27%). The greatest changes were for moxidectin, and these were significantly greater for goats than for sheep ($p < 0.0001$). The first cases of moxidectin resistance diagnosed on goat and sheep farms were in 2001 and 2002, respectively. Prevalence of resistance to moxidectin progressively and dramatically increased over the next two time periods (2006-2010, 2011-2016) on both goat farms (40%, 59%) and sheep farms (13%, 26%). Coincident with this increase in prevalence, the levels of resistance also increased from a mean IC_{50} of 395 nM and 125 nM (2000-2006) to 1845 nM and 703 nM (2011-2016) for goat and sheep farms, respectively. These data provide interesting insights into changes occurring over time, and indicate a severe situation now exists in the USA.

Keywords: Haemonchus contortus; anthelmintic resistance; DrenchRite[®] Larval development assay; sheep, goats

Abstract No: 4465

5 Sept 2017, 1415 - 1430

Evidence Based Strategies to Mitigate the Development of Anthelmintic Resistance Development in the UK

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Abstract Content

The UK's Sustainable Control of Parasites in Sheep (SCOPS) initiative promotes evidence-based, best practice guidelines to veterinarians and farmers and a 5 year study was carried out to evaluate their efficacy in practice. A factorial design with 16 farms grouped for management (SCOPS, TRADITIONAL); region (North East, South West); and type (Upland, Lowland) allowed evaluation of lamb productivity and worm burdens, anthelmintic use, and development of anthelmintic resistance. Data detailing high and low risk management practices was also collated from each farm and compared between groups and to changes in 1-BZ resistance. An analysis of the cost benefit of employing SCOPS control was also carried out. SCOPS implementation led to a significant reduction in anthelmintic use, without affecting worm burdens, or lamb productivity. However, the very low price of the older anthelmintic products meant that the benefit did not always outweigh the additional management/diagnostic costs unless an increase in production was also achieved. SCOPS farms were more likely to use grazing management, carry out targeted treatments, not dose then move, leave some animals untreated, avoid off target use of combination products, treat only when necessary , target specific infections e.g. 1-BZ for Nematodiosis control, carry out partial ewe dosing at turnout and test for anthelmintic resistance than TRADITIONAL farms. Changes in 1-BZ resistance was also associated with these factors, as well as not mixing anthelmintic products, avoiding dosing ewes at tugging and avoiding treatments on lambing pastures. Results have allowed proposals for evidence based revisions to SCOPS guidelines.

Keywords: SCOPS; anthelmintic; sheep; resistance;

Abstract No: 4372

5 Sept 2017, 1430 - 1445

Assessment of treatment failure and prevalence utilizing coproantigen ELISA – Are current protocols adequate?

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Australasia Pty Ltd/ Australia

Abstract Content

The coproantigen ELISA (cELISA; Bio K201 Bio-X diagnostics, Belgium) has been used to determine flukicide efficacy against *Fasciola hepatica* and could provide an alternative to worm egg counts for monitoring parasitism on-farm. However, the reliability of the protocol has been questioned.

Treatment of experimental multi-age *F.hepatica* infection in a controlled study demonstrated delayed antigen emergence in immature fluke, requiring re-testing at intervals of 6-7 weeks post-treatment in field situations. To compose an optimal program using FWEC or cELISA, samples from sheep and cattle on two endemic farms were taken at monthly intervals for 12 months and examined by both methods. The epidemiology of the parasite was also examined at each farm.

Diagnostic results and prevalence estimates on-farm were consistent with known epidemiology and similar trends were demonstrated by FWEC and cELISA over the study period. The cELISA detected treatment failure reliably in field conditions, consistent with experimental findings.

The cELISA was shown to be a suitable tool for epidemiological monitoring and more efficient due to reduced labour inputs. For drug efficacy, investigations with the cELISA have demonstrated greater sensitivity to detect parasite survival.

A simple and robust statistical framework for planning and analysing data from faecal egg count reduction test (FECRT) studies

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Abstract Content

Introduction. There is an urgent need for a method of analysing FECRT data that is computationally simple and statistically robust. A method for evaluating the statistical power of a proposed FECRT study would also greatly enhance the current guidelines. **Methods.** A novel statistical framework has been developed that evaluates observed FECRT data against two null hypotheses: (1) the observed efficacy is consistent with the expected efficacy, and (2) the observed efficacy is inferior to the expected efficacy. The method requires only four simple summary statistics of the observed data. Power calculations can also be obtained for any proposed study design and given set of parameter values. For illustration, the power to detect a significant deviation from 95% efficacy is estimated for a population efficacy of 90% and parameter values typical to parasitology. **Results.** Simulation studies reveal that the notional type 1 error rate of the new statistical test is accurate. Power calculations demonstrate a power of only 65% with a sample size of 20 treatment and control animals, which increases to 69% with 40 control animals or 79% with 40 treatment animals. **Discussion.** The method proposed is simple and robust, and can analyse paired and unpaired data with a range of study designs, including pooling and repeated observations. The facility for power calculations gives crucial insight into the design of more efficient FECRT studies, and suggests that the priority should be to collect more samples from the treatment group. The software has been made freely available as an online tool.

Keywords: FECRT; anthelmintic resistance; study design; statistical methods;

Abstract No: 4531

5 Sept 2017, 1500 - 1515

The impact of anthelmintic resistance on beef cattle productivity

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Abstract Content

The goal of the current study was to evaluate, in a commercial beef-cattle farm, the impact of anthelmintic resistance (AR) on the productivity of calves naturally infected with gastrointestinal nematodes resistant to ivermectin (IVM) and moxidectin (MXD). This trial included two herds grazing on different forage resources: Herd A in a maize-winter forage crop rotation (low re-infection) and Herd B in a two-year-old *Agropyrum* pasture (high re-infection). In each herd, eighty male calves were randomly allocated into four groups (n= 20): Control group (CG): without anthelmintic treatment; IVM group: treated with IVM (0.2 mg/kg); MXD group: treated with MXD (0.2 mg/kg); IVM+RBZ group: treated with IVM and ricobendazole (RBZ) (0.2 and 3.75 mg/kg, respectively). All treatments were given by the subcutaneous route. The clinical efficacy was determined by FECRT 20 days post-treatment. On days 0, 20, 35, 67 and 90, individual weights were registered. The clinical efficacies were 42% (IVM), 67% (MXD) and 99% (IVM+RBZ). *Cooperia* spp. and *Haemonchus* spp. were the genera involved in AR. In Herd A the total weight gains (TWGs) in the 90 day-period increased by 0% (IVM), 59% (MXD) and 90% (IVM+RBZ) compared with the Control group. In Herd B the TWGs increased by 64% (IVM), 97% (MXD) and 151% (IVM+RBZ) compared with the Control group. The effect of AR on live weight gains was significant (Tukey, P<0.05) in both forage resources. In conclusion, anthelmintic resistance has a high impact on beef cattle productivity.

Keywords: Anthelmintic resistance; Ivermectin; Moxidectin; Cattle; Productivity

Abstract No: 4293

5 Sept 2017, 1515 – 1530

The transcription factor NHR-8 is involved in ivermectin tolerance in the model nematode *Caenorhabditis elegans* and in the strongylid nematode *Haemonchus contortus*

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¹*Animal Health/ INRA/ France*

Abstract Content

Resistance to ivermectin jeopardizes the success of treatment of diseases caused by parasitic nematodes. There is urgent need to decipher adaptive mechanisms of nematodes to anthelmintic macrocyclic lactones (MLs) treatments. There is some evidence that ATP binding cassette (ABC) transporters play a role in resistance to MLs. In this study, we have identified the transcription factor *nhr-8* as a new key regulator of tolerance to the ML ivermectin in the model nematode *Caenorhabditis elegans*. Loss-of-function mutants, subjected to larval development assays and electropharyngeograms measurements, show a clear hypersensitivity to ivermectin. In addition, silencing of *nhr-8* with the use of RNAi increases ivermectin efficacy in ivermectin-resistant *C. elegans* strains. qPCR experiments show that *nhr-8* regulates some genes involved in the drug metabolism and transport and that some of them are upregulated in case of ivermectin resistance. Finally, we have investigated whether this mechanism is conserved in parasitic nematodes. We have rescued the loss-of-function mutant *C. elegans* strain with the homolog of this key factor from the sheep parasite *Haemonchus contortus*. The transgenic *C. elegans* lines expressing *Hco-nhr-8* show a total rescue of the wild-type phenotype regarding the susceptibility to ivermectin, thus indicating a functional homology of *nhr-8* between *H. contortus* and *C. elegans*. All these results could help to better understand mechanisms underlying failure in drug efficacy and opens possibilities for managing anthelmintic resistance in parasite nematodes in the field.

Keywords: Macrocyclic lactones; Ivermectin; resistance; C. elegans; H. contortus

Gastrointestinal parasitism and anthelmintic resistance in sheep farms in Quebec, Canada

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Abstract Content

A study was conducted on 23 sheep farms in Quebec which utilize pasture over the summer. On each farm, animals grazing together were randomly allocated to 3 groups of 15 sheep. Faecal samples were individually collected immediately prior to anthelmintic treatment. Group 1 sheep were treated with fenbendazole and Group 2 with ivermectin oral, as recommended. Group 3 sheep were untreated. Faecal samples were taken 14 days post treatment for establishing the faecal egg count reductions (FECR). The proportion of *Haemonchus contortus* eggs in the samples were estimated by fluorescence. Following flotation, eggs were separated and DNA extracted. The presence of polymorphism in codons 167, 198 and 200 in β -tubulin, which is associated with benzimidazole resistance, and polymorphism at positions 141, 234 and 438 in the *dyf7* gene, possibly associated with ivermectin resistance, were assessed by pyrosequencing. Pre-treatment egg counts on most farms were not high. However, *H. contortus* eggs predominated. Based on FECR, most farms showed fenbendazole resistance and a high proportion also showed ivermectin resistance. Widespread benzimidazole resistance was also predicted by the β -tubulin genetic analysis. Polymorphism was found in the *dyf7* gene. However, the level of polymorphism seen in *dyf7* was insufficient to account for the loss of efficacy of ivermectin shown by FECR, suggesting that other genes may be important for ivermectin resistance. In conclusion the study shows widespread benzimidazole resistance and considerable ivermectin resistance, and that molecular analysis can predict the presence or absence of benzimidazole resistance. Supported by Agriculture & Agri-Food, Canada. *Haemonchus contortus*

Keywords: fenbendazole, ivermectin, anthelmintic resistance, sheep, *Haemonchus contortus*, Canada, egg count, molecular analysis, β -tubulin, *dyf-7*

Heartworm preventive drug 'lack of effectiveness' claims submitted to the FDA: an analysis of reports, 2004 – 2015

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²*Center for Veterinary Medicine/ U.S. Food and Drug Administration/ United States,* ³*Department of Infectious Diseases/ University of Georgia College of Veterinary Medicine/ United States*

Abstract Content

In 2005, Hampshire published the first peer-reviewed article on the potential emergence of macrocyclic lactone (ML) resistance in *Dirofilaria immitis* based on lack of effectiveness (LOE) claims submitted to the Center for Veterinary Medicine (CVM) within the U.S. Food and Drug Administration (FDA). Over the next 10 years, the veterinary community would continue to see increasing numbers of suspect ML LOE cases, predominantly focused in the Lower Mississippi Delta region. Currently, however, the extent of ML resistant heartworms (HWs) is unknown, with information lacking on prevalence and geographic range of such cases. The objective of this study is to analyze ML LOE reports submitted to CVM since 2005, in order to identify possible changes, patterns, and interactions found between and within cases. To date, approximately 45,000 ML LOE claims have been reported to CVM, with each report assigned a causality score, ranking the probability of true ML drug ineffectiveness. A comprehensive analysis is underway searching for epidemiologic trends and geospatial patterns among the reports, with a focus on animal signalment, space-time relationships, and the identification of possible 'hot spots.' A statistically significant random sample of reports has been selected, and an analysis of these will be presented using the same techniques that will be applied to all 45,000 reports once they have been finalized. The outcome of this project will not only help elucidate trends of ML resistance in HWs in the USA, but similar approaches can be applied to monitor drug resistance in other parasites affecting other countries.

Keywords: Dirofilaria immitis; macrocyclic lactone; drug resistance; USA

Abstract No: 4226

5 Sept 2017, 1630 – 1645

Moxidectin is present in new born lambs at high concentrations when dams are treated during pregnancy

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Abstract Content

The administration of long-acting anthelmintics to pregnant ewes is common practice in New Zealand and some other countries. The majority of these products contain macrocyclic lactone (ML) actives, which due to their lipophilic nature are absorbed into milk of treated ewes and can be detected in the plasma of their suckling lambs. In a previous indoor trial, it was shown that after a pre-lambing treatment of ewes with Moxidectin, the drug was detected in the plasma of suckling lambs as early as 10 days after birth and over the lactation period this reduced the establishment of susceptible *Teladorsagia circumcincta* L3 by 70%. To extend our initial findings, the present study was conducted to establish the levels of moxidectin in lambs immediately after birth, and where animals were grazed outdoors on pasture. Twelve pregnant ewes were treated with one injection of long acting moxidectin and blood samples were collected at regular intervals commencing one day post treatment. After lambing, blood and milk samples from ewes and blood samples from lambs were collected immediately after birth and at regular intervals until 68 days post treatment. Plasma and ewe's milk were analysed for drug concentration using mass spectrophotometry. Plasma levels indicate that the lambs are receiving high doses of moxidectin at a very young age which raises the possibility of adverse health effect.

Prevalence of anthelmintic resistance on sheep and goat farms in Lithuania

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¹*Department of Veterinary Pathobiology, Veterinary Academy/ Lithuanian University of Health Sciences/
Lithuania*

Abstract Content

The aim of the performed study was to determine the prevalence of anthelmintic resistance (AR) in parasitic nematodes on sheep and goat farms in Lithuania. To estimate AR - egg hatch test (EHT) and larval development test (LDT) were used. Data were analysed using a threshold discriminating concentration of 21.6 ng/ml⁻¹ for ivermectin-aglycone, 2 µg/ml⁻¹ for levamisole in LDT and 0.1 µg/ml⁻¹ for thiabendazole in EHT. Twenty one sheep and 9 goat farms were tested for AR to ivermectin, 6 sheep and 8 goat farms to levamisole and 25 sheep and 9 goat farms to benzimidazoles. The *in vitro* survey showed the anthelmintic resistance to ivermectin in 13 sheep farms (61.9%), to levamisole in 2 farms (33.4%) and to benzimidazoles in all sheep farms investigated (100%). In goat farms, the resistance to ivermectin were detected in 9 farms (100%), to levamisole in 2 farms (25%) and to benzimidazoles resistance were detected using only LDT in 2 farms (22.2%). The study showed that the main genus of anthelmintic resistant gastrointestinal nematode identified in sheep and goat farms were *Teladorsagia* (P<0.05). The results of *in vitro* survey showed that sheep and goat farms already have significant problem with AR in Lithuania. Resistance to benzimidazoles is higher in sheep farms, however to ivermectin in goat farms.

Keywords: anthelmintic resistance, nematodes, sheep, goat

Genome and genetic approaches to identify loci linked to the anthelmintic resistance in *Haemonchus contortus*

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Abstract Content

Genetic crossing were undertaken between the anthelmintic sensitive genome strain-MHco3 (ISE) and a multi-drug resistant strain-MHco18 (UGA) derived from southern USA. Day 14, one hundred female (MHco3) and 100 male (MHco18) and *vice versa* were harvested from donor sheep and surgically transferred to the abomasum of a recipient sheep. The eggs of F1 and F2 released from these worms were further incubated to form L3 stage and then used to orally infect another donor sheep to obtain F2 progeny treated with three broad spectrum anthelmintic drugs [Ivermectin (IVM), Benzimidazoles (BZ) and Levamisole (Lev)].

A panel of six microsatellite markers were used to monitor the success of genetic crossing procedure of F1 progeny. Microsatellite markers were also used to monitor the IVM treated F2 genetic crossing procedure based on the presence and absence strain specific alleles, there was one marker (Hcms 8a20) in the chromosome V, which alleles specific to parental resistance strain, was retained after IVM treatment give us a strong evidence of the linkage of IVM resistance conferring locus. We are further investigating with the panel of 17 microsatellite markers located in chromosome V from IVM drug selected F2 progeny to look for the detail evidence of genetic linkage to IVM resistance conferring locus providing a starting point for more detailed studies to identify the mutations linked to IVM resistance. Sensitivity of the BZ drug against F1 and F2 have been phenotypically and genotypically assessed using the egg hatch assay and pyrosequencing assay of known isotype-1 β tubulin SNPs.

Abstract No: 4595

5 Sept 2017, 1715 – 1730

Utilization of composite fecal samples for detection of anthelmintic resistance in gastrointestinal nematodes of cattle

Melissa George^{*1}; Kelsey Paras¹; Sue Howell¹; Ray Kaplan¹

¹*Department of Infectious Diseases/ The University of Georgia/ United States*

Abstract Content

Anthelmintic resistance in gastrointestinal nematodes of cattle is becoming increasingly prevalent worldwide. Presently, the fecal egg count reduction test (FECRT) is the only means available for detection of resistance to anthelmintics in cattle herds at the farm level. However, the FECRT is labor and cost intensive, and consequently is only rarely performed on cattle farms unless for research purposes. If costs could be reduced, cattle producers might be more likely to pursue drug resistance testing on their farms. One approach to reducing the cost and improving the acceptance of the FECRT among cattle producers is the use of composite fecal samples for performing fecal egg counts (FEC), rather than the recommended protocol of conducting FEC on fecal samples from 15-20 individual animals. In this study FECRT were performed on 14 groups of cattle using both individual and composite FEC methods. Results demonstrated that there was little difference between the approaches with 98% agreement found between methods. The use of composite samples was shown to reduce the number of FEC required by 79%. These data demonstrate that pooling fecal samples from a group of cattle and then performing repeated FEC on that composite sample yields results similar to performing individual FEC on those same animals, while substantially reducing the cost of performing a FECRT as compared to individual fecal samples. Furthermore, we have developed suggested methods for using composite samples in a FECRT and described potential issues associated with the use of composite samples that must be considered.

Keywords: fecal egg count; reduction test; anthelmintic; resistance; composite samples

Abstract No: 5097

5 Sept 2017, 1730 – 1745

Dairy and meat sheep farmers' beliefs on intensity of strongyle infection and anthelmintic resistance as interpreted from semi-directive interviews

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Abstract Content

Prescribers and hence prescriptions are many for managing efficiently sheep strongyle infection. The management decisions are in the hands of farmers and there can be a large discrepancy between prescriptions by external experts and actual strongyle control on a farm. These prescriptions may be modified due to: i) absence of importance for the farmer of strongyle infection, ii) a feeling that the economic return on investment is not worthy, and / or iii) the proposal is in conflict with beliefs or other planned activities. Semi-directive interviews were implemented in 16 dairy (Basque country) and 16 meat sheep farms (Massif Central) in France, either organic or not. A new grid for analysing farmers responses was build up and the semi-quantitative variables (importance of strongyle infection, number of treatments, etc.. coded from one- low to three-high) were subjected to cluster analysis. This was intended for researchers in biology not familiar with sociological investigations. The results will be compared with text analysis (Tropes software) or classical sociological interpretation. It was shown that observance to expert proposals was very variable between farms and only meat sheep producers were relying on veterinary proposals. The organic farmers rather relied on their (or other farmer or family member) experience using alternative and complementary products. The strongyle resistance to synthetic anthelmintics was not considered by farmers. Only larger organisations regrouping farmers may play a role on avoiding the spread of anthelmintic resistance

Keywords: Sheep, strongyle, anthelmintic resistance, sociology

Abstract No: 4946

5 Sept 2017, 1745 – 1800

Macrocyclic lactone (ML) anthelmintics lack meaningful in vitro activity against L3 and L4 stages of both ML-susceptible and ML-resistant *Dirofilaria immitis*

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Abstract Content

Macrocyclic lactone (ML) anthelmintics are the only treatment available for preventing vascular infection with *Dirofilaria immitis* (Di). However, the mechanism of action of ML drugs against Di remains unclear. In this study, we tested the in vitro dose response of both L3 and L4 stages of ML-susceptible (Missouri strain) and ML-resistant (Metairie-2014 strain) Di, using both ivermectin (IVM) and eprinomectin (EPR) at concentrations ranging from 0.625 to 20 μM . L3 were isolated from mosquitoes and used directly, or were cultured for 6 days to obtain L4 before adding drug. Motility measurements were made every 24hr for 4 days using computer processed video imaging (Worminator system), and a nonlinear regression model was fit to the dose response data. Mean IC₅₀ values for IVM and EPR were 12.8, 5.37, and 11.36, 9.53 μM for the susceptible and resistant strain, respectively for L3, and 6.97, 8.79, and 9.38, 4.64 μM , respectively for L4. Maximal inhibition of motility varied from 68-92% with no apparent biological differences between strains or larval stages. In vitro exposure to ML failed to achieve complete inhibition in the ML-susceptible strain, even at the highest concentration tested (20 μM), which is >5,000X times higher than the in vivo plasma concentration following a preventive dose of IVM. Additionally, there were no apparent differences in dose response between ML-susceptible and ML-resistant strains. These data strongly suggest that ML action and resistance in Di are not mediated through paralytic effects and that ML drugs do not demonstrate meaningful in vitro activity against Di.

Keywords: Macrocyclic lactones; Dirofilaria immitis; L3; L4; motility

Abstract No: 4641

5 Sept 2017, 1800 – 1815

Re-Orientation of Helminth Control in Adult Horses in Switzerland

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Abstract Content

The epidemiological situation of helminth infections in adult horses in Switzerland is characterized by a strong dominance of small strongyles (Cyathostominae) and an overall low level of egg shedding in the faeces. The prevailing strategy, usually comprising 3 to 4 annual treatments, considers neither husbandry conditions nor pastures management. With respect to the increasing problem of anthelmintic resistance a re-orientation of the prevailing concept seemed to be mandatory. In 2011 a consensus has been agreed on between equine parasitologists and clinicians of the Vetsuisse Faculty in Zurich and Berne to focus on the concept of a selective control approach, based on individual faecal egg counts as the central element. Since then it is recommended that clinically healthy horses (≥ 4 y) are treated only when their strongyle egg count is equal to or higher than 200 eggs per gram of faeces, or in the case of detection of *Parascaris* sp., *Strongylus* sp. or cestode infection. A yearly analysis of the strongyle population based on coprocultures, the regular control of drug efficacy with the faecal egg count reduction test and quarantine measures for new horses are mandatory components of the concept. For horses that did not receive any anthelmintic during the current season, a 'safety' treatment is recommended at the end of the grazing period. After 5 years the approach has been established in practice very successfully, indicating that maintaining health in adult horses is feasible with a considerably lower input of anthelmintics.

Keywords: horse; cyathostominae; Parascaris; control; Switzerland

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Fasciolosis: either all or nothing on even adjacent Tsitsikamma dairy farms in South Africa

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Abstract Content

INTRODUCTION: Serious regional losses from fasciolosis and threat of flukicide resistance led to preliminary investigations on four dairy farms in the Tsitsikamma region. **AIMS:** (i) Longitudinal investigation of seasonal cycling of *Fasciola hepatica* to inform sustainable fasciolosis management; (ii) Farm survey facilitation by developing a rapid method for evaluating the potential to harbour *Fasciola* sp. **METHODS: Farms:** Three of four farms (farm 1-3) were selected on farmer opinion for high potential for fasciolosis, but the fourth (farm 4), with a long common boundary with one of the other three, was selected as control, reported by the farmers as free from fasciolosis. **Monthly monitoring per farm over 21 months:** (i) Intermediate snail host surveillance on six marshy patches; (ii) Sixty cows/heifers sampled for faecal worm egg counts and liver enzymes in serum. **RESULTS:** There were clear seasonal variations in faecal egg counts and recovered intermediate host snails, with significant differences between farms. Totals of recovered *Galba truncatula* per farm were **zero on control Farm 3** and 884, 1345 and 1725, respectively, on farms 1, 2 and 4. On farm 4, with the highest number of *G. truncatula*, clinical fasciolosis as well as significant increases in liver enzyme levels in serum were observed. Farmer-instigated earthworks on *Fasciola*-affected farms were only temporarily successful in many snail habitats, as repopulation with *G. truncatula* was mostly observed within 6 months. **DISCUSSION:** Dramatic differences were demonstrated in potential for fasciolosis between both adjacent farms and marshy patches and ongoing investigations are aimed at discerning reasons for the differences.

Keywords: Fasciola; Epidemiology; Worm management

Does the epidemiological benefit from resistant animals outweigh the cost?

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Abstract Content

Selection of animals that are genetically either resistant or resilient to gastro-intestinal nematodes is a debated option for sustainable parasite control strategies. Although resistance confers an epidemiological advantage, it is not clear whether this outweighs the reported poorer growth potential. In a replicated farmlet study, the benefit of selecting for resilience or resistance to gastrointestinal nematodes was evaluated using Romney sheep from established selection lines. Three farmlets contained only resistant animals without any anthelmintic use, three contained only resilient animals without any anthelmintic use and two contained resistant and resilient animals grazed together but receiving suppressive long-acting anthelmintic treatment to determine the growth potential in the absence of parasite challenge. Post-weaning performance was assessed monthly until 24 weeks-of-age. Overall, for the suppressive anthelmintic treated groups, cumulative liveweight gain was greater in resilient (20.2 ± 1.19 kg) compared with resistant (17.2 ± 0.88 kg) animals ($P=0.02$) indicating a difference in the growth potential of the lines in the absence of parasite challenge. For lines run separately, mean faecal egg counts were greater in resilient compared with resistant animals, viz, 621 ± 137 eggs per g c.f. 30 ± 10 eggs per g ($P<0.001$) with cumulative liveweight gains being similar, viz, 14.1 ± 2.54 kg and 14.4 ± 1.01 kg, respectively ($P>0.05$). Although differences in the growth potential of the lines exist, in the absence of anthelmintic the epidemiological advantage conferred by resistance reduces the impact of infection to the extent that resistant and resilient lines have similar levels of production.

Keywords: resistance; resilience; selection; sheep; epidemiology

Evaluation of a refugia-based strategy in pastured stocker cattle treated with LongRange®

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United States

Abstract Content

Anthelmintic resistance in gastrointestinal nematodes of cattle is becoming increasingly prevalent worldwide. Refugia-based strategies have proven effective in slowing the development of resistance in parasites of sheep, but few studies have examined this strategy in cattle. In this study, we examined the use of a selective non-treatment strategy in stocker cattle. 180 stocker calves ranging in weight from 184-334kgs were allocated randomly into three treatment groups; 100% treated with Dectomax®, 100% treated with LongRange®, and 90% treated with LongRange®. Following treatment cattle were placed on newly sown wheat/rye grass pastures for 112 days. Cattle were weighed and fecal samples collected for egg counts and coprocultures at the time of treatment and then monthly for 4 months. After 112 days of grazing, there were no significant differences between the three treatment groups in total weight gain ($p=0.74$), average daily gain ($p=0.83$), or in numbers of cases bovine respiratory disease ($p=0.44$). Mean FEC at treatment was 514 EPG, and FEC reductions were, 70.4%, 92.1%, 87.9% for the Dectomax®, 100% LongRange®, and 90% LongRange® groups, respectively. By 112 days the EPG of all groups were low, averaging 12.8 across the three groups. *Cooperia*, *Haemonchus*, *Ostertagia*, and *Oesophagostomum* were observed on coprocultures. Treating only 90% of the herd with LongRange® had minimal impact on parasitological parameters, and did not significantly affect group weight gain, suggesting that a selective non-treatment strategy may be a sound approach for integrating a refugia-based strategy for parasite control in cattle.

Keywords: Refugia; cattle management; anthelmintics; trichostrongyles

Application of molecular methods and conventional parasitology to understand ovine nematode parasite co-infections, in the absence of intervention.

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Abstract Content

Host fitness is influenced by the presence of co-infecting parasites, and understanding intraspecific dynamics is important in gaining an insight into parasite transmission and host health. Previous methods of nematode burden assessment are low-throughput and have limited sensitivity, which prevents fine-scale partitioning of species. Researching the nematode biome structure in the absence of control measures is required to recognise the impact of management decisions on sustainable control. The unmanaged, feral population of Soay sheep on St Kilda (Scotland) provides an ideal study population. Faecal samples were collected over 8 sampling months, from 9 different sex-age groups. These were used for faecal egg counting using a cuvette salt floatation method and were incubated to grow 3rd-stage larvae for molecular and morphological analyses. The development of a deep sequencing assay of the ITS-2 region of the rDNA cistron has enabled the accurate identification and quantification of clade V species, with this being the first field application of this method. Preliminary analyses of the sequencing data recovered all five species previously identified on St Kilda, with seasonal, age and sex differences in species composition. Additionally, cyclic trends in co-infections have been observed, with species sequentially peaking throughout the year. These seasonal differences appear to correspond with the sheep's dynamic life-history. Correcting for species-specific sequencing biases, amplicon repeatability and species detection threshold ensures this method is repeatable.

Keywords: co-infection; biome; Soay sheep; St Kilda; strongyle; nematode; MiSeq

Abstract No: 4496

5 Sept 2017, 1500 – 1515

Use of a stochastic production frontier approach to examine impact of GIN management in beef cow-calf herds in Canada.

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Abstract Content

Introduction: Blanket treatment at set times of the year of all cattle for gastrointestinal (GIN) parasites is likely to lead to anthelmintic resistance. Understanding how producers make these decisions would be helpful to change, although there is little information on how that takes place. This research developed a basic spreadsheet tool to allow beef producers to estimate changes in economic impact from use of various anthelmintic options, based on production and market price knowledge.

Methods: A basic stochastic production frontier (SPF) approach was applied for cow-calf herds using data from records in Western Canada. Functional form was based on a standard Cobb-Douglas approach. Data were also collected regarding anthelmintic useage, production demographics, and ecozone. These variables were used to define expected differences from the mean for production. Output was valued using current market prices. Users are able to modify data to fit their system.

Key results: A spreadsheet tool to estimate impact from anthelmintic application was developed and tested based on concordance with producer records. Sensitivity analysis yielded a deviation of +/- 15-20% from expected outcomes.

Discussion: Previous use of an SPF approach to cow-calf herd GIN management was not located in the literature. This approach accepts modest deviations from perfectly efficient expectations and allows producers to enter their own production details to estimate economic gains from changing GIN management.

Conclusions: An interactive tool is helpful for discussion with producers regarding options for reducing use of GIN antiparasitics. This will be a valuable contribution to sustainable prudent use of anthelmintics.

Keywords: *beef cow-calf; anthelmintic resistance; GIN; stochastic production function; economic impact analysis;*

Abstract No: 4258

5 Sept 2017, 1515 – 1530

Parasites of Philippine native swine: revisiting low-cost farming systems in the Philippines

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Abstract Content

Philippine native swine industry has increased in the past recent years in the country and common in smallholder areas where it is kept under low-cost farming system. At present, there is an increasing demand for native swine meat due to its nutraceutical value and palatability. However, there is a dearth of information regarding the potential threats of pathogens, such as parasites, in low-cost native swine farming. Hence, this study aimed to assess the prevalence of parasites in Philippine native swine among smallholder farms and the extent of contamination in the environment. Results revealed the occurrence of 12 different parasite species in Philippine native swine and their respective prevalence namely *Strongyloides ransomi* (78.9%), *Entamoeba* spp. (63.6%), *Balantidium* sp. (54.55%), *Ascaris suum* (42.1%), *Oesophagostomum* sp. (36.8%), *Trichuris suis* (36.8%), *Cryptosporidium* sp. (27.3%), *Iodamoeba* sp. (27.3%), *Blastocystis* sp. (18.2%), *Endolimax* sp. (18.2%), *Metastrongylus* sp. (10.5%) and other coccidian oocysts (26.3%). Some parasites were also found contaminating the soils from the farm vicinities such as *Ascaris* sp., coccidian oocysts, strongylids, *Trichuris* sp., *Toxocara* sp. and *Hymenolepis nana*. *Cryptosporidium* sp. and *Giardia* sp. were also recovered from water samples using immunofluorescence assay. The results, thus, indicate high susceptibility not only of the native swine, but of humans and other animals, to a wide range of parasite infections associated with low-cost farming systems. The current data will be useful in improving agricultural practices in marginalized sectors and facilitate effort from various stakeholders in order to mitigate the adverse effects of parasitic infections in native swine.

Keywords: Philippine native swine, parasites, zoonoses, low-cost farming

Abstract No: 3832

5 Sept 2017, 1600 – 1615

Investigation of association between *Toxoplasma gondii* and early pregnancy and abortion rates in New Zealand farmed red deer

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Abstract Content

Reproductive performance in farmed red deer in New Zealand is suboptimal. This study examined association between *Toxoplasma gondii* sero-status, as determined by ELISA, mid-pregnancy abortion, and DNA evidence from maternal and fetal tissue, in young (R2) and adult (MA) hinds on 85 deer farms. At ultrasound pregnancy scanning early in gestation (Scan-1), 31.1% of 861 R2 and 28.3% of 357 MA hinds were sero-positive for *T. gondii*. There was no association between Scan-1 sero-status and non-pregnancy at animal (R2 $P=0.05$, MA $P=0.43$) or herd level (R2 $P=0.37$). *Toxoplasma gondii* DNA was detected in 16% of placenta and 22% fetal brain samples from aborting R2 hinds and 10% of uteri from non-pregnant hinds at Scan-1. Later in gestation at Scan-2, the sero-prevalence in R2 hinds aborted since Scan-1 (34.3%) was significantly higher than in non-aborted R2 hinds (23.5%) (OR=1.6, $P=0.032$, $n=714$). However no difference in sero-prevalence was observed in aborted (32.9%) or pregnant (31.4%) MA hinds ($P=0.21$, $n=372$). The within-herd sero-prevalence at Scan-2 was positively associated with daily abortion rate in R2 herds having aborted hinds ($P=0.0003$) but not in MA herds ($P=0.07$). *Toxoplasma gondii* DNA was detected in 16% of uteri, 10% coyledons, and 1/5 fetal brains from aborted hinds at Scan-2 and in 15% of uteri from hinds not rearing a calf to weaning. In R2 hinds, 7.9% of abortions could be attributed to *T. gondii*. These findings provide serological and molecular evidence that *T. gondii* causes abortion in deer, possibly in all three trimesters.

Keywords: Toxoplasma, deer, reproduction

Abstract No: 4332

5 Sept 2017, 1615 - 1630

Greater intensity and frequency of *Cryptosporidium* and *Giardia* oocyst shedding is associated with reductions in growth, carcass weight and dressing efficiency in sheep

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Abstract Content

Associations between intensity and frequency of *Cryptosporidium* and *Giardia* shedding with growth, carcass weight and dressing % were investigated using a longitudinal study of 1,182 lambs on eight Australian farms. Faecal samples were collected from each lamb on 3 occasions (weaning, post weaning and pre-slaughter) and screened for *Cryptosporidium* and *Giardia* using qPCR. Weight was recorded on 3 occasions. Carcass weight and dressing % were determined at slaughter. Lambs were categorised for shedding intensity (high - above median for positive sheep, low - below median, or not detected), shedding type (not detected, single *Giardia*, single *Cryptosporidium*, concurrent *Giardia* and *Cryptosporidium*) and shedding frequency (0-3 positive samples). Associations with lamb production were assessed using general linear models and linear mixed effects models. High *C. parvum* shedding was associated with lower live weight, carcass weight (post-weaning and pre-slaughter only) and dressing % (post-weaning only). *Cryptosporidium* (all species) shedding pre-slaughter was associated with lower dressing %. *Giardia* shedding post-weaning was associated with lower carcass weight. Repeated detection of *C. parvum* and *Giardia* shedding in a specific animal were associated with reduced carcass weight and dressing %. Concurrent *Giardia* and *Cryptosporidium* shedding pre-slaughter was associated with lower dressing %. The findings suggest naturally acquired *Cryptosporidium* and *Giardia* infections beyond the neonatal period are associated with depressed growth, carcass weight and dressing efficiency in grazing sheep. These findings have implications for sheep farmers and sheep meat processors, as the protozoan parasites are widespread, and these measures are important drivers of productivity.

Keywords: protozoan; sheep meat;

Abstract No: 4040

5 Sept 2017, 1630 – 1645

Host-pathogen interactions in neonatal calves experimentally infected with *Cryptosporidium parvum*

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Abstract Content

Calves infected with *C. parvum* can suffer from profuse watery diarrhoea, dehydration and in severe cases death may occur. Neonatal calves are highly susceptible to infection however; older calves become infected but do not show clinical signs. Our understanding of the host-pathogen interactions that determine disease outcome and host resistance in cattle is very limited. Examine the *in vivo* host response to experimental infection with *C. parvum* in neonatal calves. Twenty-four calves were infected at 3 or 4 days of age with 2.3E7 *C. parvum* oocysts. Twelve uninfected age-matched controls were used. Regular blood and faecal samples were taken from all calves and groups of calves were culled at defined time points (days 3, 6, 9, 12, 18 and 24 post-infection). Ileum, lymph nodes, faeces and blood were collected at each time point. Sections of ileum and lymph node were collected for histological examination and immunohistochemistry. Clinical data was recorded and sera were used to measure specific antibody responses throughout. Preliminary results showed that calves infected with *C. parvum* displayed inappetence, diarrhoea and lethargy from day 3 to 15 post-infection. Severe clinical disease occurred in some animals. Analysis of faeces confirmed that challenged calves were infected with *C. parvum*. Histological examination of ileum sections showed mild to moderate changes to the villi and infiltration of eosinophils. In the Peyer's patches large numbers of apoptotic and mitotic figures were present. Pathological findings and distribution of the parasite from time points throughout the study will be presented.

Keywords: C. parvum, calves, histology, ileum, lymph nodes

***Besnoitia besnoiti* successful replication depends on the modulation of the endothelial host cell cholesterol metabolism**

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Abstract Content

Bovine besnoitiosis is an emerging disease in Europe caused by the apicomplexan *Besnoitia besnoiti*, which replicates in host endothelial cells during acute infection and causes severe clinical signs: dermatitis, orchitis and vulvitis. During parasite proliferation, *B. besnoiti* is in considerable need for energy and building blocks. Especially cholesterol is indispensable for offspring formation. Given that apicomplexan parasites are generally considered as deficient in *de novo* cholesterol synthesis they have to scavenge this molecule from their host cells. We analyzed the influence of *B. besnoiti* on host cellular cholesterol metabolism. Staining of *B. besnoiti*-infected endothelial host cells with filipin, Nile red and Bodipy 493/503 revealed enhanced contents of total cholesterol, neutral lipids and lipid droplets. The key role of lipid droplets for optimal parasite proliferation was confirmed by an artificially enhanced lipid droplet disposability, which boosted tachyzoite production. In accordance, enhanced levels of esterified cholesterol (generally stored in lipid droplets) were detected in infected cells and chemical blockage of cholesterol esterification via CI976 treatments significantly reduced parasite proliferation. Furthermore, biochemical analyses indicated an enhanced cholesterol *de novo* synthesis since several precursors of cholesterol were found enhanced in infected cells. Accordingly, lovastatin treatments significantly inhibited tachyzoite production. RT-PCR analyses revealed an up-regulation of the scavenger receptor LOX-1 in *B. besnoiti*-infected cells indicating a potential role of LDL-mediated cholesterol up-take for parasite proliferation. In accordance, supplementation with non-modified- and acetylated-LDL boosted *B. besnoiti* replication. The current data indicate that *B. besnoiti* significantly exploits the host cell cholesterol metabolism to guarantee its successful replication.

Keywords: *Besnoitia besnoiti*; endothelial host cell; cholesterol metabolism; modulation; *de novo* synthesis

Abstract No: 4081

5 Sept 2017, 1700 - 1715

***Stomoxys calcitrans* (Linnaeus, 1758) (Diptera: Muscidae), mechanical vector of virulent *Besnoitia besnoiti* (Henry, 1913) (Apicomplexa, Sarcocystidae)**

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Abstract Content

Cattle besnoitiosis, due to *Besnoitia besnoiti*, is an emergent disease in Europe. Blood sucking arthropods are probably mechanical vectors of this parasite but definitive proofs are lacking. The aim of this study was to explore the capacity of *Stomoxys calcitrans* to transmit live *B. besnoiti* from chronically infected cows (showing high numbers of cysts in derma) to rabbits which are known to be susceptible hosts. Two rabbits were included into a control group ("C") and two rabbits were exposed each to the bites of 300 non infected *S. calcitrans* (group "S"). Three batches of 300 stable flies were allowed to take an interrupted blood meal on chronically infected cows and then completed immediately their blood meal on three rabbits of group "B". Blood qPCR analyses, clinical follow-up, serological and haematological surveys were performed in the three groups during 21 weeks until euthanasia. Quantitative PCR examination was performed on several tissue samples (skin and organs) per rabbit. Only one rabbit of group "B" exhibited hyperthermia from day 7 to day 14, with marked weight loss, anaemia, neutropenia, lymphopenia, positive qPCR in blood and specific antibodies from day 14 until euthanasia. Positive threshold values were found in the trachea and the vagina. These results show that *S. calcitrans* acts as a mechanical vector of *B. besnoiti*. The failure of infection in the two other rabbits of group "B" indicates that many factors could interfere with the efficacy of the mechanical transmission of this parasite.

Keywords: Cattle besnoitiosis; vector competence; experimental transmission; stable fly

Abstract No: 3935

5 Sept 2017, 1715 – 1730

Detection of *Theileria* and *Anaplasma* species in ticks collected from cattle in central part of Tamil Nadu, India

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Abstract Content

The present investigation was carried out to detect *Theileria* and *Anaplasma* in tick species that infested cattle in Central Tamil Nadu, India. A total of 228 cattle, comprised of 123 crossbreed and 105 native breed, were examined for tick infestation. Of the 228 cattle, 41 cross breed (18%) and 29 native breed (12.7%) were infested with ticks. A total of 960 ticks belonging to 4 Ixodid genera were collected from the infested animals. The most prevalent tick was *Hyalomma anatolicum anatolicum* (428) followed by *Boophilus microplus* (213), *Haemaphysalis intermedia* (165) and *Rhipicephalus haemaphysaloides* (74). The tick species collected in the order of prevalence were separated into 88, 20, 12 and 8 pools respectively. These pools were examined for *Theileria* and *Anaplasma* species using semi-nested PCR. In this study, 34 and 43, 8 and 12, and 2 and 4 pools of *Hyalomma*, *Boophilus*, and *Haemaphysalis* ticks were found infected with *Theileria* and *Anaplasma* species respectively. Whereas, in *Rhipicephalus* only *Theileria* was detected in 50 per cent of the tick pools examined. Mixed infection of *Theileria* and *Anaplasma* was recorded in 14 pools of *Hyalomma* and out of 18 pools of *Hyalomma* ticks collected from native breed, 9 were found to be infected with *Theileria* and *Anaplasma*. This indicates that native breeds usually harbour haemoprotozoan infections without clinical manifestations, but they act as carrier and source of infection to the tick vectors. A higher prevalence of *Anaplasma* species endorses the fact that it could be transmitted by any haematophagous arthropod vectors.

Keywords: Theileria; Anaplasma; tick; PCR; Tamil Nadu

Abstract No: 3178

5 Sept 2017, 1730 - 1745

Prevalence and molecular diagnosis of *Theileria annulata* in bovine from three distinct zones of Khyber Pakhtunkhwa Province, Pakistan

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Abstract Content

Tropical theileriosis is a tick-borne hemoparasitic disease and is responsible for huge economic losses in livestock sector of Pakistan. Bovine of three distinct zones of Khyber Pakhtunkhwa (KPK) province were examined to determine the molecular prevalence of *T. annulata* along with associated risk factors. A total of 900 blood samples (n=479 cows; n=421 buffaloes) were collected and examined; 170(18.88%) were found positive for *T. annulata*. The central zone showed greater prevalence 65/300 49/300 (21.66%), followed by southern zone 56/300 (18.66%) and northern zone 49/300 (16.33%). Significant difference ($P < 0.05$) was observed in cows as compared to buffalo population ($P > 0.05$). Univariate analysis of risk factors included temporal zone, specie, breed, sex, age, management system, tick infestation, previous tick history, tick control, type of acaricide used, and interval of acaricide usage showed a significant ($P < 0.05$) association with prevalence of *T. annulata* in bovine. This study will help in developing more effective control of *T. annulata* in bovine of Pakistan. The results revealed here will help in developing more effective control strategies in future for dairy farmers in Pakistan.

Keywords: Theileria annulata; Prevalence; Risk factors; Temporal Zones; Khyber Pakhtunkhwa.

Abstract No: 2626

5 Sept 2017, 1745 – 1800

Ticks and tick-borne pathogens of livestock and Wildebeests at the Maasai Mara interface, Kenya

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Abstract Content

Wildlife-livestock interfaces are hotspots for tick-borne livestock diseases which cause high mortalities and morbidity. Wild bovidae in these interfaces play a major role in maintaining tick vectors and tick-borne pathogens. We studied the diversity and genetic relatedness of ticks and tick-borne pathogens in blue wildebeest (*Connochaetes taurinus*), cattle and sheep populations in the Maasai Mara wildlife – livestock interface in Kenya. Ticks were directly collected from cattle and wildebeests, identified and genetic diversity determined by amplification of cytochrome c oxidase subunit 1 (COI), Internally Transcribed Spacer 2 (ITS2) and 12S DNA. Pathogen diversity was studied by amplification of 18S rDNA. Of the 165 ticks collected, 69 were *Rhipicephalus appendiculatus* and 74 were *Rhipicephalus evertsi*. All the *R. appendiculatus* ticks were obtained from wildebeests while *R. evertsi* infested cattle, sheep and wildebeests. Eight *Rhipicephalus evertsi* ticks from wildebeest harboured *Theileria parva*, while two were found to be infected by *Theileria equi* and one harboured *Theileria ovis*. *Theileria parva* was found in ten *R. appendiculatus* ticks while *Theileria equi* was found in two *R. appendiculatus* ticks. Phylogenetic analysis showed that *T. parva* from both *R. evertsi* and *R. appendiculatus* were significantly similar across domestic and wild hosts. Wildebeest, cattle and sheep populations in the Maasai Mara ecosystem harbour ticks and tick-borne pathogens of similar species and significant genetic relatedness suggesting circulation of ticks and pathogens responsible for bovine, equine and ovine theilerioses. This warrants intensified disease surveillance to mitigate transmission and disease outbreaks in livestock.

Keywords: Wildlife; Livestock; Parasites; Diseases; Interfaces

Point prevalence of tick infestation among livestock population along Karakorum highway from Mansehra to Gilgit, Pakistan

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Abstract Content

Ticks (Acari: Ixodidae) are among those blood imbibing ecto-parasites of mammals that can cause colossal economic losses both in terms of production, reproduction and transmission of diseases. They have worldwide distribution from tropics to temperate regions. This study reports the point prevalence of ticks in free range livestock population of hilly areas of Gilgit Baltistan (Diامر, Gilgit, Astor) along the Karakoram highway and Khyber Pakhtunkhwa (Mansehra, Haripur, Shangala, Kohistan). A total of 813 animals consisting of 365 goats, 232 sheep, 163 cattle, and 53 buffaloes were screened for tick infestation through convenient and snowball sampling techniques. Overall prevalence of tick infestation recorded among screened livestock was 75.03% with the highest distribution in sheep (81.47%) followed in order by cattle (77.91%), goat (72.05%) and buffalo (58.49%). District wise prevalence of ticks was highest in Haripur (85.58), followed in order by Gilgit (83.10%), Mansehra (81.14%), Batagram (81.05%), Shangala (77.78%), Kohistan (75.38%), Diامر (72.28%) and Astor (32.22%). Prevalence of ticks was found to be higher (85.67%) in young livestock than adults (66.44%), and in females (80.33%) than males (66.44%). The observed prevalence of tick infestation among livestock population was statistically ($P>0.05$) insignificant. This study provides the first report of tick distribution in high altitude areas of northern Pakistan as well as confirms the acclimatization of ticks to the hilly geo-climate of country. Furthermore, the data on associated risk factors allows jotting down recommendations which can help modulating the existing husbandry system in northern areas of Pakistan.

Keywords: Ticks; Prevalence; Livestock; Northern Pakistan

Validation of FAMACHA® system in different sheep breeds of Pakistan

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Abstract Content

Sheep and goats are of utmost importance in Pakistan and they are reared all over the country mostly by poor and landless farmers. Unfortunately gastrointestinal nematodes particularly *Haemonchus contortus* (*H. contortus*) and development of anthelmintic resistance are the major issues for sheep industry. Use of FAMACHA® system can help to slow down the development of anthelmintic resistance. The objective of present study was to check the sensitivity and specificity of FAMACHA® system in Lohi and Kajli sheep breeds located in different geographical regions of Pakistan. The eye color scores of 60 and 80 Lohi and Kajli sheep were checked by the same trained person and experiment was performed in triplicates. The packed cell volume (PCV) served as the gold standard for clinical evaluation of FAMACHA® system. For calculation of sensitivity and specificity, two different criteria were adopted: animals classified as FAMACHA® (F[®]) 4 and 5 or 3, 4 and 5 were considered anaemic (positive test) and animals classified as F[®] 1, 2 and 3 or 1 and 2 were considered to be non-anaemic (negative test). The highest sensitivity in Kajli breed was found at PCV value < 17% in both evaluation criteria. But in Lohi breed, the highest sensitivity was found at PCV values < 22% and ≤ 12% in first and second evaluation criteria respectively. This study suggests that animals in the FAMACHA® category 3 should be drenched in order to increase the sensitivity of the method. Therefore, FAMACHA® system is a very valuable additional tool of TST.

Keywords: FAMACHA, anthelmintic, sheep, goat

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Addressing canine deworming guidelines in the tropics – not as simple as it seems!

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Abstract Content

The Tropical Council for Companion Animal Parasites Ltd. (TroCCAP) is a non-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 'Guidelines for the Diagnosis, Treatment and Control of Canine 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 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 manufacturer 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 canine endoparasites in the tropics.

Keywords: TroCCAP; Companion; Tropics; Council; Canine; Guidelines

Large scale overview of parasitism of cats in Greece

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Abstract Content

A cross-sectional study was conducted to investigate the level of ecto and endo-parasitism of cats in various locations within Greece, to identify the parasitic species present and risk factors. In total, 1093 household and shelter/stray cats from all over Greece were included in the study during a time period of two years. Faecal samples (n=900) and ear cerumen (n=845) were collected. Hair coat was also macroscopically examined for the collection of fleas and/or ticks. A direct immunofluorescence assay was performed for the detection of *Giardia* spp and *Cryptosporidium* spp (Merifluor *Cryptosporidium/Giardia* kit). InPouch™ TF Feline test was used for the detection of *Trichomonas foetus*, and for the other gastrointestinal parasites sedimentation and a flotation technique were performed. The main endoparasites found were *Giardia* (18.7%), *Toxocara* spp (14.2%), hookworms (4.9%), *Cystoisospora* spp (4.8%), *Cryptosporidium* (4.7%), *Capillaria* spp (0.9%) and *Echinococcus/Taenia* (0.7%). Only one positive sample with *T. foetus* was diagnosed. *Giardia* and *Cryptosporidium* positive samples were genotyped to assess their zoonotic potential. PCR protocols were followed targeting the 18S rDNA, β -giardin, tpi and gdh genes for the molecular identification of *Giardia* isolates, and the 18S rDNA and the HSP70 genes for the molecular identification of *Cryptosporidium* isolates. Sequencing results revealed the presence of cat-specific *G. duodenalis* assemblage F and the potentially zoonotic assemblages A and B. As for *Cryptosporidium*, the cat-specific *C.felis* was detected. Regarding the ectoparasites, fleas were collected from 237 cats(28%) and on top of that 18.9% animals had flea faeces. Finally, 25.9% cats were infected with *Otodectes cynotis*.

Troglostrongylosis: a feline disease of paediatric concern?

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Abstract Content

The immature immune system of cats in their paediatric age is one of the causes favouring the establishment of infectious diseases. Viral diseases are frequently diagnosed in kittens, whereas parasitic infections are less investigated when respiratory disorders occur. Recently, *Troglostrongylus brevior* has been found affecting the respiratory tract of cats, along with the better-known *Aelurostrongylus abstrusus*. Nevertheless, the occurrence of paediatric lungworm-infections in cats has been scantily investigated. A total of 291 domestic cats living in an endemic area for feline lungworms were diagnosed for lungworms infection by morphological and molecular means. Of 291 tested animals, 133 (45.7%) were younger than 6 months. Among 39 (13.4%) animals positive for lungworms, *T. brevior* was the nematode detected most frequently (n=31; 79.5%) followed by *A. abstrusus* (n=3; 7.7%), with 5 cats co-infected by both species (12.8%). The diagnosis of *T. brevior* infection was significantly associated with animals aging ≤ 6 months (90.3% $p < 0.01$) compared to older cats (9.7%). At the clinical examination, 14 out of 31 (45.2%) *T. brevior*-infected kittens displayed respiratory signs (e.g. sneezing, coughing, dyspnoea) and fatal troglostrongylosis occurred in two 2-month-old animals. Additionally, a retrospective analysis of reported cases of troglostrongylosis confirmed the occurrence of the disease in kittens. Results of this study indicate *T. brevior* as a feline lungworm of paediatric concern. Furthermore, considering that parasitic treatments are limited or unavailable in pregnant cats and kittens, an increased awareness and control strategies against troglostrongylosis in young animals are highly advocated.

Keywords: troglostrongylus; pediatric; cat

High prevalence of hookworm and detection of neglected parasitic infections in clinically-healthy cats in Bangkok and vicinities, Thailand

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Abstract Content

Ancylostoma ceylanicum and *Ancylostoma caninum*, commonly found in cats, are zoonotic hookworms potentially causing cutaneous larva migrans and eosinophilic enteritis in human. Also, certain assemblages of *Giardia* can lead to zoonotic infection in human but little is known about prevalence of this pathogen in clinically-healthy cats that may serve as a reservoir. A total of 840 fecal samples of clinically-healthy cats from Bangkok, Nonthaburi province and vicinities were collected between year 2014 and 2016. Samples were examined for the presence of parasitic stages using conventional microscopic methods including wet fecal smear, PBS-ethyl acetate centrifugal sedimentation and/or ZnSO₄ centrifugal flotation. 40.8% of tested samples (343/840) were positive for any parasite by microscopic examination. Hookworm eggs were found in 28.7% (241/840) of all tested samples, thus making hookworm the most predominant among enteric parasite-infected cats, 70.3% (241/343). For other enteric parasites tested from 840 samples, the positive test included 53 *Toxocara cati* (6.3%), 39 *Cystoisospora* spp. (4.7), 34 *Platynosomum fastosum* (4.1%), 20 *Taenia taeniaeformis* (2.4%), 12 Diphyllbothriidean tapeworm (1.4 %), 10 *Strongyloides* larvae (1.2%), 5 *Dipylidium caninum* (0.6%), 1 *Eucoleus aerophilus* (0.1%) and 1 *Opisthorchis*-like egg (0.1%). Furthermore, the SNAP[®] *Giardia* Test was used to detect *Giardia* cyst wall protein from fecal specimens in which 4.7% (26/559) were detected. Additional molecular characterization of assemblages will help to address if zoonotic *Giardia* is involved. Therefore, this work emphasized the need for owner awareness in routine prevention of both common and neglected endoparasites in clinically-healthy cats in order to control possible zoonotic infections.

Keywords: Cats, Enteric parasites, *Giardia*, Bangkok, Nonthaburi, Thailand

Uncovering the intermediate host of *Angiostrongylus chabaudi*

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Abstract Content

Nematodes in the Angiostrongylidae family may cause potentially life-threatening diseases in several mammal species. Alongside with the well-known *Angiostrongylus vasorum* infecting dogs and *Angiostrongylus cantonensis*, which display a major zoonotic potential, other nematodes have been recognized as infectious agents of animals. *Angiostrongylus chabaudi*, firstly reported six decades ago as a parasite of wildcats, has been recently found affecting the cardiopulmonary system of a domestic cat. Thereafter, this parasite has been diagnosed in domestic and wild cats from Italy, Germany, Greece, Romania and Bulgaria, leading to an increased awareness on feline angiostrongylosis. Nonetheless, significant gaps in the understanding of *A. chabaudi* epidemiology include the lack of information of species acting as intermediate host and of the morphological description of larval stages. *Cornu aspersum* (n=30) land snails were infected each with single infective doses of 100 first stage larvae of *A. chabaudi* collected from a naturally infected wildcat in Romania. A total of 311 larvae were collected from infected snails, and larval stages were morphologically described and molecularly identified. Second and infective third stage larvae were detected at 6 and 10 days post-infection, respectively. Here we demonstrate for the first time the development of *A. chabaudi* in snails and report *C. aspersum* as intermediate host for this parasitic nematode. Findings of this study are central to understand morphological and ecological features of feline angiostrongylosis, instrumental to support the design of epidemiological surveys in gastropods and feline definitive hosts. This information will be pivotal toward the identification of control strategies of *A. chabaudi*.

Keywords: *Felines; Cardiopulmonary Nematode; Intermediate Host; Epidemiology; Lungworms*

Abstract No: 4204

5 Sept 2017, 1515 – 1530

Molecular detection of canine tick borne pathogens in stray dogs residing in temples in Bangkok, Thailand

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Abstract Content

Canine tick borne pathogens (CTBP) such as *Babesia* spp., *Ehrlichia* spp., *Anaplasma* spp., *Hepatozoon* spp. and haemotropic mycoplasmas are significant pathogens of dogs worldwide. *Rhipicephalus sanguineus*, the main vector for several CTBP, is the most common tick species found in dogs in Thailand. The objective of this study was to characterize CTBP (and their vectors) which infect dogs; 360 blood and 85 individual tick (*R. sanguineus*) samples were collected from stray dogs residing in 38 temples from 24 districts in Bangkok and screened for CTBP using molecular techniques. The most common CTBP found infecting dogs in this study was *Ehrlichia canis* (38.3%) followed by *Mycoplasma haemocanis* (34.2%), *Hepatozoon canis* (19.7%), *Babesia vogeli* (18.1%) and *Anaplasma platys* (13.9%), respectively. While, *A. platys* (22.4%) was the most common CTBP in ticks followed by *M. haemocanis* (18.8%), *B. vogeli* (9.4%), *H. canis* (5.9%), and *E. canis* (2.4%), respectively. The detection of CTBP from stray dogs residing in Bangkok temples and their ectoparasites highlights the potential risk of infection that may occur between dogs with these pathogens. These findings introduce a change of pattern on CTBP distribution and underline the importance of performing active surveys to understand the complexity of distributions of CTBP and their vectors in Thailand.

Keywords: canine tick borne pathogens (CTBP); stray dogs; temple; Bangkok; Thailand

Worldwide clinic-based serologic survey of heartworm disease in dogs, 2011-2016

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Abstract Content

Canine heartworm disease is a globally distributed and deadly form of dirofilariasis caused by mosquito-borne *Dirofilaria immitis* infection. Infections are most commonly diagnosed using rapid tests that detect the presence of heartworm antigen in blood. The introduction of commercially-available chemoprophylactic drugs to prevent heartworm disease has reduced the incidence of infections in regions where employed, however the risk of infection for unprotected dogs persists worldwide. The goal of this study was to evaluate macrogeographic and temporal trends for heartworm antigen test results in pet dogs over a six-year period (2011-2016). The data were obtained from an international database of SNAP[®] Heartworm RT, SNAP[®] 4Dx[®], and SNAP[®] 4Dx[®] Plus field results and used to assess the frequency of suspect heartworm infection in nearly 19 million dogs. Results were reported from more than 11,000 unique postal codes spanning 62 countries grouped into eight regions. The positive rates (with sample size and 95% confidence intervals) by region were: Caribbean, 8.81% (n=44,911;8.55-9.08%); Asia, 4.05% (n=457,261;3.99-4.11%); Southern Europe, 2.75% (n=87,284;2.64-2.86%); Latin America, 1.88% (n=23,992;1.71-2.06%); Middle East-North Africa, 1.40% (n=2,709;0.99-1.92%); North America, 1.38% (n=18,238,710;1.37-1.38%); Northern Europe, 0.66% (n=59,725;0.60-0.73%); and Australia, 0.46% (n=8,298;0.32-0.63%). Among 41 countries with at least 1,000 results, the median positive rate was 1.53% with a range of 0.12% (Norway) to 18.56% (Sint Maarten). Results from this study confirm that dogs continue to be at risk for heartworm infection worldwide. Furthermore, the scale of this data has potential to support the development of more powerful models to monitor and predict changes in disease prevalence.

Keywords: Heartworm; CVBD; Canine; Dirofilaria

Abstract No: 4600

5 Sept 2017, 1615 – 1630

A statistical approach for evaluating the efficacy of heartworm drugs: what does 100% really mean?

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Abstract Content

Initial studies of heartworm preventive drugs all yielded an observed efficacy of 100% with a single dose, and based on these data all products were required to meet this standard for approval. However, those initial studies were performed on just a few strains of parasites, and therefore not representative of the diversity of circulating biotypes. This has come to light in recent years, as several studies have yielded <100% efficacy. This raises new issues because heartworm efficacy studies lack the statistical power to conclude that finding zero worms is different from finding a few worms. To address this issue, we developed a novel statistical model, based on a hierarchical modeling and parametric bootstrap approach that provides new insights to assess multiple sources of variability encountered in heartworm drug efficacy studies. Using the newly established metrics we performed data simulations and analyzed actual experimental data. Our results suggest that an important source of modeling variability arises from variability in the parasite establishment rate between dogs; not accounting for this can over-estimate efficacy in more than 40% of cases. We demonstrate conclusively that ZoeMo-2012 and JYD-34, which both were established from the same source dog have differing levels of susceptibility to moxidectin. Additionally, we demonstrate conclusively that the differences in efficacy seen in two published studies using the MP3 strain were not due to randomness, and thus must be biological in nature. Our results demonstrate how statistical modeling can provide new insights and improve the interpretation of data from heartworm efficacy studies.

Keywords: Dirofilaria immitis; macrocyclic lactone; canine heartworm; parametric bootstrap; statistical

New insights into the periodicity of microfilariaemia in dogs naturally co-infected with *Dirofilaria immitis* and *D. repens*

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Abstract Content

Dirofilaria immitis and *D. repens* are mosquito-borne zoonotic filarioids typically infecting dogs. The microfilariae of both species show a circadian periodicity in the blood. All previous studies evaluated the microfilariaemia in the peripheral venous blood and only in dogs with mono-infections. The aim of our study was to assess the circadian periodicity of *D. immitis* and *D. repens* in naturally co-infected dogs, to investigate the influence of the microfilariaemia level on the molecular diagnostic accuracy and to evaluate comparatively the level of microfilariaemia and its circadian variation in capillary (CB) vs. peripheral venous blood (PVB). Dogs harbouring natural co-infection with *D. immitis* and *D. repens* were sampled every two hours for two consecutive days. Knott's test followed by molecular (single and duplex PCR) were performed. The dynamics of microfilariaemia in naturally co-infected dogs showed similar patterns for both *Dirofilaria* species. Single species-specific PCR reactions were positive for both *D. immitis* and *D. repens* in all collected samples, while duplex PCR failed to amplify *D. repens* DNA in many cases. For the comparative evaluation of microfilariaemia in the CB vs. PVB, further samples were collected from the skin capillaries using *Triatoma rubida* insects. The microfilariaemia values were higher in the CB than in the PVB for *D. repens* and lower for *D. immitis*. Both *Dirofilaria* species microfilariae are subperiodic, following a similar variation pattern. Duplex PCR fails to identify the infection with *D. repens* in co-infected dogs when the ratio of microfilariaemia is in favour of *D. immitis*.

Keywords: co-infection, periodicity, Dirofilaria, circadian, Romania

Parameters impacting the periodicity of microfilariae in *Dirofilaria immitis*-infected dogs

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Abstract Content

Circadian and seasonal variation of microfilaria (mff) numbers in the peripheral blood has been described for many filarial infections and seems to correlate with the necessity for these parasites to be ingested by the blood feeding vector before they can be transmitted to a new vertebrate host. The mff of the dog heartworm *Dirofilaria immitis* are described to have a circadian and seasonal pattern, but for the former the peaks do not seem to appear in a consistent way. Sixteen puppies were experimentally infected with *D. immitis* isolates. The dogs were housed indoors with a natural light source (windows) and heating that prevented temperature-drops below 20°C during winter. When patency was reached, blood samples were collected in regular intervals over a period of up to 3 years in order to determine seasonal, as well as daily variation of microfilaremia. Despite the fact that the dogs were kept indoors, there was a clear seasonality of the *D. immitis*-microfilaremia, with peaks in summer and 5 to 49-times lower counts in winter. This ratio remained constant over the years, regardless the fact that the mff-counts increased from the first to the second year of patency. Interestingly, one of the used-isolates lost the seasonality after three passages of experimental infections in dogs. The circadian cycle of mff in the peripheral blood varied considerably between dogs and season. There was no consistent and apparent pattern. Potential individual and environmental parameters that might play a role for this observation were evaluated and discussed.

Keywords: Dirofilaria immitis; dog; microfilaria; seasonality; circadian pattern

Abstract No: 4950

5 Sept 2017, 1700 – 1715

The *Wolbachia*: a unique nematode-bacterium relationship in *Dirofilaria immitis*

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Abstract Content

Application of transmission electron microscopy to elucidate the ultrastructure of nematodes revealed that many filariae of medical and veterinary importance harbor intracellular, endosymbiotic bacteria now identified as belonging to the C, D, J clades of the *Wolbachia* complex. The *Wolbachia* of filariae and *D. immitis* are pleomorphic, are confined to the cytoplasm and appear to have two modes of reproduction: by a life cycle similar to that of the *Chlamydiae*. The forms observed include a small, dense granule (elementary body ?), a larger coccoid or a bacillary form with a dense, central inclusion (intermediate body ?), and a bacterial form (initial body ?) which measures approximately 0.3 x 1.5 µm and can also divide by binary fission. They are transovarially transmitted and their intracellular presence can be traced through oogenesis and embryogenesis of microfilariae. Their presence is limited to hypodermis of both sexes and of all larval stages, and microfilariae, to the germinal tissues and female and male filariae, but are not present in mature sperm cells. They are also present in the rachis, and in the epithelial cells of the ovary. Unlike the rickettsiae, the *Wolbachia* of filariae are not found free in the cytoplasm, but are enclosed in membranous vesicles of host origin. Their complete role in development and survival of filariae is still to be elucidated, but they apparently contribute to the inflammatory process during filarial infections and their susceptibility to antibiotics identifies them as a target for developing novel chemotherapeutic agents.

Keywords: Wolbachia; filariae; Dirofilaria immitis; intracellular endosymbionts; therapy

Abstract No: 4449

5 Sept 2017, 1715 – 1730

Comparative pathogenicity of Brazilian, Caribbean and European field isolates of *Toxoplasma gondii*

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Patrick Kelly¹ ; Frank Katzer¹

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Abstract Content

Toxoplasma gondii is a ubiquitous protozoan parasite capable of infecting all warm-blooded animals, including humans. Disease outcome can vary depending on a number of factors, including genetic diversity of the infecting strain. The aim of this study was to investigate the pathogenicity of eight genotypically distinct isolates of *T. gondii*. Eight groups of 15 Swiss Webster mice were inoculated intraperitoneally with 200 *T. gondii* tachyzoites (one isolate per group) – six were atypical isolates previously isolated from free-roaming chickens in St. Kitts (Caribbean), one isolate was the Type II Moredun strain (M4) and one isolate was an atypical Brazilian strain (BrI). Mice were monitored for signs of toxoplasmosis and euthanized when they reached a defined end point or at 4 weeks post-infection. Percentage mortality was recorded for 10 mice per group, and 5 mice per group were euthanized at day 8 p.i. and tissues were collected for parasite quantification, histopathology and RNA extraction and quantification of cytokines. Three of the isolates were acutely virulent for mice (100% mortality), 3 isolates were moderately virulent (30-70% mortality) and 2 isolates were non-virulent (0-20% mortality). The acutely virulent and moderately virulent strains had Type I and Type III ROP5 alleles, respectively, which are associated with virulence. Mice infected with acutely virulent isolates had significantly higher levels of parasite DNA in their lungs at day 8 p.i. Differences in histology and immunology are currently being investigated and will be discussed.

Keywords: Toxoplasma gondii, pathogenicity, genotyping, immunology

Abstract No: 4349

5 Sept 2017, 1730 – 1745

Field evaluation of Frontline Tri-Act® spot-on in reducing the transmission of *Leishmania infantum* and *Dirofilaria immitis* in dogs

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Abstract Content

European countries of the Mediterranean basin are highly endemic for canine vector borne diseases. The risk of pathogen transmission is reduced by the use of effective repellent insecticides. The objectives of this serological field study, consisted of 2 trials, in a highly endemic area of canine vector borne diseases in Greece, were to assess the effectiveness of a topical formulation of fipronil/permethrin (Frontline Tri-Act® / Frontect®) for the prevention of *Leishmania infantum* and *Dirofilaria immitis* transmission. In trial 1, a total of 31 dogs were treated monthly with the topical fipronil/permethrin formulation and followed from May to October 2015 with monthly blood samples and serological tests based on Snap Leish® and Snap 4Dx®. None of the dogs after 5 months seroconverted for both *L. infantum* and *D. immitis*. Due to the long prepatent period of dirofilariosis, trial 2 was conducted using a new group of 28 *D. immitis* antigen negative dogs treated as in trial 1 and followed for 12 months (April 2016 to March 2017), a longer period than the prepatent one, in order to demonstrate any new infection. All dogs remained negative. This study, conducted in a highly endemic area for vector-borne pathogen transmission, confirmed that the use of permethrin is a suitable approach to reduce the risk of canine *L. infantum* and *D. immitis* infection.

Keywords: Leishmania infantum; Dirofilaria immitis; fipronil+permethrin; vectorborne diseases; Greece

Abstract No: 4529

5 Sept 2017, 1745 – 1800

Lessons from *Litomosoides sigmodontis*: Filarial larvae entry into lymphatics and cardiopulmonary migration.

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¹UMR7245/ Museum National d'Histoire Naturelle/ France, ²UMR BIPAR, Anses/ Laboratoire Sante Animale/ France, ³Institute for Molecular Engineering/ EPFL/ Switzerland, ⁴Institute of Laboratory Animal Science/ University of Zurich/ Switzerland, ⁵National Heart & Lung Institute/ Imperial College London/ United Kingdom

Abstract Content

Filarial infections are tropical diseases caused by nematodes of the Onchocercidae family such as *Dirofilaria immitis*. The infective larvae (L3) are transmitted into the skin of vertebrate hosts by blood-feeding vectors. Since its discovery the *Litomosoides sigmodontis* infection model has provided an elegant tool to help us define immunity to filarial parasites. Here we use *L. sigmodontis* to analyze the early phase of the parasite life cycle, i.e. the L3 migration processes. Firstly we investigate the mechanism of lymphatic vessel targeting. To do that, we developed a method for non-toxic covalent modification of surfaces in live worm that was used to attach functional fluorescent groups. We showed absolute requirement of functional lymphatic for invasion of filarial into the circulation that accrued at the level of collecting vessels within minutes after inoculation. Infective larvae appear to move stochastically in the tissue, with possible guidance towards lymphatics from the interstitial fluid currents. Worms entered the lymphatic collecting vessel by physically disrupting the lymphatic basement membrane. Within lymphatics, filaria were guided by the system of valves and rapidly migrated to subcapsular sinus of draining lymph node. Secondly we reveal a pulmonary phase associated with lung damages characterized by haemorrhages and granulomas suggesting L3 reach the lungs via pulmonary capillaries and damage the endothelium and parenchyma by crossing them to enter the pleural cavity. This study also provides evidence for a transient inflammation in the lungs characterized by a very early recruitment of neutrophils associated with high expression levels of S100A8 and S100A9 proteins.

Keywords: Filariasis, Lymphatics, Lungs

Determination of feeding characteristics of 7 dog ticks: Implication for control and prevention of pathogen transmission

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Veterinary Scientific Affairs/ Bayer Animal Health GmbH/ Germany

Abstract Content

Ticks are obligate hematophage ectoparasites of great epidemiological importance as they transmit a variety of pathogens to wildlife, domestic animals and humans. Most of the transmission routes of these pathogens are well known as well as the life cycles of the pathogens and tick vectors but surprisingly little is known about the feeding characteristics of the tick vectors. To fill this gap of knowledge, the feeding characteristics of seven ticks frequently parasitising dogs were determined: *Rhipicephalus sanguineus*, *Ixodes ricinus*, *Dermacentor reticulatus*, *Amblyomma americanum*, *Dermacentor variabilis*, *Ixodes scapularis* and *Haemaphysalis elliptica*. For each species attachment rate was determined, weight increase and onset of blood uptake up to 120 hours after application and this to determine the possible transmission time of pathogens. Information on feeding characteristics of tick species in combination with life cycle and pathogen characteristics would allow us to determine specific control measures. To prevent or minimize pathogen transmission to dogs, tick control is often practiced by applying topical or systemic acaricides. Their action is however completely different and depending on the mode of action might fail to prevent transmission of the pathogens. Results indicate a species-specific attachment and feeding profile, with the *Ixodes* species being the fastest feeding followed by the *Dermacentor* species and *R. sanguineus*. The species feeding slower were *A. americanum* and *H. elliptica*. The attachment success was however not linked to the feeding performance. The results and possible implication of applying systemic and topical acaricides will be discussed in relation to the risk of pathogen transmission.

Keywords: Tick; feeding; dog; transmission; control

Abstract No: 5124

5 Sept 2017, 1815 – 1830

Successful vaccination of dogs against *Babesia canis* using recombinant protein

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Abstract Content

Animals can be successfully vaccinated against clinical *Babesia* infection using serum from acutely infected hosts, which contains soluble parasite antigens (SPA). Based on SPA from *in vitro* cultures of *B. canis* and *B. rossi* parasites, a commercial vaccine was developed for canine babesiosis, and successfully introduced in the field. Pulse-chase labelling experiments showed that a parasite-derived 40kDa doublet was detectable in the supernatants of *in vitro* cultures of *B. canis*, as early as 15 minutes after the pulse. The 40kDa doublet was recognized by serum from immunized dogs, and subsequently purified using immunobiological techniques to quantities that allowed amino acid sequencing. Using this information, the gene encoding the 40kDa protein in the *B. canis* genome was discovered. The 40kDa doublet is encoded for by a single gene. In addition, a GPI-anchoring site was predicted, which suggests that the molecule is a membrane protein. Indeed, antiserum raised against the recombinant 40kDa antigen reacted mainly with the surface of *B. canis* merozoites. We hypothesize that the doublet is in fact the native protein with, and without a GPI-anchor. This is corroborated by the fact that the larger molecule separates in the hydrophobic phase of a Triton-X114 extract, whilst the smaller molecule separates in the aqueous phase. Dogs that were vaccinated with the *E. coli* expressed SPA1 recombinant protein were protected against virulent *B. canis* challenge infection. Protection was reflected by a decrease of >90% in parasitaemia, and absence of clinical signs.

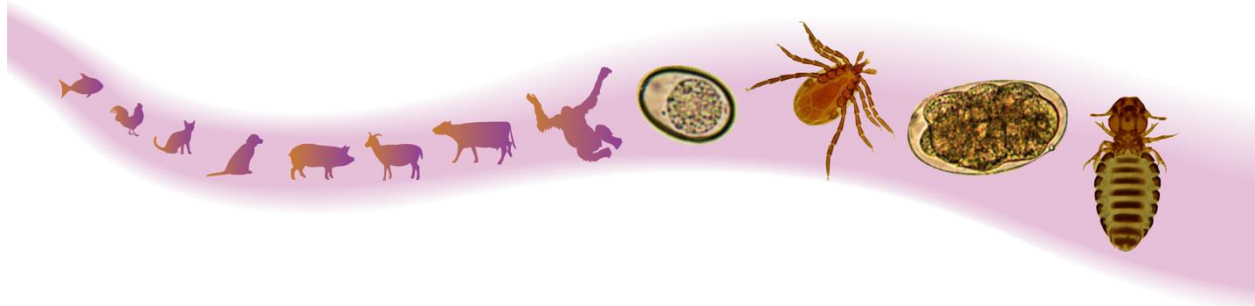
Keywords: Babesia; canis; recombinant; vaccine,

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The epidemiology of *Opisthorchis viverrini* in Central Vietnam is complicated by the presence of a sister species in ducks

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Abstract Content

Opisthorchis viverrini is a zoonotic fish borne liver fluke in SE Asia, including Central and South Vietnam, causing important morbidity and mortality in humans. A morphologically related trematode was found in the liver of ducks in Central Vietnam. We conducted cross sectional studies aiming to define prevalence and risk factors of *O. viverrini* and this duck genotype in humans and ducks, and to identify snail and fish intermediate hosts. In addition, we sequenced mitochondrial and ribosomal nuclear genes to define the phylogenetic position of the duck genotype. The prevalence of *O. viverrini* in a fish eating population was 11.4% and of the duck genotype in farmed duck was 34.3%. *Bithynia siamensis goniomphalos* and *B. funiculata* were the first intermediate hosts of *O. viverrini*, whereas 10 fish species, mainly Cyprinidae acted as the second intermediate hosts. The highest prevalence was found in *Carassius auratus*, a fish species often consumed raw in the region. *O. viverrini* and the duck genotype shared some of the intermediate snail and fish hosts. The genomic analysis, based on the sequence and phylogenetic analyses of the ribosomal transcription unit (rTU), ie. 18S and 28S rDNA, and mitochondrial 12S, *nad1*, *cytB* and *cox1* (mtDNA), classified the duck genotype as a novel species, in a sister position closely related to *O. viverrini*. Although the duck genotype has not yet been found in humans, the sympatric distribution of two related species and the sharing of the same intermediate hosts may have epidemiological consequences and result in hybridization/introgression.

Keywords: Opisthorchis viverrini; epidemiology; new species; duck; Vietnam

Neglected tropical zoonotic diseases: a perspective on the control

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Abstract Content

With the advancement in the control of Neglected Tropical Diseases (NTD), WHO and donor agencies have included several “Zoonotic Diseases” of Public Health Importance into Global Agenda for control and elimination. Thus NTD initiatives have focussed on “Neglected Zoonotic Diseases”, which are a subset of the neglected tropical diseases. These include rabies, echinococcosis, taeniasis/cysticercosis, foodborne trematodiasis etc. There is growing evidence of the importance of zoonotic causes of non-malarial febrile illness and diseases such as anthrax, brucellosis and leptospirosis. Addressing these requires collaborative, cross-sectoral efforts of human and animal health systems and a multidisciplinary approach that considers the complexities of the ecosystems where humans and animals coexist. Preventing and mitigating their occurrence in humans requires control and, where feasible, elimination of the diseases in their animal reservoirs. High priority is also given to “Emerging zoonoses”; which have potentially serious human health and economic impacts. These include Avian influenza and others. Some of the “lingering” zoonoses are re-emerging in some regions; for example brucellosis, dog rabies and parasitic diseases such as cysticercosis/taeniasis and echinococcosis/hydatidosis. Many factors such as environmental changes, human and animal demography, pathogen changes and changes in farming practice influence the occurrence, apart from socio-cultural factors. The formidable challenges ahead in Global approaches to bring the zoonotic diseases under control and sustain the same cannot be possible without Global International, National and local Collaborations and commitments. Several avenues have opened up in the recent years and we may hope to progress.

Keywords: NTD, Zoonosis, control, elimination, challenges, perspective

Emergence of *Echinococcus* spp. in North America

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Abstract Content

Echinococcus cestodes are zoonotic parasites that infect companion animals, people, livestock, and wildlife. Three species are present in Canada and the United States of America (USA), *E. canadensis*, *E. granulosus*, and *E. multilocularis*. In 2014, *E. canadensis* (genotype G8) was reported in moose (*Alces alces*) in the state of Maine (USA) for the first time. This cestode is widely distributed in Canada, Alaska, and several northern states in the contiguous USA, but was believed absent on the Atlantic coast due to the historical absence of wolves. As well, the related cestode *E. multilocularis* has recently been detected beyond its western, northern, and eastern distributional limits in central Canada, suggesting range expansion, possibly of introduced European haplotypes. Increasing recognition of alveolar echinococcosis cases in dogs in Canada may signal high levels of environmental contamination with this potentially zoonotic parasite. Therefore, we collected intestinal tracts of 43 coyotes (*Canis latrans*) in Maine, and 79 coyotes, 165 foxes (*Vulpes vulpes*) and 23 wolves (*Canis lupus*) in the neighboring Canadian province of Québec. Adult cestodes were collected from intestinal tracts using a modified sedimentation, filtration, and counting technique. Species and genotypes of *Echinococcus* will be identified based on characterization at several mitochondrial DNA loci. Preliminary results suggest that coyotes may be serving as definitive hosts for sylvatic cycles of *Echinococcus* spp. in Maine. This data will update our understanding of *Echinococcus* diversity and distribution, facilitating the development of evidence-based strategies to reduce risks posed by *Echinococcus* spp. for veterinary and public health.

Keywords: *Echinococcus*, *granulosus*, *multilocularis*, wildlife, surveillance

Prevalence and geographic distribution of *Echinococcus multilocularis* in wild canids across southern Ontario, Canada

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Abstract Content

Alveolar echinococcosis, disease due to the intermediate stage of *Echinococcus multilocularis*, is potentially fatal in humans and dogs when left untreated. Transmission occurs when eggs of the tapeworm, shed by definitive hosts, are ingested. Prior to 2012, Ontario was considered free of this parasite. Since then, alveolar echinococcosis has been reported in five dogs and two lemurs in southern Ontario. Of these cases, six had no travel history, raising concern that wild canids may be shedding eggs into the Ontario environment. In order to determine the prevalence and distribution of *E. multilocularis* in wild canids across southern Ontario, a 2-year study was initiated in 2015; coyote and fox carcasses were collected from hunters and trappers across the region. From November 2015 to August 2016, 204 wild canids (182 coyotes and 22 foxes) were collected. Rectal fecal samples were collected during post-mortem and analyzed via a semi-automated magnetic capture probe DNA extraction and real-time hydrolysis PCR method for the presence of *E. multilocularis* DNA. Overall, 16.2% (95% confidence interval: 11.4%- 22.0%) of wild canids from the western, central, and eastern regions of southern Ontario tested positive. Using a spatial scan test, a spatial cluster of high prevalence of infection was identified (relative risk=6.86; $p < 0.001$) that covered six contiguous public health units in the western and central regions of southern Ontario, a region with a high human population density. The location of this spatial cluster relative to a densely populated human region suggests that zoonotic transmission should be a public health concern.

Keywords: Echinococcus multilocularis; coyote; fox; Ontario

Molecular detection of zoonotic Rickettsiae and Anaplasma spp. in domestic dogs and their ectoparasites in Bushbuckridge, South Africa

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Abstract Content

Members of the order Rickettsiales are small, obligate intracellular bacteria that are vector-borne and can cause mild to fatal diseases in humans worldwide. There is little information on the zoonotic rickettsial pathogens that may be harbored by dogs from rural localities in South Africa. To characterize rickettsial pathogens infecting dogs, we screened 141 blood samples, 103 ticks, and 43 fleas collected from domestic dogs in Bushbuckridge Municipality, Mpumalanga Province of South Africa, between October 2011 and May 2012 using the reverse line blot and *Rickettsia* genus and species-specific quantitative real-time PCR assays. Results from RLB showed that 49% of blood samples and 30% of tick pools were positive for the genus-specific probes for *Ehrlichia/Anaplasma*; 16% of the blood samples were positive for *Ehrlichia canis*. Hemoparasite DNA could not be detected in 36% of blood samples and 30% of tick pools screened. Seven (70%) tick pools and both flea pools were positive for *Rickettsia* spp; three (30%) tick pools were positive for *Rickettsia africae*; and both flea pools (100%) were positive for *Rickettsia felis*. Sequencing confirmed infection with *R. africae* and *Candidatus Rickettsia asembonensis*; an *R. felis*-like organism from one of the *R. felis*-positive flea pools. *Anaplasma* sp. South Africa dog strain (closely related to *Anaplasma phagocytophilum*), *A. phagocytophilum*, and an *Orientia tsutsugamushi*-like sequence were identified from blood samples. The detection of emerging zoonotic agents from domestic dogs and their ectoparasites in a rural community in South Africa highlights the potential risk of human infection that may occur with these pathogens.

Keywords: *Anaplasma*; *Ctenocephalides*; *Haemaphysalis*; *Rickettsia*, South Africa

High infection frequency with *Wolbachia pipientis* and potentially transmissible *Rickettsia bellii*-like bacteria in *Tunga penetrans* from Uganda and Kenya

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Abstract Content

Tungiasis is a common but highly neglected tropical zoonosis caused by female sand fleas penetrating their host's skin. In Africa, only *Tunga penetrans* is endemic but information regarding population genetics is missing. *Wolbachia* were detected in some but not all *T. penetrans* populations and these fleas were once found positive for *Rickettsia felis*. Here, *T. penetrans* genotypes from different hosts and regions in East Africa and associated Rickettsiales were analysed. Fleas collected from Uganda (larvae, neosomic females from humans, pigs, dogs and goats) and Kenya (from humans) were analysed using PCRs targeting two flea mitochondrial (*cox2*, *cytb*) and three bacterial (*gltA*, *groEL*, 16S rRNA) loci. The *cytb* and *cox2* sequences from 80 fleas were 100% identical and the latter were also identical to a sequence from Madagascar. Comparison with sequences from South America and Africa revealed only minimal genetic variation within *T. penetrans*. Using genus-specific *gltA* PCRs, *Wolbachia* sp. and *Rickettsia* sp. were detected in 51/74 (68.9%, 95% confidence interval 57.7-78.3%) and 61/80 (76.3%, 95% confidence interval 65.9-84.2%) neosomic fleas, respectively. Phylogenetic analyses identified a *Rickettsia bellii*-like and two distinct *Wolbachia* genotypes not closely related to *Wolbachia* described from other fleas. Both *Wolbachia* potentially represent new supergroups. Genotype-specific PCRs identified both genotypes in all 32 *Wolbachia*-positive fleas tested. Pathogenicity of *R. bellii* is unknown but in insects, they are generally considered endosymbionts. However, in blood-feeding fleas and ticks transmission to mammalian hosts cannot be excluded. Effects of *Wolbachia* and *Rickettsia* on the flea physiology should be investigated in the future.

Keywords: Tunga penetrans; Wolbachia; Rickettsia; genotyping; zoonoses

Abstract No: 4921

5 Sept 2017, 1600 – 1615

Diagnosis of Cystic Echinococcosis (CE) in Animals and Man

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Abstract Content

Echinococcosis is a zoonotic infection caused by adult and Cystic echinococcosis (CE) by larval (metacestode) stages of cestodes belonging to the genus *Echinococcus*. The parasites are perpetuated in life-cycles with carnivores as definitive hosts, which harbour the adult worms in the intestine, and intermediate host animals, in which the infective larval stage develops after oral infection with eggs. The larval stages may incidentally also develop in humans causing various forms of echinococcosis and this may also occur in various animal species. The diagnosis of CE in intermediate hosts of *E. granulosus* is mainly based on necropsy findings. Clinical symptoms with mild manifestations, is normally overlooked. Currently, there is no suitably sensitive and specific serological test available for ovine hydatidosis or for any other livestock species. Identification of exposure to *E. granulosus* at the flock or herd level by detecting serum antibody is possible using hydatid cyst fluid antigens in ELISA and may be useful in hydatid screening and surveillance programmes. Recombinant antigens may improve specificity, but sensitivity problems are likely to remain. Studies on a range of parasites, including *Echinococcus*, have shown that the ITS region (internal transcribed spacer) of rDNA can provide a useful diagnostic marker for taxonomic purposes. The rDNA ITS1 region has been shown to be a very useful genetic marker for distinguishing strains and species of *Echinococcus* and small quantities of *Echinococcus* material can be characterised using a PCR-RFLP 'fingerprinting' technique.

Keywords: Cystic Echinococcosis; Diagnosis; Animals; Man

Abstract No: 4297

5 Sept 2017, 1615 – 1630

The prevalence of intestinal parasitic protozoan among patients in Ad-Dawadimi General Hospital, Saudi Arabia

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²Department of Zoology, College of Science/ King Saud University/ Saudi Arabia ³Department of Microbiology and Medical Parasitology, Faculty of Medicine/ King Faisal University/ Saudi Arabia

Abstract Content

Intestinal parasitic protozoan diseases in Saudi Arabia are a significant public health problem with prevalence ranging from 4.1% to 42%. This study was carried out to determine the risk factors associated with the prevalence of intestinal parasitic protozoan infections among patients in Ad-Dawadimi General Hospital, Saudi Arabia. This study was conducted from the 1st of January to the end of December 2015. Faecal specimens from 4,000 patients who were admitted to Ad-Dawadimi General Hospital during the study period were analyzed by a wet mount preparation after formal-ether concentration technique for trophozoites and cysts of *Giardia lamblia* and *Entamoeba histolytica*. Ziehl-Neelsen staining was used to detect *Cryptosporidium* oocysts. Overall, intestinal parasitic protozoan were found in 470 patients (11.75%). The infection rate of *Giardia lamblia*, *Cryptosporidium* spp and *Entamoeba histolytica/dispar* was 5 %, 4.275% and 2.475 %, respectively. Infections among males was (8.357%), while among females was (3.357%). Age, gender and season were highly significant factors on the prevalence of parasites infection at $P < 0.005$. However, the present study indicated that intestinal parasitic protozoan infections are still a public health problem in Saudi Arabia.

Keywords: Intestinal protozoan, Giardia lamblia, Cryptosporidium spp, Entamoeba histolytica/dispar

Occurrence of neurotropic parasite stages in wild rodents

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¹Institute for Parasitology/ University of Veterinary Medicine Hannover, Foundation/ Germany,

¹Department of Parasitology/ Ankara University, Faculty of Veterinary Medicine/ Turkey, ²Institute of Animal Hygiene and Veterinary Public Health/ University of Leipzig/ Germany ³Department for Companion Animals/ University of Veterinary Medicine Vienna, Small Animal Clinic/ Austria

Abstract Content

Wild rodents may serve as intermediate or paratenic hosts for various helminths and protozoans including zoonotic agents like *Toxocara* spp. and *Toxoplasma gondii*, contributing to distribution of those parasites as a source of infection for respective definitive hosts. Neural affinity of several zoonotic parasites within infected hosts has been described. Therefore, the occurrence of neurotropic parasite stages in brains of 724 wild rodents was evaluated. Brains were investigated for coccidian cysts and roundworm larvae by microscopic analysis of native tissue and artificial digestion, respectively. Detected cysts were differentiated by amplification of the 18S rRNA gene and subsequent sequencing. No larvae were detected in examined brains, possibly indicating low susceptibility or low affinity to the CNS in investigated rodent species. Contrary, coccidian cysts were detected in 9.9% (72/724) of brains and morphologically classified as small (~20-30 µm; 15.3%, 11/72) and large cysts (~150-180 µm; 84.7%, 61/72). Sequencing of 18S rRNA amplicates of small cysts was solely successful for two samples, showing 100% identity with *T. gondii*. Sequences of large cysts revealed partial identities with the closely related species *Frenkelia glareoli* and *Sarcocystis* spp. (68.1%; 49/72). Based on species-specific predilection sites, predominant infections of rodents with *F. glareoli* are presumed. Detailed analyses of obtained sequences are currently in progress and will be presented. In general, investigated rodent species appear to be of minor importance in the epidemiology of zoonotic, neurotropic parasites; however, further investigations are required as merely brain tissues were included in analyses.

Keywords: zoonoses; wild rodents; neurotropic parasites; roundworms; coccidian

Preliminary assessment of an integrated *Taenia solium* elimination program in eastern Zambia

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Abstract Content

The zoonotic tapeworm *Taenia solium* causes significant health and economic burdens worldwide. A large-scale interventional study is being conducted in the highly endemic eastern region of Zambia, to assess the effectiveness of an integrated *T. solium* elimination program targeting both human and porcine hosts. Human taeniasis and porcine cysticercosis prevalence in the Nyembe neighbourhood community were determined at baseline. All eligible humans and pigs were included in the study, and received four-monthly interventions including human mass drug administration (praziquantel) and health education, combined with porcine mass drug administration (oxfendazole) and vaccination (TSOL 18). Human stool and pig blood samples were collected to assess the impact of the interventions on taeniasis and porcine cysticercosis prevalence after one year, including three interventions. Baseline prevalence by AgELISA of human taeniasis in Nyembe was 16%, and of porcine cysticercosis was 41%. Carcass dissection at baseline revealed 43% (16/37) prevalence of porcine cysticercosis. Results of the mid-intervention survey will be presented and discussed.

Keywords: *Taenia solium* cysticercosis; Neglected tropical diseases; One Health; Parasitic zoonoses; Zambia

Abstract No: 3068

5 Sept 2017, 1700 – 1715

Arthropods, microbes, and soil chemistry dynamics associated with delayed carrion decomposition: Implications to public health

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Abstract Content

The objectives of this study were to determine the sensitivity of microbial metabolic community profiles, terrestrial and soil arthropod community structures, and soil chemistry dynamics associated with carrion experiencing delayed decomposition in the absence of Diptera for 7 days and 14 days. Bacterial metabolism profiles indicate a significant difference between carrion with immediate insect access (Control) and carrion with delayed insect colonization for 7 days and 14 days (Treatments). In contrast, soil samples demonstrated a stochastic response in the soil microbial ecosystem. Soil chemistry profiles were significantly different between Control and Treatment carcasses. Furthermore, significant differences were found between days of decomposition (temporal sensitive) and soil regions (spatial sensitive). Soil nutrients such as ammonium, phosphate, non-purgeable organic carbon and total nitrogen were sensitivity to treatment effects, but not demonstrated in nitrate. Soil arthropod (including acari) community structures were sensitive to treatment effects only at the Family level. The total abundance of acari was not significantly different across treatments in all-sampling days. For aboveground arthropod community structure and function trapped by sticky traps, significant different in treatments was detected at the Order level, and Genus level for both pitfall traps and sweep nets. The present study demonstrated that insect succession on carrion by family level is predictable. However, insect succession by genus level demonstrated stochasticity when dealing with disturbances. The effects of delayed carrion decomposition to public health which include proliferation of microbe community and environmental dissemination will be discussed in this presentation.

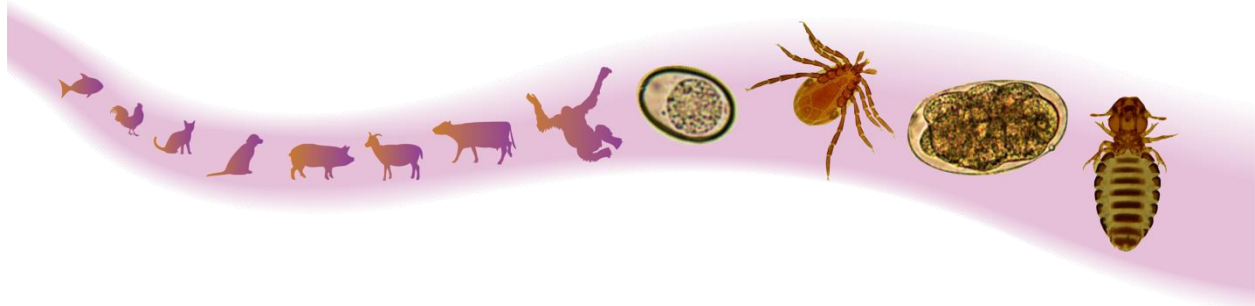
Keywords: carrion ecology; arthropods; microbes; mites; soil chemistry; public health

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Abstract No: 4922

5 Sept 2017, 1400 – 1415

Identification and characterisation of secreted proteins from *Eimeria* parasites

Kiew-Lian Wan^{*1}; Intan Azlinda Ramlee¹; Mohd Firdaus-Raih¹

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Abstract Content

Eimeria parasites are known to cause coccidiosis that brings about significant losses to the poultry industry. The development of more effective control methods will benefit from understanding the function of proteins that are secreted by *Eimeria* parasites as these molecules are part of the parasite-host interface and interact with host cell components. In this study, secreted proteins from *Eimeria* species infecting chickens were identified and characterised. For this work, secreted proteins were defined as protein sequences that possess a signal peptide, do not localise in organelles and are without transmembrane domains or a glycosylphosphatidylinositol (GPI) anchor. From a total of 56,547 protein sequences from seven *Eimeria* species, 5,194 proteins were found to have signal peptides. Further screening eliminated 83 apicoplast and mitochondrial proteins, 1,896 transmembrane proteins, 1,010 GPI anchored proteins and 190 surface antigens, mitochondrial and apicoplast proteins that were missed out during the initial screening. In total, 2,436 secreted proteins were successfully predicted. Sequence similarity search by BLAST showed that a portion of the *Eimeria* secreted proteins matched with annotated proteins in public databases including microneme, rhoptry and dense granule proteins. The majority of the *Eimeria* secreted proteins correspond to signalling pathways such as cGMP-PKG, calcium, PI3K-Akt and MAPK signalling pathways from the KEGG database. Gene Ontology prediction by Blast2GO showed that the secreted proteins may be associated to metabolic processes and protein binding as well as localised in cell compartments. Further characterisation of these *Eimeria* secreted proteins may unveil attractive targets for coccidiosis intervention.

Keywords: Protozoan parasites, secretome, coccidiosis, signalling pathway

Abstract No: 4343

5 Sept 2017, 1415 – 1430

Cloning, expression and characterization of Pfr 2 gene of *Trypanosoma evansi* of Indian cattle isolate

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Abstract Content

Paraflagellar rod (PFR) is the major structural component of the flagellum and is identified as a complex lattice of filaments which runs parallel to the axoneme throughout most of the length of the flagellum of Trypanosomatids. *Trypanosoma evansi* isolate collected from naturally infected local cow was multiplied in Wistar rats. Complementary DNA (cDNA) was synthesized from the RNA of host cell free *T. evansi* parasites by reverse transcription using oligo dT primers. The gel purified PCR product (PFR 2 gene of *T. evansi*) was cloned into the pTZ57R/T vector system. The presence of the inserts was confirmed initially by Colony-PCR and then by Plasmid-PCR. Nucleotide sequence of the PFR 2 gene of *T. evansi* (Accession No. KT277497, S.V.V.U. isolate) of the present study revealed 100 % homology with *T. evansi* China isolate and 99% homology with *T. evansi* Izatnagar and Bikaner isolates. The recombinant protein was sub-cloned into pET 32a and expressed in the BL21 (DE3) pLysS expression system. PFR 2 gene of *T. evansi* S.V.V.U. isolate was further characterized by determination of its protein profile with SDS-PAGE and western blotting against hyper immune serum. Indirect ELISA was optimized for detection of specific antibody titre against recombinant protein of PFR 2 gene of *T. evansi*. PFR 2 gene is highly conserved in the kinetoplastid species. Therefore PFR 2 gene could be a novel diagnostic as well as vaccine candidate against surra caused by *Trypanosoma evansi* in domestic and wild animals.

Keywords: Indian isolate - Trypanosoma evansi - PFR-2 gene – Cloning - Expression

Abstract No: 4648

5 Sept 2017, 1430 - 1445

Biogenesis of the flagellar pocket cytoskeleton in the sleeping sickness parasite *Trypanosoma brucei*.

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Abstract Content

The flagellar pocket (FP) of *Trypanosoma brucei* is an essential, single-copy structure that is formed by the invagination of the pellicular membrane. It is the unique site of endo- and exocytosis and is required for parasite pathogenicity. The FP consists of distinct structural sub-domains with the least explored being the flagellar pocket collar (FPC). To date the only known component of the FPC is the protein BILBO1, a cytoskeleton protein that has a N- terminus that contains an ubiquitin-like fold, followed by two EF-hand domains, plus a large C- terminal coiled-coil domain. BILBO1 binds calcium, and we demonstrate that mutating either or both calcium-binding domains prevents calcium binding. The expression of deletion or mutated forms of BILBO1 in trypanosomes and mammalian cells demonstrate that the coiled-coil domain is necessary and sufficient for the formation of BILBO1 polymers. This is supported by Yeast Two-Hybrid analysis. Expression of full-length BILBO1 in mammalian cells induces the formation of linear polymers with comma and globular shaped termini, whereas mutation of the canonical calcium-binding domain resulted in the formation of helical polymers and mutation in EF-hand domains prevented the formation of linear polymers. This data indicates that BILBO1 has intrinsic polymer forming properties and that binding calcium can modulate the form of these polymers. We discuss whether these properties can influence the formation of the FPC and the potential role of BILBO1 partners.

Keywords: Trypanosome; Cytoskeleton; Biogenesis; Flagella Pocket Collar

Abstract No: 4105

5 Sept 2017, 1445 - 1500

Mining the *Babesia canis* genome and analysis of gene expression and protein secretion during virulent infection identifies potential pathogenicity factors.

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Abstract Content

Infections of dogs with virulent strains of *Babesia canis* are characterized by rapid onset, severe clinical signs and high mortality. As in other apicomplexan parasites, most *Babesia* virulence factors responsible for survival and pathogenicity are secreted to the host cell surface and beyond, where they remodel and biochemically modify the infected cell by direct and specific interaction with host proteins. We investigated the *B. canis* secretome during acute infection in dogs and report on *in silico* predictions and experimental analysis of the parasite's exported factors. As a backdrop for this analysis, we generated the first fully annotated *B. canis* genome sequence of a virulent Hungarian field isolate (strain BcH-CHIPZ). Read assembly produced a ~7 Mb reference genome consisting of 43 high quality contigs. Annotation revealed 3467 gene models showing high synteny to the 4 chromosomes of the related *B. bovis* T2Bo genome. Underpinned by genome-wide RNA-seq and mass spectrometry analyses of the parasite exported proteins, we identified conserved factors in apicomplexan hemoparasites involved in immune-evasion (e.g. VESA-protein family), proteins secreted across the iRBC membrane into the host bloodstream (e.g. SA- and Bc28 protein families), potential moonlighting proteins (e.g. profilin and histones), and uncharacterized antigens present during acute crisis in dogs. The combined data provide information on a first predicted and validated set of potential virulence factors exported during fatal infection, which can be exploited for urgently needed innovative intervention strategies aimed at facilitating diagnosis and management of canine babesiosis.

Keywords: Babesia canis, genome, pathogenicity factors, parasite-host interaction, secretome

Exclusive Phospholipid Expression and Autonomous Membrane Biogenesis in *Eimeria* Indicate a Host-independent Lifestyle of Apicomplexan Sporozoites

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Abstract Content

Successful inter-host transmission of most apicomplexan parasites begins with the infective sporozoite stage developing within the oocyst. Unlike all other infective stages that are strictly intracellular and depend on host resources, sporozoite is the only stage occurring outside host cells during the lifecycle of apicomplexan parasites, but little is known about its metabolism. Besides, it offers an excellent model to determine the “true” metabolic potential of otherwise host-dependent organisms. This study deployed *Eimeria falciformis*, a parasite infecting the mouse as its natural host, to investigate the process of membrane biogenesis in sporozoites. Lipidomic analyses demonstrated the occurrence of prototypical phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and phosphatidylglycerol) along with abundant expression of at least two exclusive lipids phosphatidylthreonine and inositol phosphorylceramide in sporozoites. To produce them all, the parasite harbors nearly the entire biogenesis network, which is an evolutionary mosaic of eukaryotic- and prokaryotic-type enzymes. Many have no phylogenetic counterpart or functional equivalent in the mammalian host. Using *Toxoplasma gondii* as a gene-tractable surrogate to examine the *Eimeria* enzymes, we show a highly compartmentalized network of lipid synthesis spread primarily in the apicoplast, endoplasmic reticulum, mitochondrion and Golgi complex. Likewise, trans-species complementation of a *Toxoplasma* mutant with a PtdThr synthase from *E. falciformis* suggests a convergent function of PtdThr in promoting the lytic cycle of coccidian parasites. Our work establishes a model of self-governing membrane biogenesis involving significant inter-organelle cooperation and lipid trafficking in sporozoites. Phylogenetic divergence of certain pathways offers attractive drug targets to block the sporulation and subsequent transmission.

Keywords: Apicomplexan Parasites, Lipidomics, Membrane Biology, Inter-host transmission

Determination of sequence descriptions and predicted functions of selected *Theileria parva* hypothetical proteins

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Abstract Content

In an effort to understand mechanisms responsible for the variation observed in East Coast fever and Corridor disease, cattle theileriosis syndromes caused by *Theileria parva*, a transcriptome study was undertaken which detected differentially expressed genes (1089), from which 867 were hypothetical proteins (HPs). For the purpose of this study it became crucial to determine the biological roles of HPs. Consequently, the aim of the current study was to predict function of HPs using a combination of *in silico* methods. Sequence description hold important information pertaining to the protein function(s). Thus, 397 HPs, which were assigned sequence descriptions following initial analysis based on sequence similarity, were selected for investigation. Sequence descriptions for 252 HPs were confirmed by two other sequence similarity search database, sequence homology and domain identification. Additionally, predicted functions of 202 HPs were supported by gene ontologies. Metabolic pathway reconstruction provided possible protein functions for four *T. parva* HPs. Protein interaction networks revealed interacting partners for three HPs; protein interrelation allows prediction of function for HPs from assuming that they perform similar roles as their partners. Essential genes were also identified (104 HPs) and 23% non-homologous to host proteins implicating parasite-specific roles; these can make good candidates as drug targets. Overall, a range of functions including enzymes, structural components, binding proteins and general information processing components, were assigned to various HPs and it is anticipated that the predicted functions of HPs will provide a better understanding of the biology of *T. parva* and how the parasite affects its host.

Keywords: Hypothetical proteins, Functional annotation, Theileria parva, east coast fever, corridor disease

Abstract No: 4292

5 Sept 2017, 1600 – 1615

Molecular mechanism of progesterone and estradiol on *Toxoplasma gondii* infection and pathogenesis

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Abstract Content

Toxoplasma gondii is more susceptible to pregnant female, and causes reproductive disorders. Dramatic changes of estrogen levels during pregnancy may be an important factor for high infectivity and pathogenicity of *Toxoplasma* in this period. Our data showed that estradiol promoted *Toxoplasma* infection and significantly contributed to the pathogenicity in mice. Estrogen antagonists can inhibit these effects, suggesting this phenomenon may relate to the interplay of *Toxoplasma* and estradiol. Further studies showed that estradiol can enter the cytoplasm of *Toxoplasma* and induce "Non-genomic" effect rapidly, causing Ca²⁺ fluctuations, and activating IP3 and cAMP to promote *Toxoplasma* secretion, movement and egress. Meanwhile, we found that estradiol may bind to a specific sequence on *Toxoplasma* promoters to induce transcription changes and we called it "Genome" effect. In summary, estradiol may affect *Toxoplasma* invasion, proliferation and mice pathogenicity through "Genome" and "Non-genomic" pathways. Progesterone failed to promote *Toxoplasma* infection as estradiol. On the contrary, physiological concentration of progesterone becomes a kind of "Toxoplasma inhibitor". Further studies showed that progesterone can still induce the "Non-genomic" effect of *Toxoplasma*, but the signal pathway is slightly different with estradiol, and ultimately induce *Toxoplasma* abnormal division, disordered endometrial complex and decreased moving, secretion and egress capacities. We speculated that these phenomena may due to a progesterone receptor membrane component (PGRMC) induced mitochondrial disorders and Tg-ATG8 autophagy protein aggregation. Estradiol and progesterone is associated with *Toxoplasma* infection and pathogenesis, and further research is still underway.

This study was supported by National Natural Science Foundation of China (No.31372424, No.31672544)

Keywords: Toxoplasma gondii; Estradiol; Progesterone; Pathogenesis; molecular mechanism

The immunological responses of pigs following vaccination and challenge with *Toxoplasma gondii* parasites

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Abstract Content

Toxoplasma gondii has a worldwide distribution and is capable of infected almost all warm blooded animals, including humans and pigs. The ingestion of viable tissue-cysts in raw-undercooked meats is considered a major route of infection, with infected pork considered one of the main meat sources of *T. gondii* infections in humans. In this study we examined the immune responses (CD4, CD8, CXCR3, IFN- γ , IL-12 and MyD88 gene expression (SYBR-qPCR)) in the retropharyngeal lymph node (LN), mesenteric LN and spleen of pigs following vaccination and challenge with *Toxoplasma gondii* parasites. Group (G)1 (n=5) non-vaccinated and challenged, G2 (n=5) vaccinated and challenged, G3 (n=5) vaccinated non-challenged, G4 (n=3) non-vaccinated and non-challenged. G2 and G3 were vaccinated 1.2×10^5 S48 tachyzoites on day 0. Animals in G1 and G2 were orally challenged with 1×10^3 *T. gondii* oocysts (M4 isolate) on day 28, all animals were then culled on day 70. The results from the RLN show increases in gene expression in CD8, CXCR3, IFN- γ and MyD88 from G1 and G2, with lower rises in IFN- γ and MyD88 being seen in G3. Similar responses are seen for CD8, CXCR3, IFN- γ and MyD88 in the MLN of G1 and G2, while CXCR3, IFN- γ and MyD88 increases were seen in G3, though these responses were generally not as strong as those seen in the RLN. In the spleen, increases were only seen in IFN- γ and MyD88 for G1, G2 and G3. These results show that cellular and innate responses are involved in immunity against *Toxoplasma* in pigs.

Keywords: Toxoplasma gondii, pigs, vaccination, immunology

Abstract No: 4249

5 Sept 2017, 1630 – 1645

Immunization with *Toxoplasma gondii* GRA17 deletion mutant confers protective immunity against toxoplasmosis

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Abstract Content

No effective vaccine is currently available to prevent *Toxoplasma gondii* infection, since the mechanisms of pathogenesis are not well understood. Here, we created a Δ GRA17 deletion mutant by disrupting the virulence factor *GRA17* using CRISPR-Cas9 method. Then, we tested whether Δ GRA17 tachyzoites can be used as a live-attenuated vaccine against acute, chronic and congenital *T. gondii* infection. Immune response evoked by Δ GRA17 immunization suggests a sequential Th1 and Th2 T cell response, indicated by high levels of Th1 and a mixed Th1/Th2 cytokines at 28 and 70 days after immunization, respectively. Δ GRA17-mediated immunity significantly protected mice against lethal infection with wildtype (wt) RH strain and heterologous challenge with PYS and TgC7 strains of the Chinese ToxoDB#9 genotype. In latently infected mice, parasite cyst burden in the brain was significantly reduced ($P < 0.05$) in immunized mice compared to non-immunized mice. In respect to congenital infection, the litter size, survival rate and mean body weight (BW) of pups from immunized dams were not significantly different compared to pups from naïve control dams ($P = 0.24$). In contrast, significant reduction was detected in the litter size ($P < 0.001$), survival rate and mean BW ($P < 0.01$) of pups born to non-immunized and infected mice. After birth, immunized dams did not develop clinical disease, while non-immunized dams exhibited signs 5 days after challenge with wt RH strain. Also, immunized dams infected with type II Pru strain showed significantly ($P < 0.001$) less brain cyst burden than the non-immunized and infected dams.

Keywords: *Toxoplasma gondii*; congenital infection; Δ GRA17; immunization; live-attenuated vaccine; toxoplasmosis

Abstract No: 4404

5 Sept 2017, 1645 – 1700

***Toxoplasma gondii* acyl-CoA transporters play key roles in lipid metabolism**

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Abstract Content

Acyl coenzyme A-binding protein (ACBPs) can bind, store and transport acyl-CoA esters with high specificity and affinity to play multiple roles in biological functions. We identify a novel ACBP in *Toxoplasma*, TgACBP2, which contains a N-terminal signal peptide and two ankyrin repeats in addition to a highly conserved acyl CoA binding domain. Recombinant TgACBP2-GST was able to bind NBD-C16:0-CoA in a vitro fluorometric assay. Moreover, heterologous expression of TgACBP2 in yeast ACBP mutant could rescue the multi-lobed vacuole phenotype, demonstrating that TgACBP2 is active in vivo. By endogenous gene tagging and biochemical techniques, we show that TgACBP2 localizes to the outer membrane of mitochondrial. Furthermore, TgACBP2 was phosphorylated in extracellular tachyzoites and dephosphorylated in intracellular tachyzoites in an alkaline phosphatase assay. We found that extracellular TgACBP2 could be dephosphorylated under high potassium stress. We also investigated the roles of TgACBP2 in regulating mitochondrial by generating a knockout mutant. Under high potassium stress, loss of TgACBP2 lead to increased mitochondrial reactive oxygen species (ROS), reduced mitochondrial inner membrane potential and subsequently weakened proliferation, indicating that TgACBP2 is required for maintaining mitochondrial homeostasis under high potassium stress and thus for the intracellular replication of tachyzoites. This work characterizes key roles of TgACBP2 in stress response and its potassium mediated phosphorylation-dephosphorylation modification.

This study was supported by National Natural Science Foundation of China (No.31372424), Beijing Municipal Natural Science Foundation (Grant No. 6172023)

Keywords: Toxoplasma gondii; acyl-CoA binding protein; high potassium stress; mitochondrial homeostasis

Proteomic Differences Between Developmental Stages of *Toxoplasma gondii* Revealed by iTRAQ-based Quantitative Proteomics

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Abstract Content

Toxoplasma gondii has a complex life cycle. Proteomic differences underlying the different stages of *T. gondii* life-cycle may improve understanding of the molecular adaptation mechanism of *T. gondii* across life-cycle stages, and have implications for the development of new treatment and prevention approaches against *T. gondii* infection. We applied iTRAQ technology integrated with LC-MS/MS analysis to identify differentially expressed proteins (DEPs) specific to tachyzoite (T), bradyzoites-containing cyst (C) and sporulated oocyst (O) stages of the cyst-forming *T. gondii* Prugniud strain. A total of 6285 proteins were identified. We obtained 875, 656 and 538 DEPs in group O vs T, T vs C and C vs O, respectively. The up- and down regulated proteins were analyzed by GO enrichment, KEGG pathway and STRING analyses. The virulence factors expressed in sporulated oocysts and the number of up-regulated virulence factors in cyst stage was about twice as in tachyzoites. Of the 79 ribosomal proteins in *T. gondii*, the number of up-regulated ribosomal proteins was 33 and 46 in sporulated oocysts and cysts, respectively, compared with tachyzoite. Both oocyst and cystic stages are more adapted to withstand adverse environmental conditions and selection pressure caused by immune responses. A number of virulence-related factors and ribosomal proteins exhibited distinct expression patterns across the life-cycle stages. This firstly provides the proteomic variations across the life-cycle stages of *T. gondii*, and has important implications for the understanding of the developmental biology of *T. gondii*, which will facilitate the discovery of novel therapeutic targets and vaccines to control toxoplasmosis.

Keywords: *Toxoplasma gondii*; Life-cycle; Mass spectrometry; Proteomics; iTRAQ; Differentially expressed protein (DEP)

Abstract No: 4387

5 Sept 2017, 1715 – 1730

Conventional and next-generation sequencing provide insights into the bacterial and protozoal microbiome of Australian companion animal ticks

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Abstract Content

Little is known about the microbiome of ticks that parasitise companion animals and their pathogenic potential in Australia. Companion animal ticks were collected in Australia from 2012-15 and this study aimed to identify species of bacteria and protozoa within their microbiome. Ticks collected from horses ($n = 70$), cats ($n = 150$), and dogs ($n = 500$) were morphologically identified and genomic DNA was isolated and amplified with universal bacterial 16S rRNA primers for next-generation sequencing (NGS) on the Illumina MiSeq platform. Protozoal DNA was amplified with 18S rRNA primers using conventional PCR and positives were sequenced using Sanger sequencing. The most abundant bacteria identified were endosymbiotic Proteobacteria, including *Coxiella* spp. in *Haemaphysalis longicornis* and *Rhipicephalus sanguineus*, *Francisella* in *Amblyomma triguttatum*, and "*Candidatus* Midichloria mitochondrii" in *Ixodes holocyclus*. Other bacterial species of interest included *Rickettsia* spp. in *I. tasmani* and *R. australis*, the Q fever pathogen *Coxiella burnetii* in *I. holocyclus*, *Anaplasma bovis* in *H. longicornis*, and the recently described "*Candidatus* Neoehrlichia australis" and "*Candidatus* Neoehrlichia arcana" in *I. holocyclus*. Furthermore, NGS identified a novel *Coxiella* sp. and *Francisella* sp., and two new species of protozoa (*Babesia* sp. and *Hepatozoon* sp. in *Haemaphysalis* spp.) were characterised using Sanger sequencing. This study demonstrates the ability of 16S NGS to broadly screen the tick microbiome for known and novel bacterial species that may be of medical and veterinary importance. Sanger sequencing of the 18S gene was shown to be a useful tool for protozoa discovery and characterisation.

Keywords: Next-generation sequencing; Ticks; Microbiome; Bacteria; Protozoa

Abstract No: 4422

5 Sept 2017, 1730 – 1745

Molecular characterisation of '*Candidatus Borrelia tachyglossi*' in echidna ticks in Australia

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Abstract Content

In recent years, tens of thousands of Australians have reported suffering from an ill-defined illness, thought to be a manifestation of locally-acquired Lyme Disease (LD). However, the presence of the causative agent, *Borrelia burgdorferi* sensu lato (Bbsl), in non-travelled Australians, native wildlife or their ticks has not been demonstrated. Recently a novel *Borrelia* sp. was identified in *Bothriocroton concolor* and *Ixodes holocyclus* ticks, which had parasitised echidnas (*Tachyglossus aculeatus*). Preliminary analyses of 16S rRNA (16S) and *flaB* genes, identified three closely related genotypes of this bacterium (*Borrelia* sp. Aus A-C), that were unique and distinct from previously described borreliae. Further phylogenetic analyses of multiple loci: *flaB* (763 bp), *groEL* (1,537 bp), *gyrB* (1,702 bp), and *glpQ* (874 bp) gene sequences and concatenated sequences (3,585 bp) of three gene loci (16S, *flaB*, and *gyrB*), confirmed that this novel *Borrelia* species was more closely related to, yet distinct from, the Reptile-associated (REP) and Relapsing Fever (RF) groups. At the *flaB* locus, genotypes A, B, and C shared the highest percentage similarities (87.9%, 88%, and 87.9%, respectively) with *B. turcica* (REP), whereas at the *groEL* and *gyrB* loci, these genotypes were most similar (88.2-89.4%) to *B. hermsii* (RF). At the *glpQ* locus, genotypes A and B were most similar (85.7% and 85.4% respectively) to *Borrelia* sp. Tortoise14H1 (REP). We propose the name '*Candidatus Borrelia tachyglossi*', and hypothesise that this novel *Borrelia* species may be endemic to Australia. The pathogenic potential of this bacterium is not yet known.

Keywords: Australian ticks; Echidna; *Candidatus Borrelia tachyglossi*; *Bothriocroton concolor*; Spirochaetaceae

Abstract No: 3922

5 Sept 2017, 1745 – 1800

Genetic variability of *Anaplasma phagocytophilum* strains circulating in diverse hosts in Bushbuckridge, Mpumalanga, South Africa

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Abstract Content

Anaplasma phagocytophilum is a zoonotic, tick-borne, obligate intracellular bacterium capable of causing disease in diverse hosts, including humans, dogs, cattle and horses. It has not often been detected in Africa but recent research suggests that it is present in the Mnisi community, a rural community nestled in the heart of a human/livestock/wildlife interface in Bushbuckridge Municipality, Mpumalanga Province, South Africa. The aim of this study was to explore the genetic diversity of *A. phagocytophilum* in different hosts in order to better understand its circulation in the study community. To achieve this, DNA extracted from blood samples from 282 wild rodents from five different habitat areas, 74 humans diagnosed with non-malarial undifferentiated acute febrile illness at the community clinics, 100 cattle and 56 domestic dogs, and 20 pools of *Rhipicephalus sanguineus* ticks collected from domestic dogs (1 pool=8 adult male ticks), were screened for *A. phagocytophilum* using a quantitative real-time polymerase chain reaction (qPCR) assay that targets the *msp2* gene. Results revealed that 59% of wild rodents, 11% of humans, 10% of cattle, 82.9% dogs, and 85% *R. sanguineus* ticks were positive for *A. phagocytophilum*. Characterization of different strains by targeted sequencing of the 16S rRNA, *msp2*, *msp4* and *ankA* genes from positive samples revealed the presence of different and unique genotypes of *A. phagocytophilum* circulating within the community. This is the first detailed report of *A. phagocytophilum* in humans and other hosts in South Africa and highlights its possible importance as a cause of acute febrile illness in the country.

Keywords: Zoonoses; tick-borne; Anaplasma phagocytophilum; PCR; Characterization

Abstract No: 4547

5 Sept 2017, 1800 – 1815

Genetic diversity of *E. canis* Tandem Repeat containing Proteins (TRP36) gene isolates from different geographically dispersed isolates

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Abstract Content

In order to further understand the genetic variation of *Ehrlichia canis* TRP36 gene with respect to the N-Terminal pre-repeat region as well as the tandem repeat and C-Terminal post-repeat regions among *E. canis* isolates globally, 17 representative *E. canis* TRP36 gene sequences amplified from Malaysia, Brazil, Cameroon, Czech Republic, Israel, Nigeria, Taiwan, Thailand and USA were aligned using the MEGA 6 software. Analysis of N-terminal pre-repeat region consisting of 143 amino acids was found to be highly conserved among all the other isolates with few numbers of amino acid differences ranging between 1 and 24 numbers of amino acids. Sequences from Czech Republic and Brazil showed a unique pre-repeat region at position 148 and 144 amino acid sequences respectively. Repeat regions from the Malaysian isolates showed a completely conserved amino acid sequence (TEDSVSAPA) with those from Brazil, Cameroon, Nigeria, Taiwan and USA. In addition to the amino acid sequence TEDSVSAPA, two of the Malaysian isolates showed an entirely different tandem repeat sequence 'QLLKILFLL' which is entirely unique from all other repeat sequences reported from other regions and identified the presence of two entirely different tandem repeat sequences in one isolate for the first time in this study among the Malaysian *E. canis* isolates. This research reported an entirely new tandem repeat sequence among *E. canis* strains and also the presence of two different repeats on one sequence.

Keywords: *E. canis*, Genetic diversity, Tandem Repeats, GP/TRP36 gene, geographically dispersed isolates

Abstract No: 5222

5 Sept 2017, 1815 – 1830

Morphological and molecular identification of *Haematopinus* sp. on cattle in Java, Indonesia

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Abstract Content

Haematopinus sp. is an ectoparasite that causes pediculosis (ptiriasis) in cattle, and is a vector of babesiosis, theileriosis, and anaplasmosis disease. The aim of this research to identify the morphology, genetic diversity and phylogenetic relationship of *Haematopinus* specimens collected from four cattle breeds in central locations in Java. At each location, 5 – 10 samples of *Haematopinus* were collected from the tip of the tail, perineum vulva, ears and around the eyes from one individual representing each of four cattle breeds: Ongole (PO), Simental, Limousin, and Fries Holland (FH). Morphological identification was supported scanning electron microscopes. The molecular identification using a universal primer 18S region with forward primer (5'-TCATTACGAGGCTCTGCAAT-3') and reverse primer (5'-TTCAAAGTAAACGTGTCGGC-3'), followed by sequencing. Morphological and surface ultrastructure were analysed descriptively. Molecular data were analyzed using MEGA software and we constructed phylogenetic trees using the Neighbor Joining method and maximum parsimony. Based on morphological similarity and molecular results, we found *Haematopinus* samples from Simental, Limousin, and PO in all locations as *Haematopinus quadripertusus*. Molecular data indicated that FH samples was clustered with *Haematopinus quadripertusus*. *Haematopinus* sp. from FH cattle in Yogyakarta and Jember show differences in ultrastructures of the genital median plate.

Keyword: *Haematopinus* sp., PCR, surface ultrastructure, pediculosis

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Abstract No: 4888

5 Sept 2017, 1715 – 1730

Evaluation of the efficacy and safety of PolyVar Yellow® (flumethrin 275 mg bee-hive strips) for the treatment of varroosis in honeybees caused by flumethrin-sensitive *Varroa destructor* mites in a multicentre field study in Europe

Klaus Hellmann¹ ; Gabriele Braun¹ ; Gertraut Altreuther¹ ; Klemens Krieger¹
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Abstract Content

A controlled, randomised and partly-blinded multicentre clinical field study, *VICH GL9 Good Clinical Practice* compliant, was conducted in 2014 and 2015 in Germany, Hungary, Spain and the Netherlands, to evaluate the efficacy and safety of PolyVar Yellow®. In total, 277 honey bee colonies received a late summer/ autumn treatment either with PolyVar Yellow® (as gate at the hive entrance) for 92 to 122 days, or the control product (flumethrin bee-hive strips for in hive use, Bayvarol®) for 42±3 days. Between day 92 and 122 a follow-up treatment with coumaphos (Perizin®) was applied to both groups. Dead mites were counted in suitable intervals during treatment until day 7 after reference treatment. After treatment, safety was evaluated at different times during the study until the following summer. Safety parameters were colony survival until the following summer, colony strength, presence of queen, presence and amount of open, capped and drone brood, and honey production in the following spring and summer, as well as frequency of adverse events. In total, 150 adequately infected colonies treated according to the protocol were included in the efficacy calculation. For PolyVar Yellow® the percent mite reduction was 98.2%, exceeding the threshold of 95% stipulated in the 'guideline on veterinary medicinal products controlling *Varroa destructor* parasitosis in bees' (EMA/CVMP/EWP/459883/2008). For none of the safety parameters a significant difference between groups was observed ($P \geq 0.111$). Thus, PolyVar Yellow® was efficacious and safe in the treatment of Varroosis in honeybees caused by *Varroa destructor* under field conditions.

Keywords: Flumethrin, PolyVar Yellow, varroosis, honeybees

Abstract No: 4515

5 Sept 2017, 1730 – 1745

Control of varroosis in Western Honey Bees (*Apis mellifera* L.1758) by Flumethrin containing polymer matrix strips applied at the beehive entrance

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Abstract Content

Varroa destructor and varroa-transmitted viruses are considered the biggest threat for beekeeping with *Apis mellifera*. Only four acaricides have been approved for use in honeybees: amitraz, coumaphos, flumethrin and tau-fluvalinate. As development of resistance but also reversion have been reported rotational use of acaricides with different modes of action is recommended. Available products so far do not cover the complete period between end of nectar flow and end of flight activity towards winter so that significant re-infestation can occur despite of a successful Varroa treatment. PolyVar Yellow® (Flumethrin 275 mg beehive strips) was developed for a treatment duration of up to four months. The strips are applied at the hive entrance and have holes through which the bees enter and leave the hive thereby getting exposed to the active ingredient. Exposure of single bees was investigated in laboratory experiments. Mean flumethrin exposure was 7 ng/bee (LoQ by HPLC-MS/MS: 0.1 ng/bee). To evaluate distribution of flumethrin by social contact directly exposed bees were housed with non-exposed bees for 6 hours. Mean amounts of 0.59 ng/bee and 0.34 ng/bee were measured in exposed and non-exposed bees, respectively, with no statistically significant differences between the groups so that flumethrin was quickly distributed by social contact. Studies in honeybee colonies showed that flumethrin levels applied through PolyVar Yellow provide sufficient efficacy and are safe. This indicates that exposure of bees only at the beehive entrance results in successful treatment of the colony. Thus, PolyVar Yellow can be integrated in a sustainable Varroa control program.

Keywords: Varroa destructor; Apis mellifera; flumethrin, integrated control

Abstract No: 4514

5 Sept 2017, 1745 – 1800

Clinical development of Polyvar Yellow® (flumethrin 275 mg bee-hive strips) for the treatment of varroosis in honey bees caused by flumethrin-sensitive *Varroa destructor* mites.

Gertraud Altreuther^{**1}; Klemens Krieger¹
¹Development/ Bayer Animal Health GmbH/ Germany

Abstract Content

A controlled, randomized and partially blinded study was conducted to evaluate target animal safety and efficacy of flumethrin 275 mg bee-hive strips (PolyVar Yellow) against natural infestation with *Varroa destructor* in honey bee colonies. Thirty honey bee colonies were treated either with the test formulation applied as a “gate” at the hive entrance over 116 days, a positive control product (flumethrin bee-hive strips for in hive use, Bayvarol®) over 42 days or remained untreated as a negative control. On day 117 a follow-up treatment with coumafos solution (Perizin®) was applied to all three groups. Dead mites were counted during treatment up to 2 weeks after application of the reference treatment. For the safety evaluation dead bees were collected by dead bee traps and several colony examinations were conducted during treatment as well as in the following spring and early summer. Mite count reduction after treatment was 99.9% and superiority over the untreated control was demonstrated ($p=0.0008$). Safety was confirmed by high survival, lower numbers of dead bees of treated groups compared to the untreated control, and no differences in colony development that were considered clinically relevant. The results were further confirmed by a multicentre field study with 277 colonies in Germany, the Netherlands, Hungary and Spain, demonstrating safety and an overall efficacy of 98.2%. Thus PolyVar yellow was shown to be an efficacious and safe alternative for treatment of varroosis in honey bees caused by flumethrin-sensitive *Varroa destructor* and hence may significantly contribute to integrated varroa control programmes.

Keywords: Varroa destructor, Apis mellifera, flumethrin, integrated varroa control

Distribution of mutations associated with resistance to pyrethroids in *Varroa destructor* samples collected in Europe and the USA.

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Abstract Content

Varroa destructor (Anderson and Trueman) is an ectoparasitic mite responsible for a significant share of the Western honeybee (*Apis mellifera* L.) colony losses every year. This mite feeds directly from the haemolymph of immature and adult bees. It also vectors a series of viruses that will decimate the colony and cause collapse if there are no effective control measures in place. The rational use of acaricides is still the best way to control the parasite; however beekeepers have relied almost exclusively on them leading to many reports of resistance. We have shown recently that resistance to certain synthetic pyrethroids correlates directly with the presence of certain mutations in the target site of these compounds, the Voltage-Gated Sodium Channel. Analysis of sodium channel sequences from resistant samples collected in Europe and the USA has identified three different amino acid substitutions of leucine 925 (L925V, L925I and L925M), which is an important residue located in the putative pyrethroid binding site. Our data indicates that resistance has evolved independently in these different locations since L925V is present only in European mites, while L925I and L925M are found only in American samples. We have used a high-throughput genotyping assay to screen mite samples from many locations in Europe and the USA. Our results show that these mutations are present in the vast majority of locations and that their presence correlates with recent treatments with pyrethroids. Stakeholders can use this information to design resistance management strategies to maintain a more effective control of the parasite.

Keywords: Varroa; honeybee; pyrethroids; acaricide; resistance

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Best-bet integrated strategies improving the effectiveness of trypanocides and minimizing the development of trypanocide resistance in village cattle populations of northern Togo

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Abstract Content

The study aimed at improving trypanosomosis management in village cattle populations in tsetse-infested northern Togo. Baseline data collection comprised trypanosome prevalence, trypanocide resistance status, the quality of trypanocides and farmers' knowledge and attitude toward the disease. Based on these findings, a herd management program was implemented in three villages with 60 to 100 calves per village for a duration of 18 months starting in June 2014 (three other villages served as controls). Best-bet strategies consisted of rational trypanocidal drug use in symptomatic cattle (diminazene aceturate, Trypadim[®], Merial, 7.0 mg/kg b.w.), strategic use of anthelmintics (albendazole, Benzal[®]10%, LAPROVET, 7.5 mg/kg b.w.), restricted insecticidal spraying of the lower body parts of cattle (deltamethrin 5%, Vectocid[®] 50, CEVA) and, in 3 pilot studies, the use of insecticide treated nets (ZeroFly[®] Livestock, Vestergaard). Trypanosome prevalence decreased significantly ($P < 0.01$) in the intervention herds from 20.5% to 3% while it remained consistently high ($> 10\%$) in control herds. The mean PCV% of calves in the intervention herds remained constant (29.0 ± 0.9 ; 29.9 ± 0.7 , before and after intervention), while in the control animals mean PCV percentages decreased from 28.4 ± 1.0 to 26.7 ± 1.2 . Fecal egg count reduction (FECR) tests showed the efficacy of albendazole with a mean egg count reduction of 98.2-98.7% (CI 95%). The calves of the intervention herds displayed a good health status despite a significantly reduced number of trypanocidal treatments compared to the control herds. The European Union funded this study through the 'Trypanosomosis Rational Chemotherapy' (TRYRAC) project (<http://www.trypanocide.eu/>).

Keywords: African animal trypanosomosis; trypanocide resistance; rational drug use

Abstract No: 4310

6 Sept 2017, 0915 – 0930

Responsible use of anthelmintics in the lamb supply chain

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Abstract Content

With the current emphasis on antimicrobial resistance and its link to food production, the meat supply chain is under scrutiny to reduce chemical usage in meat producing animals. Consumers are also becoming more conscientious about the origin, traceability and production methods of the meat they purchase. Leading UK supermarket, Sainsbury's, initiated a project to engage members of their lamb supply group with the view of improving on farm control of intestinal roundworms. The aim of the Sainsbury's FECPAK^{G2} project was to improve the on-farm diagnostics and management of worms and enable a targeted approach to the use of anthelmintic interventions. The project piloted the new on-line FECPAK^{G2} platform that enables remote location diagnostics while connecting the supplier with expertise and local decision support. The results showed that farmers who engaged in the project: a) made more use of regular FEC monitoring, b) in most cases reduced the use of anthelmintic, c) changed the timing of treatments, d) determined resistance status so only used effective anthelmintics, e) in many cases reported an improvement in animal performance and f) had far better awareness of the parasite issues they faced. Resistance testing indicated that 93% of UK and 53% of NZ producers had been using wormers with reduced efficacy. Technology adoption remains a challenge, but opportunities for the wider Sainsbury's production group are significant. This project demonstrates how the supply chain can positively influence behaviour change in primary production using a precision medicine based approach.

Keywords: Sustainable; Responsible; Resistance; Anthelmintic; Monitoring

Field evaluation of anticoccidial efficacy in sheep based on oocyst excretion

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Abstract Content

Coccidiosis in lambs caused by *Eimeria* spp. leads to reduced welfare, increased mortality and substantial economic losses. Prevention of outbreaks is largely based on chemoprophylaxis with anticoccidials. Anecdotal reports of reduced anticoccidial efficacy in Norwegian lambs necessitate the formal evaluation of anticoccidial efficacy, which requires appropriate field methods. The main aim of this study was to assess anticoccidial efficacy in Norwegian lambs. A total of 41 flocks were recruited based on questionnaire data indicating signs of coccidiosis in lambs treated with anticoccidials. Two faecal samples were collected from 8 twin pairs (≥ 14 days old at turnout) in each flock. Sample 1 was taken 6-8 days after turn out, and one twin was treated with 20 mg/kg toltrazuril (Baycox® Sheep vet, Bayer Animal Health). Sample 2 was taken 7-11 days post treatment. Oocyst excretion (McMaster with a sensitivity of 5 oocysts per gram (OPG)), faecal score and weight gain were measured, and speciation was performed. Observed efficacy was below 65% in 5/41 flocks based on the following formula adapted from FECRT-calculations: $100 \times (1 - T/C)$, where T = arithmetic mean in treated group after treatment, and C = arithmetic mean in control group after treatment. However, this formula disregards the initial OPG and does not take into account that there will be no oocyst reduction in lambs treated metaphylactically. Our study highlights the difficulties associated with estimating anticoccidial efficacy, and demonstrates a need for a specific test aimed at evaluating field efficacy of anticoccidials.

Keywords: Eimeria spp.; anticoccidial efficacy; field evaluation; sheep

Abstract No: 4625

6 Sept 2017, 0945 – 1000

Anthelmintic resistance in feedlot cattle in Southern Australia detected using Mini-FLOTAC.

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Abstract Content

Australian beef cattle are routinely treated with anthelmintics on feedlot entry. This study tested efficacy of popular drenches using Faecal Egg Count (FEC) Reduction Tests. During the period Nov 2016 to April 2017, cattle at two feedlots in New South Wales, with a capacity of 35,000 and 50,000 head, were treated at induction with 2 commercially available anthelmintic products: oral albendazole (OA) and ivermectin pour-on (IPO). Faecal samples x15 were taken from each pen of animals at induction as well as 14 days later and submitted to the Dawbutts laboratory for FEC and larval differentiation (n=20). Mini-FLOTAC was used for individual FECs, with a sensitivity of 5 eggs per gram. FEC reduction for each nematode genus was calculated using RESO5 on Excel. Average FEC at induction was 133 (0-1150) epg on Feedlot A and 61 (0-475) epg on Feedlot B. Four major genera of worms were identified: *Cooperia*, *Haemonchus*, *Trichostrongylus* and *Ostertagia*, with small numbers of *Oesophagostomum*. Average efficacy of IPO was 1.3% (-130 to 87%) with efficacy >95% only observed for *Trichostrongylus*. Average efficacy of OA on Feedlot A was 61% (-156 to 100%) and Feedlot B was 86% (58 to 100%) with apparent resistance to *Ostertagia* and *Trichostrongylus*. However, 10 out of 15 FECRTs using albendazole had efficacy >95%. This study demonstrated the usefulness of Mini-FLOTAC for efficacy testing in populations of cattle with low starting worm egg counts, as well as highlighting lack of efficacy of popular products against common worm genera of Australian beef cattle.

Keywords: anthelmintic resistance, beef cattle, Mini-FLOTAC

Genetic analysis of BZ resistance in UK *Nematodirus battus* populations

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Abstract Content

Nematodirus battus was previously believed to be refractory to the development of benzimidazole resistance until the initial case was identified in on a commercial sheep flock in 2010. This was significantly later than the emergence of resistance to these compounds in other ovine gastro-intestinal nematode species. Further investigation of the isolate identified a mutation in the β -tubulin gene (F200Y) to be highly important in conferring resistance in this species, similar to BZ-resistance in other GIN species. More recently the F167Y mutation has been found in this isolate. *N. battus*-specific pyrosequencing assays for quantifying both mutations was developed and utilised to investigate the prevalence of BZ-resistance genes in *N. battus* populations. Initial results with this BZ-R population showed a spread of genotypes at both P200 and P167 (10% SS^{P200}, 20% Sr^{P200}, 70% rr^{P200}, 71%SS^{P167}, 18%Sr^{P167} and 12% rr^{P167}) with no double resistant genotypes at both loci. Analysis of the P200 resistant mutation from populations obtained from 289 flocks from around the UK identified the resistant alleles in 107 of the 289 populations analysed with an overall low prevalence (\pm SEM) in all regions at 3% \pm 0.5. Screening for F167Y in populations is underway. The frequency of the P200 mutation within populations ranged from 2-93%. Multiple potential 'focal regions' were identified throughout the UK in which resistant allele frequency appears to be higher than the national average. Factors associated with the development and dissemination of resistance in this species and the localisation of resistance is yet to be understood.

Keywords: *Nematodirus battus*; Benzimidazole resistance; F200Y; F167Y;

Abstract No: 4100

6 Sept 2017, 1015 - 1030

Mutations in the *mptl-1* gene in a field-derived monepantel-resistant isolate of *Haemonchus contortus*

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Abstract Content

Resistance to the anthelmintic drug monepantel (Zolvix®) has emerged in parasitic worms infecting sheep and goats. The mechanism of resistance in these cases is unknown. The drug targets nicotinic acetylcholine receptors belonging to the nematode-specific DEG-3 subfamily. We examined the receptor gene, *Hco-mptl-1*, in a highly Zolvix®-resistant and a -susceptible isolate of the parasitic nematode *Haemonchus contortus*. cDNA coding for the full length receptor protein (Hco-MPTL-1) was present in all clones prepared from a pool of susceptible larvae (21/21 clones) and approximately 50% of those from the resistant isolate (17/33). On the other hand, the remaining clones from the resistant isolate showed various mutations that resulted in truncated predicted proteins, missing at least one transmembrane domain. The most common mutation (11/33 clones) resulted in the retention of intron 15, a premature stop codon, and a truncated protein. Sequencing of intron 15 genomic DNA showed very few SNPs in susceptible larvae and in 12/18 clones from resistant larvae, alongside the presence of at least 17 SNPs in the remaining resistant clones. The present study shows that the highly resistant isolate has a number of mutations in the drug target gene that would most-likely result in a non-functional receptor, thus rendering the larvae insensitive to the drug. The presence of multiple separate mutations in the *Hco-mptl-1* gene in this viable field-derived worm isolate may at least partly explain why resistance to Zolvix® has arisen rapidly in the field.

Keywords: Haemonchus contortus, monepantel, resistance, mutation, target site

Abstract No: 4553

6 Sept 2017, 1100 – 1115

Pharmacology of ATP-binding cassette transporter associated with macrocyclic lactone tolerance in *Toxocara canis* larvae

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Abstract Content

Toxocara canis, the cosmopolitan ascarid of dogs, has a complex lifecycle. It matures to adulthood in puppies after a hepato-pulmonary migratory phase. In adult dogs, somatic migration results in hypobiotic third stage larvae. These L3s are reactivated in pregnant bitches and transmitted to puppies through lactogenic and transplacental routes. Somatic larval stages show remarkable tolerance to the routinely used drugs that have adulticidal activity, and complete elimination of the somatic larval stages is not possible with macrocyclic lactones. We hypothesized that this phenomenon is related to the efflux of the drugs away from their site of action by specialized proteins, which have been demonstrated in other nematodes of veterinary importance. Our investigation revealed a P-glycoprotein from the ATP binding cassette transporter family that is highly expressed in *T. canis* larvae. In this report, we characterize the expression and pharmacological profile of this drug efflux protein. Our findings suggest that macrocyclic lactones are compartmentalized in specific nematode tissues, thereby precluding access to sites of targeted receptors.

Keywords: Toxocara; macrocyclic lactones; somatic larvae; ABC transporters

Abstract No: 4896

6 Sept 2017, 1115 – 1130

Haplotype diversity in the *dyf-7* gene of *Haemonchus contortus* from Swedish sheep farms with differing levels of anthelmintic resistance

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Abstract Content

The dye-filling protein (DYF-7) is involved in anchoring the dendritic tips during the migration of glial cells. This affects the development of amphid sensory organ in nematodes. Mutant *dyf-7* genes were originally discovered in *Caenorhaditis elegans*, where it has been shown to modulate the level of ivermectin (IVM) resistance. Likewise it has been shown by structural studies that IVM-resistant *Haemonchus contortus* has markedly shorter amphids than their susceptible counterparts. Recently, it was also suggested that IVM resistance in *H. contortus* is caused by loss-of-function due to certain mutations in the *dyf-7* gene.

In this study we have investigated *dyf-7* haplotype diversity by sequencing population samples of *H. contortus* from Swedish farms. These worm populations have been characterized by their IVM-resistance status by fecal egg count reduction test. We used adult male and female worms isolated from sheep, which were compared with additional samples from different continents and previously published sequences. In the coding regions of the gene, we identified new SNPs in exon 2 and 3 sequences. The introns were also variable, containing substitutions, insertions and deletions. We have constructed a haplotype network to investigate IVM resistance haplotype clustering. This finding provides additional insight into the role of *dyf-7* as a genetic marker for IVM resistance.

Keywords: Haemonchus contortus; dyf-7; Haplotype diversity; ivermectin resistance; sheep

Abstract No: 2990

6 Sept 2017, 1130 – 1145

Comparative efficacy of the herbal and popularly used anthelmintics against fenbendazole-resistant caprine strain of *Haemonchus contortus* in Jabalpur, India

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Abstract Content

Investigations were undertaken to assess the comparative efficacy of the anti-parasitic drugs vis-à-vis herbal extracts with known anti-parasitic activity. Seventy goats were randomly assigned to seven groups of 10 animals each. Each animal in group I was orally administered with fenbendazole @ 7.5 mg/kg whereas group II, III and IV were respectively given aqueous leaf extracts of herbal products viz. neem (*Azadirachta indica*) @ 1 g/kg BW, sitaphal (*Annona squamosa*) @ 1.5 g/kg BW and tobacco (*Nicotiana tabacum*) @ 1 g/kg BW, respectively. Animals in group V were maintained as untreated controls. Each animal in group VI was orally treated with closantel 10 mg/kg and in group VII doramectin @ 0.2 mg/kg subcutaneously. Compared to untreated control, there was no conspicuous and significant reduction in post treatment (day 10) faecal egg counts (FEC) in animals administered with the herbal extracts (Group II, III, and IV) suggestive of poor anti-parasitic activity. However judging the impact of popularly used anthelmintic, using Faecal egg count reduction test (FECRT) as index, the overall efficacy of fenbendazole (24.9%), closantel (95.64%) and doramectin (99.64%) were recorded, establishing the least anti-parasitic activity of fenbendazole and evidenced the fenbendazole resistant *in situ* gastrointestinal populations in the treated goats. The rotational use of closantel/doramectin is preferred choice over benzimidazole group of medicines and/or herbal extracts so much so to overcome the burning problem of *in situ* developing drug resistant gastrointestinal nematodes, especially *H. contortus* and to ensure increased productivity while eliminating production losses incidental to gastrointestinal nematodosis in goats.

Keywords: Benzimidazole resistance, Herbal extracts, *Haemonchus contortus*, Goat, India

Abstract No: 4316

6 Sept 2017, 1145 – 1200

The application of “Nemabiome” deep-amplicon sequencing approach to investigate nematode species composition and anthelmintic resistance status in field samples

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Abstract Content

Anthelmintic resistance is of increasing concern to sheep producers and has been reported in all continents of the World. In Canada, despite a sub-optimal climate, *Haemonchus contortus* and related nematode species are widespread, and there is evidence of resistance against the main anthelmintic drug classes used. Conventional methods of detecting resistance such as the Fecal Egg Count Reduction Test (FECRT) are still the gold standard, however, this method can be difficult to interpret. The Nemabiome deep-amplicon sequencing approach is based on a “microbiome-style” next-generation deep sequencing of ITS-2 rDNA amplicons and allows large-scale analysis of samples. The present study combined the Nemabiome with the FECRT to allow identification of nematode species present in both pre- and post-treatment field samples, thus highlighting species-specific resistance to different classes of drugs in Western Canadian sheep farms. We also applied a deep-amplicon sequencing assay to determine the frequency of mutations conferring resistance to fenbendazole. The results allowed us to identify benzimidazole and ivermectin resistant *H. contortus* on many farms enrolled in the study. Also, deep sequencing showed ability to accurately detect resistance mutations at low frequency and screen large numbers of samples in a single run. Applying this combined approach in the field will help producers and veterinarians to take evidence-based decisions and design tailored anthelmintic treatment strategies, thereby delaying the spread of anthelmintic resistance. In addition, it will increase our understanding of the origin and spread of resistance mutations in nematode species.

Keywords: Sheep nematodes, Drug resistance, Molecular approach, Nemabiome

Abstract No: 4300

6 Sept 2017, 1200 – 1215

No effects of *in vitro* thiabendazole exposure on cytochrome P450 expression in fourth stage larvae of benzimidazole-resistant and -susceptible *Haemonchus contortus*

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Abstract Content

Cytochrome P450 monooxygenases (CYPs) are important mediators of drug resistance in mammals and arthropods. Recent findings suggest that CYPs are also involved in nematode resistance against benzimidazole (BZ) anthelmintics, including the important ruminant parasite *Haemonchus contortus*. Hence, expression patterns of five CYPs in fourth stage larvae of three BZ-resistant and two susceptible *H. contortus* isolates were compared following *in vitro* exposure to thiabendazole for 3 and 6 h using qRT-PCR. The resistance status of all isolates was determined using egg hatch assays and pyrosequencing. The selected CYPs are orthologues of the BZ/xenobiotic-inducible CYP35 and the xenobiotic-inducible CYP31A families in *Caenorhabditis elegans*. Comparison of basal transcript levels revealed that CYP HCOI100383400 was significantly higher expressed in the WR isolate with the strongest resistance phenotype when compared to both susceptible HcH (2.4fold) and CAVR (2.7fold) isolates and the intermediately resistant IRE (3.7fold) isolate. Transcript levels of this CYP were slightly but not significantly elevated in the TBZ isolate in comparison to susceptible isolates. There were no significant changes in expression profiles of all investigated CYPs after BZ exposure. Essentially, these findings negate an *in vitro* thiabendazole inducibility in *H. contortus* L4 and oppose the hypothesis of a substantial contribution of CYPs to BZ resistance in this parasite, since the difference in basal transcription of HCOI100383400 in the resistant WR isolate was rather small and no differences were detectable for the other CYPs. However, this does still not exclude effects of CYPs on resistance in other parasite species, isolates or life cycle stages.

Keywords: drug resistance; cytochrome P450, Haemonchus contortus

Insecticide resistance in stable flies (*Stomoxys calcitrans*) on dairy farms in Brandenburg, Germany

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Abstract Content

The aim of this study was to assess the occurrence of insecticide resistance in stable flies (*Stomoxys calcitrans*) on dairy cattle farms in Brandenburg, Germany. In a first step, we checked the on-farm susceptibility of stable flies to deltamethrin by using the FlyBox®, a cardboard box with its interior coated with 55 mg dtm/m². Out of all *Stomoxys* populations from 40 dairy farms, an average 95% turned out to be suspicious for resistance when exposed for 10 seconds. In a second step 10 suspect stable fly colonies from 10 farms were established in the laboratory and their emerging offspring tested against the pyrethroid deltamethrin, azamethiphos, a phosphoric ester, and two insect growth regulators (IGR) (cyromazine and pyriproxyfen) under laboratory conditions. The on-farm results of the FlyBox®-method were confirmed in the laboratory, demonstrating mortalities below 80 % at the LD₉₅ of deltamethrin (2.3 ng/fly) 24 hrs after topical application. At the LD₉₅ of azamethiphos (4.9 ng/fly) all stable fly colonies also turned out to be resistant. In contrast, full susceptibility against the IGR cyromazine and pyriproxyfen was recorded at the recommended concentration of 5 mg/kg and 0.027 mg/kg, respectively. In conclusion, it is recommended to evaluate insecticide susceptibility in fly populations prior to the use of insecticides. The non-strategic use of insecticides will lead to insecticide resistance development within a few generations. Rotation of insecticides, frequent manure removal and biological means of control is expected to delay resistance development.

Keywords: Stomoxys calcitrans, insecticide resistance, dairy cattle, deltamethrin, azamethiphos, cyromazine, pyriproxyfen, FlyBox®,

Comparative efficacy of three anthelmintics for control of gastrointestinal nematodes in sheep and goats in Punjab, India.

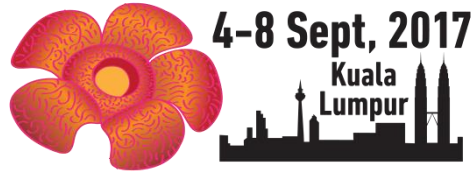
Prashant Pawar^{*1}; Lachhman Das Singla¹; Paramjit Kaur¹; Mandeep Bal¹; Mohammed Javed¹
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Abstract Content

Anthelmintic efficacy of fenbendazole, ivermectin and levamisole was evaluated on two organized and one unorganized farm in Punjab using fecal egg count reduction (FECR) test as per the WAAVP guidelines. Non-dewormed animals (≥ 8 Week) were randomly assigned three treatments and one control group (n=10). Group I was treated with fenbendazole @ 5mg/kg and @10 mg/kg. Group II with ivermectin @0.2mg/kg and 0.4mg/kg. Group III with levamisole @ 8mg/kg and 12mg/kg.BW, orally for sheep and goat, respectively. Group IV served as a untreated control. The FECR reduction percentage for fenbendazole, ivermectin and levamisole were found to be 59, 90 &100 percent at organized farm Ludhiana, 50, 48 & 49 percent at organized farm Mattewara and 83, 97 & 56 percent at unorganized goat farms, respectively. While the FECR percentage for fenbendazole, ivermectin and levamisole at Mattewara was 84, 85 & 87 percent and 51, 26 & 87 percent at Monji village, respectively in case sheep farms. There was fenbendazole resistance to GIN in all sheep and goat both at organized and unorganized farm. Levamisole was highly effective in Ludhiana goat farm (100%) and moderately effective in Mattewara and Monji village sheep farms (87%).The ivermectin was highly effective in Monji village goat farm (97%) and Ludhiana goat farm (90%).There was fenbendazole resistance on organized farm using the FECR reduction test, while no resistance was detected to levamisole at organized farms. FECR percentage reduction test and confidence limit indicated the existence of resistance or suspected resistance.

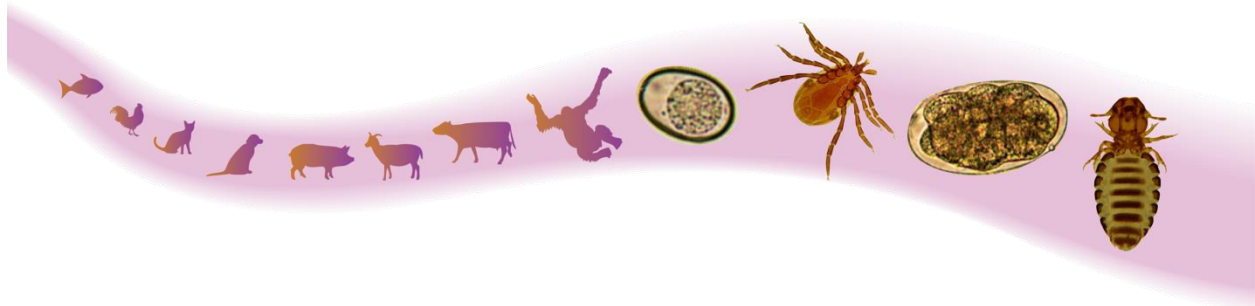
Keywords: Anthelmintic resistance, sheep, goat, FECRT, Punjab.

WAAVP



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Abstract No: 4396

6 Sept 2017, 0900 – 0915

Next Generation Sequencing uncovers within-host genetic diversity of *Cryptosporidium* gp60 subtypes

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Abstract Content

The extent of within host genetic diversity of parasites has implications for our understanding of the epidemiology, disease severity and evolution of parasite virulence. Despite this, relatively little is known about within host genetic diversity in the important enteric parasite *Cryptosporidium*. The present study compared Sanger and Next Generation Sequencing (NGS) of glycoprotein 60 (*gp60*) amplicons from *C. hominis* (n=11), *C. parvum* (n=22) and *C. cuniculus* (n=8) isolates from Australia and China. Sanger sequencing identified only one *gp60* subtype in each isolate; *C. hominis* subtype (IbA10G2) (n=11), 4 *C. parvum* subtypes belonging to IIa (n=3) and IIc (n=19) and one *C. cuniculus* subtype (VbA23) (n=8). NGS identified the same subtypes initially identified by Sanger sequencing but also identified additional *gp60* subtypes in *C. parvum* and *C. cuniculus* but not in *C. hominis* isolates. The number of *C. parvum* and *C. cuniculus* subtypes identified by NGS within individual isolates ranged from two to four and both *C. parvum* IIa and IIc subtype families were identified within the one host. The finding of the present study has important implications for *Cryptosporidium* transmission tracking as well as vaccine and drug studies.

Keywords: *Cryptosporidium*; within-host diversity; glycoprotein 60 (*gp60*); Sanger; Next Generation Sequencing (NGS)

Abstract No: 3706

6 Sept 2017, 0915 – 0930

Curating and characterizing the kinomes of parasitic worms – implications for fundamental and applied molecular explorations

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Abstract Content

The availability of many draft genomes and transcriptomes of parasitic worms (helminths), including those of veterinary importance, now enables the identification and characterization of genes that are essential for parasite survival, development and reproduction. In this context, signalling pathways are of particular significance, given their crucial roles in many developmental and physiological processes, most of which are regulated by protein kinases. However, most helminth kinase complements (kinomes) have not been curated or characterized in detail, and thus remain largely unexplored. Having available well-curated kinomes would provide a solid basis to explore fundamental biological processes and would provide an opportunity to prioritize and validate kinases as anthelmintic targets. In this context, we have designed, validated and enhanced a bioinformatic workflow to fully characterize and curate kinomes using currently available genomic and transcriptomic resources. This workflow achieves a substantially improved identification and characterization of kinase genes, and, by integrating a structural modelling approach, enables a more refined classification and functional annotation of kinases compared with sequence homology-based approaches. Using curated kinomes, we have been able to: (i) infer structural and functional information for known kinase inhibitors, small molecules and respective targets; (ii) dissect the transcriptional regulation of kinase genes during parasite life cycles; and (iii) characterize and annotate novel protein kinases of unknown function. These results and applications show that our workflow provides a useful tool for the large-scale curation and characterization of helminth kinomes as a basis for many fundamental molecular investigations and to underpin the validation of drug targets.

Keywords: helminths; kinomes; kinase; drug targets; bioinformatics

Abstract No: 4015

6 Sept 2017, 0930 – 0945

Bioactive lipid mediator profiling of *Toxocara canis*- and *T. cati*-infected brains

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¹University of Veterinary Medicine Hannover/ Institute for Parasitology/ Germany ²University of Veterinary Medicine Hannover/ Institute of Food Toxicology/ Germany, ³University of Wuppertal/ Institute of Food Chemistry/ Germany

Abstract Content

Neurotoxocarosis (NT) is induced by larvae of *Toxocara canis* or *T. cati* migrating and persisting in the central nervous system (CNS) of paratenic hosts and may be accompanied by severe neurological symptoms. Host- or parasite-induced immunoregulatory processes contribute to pathogenesis; however, solid data on involvement of bioactive lipid mediator is lacking. These regulatory lipid mediators (e.g. oxylipins, eico-/docosanoids), derived from arachidonic acid and related polyunsaturated fatty acids, play a crucial role in induction and resolution of inflammation as well as in immune response. To elucidate changes in the oxylipin pattern during the course of *T. canis*- and *T. cati*-induced NT, lipidomic profiling of cerebra and cerebella of experimentally infected C57Bl/6J mice was conducted at six different time points post infection (pi) by *liquid chromatography electrospray ionization tandem mass spectrometry* (LC-ESI-MS/MS). A total of 74 different eico-/docosanoids were successfully quantified in analysed brains. Solely minor changes were detected in the biosynthetic pathway of mostly proinflammatory prostaglandins (COX-pathway). In contrast, a significant increase of metabolites of ALOX-pathways was observed for both infection groups and brain regions starting day 14 pi, reaching a peak day 42 pi and declining gradually days 70-98 pi, implicating a predominantly anti-inflammatory driven immune response. The significant increase of anti-inflammatory oxylipins in contrast to low regulation of proinflammatory oxylipins may indicate a parasite-induced immunomodulatory effect to evade the host's immune response, facilitating persistence in brain tissues. Further in depth analyses will contribute to the characterization of the still mostly unknown pathogenesis of *T. canis*- and *T. cati*-induced NT.

Keywords: Toxocara; Neurotoxocarosis; regulatory lipids; Eicosanoids; brain infection

Gastropod-derived haemocyte extracellular traps entrap larval stages of *Angiostrongylus vasorum*, *Aelurostrongylus abstrusus* and *Troglostrongylus brevior*

Malin Lange^{*1} ; Felipe Penagos¹ ; Tamara Muñoz-Caro¹ ; Ulrich Gärtner^{1 2} ; Anika Seipp^{1 2} ; Helena Mejer^{1 2 3} ; Roland Schaper^{1 2 3 4} ; Carlos Hermosilla^{1 2 3 4} ; Anja Taubert^{1 2 3 4}
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Abstract Content

Phagocyte-derived extracellular traps (ETs) were demonstrated mainly in vertebrate hosts as an important innate effector mechanism acting against different kinds of pathogens. In the present study we aimed to characterize gastropod-derived invertebrate extracellular phagocyte trap (InEPT) formation in response to larval stages of canine and feline metastrongyloid lungworms. Gastropod haemocytes were isolated from the slug species *Arion lusitanicus* and *Limax maximus* and from the snail species *Achatina fulica*. Haemocytes were exposed to larval stages of *Angiostrongylus vasorum*, *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* and analyzed for InEPT formation. Phase contrast as well as scanning electron microscopic (SEM) analyses revealed ET-like structures to be extruded by haemocytes, thereby contacting and ensnaring the parasites. Co-localization studies of haemocyte-derived extracellular DNA and histones or myeloperoxidase in larvae-entrapping structures confirmed classical molecular characteristics of ETs. During experimental infection of slugs with *A. vasorum* larvae InEPTs were observed within the slug mucous extrapallial space, thereby indicating *in vivo* evidence of this effector mechanism. Functional larval entrapment assays demonstrated that almost half of the haemocyte-exposed larvae were contacted or even immobilized by InEPTs. As reported for mammalian-derived ETs, different types of InEPTs were here observed, i. e. aggregated, spread and diffused InEPTs. To our knowledge, this study represents the first report on metastrongyloid lungworm-triggered ETosis in gastropods thereby providing evidence of early mollusc host innate immune reactions against invading larvae. These findings will contribute to the better understanding on complex parasite-intermediate host interactions as well as on gastropod-derived innate immune mechanisms.

Keywords: Gastropod-borne diseases, Metastrongyloidea, Extracellular traps, Lungworm, Innate immune response

Abstract No: 4108

6 Sept 2017, 1000 – 1015

Biology of small secreted extracellular vesicles from hookworms and their roles in parasite-host interactions

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¹*James Cook University/ Australian Institute of Tropical Health and Medicine/ Australia*

Abstract Content

Soil-transmitted helminths composed of the three major groups - hookworms, whipworms and roundworms - form one of the most important infectious agents causing an enormous global disease burden. The adult worms live in the intestine of the host and release excretory/secretory products, representing the major host-parasite interface. While studies on parasite-host interactions have traditionally focused on soluble proteins, we focus herein on the characterization of secreted extracellular vesicles (EVs) that contain protein, lipids and nucleic acids. Recent research has shown that helminths communicate with their surrounding cells via the secretion of EVs, possibly via release of genetic material. Here, we analyse EVs from the rodent parasite *Nippostrongylus brasiliensis*, which has been used as a model for hookworm infection. We used proteomics and RNA Seq to profile the molecular composition of *N. brasiliensis* EVs and have begun to evaluate the mechanisms why which these vesicles aid the parasite in evading host immune attack. Helminths have evolved strategies to 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 and their secreted products for the development of novel therapies for autoimmune, allergic and even metabolic diseases. Indeed, we have shown that hookworm EVs confer protection against inflammation in a model of colitis. This will reveal potential applications in helminth drug development and vaccine design, as well as development of an entirely new generation of therapeutics to treat chronic non-infectious diseases.

Keywords: Hookworm, parasite-host interaction, secreted extracellular vesicles, anti-inflammatory therapeutics, drug development

**Modulation of goat monocyte function by HCcyst-2, a secreted cystatin from
*Haemonchus contortus***

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¹College of Veterinary Medicine/ Nanjing Agricultural University/ China

Abstract Content

Modulation and suppression of the host immune response by nematode parasites have been reported extensively and the cysteine protease inhibitor (cystatin) is identified as one of the major immunomodulator. In the present study, we cloned and produced recombinant cystatin protein from nematode parasite *Haemonchus contortus* (rHCcyst-2) and investigated its immunomodulatory effects on goat monocyte. rHCcyst-2 protein is biologically functional as shown by its ability to inhibit the protease activity of cathepsin L, cathepsin B and papain. Immunohistochemical test demonstrated that the native HCcyst-2 protein was predominantly localized at the body surface and internal surface of the worm's gut. We demonstrated that rHCcyst-2 could be distinguished by antisera from goats experimentally infected with *H. contortus* and could uptake by goat monocytes. The immunomodulatory effects of HCcyst-2 on cytokine secretion, MHC molecule expression, NO production and phagocytosis were observed by co-incubation of rHCcyst-2 with goat monocytes. The results showed that the interaction of rHCcyst-2 decreased the production of TNF- α , IL-1 β and IL-12p40. However, it significantly increased the secretion of IL-10 in goat monocytes. After rHCcyst-2 exposure, the expression of MHC-II on goat monocytes was inhibited. Moreover, rHCcyst-2 could up-regulate the LPS induced NO production of goat monocytes. Phagocytotic assay by FITC-dextran internalization showed that rHCcyst-2 inhibited the phagocytosis of goat monocytes. Our findings provided potential target as immunoregulator, and will be helpful to elucidate the molecular basis of host-parasite interactions and search for new potential proteins as vaccine and drug target candidate.

Keywords: Haemonchus contortus; cystatin; monocyte; immunomodulation

Abstract No: 5023

6 Sept 2017, 1100 – 1115

Expression profiles of genes involved in TLRs and NLRs signaling pathways of water buffaloes infected with *Fasciola gigantica*

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Abstract Content

Infection of ruminants and humans with *Fasciola gigantica* is attracting increasing attention due to its economic impact and public health significance. However, little is known of innate immune responses during *F. gigantica* infection. In this study, we investigated the expression profile of genes involved in Toll-like receptors (TLRs) and NOD-like receptors (NLRs) signaling pathways in water buffaloes infected by *F. gigantica*. Buffaloes were infected with 500 viable *F. gigantica* metacercariae. Serum, liver and peripheral blood lymphocytes (PBL) samples were collected from animals at 3, 10, 28, and 70 days post infection (dpi). Then, the levels of expression of 43 genes related to TLRs and NLRs signaling were determined using quantitative RT-PCR. Levels of 12 cytokines were evaluated by ELISA. At 3 dpi, immunosuppression was evident by the reduced levels of all 12 tested cytokines, which correlated with modest activation of TLR4 and TLR8 and the adaptor protein (TICAM1). At 10 dpi, NFκB and Interferon Regulatory Factor signaling pathways were upregulated along with activation of TLR1, 2, 6 and 10 in the liver, and inflammatory response with activated TLR4 and 9 in PBL. At 28 dpi, there was increase in the levels of cytokines along with induction of NLRP1 and NLRP3 inflammasomes-dependent immune responses in the liver and PBL. At 70 dpi, *F. gigantica* activated TLRs and NLRs, and their downstream interacting molecules, and induced a mixed Th1/Th2 cytokine response. These findings indicate that *F. gigantica* alters the expression of TLRs and NLRs genes to evade host immune defences.

Keywords: *Fasciola gigantica*; Gene expression; Host-pathogen interaction; Pattern recognition receptors; Toll-like receptors

Molecular characterization of *Fasciola gigantica* from Malaysia

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Abstract Content

Fasciola gigantica is widely distributed in the tropical regions including Southeast Asian countries. Recently, we have revealed the phylogenetic relationships between *F. gigantica* from Asian countries. In this study, molecular characterizations were performed for *Fasciola* flukes from Malaysia. Phylogenetic analysis for Malaysian *Fasciola* flukes together with neighbouring Asian countries was performed based on mitochondrial DNA sequences. 40 *Fasciola* flukes were collected from bile ducts of water buffaloes and cattle in seven locations of Malaysia. The flukes were fixed in 70% ethanol and transported to the laboratory and preserved until DNA extraction. For species identification, nuclear phosphoenolpyruvate carboxykinase (*pepck*) and DNA polymerase delta (*pold*) genes were analyzed by using multiplex PCR and PCR-RFLP, respectively. Regarding phylogenetic analysis, the nucleotide sequences of mitochondrial NADH dehydrogenase subunit 1 (*nad1*) gene were determined by direct sequencing. The resultant *nad1* haplotypes were compared with those of *F. gigantica* from other Asian countries available in the GenBank to reveal relationship among them. Malaysian *Fasciola* flukes were identified as *F. gigantica* because they showed the *F. gigantica* band patterns in both *pepck* and *pold* genes. The eight *nad1* haplotypes were detected on the basis of the sequencing analysis of Malaysian *F. gigantica*. They were related to those from Thailand, Indonesia, Vietnam, and China. These findings suggest the origin and dispersal route of Malaysian *F. gigantica*.

Keywords: *Fasciola*; *pepck*; *pold*; *nad1*; Asia

Abstract No: 4237

6 Sept 2017, 1130 – 1145

Molecular phylogenetic analyses on *Fasciola gigantica* from India and Asian countries suggest the spreading histories of the fluke in Asia

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Abstract Content

Fasciola gigantica is parasitic flukes in bile duct of ruminants, and causes economic loss in the livestock industry in Asia and Africa. In this study, phylogenetic analyses on *F. gigantica* in India along with six Asian countries were performed to clarify their spreading histories in Asia. One hundred sixty-four flukes were collected from bile duct of ruminant livestocks in four districts of India, and confirmed as *F. gigantica* based on two nuclear markers, *pepck* and *pold*. The flukes from India and the countries were analyzed phylogenetically using the mitochondrial *nad1* haplotypes. In the resultant phylogenetic tree and network, the *nad1* haplotypes from all the flukes were divided into three distinct groups (A, B and C), and most of the Indian flukes were categorized into group A which is predominant population in South Asia. In AMOVA, the percentage of variation among the groups indicated higher value than that among countries, suggesting that *F. gigantica* in Asia has differentiated into three genetic groups. Then, Tajima's D and Fu's *F_s* values for each group showed significant negative values, which is considered evidence of a founder effect or upsurge of the populations. Therefore, we hypothesized that genetically diversified flukes had occurred in ancient Asia, and some of them were selected, increased their population and spread through Asia along with domestication and artificial movement of host ruminants.

Keywords: Fasciola gigantica; phylogeny; spreading history; Asia; India

Characterization of *Echinococcus granulosus* haplotype network and cattle hydatid cysts infection status in Chile

Felipe Corrêa^{*1}; Caroll Stoores¹; Mauricio Jiménez¹; Christian Hidalgo¹; Pamina Horlacher¹; Cristian Álvarez²; Henrique Bunselmeyer Ferreira³; Marcela Hernández^{4,5}; Rodolfo Paredes¹

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Abstract Content

Cystic Echinococcosis (CE), a worldwide-distributed zoonosis caused by the cestode *Echinococcus granulosus*, is an infection endemic in Chile. Despite its economic and public health significance, there is lack of information on CE in Chile. Therefore the aim of this work was to characterize the status of infection in cattle and also contribute to the study of the molecular epidemiology of this parasite. From 2,961 veterinary inspected cattle, information was recorded and hydatid cysts (HC) from infected animals were collected. Genomic DNA was extracted from germinal layer/protoscoleces and a fragment of the mitochondrial *cox1* gene was amplified. PCR products were sequenced and compared with reference sequences retrieved from GenBank. The prevalence of CE was 19%. From 558 cattle with HC, 51% had cysts only in their lungs, 19% only in their liver, and 30% in liver and lungs; also, 79% were infertile, 6% fertile and 15% smaller than 3cm. DNA sequencing revealed the existence of 59 *E. granulosus sensu stricto* (G1-G3) and 2 *E. ortleppi* (G5) samples in the studied area. The Chilean *E. granulosus* s.s. parsimony network displayed 11 haplotypes and showed the presence of a common *E. granulosus* haplotype described in Egypt, Mongolia and Russia. Further studies using a larger number of hydatid isolates from various locations across Chile and different intermediate hosts will provide molecular information of *E. granulosus* s.s. CE is widespread in cattle in the central area of Chile and here we show the first report of the presence of *E. ortleppi* (G5) strain in Chile.

Keywords: Echinococcus granulosus; genotype; cystic echinococcosis, Echinococcus ortleppi, Chile

Abstract No: 3202

6 Sept 2017, 1200 – 1215

Random Amplified Polymorphic DNA (RAPD) and Internal Transcribed Spacer 2 (ITS-2) rDNA study of some paramphistome isolates from Southern Africa.

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Abstract Content

Paramphistomes are parasites of both domestic and wild ruminants whose effects still remain underestimated. Limited studies in Africa have been done using molecular techniques to resolve problems associated with taxonomical groupings. In this study, the genetic variability of nine representative paramphistome isolates collected from different geographical locations in Southern Africa was assessed using RAPD data analysis and ITS 2 sequence data. All isolates were sectioned for morphological characterisation and for molecular analyses; DNA was extracted, amplified and purified. Sagittal sections revealed three species of paramphistomes belonging to three different sub-families: one *Stephanopharynx compactus* isolate, a member of the Stephanopharyngidae sub-family, one *Carmyerius dollfusi* isolate, a member of the Gastrothylacidae sub-family and seven *Calicophoron microbothrium* isolates belonging to the Paramphistomidae sub-family. Phylogenetic reconstruction of the paramphistome isolates based on the ITS 2 rDNA sequence data obtained using Mega 6 separated them into three clades representing the three species. The low divergence values on the ITS 2 sequences of the *C. microbothrium* isolates indicate that ITS rDNA sequence data can be used as a molecular tool to infer knowledge for resolving taxonomic groupings. RAPD data analysis using Popgene 32 clustered the paramphistome isolates according to their geographic origin in spite of the differences in species of the isolates. There was significant variability between the isolates with an average genetic distance value of 0.4381. These results show that RAPDs can be used as a molecular marker for epidemiological studies of *C. microbothrium* and could possibly show that cross fertilisation between species does occur.

Keywords: Calicophoron microbothrium; Stephanopharynx compactus; Carmyerius dollfusi; RAPD; ITS 2

Abstract No: 4456

6 Sept 2017, 1215 – 1230

Molecular characterization of amphistomes affecting cattle from selected areas in South Africa and Zimbabwe

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¹*School of Life Sciences/ University of KwaZulu-Natal/ South Africa*

Abstract Content

Amphistomiasis is a disease of wild and domestic ruminants caused by infection with digenetic trematode flukes from the superfamily Paramphistomoidea Fischöder, 1901. These flukes have also been reported to infect humans. They constitute a group of neglected parasites of economic importance in Sub-Saharan Africa as they cause weight loss, reduced milk yield and, if untreated, death in infected ruminants. Most studies on the characterization of amphistomes have used morphological identification techniques, which can be unreliable as amphistomes contain cryptic species that are difficult to identify morphologically. The aim of this study was to use molecular approach based on analysis of nuclear ribosomal internal transcribed spacer 2 (ITS-2) and mitochondrial cytochrome oxidase I (COI) sequences to identify and characterize amphistome species collected from cattle in the KwaZulu-Natal and Mpumalanga provinces of South Africa and from Zimbabwe. Findings from ITS and COI are presented and the novel haplotypes thereof. It was also noted from the results of the study that there are a lot of misidentified samples deposited in the Genbank, which can be problematic when using this information to identify experimental samples based on their phylogenetic position in comparison with Genbank samples

Keywords: Amphistomes, molecular analysis, haplotypes, ITS2, COX1, South Africa, Zimbabwe

Abstract No: 4492

6 Sept 2017, 1230 – 1245

Characterization and functional studies of Serine/Threonine Protein Phosphatase 1 (PP1) encoding genes from *Schistosoma japonicum*

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¹College of Veterinary Medicine/ Huazhong Agricultural University/ China

Abstract Content

In the present study, three Serine/threonine protein phosphatase 1 encoding genes (*pp1c*) were identified in *Schistosoma japonicum* and their transcription and functions were investigated by real-time PCR, in-situ hybridization and RNA interference. Three PP1c proteins (*Sj-PP1-1*, *Sj-PP1-2* and *Sj-PP1-3*) belong to PP1beta, PP1 gamma and PP1 alpha subfamily, respectively. *Sj-pp1c* were all transcribed in both sexes and throughout development. They were predominantly expressed in gonad related organs such as the testis of male, the ovary or vitellarium of female as well as ootype surrounding area. RNAi silencing of three *Sj-pp1c* caused stunted growth of female and male worms, decreased cellular mitosis activities in ovary, vitellarium of females and testis and parenchyma of males, distinct morphological changes in female worms with significant smaller ovaries which were dominated by the presence of immature oocytes and low maturity of vitellarium surrounding by immature vitelline cells and a reduced diameter of the testicular lobes accompanied by a reduction of cell density in testes as well as empty seminal vesicles in male worms as well as remarkably reduced egg production and serious harassment on pairing behavior between female and male adults. In addition, a large number of differential expressed genes were found after knocking down *Sj-pp1c* which mainly involved in cell catabolism, lipid metabolism, cytoskeleton organization, muscular contraction and DNA replication. Our findings demonstrate PP1c may function in developmental and reproductive processes as well as being involved in the female-male interaction of *Schistosoma japonicum*.

Keywords: *Schistosoma japonicum*; protein phosphatase 1; RNA interference; development; reproduction

Abstract No: 4241

6 Sept 2017, 1245 – 1300

Human transportation as factor shaping the genetic structure of *Aedes albopictus* in Penang Island.

Nur Faeza Abu Kassim^{*1}; Nor Atikah Farhah Muhammad¹; Abdul Hafiz Ab Majid¹; Mustafa Fadzil Farid Wajidi²; Jamsari Amirul Firdaus Jamaluddin¹

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Abstract Content

Estimates of genetic structure of *Aedes albopictus*, the vector of dengue virus have provided insights into dengue epidemiology. To evaluate role of the vector in the changing pattern in Penang Island, Malaysia, studies on the genetic differentiation of *Ae. albopictus* inferred from microsatellite markers have been carried out. We assessed the molecular population genetic of *Ae. albopictus* in term of allelic variation, characterization of genetic diversity and population structure from differently urbanized settings. A total of 42 mosquitoes were sampled from Jelutong, Batu Maung and Balik Pulau which represented urban, suburban and rural area in Penang Island respectively and analyzed for polymorphism at six microsatellite loci. Analysis showed that there was less genetic differentiation between mosquito populations ($F_{ST} = 0.0362$). It is supported with admixture individuals observed in STRUCTURE and FCA suggested that high gene flow has been experienced between populations, suggesting that passive migrations through human transportation help to clarify this pattern of differentiation.

Keywords: Aedes albopictus; Genetic structure; Dengue; Microsatellite; Human transportation.

Abstract No: 5578

6 Sept 2017, 1600 – 1651

Parasite genomics – yea or nay?

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Abstract Content

Parasitic diseases remain a challenge to societies through their impact on human welfare and agricultural economy. Climate-related changes and the rapid development of antiparasitic drug resistance have further contributed to the exacerbation of the problem. These enormous and constant challenges create a pressing need for more effective and timely research solutions to advance our understanding of parasite biology and counteraction strategies. Since its initiation more than 2 decades ago, parasite genomics was found to present a better solution to accelerate parasitological research. The genomic data produced an overarching conceptual framework which permits the integration of biological datasets from different research disciplines in addressing important research questions and in turn, promote the rate of novel discoveries. In recent years, the advent of long-read sequencing technology which circumvents various bottleneck issues plaguing short-read sequencing genomics studies have further revolutionized the applicability of genomic approaches in comprehension of host-parasite systems. With careful data analysis design, the growth of parasite genomics will undoubtedly pave ways to exciting discoveries and illuminate many unexplored aspects of parasitological research. The question is, are more researchers willing to embrace this new research paradigm?

Keywords: long-reads sequencing technology: genomics

Abstract No: 4939

6 Sept 2017, 1615 – 1630

The battle against flystrike – past research and new prospects through genomics

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Abstract Content

Lucilia cuprina is a parasitic blowfly of major global importance to the livestock industry. Maggots of this fly parasitize the skin of animal hosts, feed on excretions and tissues, and cause severe disease (flystrike or myiasis). In Australasia alone, flystrike in sheep causes productivity losses estimated at \$320 million per annum. Although there has been considerable research on flystrike, little is understood about the molecular biology, biochemistry and genetics of this parasitic fly as well as its relationship with the host animal. No vaccine is available and resistance in blowfly against almost all available treatments demands new and innovative interventions. The sequencing, annotation and analyses of the draft genome of *L. cuprina* was an exciting possible answer to blowfly control and provided a critical foundation for which to build upon. Recent improvements to the genome, incorporating Dovetail Genomics Chicago sequencing + HiRise data, PacBio sequencing, and transcriptomic data from multiple life cycle stages and conditions, has resulted in a drastically refined 'draft 2' genome. In the near future, we will be able to test the function of genes and gene products of major biological significance using CRISPR, which should facilitate the development of novel interventions against flystrike.

Keywords: Lucilia; blowfly; myiasis; genome; transcriptome

Abstract No: 4601

6 Sept 2017, 1630 – 1645

Molecular characterization of matrix metalloproteinase gene (MMP-9) in *Oestrus ovis* larvae by Reverse Transcription Polymerase Chain Reaction (RT-PCR)

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Abstract Content

Oestrus ovis (sheep nasal fly) is responsible for nasal myiasis in sheep and also it is the cause of ophthalmomyiasis in human beings. Matrix metalloproteinases play a vital role in tissue penetration and migration of parasites. Hence, the present study was undertaken to detect the presence of matrix metalloproteinase gene (MMP-9) in larvae of *Oestrus ovis* by reverse transcription polymerase chain reaction. Mature larvae of *Oestrus ovis* were collected from sheep slaughtered at local abattoir in Thanjavur, Tamil Nadu. Live, intact mature larvae were washed thoroughly with Phosphate buffered saline (PBS, pH 7.4). Total RNA was isolated from the larvae of *Oestrus ovis*. Using standard protocol. The concentration and purity of RNA from 50 milligram of tissues was 0.184 and the ratio of absorbance A_{260} / A_{280} was 1.82 indicating that the isolated RNA was reasonably pure. Suitable primer was designed and used in the assay. RT-PCR was carried out and the PCR product was subjected to 1 per cent agarose gel electrophoresis. A 1080 base pair catalytic domain of MMP-9 gene was detected in RT-PCR. Based on this study, it was concluded that the activity of the MMP-9 gene in larvae of *O. ovis* was found to be very strong, and helping in the migration of the larvae from one site to the other.

Keywords: Oestrus ovis; MMP; RT-PCR;

Abstract No: 5019

6 Sept 2017, 1645 – 1700

MicroRNA-275 and its target Vitellogenin are crucial in ovary development and blood digestion of *Haemaphysalis longicornis*

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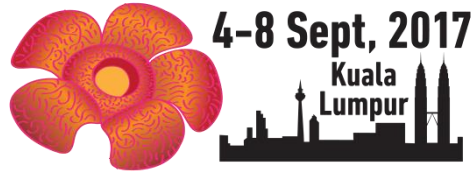
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Abstract Content

Haemaphysalis longicornis is widely distributed in eastern Asia, New Zealand and Australia and is considered the major vector of *Theileria* and *Babesia*, harmful to humans and animals. Female ticks have an important role in the tick life cycle. Therefore, elucidation of the underlying molecular mechanism of *H. longicornis* development and reproduction is needed. Luciferase assays were used to identify the targets of miR-275 *in vitro*. RNAi of Vitellogenin (Vg) was used in phenotype rescue experiments of ticks with miR-275 inhibition, and these analyses were used to identify the authentic target of miR-275 *in vivo*. The expression of miR-275 in different tissues and developmental stages of ticks was assessed by qPCR. To elucidate the functions of miR-275 in female ticks, we injected a miR-275 antagomir into female ticks and observed the phenotypic changes. We identified Vg as an authentic target of miR-275 both *in vitro* and *in vivo* by luciferase assays and phenotype rescue experiments. MiR-275 is often regulated in a developmental stage- and tissue-specific manner. Silencing of miR-275 resulted in fewer eggs ($p < 0.0001$), blood digestion problems and substantially inhibited ovary development. Furthermore, RNAi silencing of Vg not only impacted the blood meal ($p < 0.05$) but also the number of eggs laid ($p < 0.05$). Vg RNAi in a miR-275 inhibition background partially recovered the phenotype observed in miR-275-depleted ticks. This study is first to demonstrate that miR-275 targets Vg in *H. longicornis* and regulates blood digestion and ovary development. These findings may contribute to elucidating the underlying molecular mechanisms of development and reproduction in ticks.

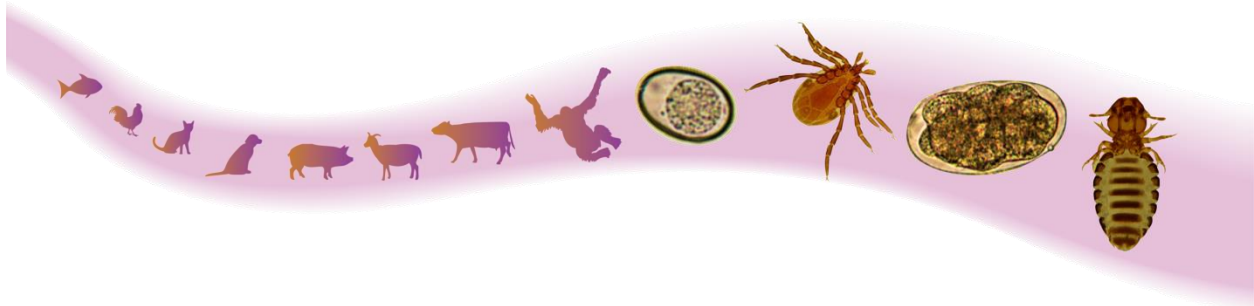
Keywords: *Haemaphysalis longicornis*; MicroRNA; Vitellogenin; Blood digestion; Ovary development

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Seasonal variation in *Angiostrongylus vasorum* in red foxes (*Vulpes vulpes*) in the Greater London area

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Abstract Content

The red fox is an important wild animal reservoir of *Angiostrongylus vasorum* for domestic dogs though little is known of the seasonal variation in prevalence and intensity of parasite infection. One hundred and seventy-six foxes, culled routinely by licenced local authority pest control officers in Greater London between January and December 2016, were examined at necropsy. The adult parasite burden was established by careful dissection of the right side of the heart and pulmonary artery and the larval burden by lung perfusion and Baerman examination of alimentary tract contents. The overall prevalence of infection was 74.4% (CI 69.3%-80.1%); there was no significant difference in prevalence during the year. The median number of adult parasites was 6 (range, 1-157), of larvae recovered from the lungs of infected foxes was 19 (range, 0-136,724) and from alimentary tract contents was 1.2 per g (range, 0-3007). There was a significant association between parasite prevalence and host age (cubs, 50.0%; juveniles, 84.1% and adults, 78.2%; $P < 0.001$) though none with host gender. Indirect evidence for the development of an acquired immunity with age included a lower intensity of infection and impaired growth of adult worms in adult foxes compared with juveniles and cubs; fecundity was unaffected by host age. Preliminary molecular analysis revealed two cytochrome oxidase subunit I genotypes, one previously detected in *A. vasorum* recovered from foxes in Denmark, the second described for the first time. This study confirms the importance of red foxes as a source of *Angiostrongylus* infection for dogs throughout the year.

Keywords: Angiostrongylus; Fox; London; Prevalence; Seasonal

Abstract No: 4347

6 Sept 2017, 0915 – 0930

The hidden faces of a biological invasion: parasite dynamics of invaders and natives

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Abstract Content

One of the primary drivers of emerging infectious diseases (EIDs) is human intervention via host or parasite translocations. A unique opportunity to study the processes involved in EIDs, currently exists in Ireland due to the introduction of the bank vole (*Myodes glareolus*), via Germany in the 1920's. The continuing range expansion of the bank vole within Ireland presents a natural large-scale perturbation experiment, with the bank vole currently established in one third of the country. The primary objective of this study is to use the Irish bank vole model to conduct a spatiotemporal study analysing the parasite dynamics of native and invasive species throughout their range, with particular emphasis on the invasion front. Bank voles and native woodmice have been trapped in woodlands throughout Ireland and surveyed for their macroparasites. 16SrRNA sequencing was used to detect major genera of pathogenic bacteria. Bank voles in Ireland were found to have much less parasite diversity and a smaller community of pathogenic bacteria in comparison to bank voles from across Europe and the native woodmice. Furthermore voles at the expansion front are less parasitised than those from the core population. This "enemy release" is believed to be mediating their continued successful spread across Ireland. Results also demonstrate the presence of the bank vole has impacted the parasite dynamics of the native woodmouse through the processes of parasite dilution, spill-over and spillback. The study demonstrates how a bio-invasion alters the disease dynamics of a system, influencing the invasion and leading to EIDs.

Keywords: Infection dynamics; Emerging Infectious Diseases; invasive species

Increasing prevalence of *Angiostrongylus vasorum* in wild Swiss red foxes between 2012-2017 and evaluation of serological procedures

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Abstract Content

Angiostrongylus vasorum is a cardiopulmonary nematode increasingly found in dogs and foxes throughout Europe. We investigated the prevalence, worm burden and regional distribution of lungworms in wild Swiss red foxes and evaluated ELISAs for detection of circulating *A. vasorum* antigen and specific antibodies, which had been previously developed for dogs. The necropsy of totally 467 foxes revealed an increasing prevalence for *A. vasorum* from 20.5% (WB: 1-30, mean 7.3) in 2012 to 75.5% (WB: 1-50, mean 11.3) in 2017, while the prevalence of *Capillaria aerophila* and *Crenosoma vulpis* was fluctuating between 33.3% and 74.7% (WB: 1-99, mean 6.4) and between 3.6% and 14.9% (WB: 1-48, mean 6.2), respectively. Antigen detection in naturally infected wild Swiss foxes had 91.2% sensitivity and 89.4% specificity, whereas the corresponding figures for antibody detection were 42.2% and 92.0%. Serological findings obtained from experimentally inoculated farmed foxes confirmed a trend comparable to dogs for antigen detection, while the antibody responses were highly variable, with decreasing optical density values despite persistent infections with high worm burdens. Conclusively, ELISAs are reliable and quick methods to detect *A. vasorum* in foxes. The parasite is established in the Swiss fox population with yearly increasing prevalence. We hypothesise that infected foxes develop a variable and non-protective immunity, allowing long term survival of *A. vasorum* in this definitive host, considered relevant for parasite transmission. This may explain the epidemiological dynamic of *A. vasorum* that is evident in many parts of Europe over the last decades.

Keywords: Angiostrongylus vasorum, foxes, serology, epidemiology, immunology

New insights in the epidemiology and diagnostics of three species of *Angiostrongylus* infecting wild carnivores in Europe

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Abstract Content

Three species of *Angiostrongylus* are parasitic in European carnivores: *A. vasorum*, *A. daskalovi* and *A. chabaudi*. In various European countries, the epidemiology of *A. vasorum* has been studied in detail due to its significant clinical impact on domestic dogs. So far, no data about its presence in Romania has been documented. Additionally, the other two species are generally poorly known. The aim of our study was to investigate the epidemiology of these three species of *Angiostrongylus* in wild carnivores in Romania. Moreover, due to the possibility that the host specificity of *Angiostrongylus* spp. in European carnivores might be relatively low, our aim was to evaluate the cross-reactivity of *A. chabaudi* and *A. daskalovi* with *A. vasorum* using a commercial serologic test developed for domestic dogs. Overall, 602 foxes, 11 badgers, 49 wildcats and 81 jackals have been examined by full necropsy and vascular parasites were collected. Additionally, from each badger and wildcat, a serum sample has been examined using IDEXX, AngioDetect. The prevalence of infection was as follows: *A. vasorum* in foxes (3.9%), *A. daskalovi* in badgers (45.4%) and *A. chabaudi* in wildcats (6.1%). All jackals were negative. Serological examination of the badgers and wildcats revealed the positivity of the same samples as in necropsy. Although this commercial test was used off-label, our results bring evidence for a possible immunological cross reactivity between *A. vasorum* and other *Angiostrongylus* species infecting European carnivores. Moreover, this is the first report of *A. vasorum* in Romania.

Keywords: Angiostrongylus vasorum, Angiostrongylus daskalovi, Angiostrongylus chabaudi, wildlife, Romania

Parasitic infections of veterinary importance in foxes in the Netherlands.

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Abstract Content

From the North Eastern part of the Netherlands carcasses from free ranging red foxes (*Vulpes vulpes*) were examined for parasitic infections. The foxes were shot by hunters and their complete carcasses were sent to the Dutch Institute for Public Health and the Environment. The carcasses were stored at -80 degrees Celsius for at least one week. After thawing their faeces was quantitatively investigated for eggs of *Toxocara canis* and qualitatively for other parasite eggs. Adult *Toxocara* worms in the small intestines were collected and counted. The hearts and lungs were flushed for the detection of *Angiostrongylus vasorum*, *Crenosoma vulpis* and *Eucoleus aerophilus*. Nasal cavities were opened for detection of *Eucoleus boehmi* and the urine bladder for *Pearsonema plica*. The study is still ongoing and prevalence of these parasites of veterinary importance and the intensity of infection with the zoonotic roundworm *T. canis* will be presented. So far, 159 foxes have been examined with preliminary prevalences of 64% (*T. canis*), 34% (*A. vasorum*), 35% (*C. vulpis*), 86% (*E. aerophilus*), 98% (*E. boehmi*) and 85% (*P. plica*). Where living areas of foxes are shared by walking areas of dogs transmission of these parasites to dogs can occur. The prevalence of *A. vasorum* in dogs from this part of the Netherlands is unknown and this study will provide useful epidemiological information about this parasite that can lead to severe illness in dogs.

Keywords: Foxes, intestinal parasites, lungs, nasal cavities, lungs, EPG

First Report of *Cryptosporidium* species in Captive Wildlife of India

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Abstract Content

Seven hundred and eighty eight faecal samples were collected from 127 captive wildlife species of three national zoological parks viz., Sri Venkateswara Zoological Park, Tirupati (n=242); Indira Gandhi Zoological Park, Visakhapatnam (n=218); Nehru Zoological Park, Hyderabad (n=328), in Andhra Pradesh and Telangana states of India for detection of *Cryptosporidium* infection. Modified Ziehl-Neelsen (mZN) staining method was adopted for preliminary screening, and then mZN staining positive isolates (n=57) were confirmed by nested PCR targeting 18S rRNA *Cryptosporidium* gene. Nested PCR amplicons were sequenced to determine the *Cryptosporidium* species. The highest prevalence of *Cryptosporidium* infection was detected in the faecal samples of wildlife at Nehru Zoological Park, Hyderabad (8.23%), followed by Sri Venkateswara Zoological Park, Tirupati (7.44%) and Indira Gandhi Zoological Park, Visakhapatnam (5.50%). The highest prevalence of *Cryptosporidium* was observed in rodents (18.18%) followed by reptiles (11.54%), primates (11.11%), herbivores (9.29%), birds (7.79%) and the lowest was recorded in carnivores (1.54%). Randomly selected nested PCR *Cryptosporidium* positive amplicons were subjected to sequencing and determined five species viz., *Cryptosporidium parvum*, *C. ryanae*, *C. suis*, *C. muris* and *Cryptosporidium* avian genotype III. The zoonotic species, *C. parvum* was recorded in one rescued elephant calf of Sri Venkateswara Zoological Park, Tirupati. The observations in the current study conclude that, for the first time *Cryptosporidium* infection was reported in the captive wildlife of India and also alerts the zoo authorities to take up the suitable preventive measures in spreading of this infection keeping in view of its public health significance.

Keywords: *Cryptosporidium*; Captive wildlife; India

Abstract No: 3838

6 Sept 2017, 1100 – 1115

Occurrence of *Babesia rossi* in black-backed jackals, African wild dogs and domestic dogs in South Africa

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Abstract Content

Numerous studies have reported on the occurrence of *Babesia rossi* in domestic dogs in South Africa, but information on occurrence of *B. rossi* in free-ranging indigenous canids, e.g. African wild dogs and black-backed jackals, is lacking. This study aimed at investigating the occurrence of *B. rossi* in black-backed jackals (n = 104) from Mogale's Gate Biodiversity Centre (Gauteng province) and S.A. Lombard Nature Reserve (North West Province), African wild-dogs (n=36) from DeWildt Cheetah and Wildlife Centre (North West Province) and also in domestic dogs (n=75) presented as patients at the Onderstepoort Veterinary Academic Hospital, South Africa. Preliminary results of Reverse Line Blot hybridization assay revealed an overall *B. rossi* occurrence of 53.07%, with the highest occurrence (88%) detected in domestic dogs. Mixed infection of *B.rossi* (sensu stricto) and *Hepatozoon* sp has been observed in the wild canine specimens. The sequencing results of the 18S rRNA gene confirmed the detection in black-backed jackals, African wild-dogs and domestic dogs to be *B. rossi* (sensu stricto). Future studies will focus on investigating the genetic diversity of *B. rossi* in domestic dogs, African wild dogs and black-backed jackals, with emphasis on the relatedness of the strains occurring in the three canine hosts.

Keywords: Babesia rossi; domestic dogs ; wild canine hosts ; South Africa

Detection of *Babesia* spp. DNA in British wild carnivores

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Abstract Content

Babesia are small piroplasmid parasites with a worldwide distribution, that can be transmitted to hosts through the bite of infected ticks. Red foxes (*Vulpes vulpes*) and Eurasian badgers (*Meles meles*) are found throughout Great Britain. This study aims to demonstrate the prevalence and species of *Babesia* found in these two carnivorous species. Bloody lung exudate samples were collected from 316 foxes, while 47 blood and spleen samples were collected from badgers. DNA was extracted from all samples and tested by PCR for the presence of *Babesia* 18S rRNA gene DNA. In the fox samples 46/316 (14.6%) tested positive for the presence of *Babesia annae*. Sequence analysis of the *B. annae* DNA in the British fox samples showed it to have 100% sequence identity to *Babesia* sp.-'Spanish Dog'. In the badgers, 28/47 (59.6%) blood and 14/47 (29.8%) spleen samples tested positive for the presence of *Babesia* DNA. Sequence analysis identified three distinct *Babesia* sequence types (UK-Type A, UK-Type B1 and UK-Type-B2), which were closely related to *Babesia* sp. parasites previously identified in badgers in Spain. Badgers were routinely infected with more than one parasite isolate and there was evidence of genetic recombination between the *Babesia* parasite isolates. Our results show that *Babesia* infections are well established and widespread throughout the fox and badger populations. Both species of *Babesia* show host specificity, *B. annae* for foxes and *Babesia* sp. for badgers, even though the animals were collected from overlapping locations and will carry the same tick species.

Keywords: Babesia, Foxes, Badgers, UK, sequencing

Epidemiology of gastrointestinal nematodes of alpacas in Australia

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Veterinarian/ Australia

Abstract Content

Gastrointestinal nematodes (GINs) can cause production losses and death in alpacas (*Vicugna pacos*). Very little is known about the prevalence of GINs of alpacas in Australia. This study was designed to determine the prevalence of GINs and their epidemiology in Australia. A longitudinal study was conducted on 13 selected alpaca farms located in two different climatic zones of Australia. Monthly faecal samples ($n = 15$) were collected from each farm and analysed using faecal egg counts (FEC), larval cultures and Multiplexed Tandem PCR (MT-PCR). Gastrointestinal tracts ($n = 87$) were also examined to assess worm burdens. Data revealed that 61% (1040/1692) of faecal samples were positive for GINs, with a mean (\pm standard error of mean) of 167 ± 14 for eggs per gram (EPG). The highest EPG was 15,630. The mean EPG was higher in warm summer and cold winter zones (winter rainfall) than hot and humid summer rainfall zones. Larval culture revealed the presence of *Haemonchus* spp., *Trichostrongylus* spp., *Ostertagia/Teladorsagia* spp., *Cooperia* spp. and *Oesophagostomum* spp. MT-PCR assay also identified above mentioned GINs including *Camelostrongylus* spp. Total worm counts (TWC) revealed the presence of 13 species/genera of GINs in alpacas. The main five genera/species were *Camelostrongylus mentulatus*, *Haemonchus contortus*, *Trichostrongylus* spp., *Cooperia* spp. and *Nematodirus* spp. Small numbers of *Graphinema aucheniae* were also found. Results of this study suggest that Australian alpacas harbour shared-GINs. This study provides significant information on the prevalence of GINs of Australian alpacas which could be used to develop control strategies against them.

Keywords: Alpaca; GINs; Epidemiology; MT-PCR; Prevalence

Abstract No: 4070

6 Sept 2017, 1145 – 1200

Wild deer as reservoirs of agriculturally important gastrointestinal parasites in Eastern Australia

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Abstract Content

Deer were originally introduced into Australia by Acclimatisation Societies in the 19th and early 20th centuries. Six species of deer are now established in Australia, the Asiatic species of sambar, hog, rusa and chital and European fallow and red deer. Deer have adapted well to the Australian environment, with a population that may be over 1 million animals in Eastern Australia. Despite common sightings of deer in forests, on forest-farm boundaries, in farmland and in peri-urban areas, very little is known about wild deer populations in Australia. Of concern is the potential for wild deer populations to act as disease reservoirs, contributing to the spread of disease to livestock, as demonstrated in Europe, America and Asia. Deer are protected in the south eastern Australian state of Victoria. Populations in Victoria are thought to be increasing, and interactions between deer and economically important ruminants (sheep and cattle) occur frequently through co-grazing of pastures. This research aims to investigate the presence of economically important nematodes and liver fluke in Victorian deer populations; and assess the potential for transmission to ruminant livestock. PCR assays and mitochondrial DNA sequencing will be performed on parasite eggs and worms isolated from wild deer faecal samples. Initial PCR screening has shown that 63% of wild deer are carrying a strongylid parasite burden, including *Haemonchus* species, an important sheep parasite. The presence and prevalence of other parasites, including *Fasciola hepatica*, *Ostertagia*, *Teladorsagia*, *Cooperia* and *Trichostrongylus* species in wild deer will be discussed.

Keywords: Fasciola hepatica; gastrointestinal nematodes; deer; zoonosis; ruminant

Increased genetic diversity and prevalence of co-infection with *Trypanosoma* spp. in koalas (*Phascolarctos cinereus*) and their ticks identified using Next-Generation Sequencing (NGS)

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Abstract Content

Infections with *Trypanosoma* spp. have been associated with decreased survival of koalas (*Phascolarctos cinereus*), particularly in the presence of concurrent pathogens such as *Chlamydia* and koala retrovirus. The present study describes the application of a next-generation metabarcoding assay to characterize the prevalence and genetic diversity of trypanosome communities in koalas and two native species of ticks (*Ixodes holocyclus* and *I. tasmani*) removed from koala hosts. Among 168 koalas tested, 32.2% (95% CI: 25.2-39.8%) were positive for at least one *Trypanosoma* sp. Previously described *Trypanosoma* spp. from koalas were identified, including *T. irwini* (32.1%, 95% CI: 25.2-39.8%), *T. gilletti* (25%, 95% CI: 18.7-32.3%), *T. copemani* (27.4%, 95% CI: 20.8-34.8%) and *T. vegrandis* (10.1%, 95% CI: 6.0-15.7%). *Trypanosoma noyesi* was detected for the first time in koalas, although at a low prevalence (0.6% 95% CI: 0-3.3%), and a novel genotype (*Trypanosoma* sp. AB-2017) was identified at a prevalence of 4.8% (95% CI: 2.1-9.2%). Mixed infections with up to five species were present in 27.4% (95% CI: 21-35%) of the koalas, which was significantly higher than the prevalence of single infections (4.8%, 95% CI: 2-9%). Co-infections involving *T. gilletti*, *T. irwini*, *T. copemani*, *T. vegrandis* and *Trypanosoma* sp. AB-2017 were also detected within the ticks examined, indicating they are vector candidates for *Trypanosoma* spp. The present study provides new insights on the natural genetic diversity of *Trypanosoma* communities infecting koalas and constitutes a benchmark for future clinical and epidemiological studies required to quantify the contribution of trypanosome infections on koala survival rates.

Keywords: *Trypanosoma*; Next-generation sequencing; koala; polyparasitism; 18S rRNA gene

Gastrointestinal parasites of free-ranging long-tailed macaques (*Macaca fascicularis*) in Puerto Princesa Subterranean River National Park, Palawan, Philippines

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Abstract Content

Ecotourism and forest encroachment in the Philippines created avenues where human-macaque interactions occur. This study was undertaken to investigate the presence of intestinal parasites in free-ranging long-tailed macaques (*Macaca fascicularis*) within the Puerto Princesa Subterranean River National Park, Palawan, Philippines. Thirty five fecal samples were collected opportunistically during transect walks. To ensure that one sampling unit is equivalent to one individual, sampling points with distance not less than 10 meters were considered throughout the collection. Helminth eggs, larvae, and protozoan cysts were isolated by means of formalin-ethyl acetate concentration technique. Of the 35 samples, 30 (85.71%) were positive for gastrointestinal parasites comprising of four nematodes (*Ascaris* sp., *Strongyloides* sp., *Trichuris* sp. and hookworm), one cestode (*Hymenolepis nana*), and eight protozoans (*Blastocystis* sp., *Chilomastix mesnili*, *Endolimax nana*, *Entamoeba coli*, *Entamoeba polecki*, *Entamoeba* spp. and *Iodamoeba butschlii*). Among parasitic helminths, hookworm was the most prevalent (42.86 %), followed by *Strongyloides* sp. (25.71%), *Trichuris* sp. (22.86%), *Ascaris* sp. (11.43%), and *H. nana* (2.86%). Among protozoans, *E. coli* showed the highest prevalence (40%), followed by *I. butschlii* (37.14%), *E. nana* (28.57%), *Entamoeba* spp. (25.71%), *Blastocystis* sp. (22.86%), *C. mesnili* (20%), and *E. polecki* (20%). Multiple infections were also observed in 63.33% (19/30) of those positive for gastrointestinal parasites. These findings show that wild populations of long-tailed macaques could serve as sentinels in the monitoring of infectious diseases especially where human and macaque territories overlap. This study recommends scatological and molecular research to further understand enteroparasitic infections occurring in long-tailed macaques.

Keywords: gastrointestinal parasites; long-tailed macaques; Palawan

Seasonal and socio-ecological influences on parasite communities of sympatric Malagasy lemur species (*M. murinus* and *M. ravelobensis*)

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Abstract Content

Parasitic infections of endangered wildlife species have become a major concern in conservation biology due to the potential for disease transmission and the possible negative effects on an individual and population level. This project investigates ectoparasite communities of two closely related, similar-sized, Malagasy primate species (*Microcebus murinus* and *Microcebus ravelobensis*) and evaluates seasonal and socio-ecological influences on parasite infestation. Both study species occur sympatrically in the seasonal, dry deciduous forests of the Ankarafantsika National Park in north-western Madagascar, but show distinct differences in sleeping site ecology. Mouse lemurs were regularly trapped and sampled for parasites and collected ectoparasite specimens were identified by morphological and genetic analysis. Individuals of *M. murinus* and *M. ravelobensis* were found infected with the same tick (*Haemaphysalis* sp.), lice (*Lemurpediculus verruculosus*) and mite species (Trombiculidae sp., Laelaptidae sp.). Season and host species-specific differences in sleeping site ecology and sociality were detected to influence ectoparasite prevalence. More specifically, *M. murinus*, sleeping mostly solitarily in tree holes, showed a significantly higher mite prevalence, whereas lice infestation was higher in the group-sleeping *M. ravelobensis* who preferred open nests. Seasonal variations in tick prevalence provide evidence for an univoltine life cycle of the detected *Haemaphysalis* species. Our study points to complex host-ectoparasite dynamics, and detection of the same parasite species infesting the two mouse lemur hosts indicates a potential cross-species pathway for pathogen transmission. Analyses of simultaneously collected blood samples will complement the picture and enhance our knowledge on host-parasite transmission pathways in the tropics.

Keywords: Lemurs; Ectoparasites; Seasonality, Socio-ecology

Abstract No: 5476

6 Sept 2017, 1245 – 1300

Prevalence of endoparasites on laboratory rats at the Laboratory Animal Facility and Management (LAFAM), UiTM Selangor, Malaysia

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Abstract Content

A study on prevalence of endoparasites of laboratory rats was conducted between March and May 2016 at LAFAM. The faecal samples were collected from a total of 187 adult healthy breeding stock rats (3-4 years old) which included 112 Sprague-Dawley and 75 Wistar rats. The faecal samples were examined by direct smear technique as well as faecal floatation technique for parasitic larva, eggs and/or oocysts. Out of the 187 faecal samples examined, 35.83% were found positive for endoparasites. Prevalence of endoparasites was significantly higher in Wistar (54.67%) than in Sprague-Dawley 23.21% (26 of 112) ($P=0.00$). The most prevalent nematode parasites in both species were *Syphacia muris* and *Syphacia obvelata*, with prevalence of 68.66% and 26.87%, respectively. *Aspiculuris tetraptera* was also found in Wistar (7.32%). The prevalence of different species of parasites with their respective hosts were *S. muris* 20 (76.92%) in Sprague-Dawley, 26 (63.41%) in Wistar and prevalence of *S. obvelata* 6 (23.08%) in Sprague-Dawley, 12 (29.27%) in Wistar and prevalence of *A. tetraptera* nil in Sprague-Dawley, 3 (7.32%) in Wistar. This breeding stock indicates the high indication of parasitic load. These infected rodents should not be used for research purpose as this will interfere with the blood parameters.

Keywords: Endoparasite, faecal sample, Syphacia muris, Syphacia obvelata, Aspiculuris tetraptera

Abstract No: 4212

6 Sept 2017, 1600 – 1615

Parasitism of ants by larval *Dicrocoelium dendriticum* revealed by micro-computed tomography

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Abstract Content

Larvae of the parasitic flatworm, *Dicrocoelium dendriticum*, are well known for inducing a sacrificial behaviour in their intermediate ant hosts. At low temperatures, infected ants climb to the top of flowers and other low stature vegetation to which they cling with their mandibles. This apparent tetany can be reversed as temperatures rise, but during it the ants and their parasites are exposed to a high probability of ingestion by grazing ruminants in which the adult stages of the flatworm develop. Traditional techniques used to image the parasites inside the ants are challenging because of the ants' hard cuticle and fragile brain tissues. However, X-ray micro-computed tomography facilitates a non-destructive, virtual histological dissection at any orientation to reveal the 3D structure of biological objects. Here, we stained infected *Formica aserva* with phosphotungstic acid (PTA) and then scanned their decapitated heads and separated abdomens using a ZEISS Xradia 520 Versa. Our study demonstrated that micro-CT imaging provides unparalleled novel insights into the relationship between *D. dendriticum* and the intermediate ant host, including the nature of the host-parasite interface within brain tissue. For the first time, we were able to show co-infection of an ant head with the so-called 'brain worm' and encysted metacercariae. Previously, encysted metacercariae had only been reported to reside within the abdomen. We also show infection of the brain with up to three metacercariae, providing qualitative information on the location of infection within the brain and quantitative data on the parasites themselves, including the volumes of the different parasite forms.

Keywords: trematode; intermediate host; ant

Chigger mite infestation in *Mantella baroni* frogs illegally imported from Madagascar to France

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²Laboratory/ VetDiagnostics/ France

Abstract Content

Madagascar is inhabited by a species-rich anuran fauna. Almost all species are endemic, with the family Mantellidae displaying highest diversity concerning ecological demands, reproductive diversity and species numbers. During airport inspection in Paris in April 2016, a set of 45 Mantellidae frogs (*Mantella baroni*) were seized by the customs for illegal importation. Thirty of these frogs presented erythematous cutaneous lesions on the ventral side of forelimbs and on the belly. Bacterial and mycological cultures (including *Batrachochytrium dendrobatidis*) were negative. Histological examination revealed an hyperplasic and granulomatous dermatitis with numerous intralesional arthropods. Direct microscopic examination of skin scrapings indicated that the arthropods were chigger mites and more precisely larvae of the genus *Endotrombicula*. To control the disease, different strategies were combined: (i) manual removal of the mites; (ii) bath in a solution containing 10mg/L ivermectin during 1h, every two weeks and (iii) disinfection of the environment. Complete recovery was obtained after 23 weeks. Chigger mites are uncommon parasites of amphibians. The genus *Endotrombicula* Ewing, 1931 is known from larvae only and originally included the type species *E. penetrans*. The geographic distribution of *Endotrombicula*, restricted to Africa, the Arabian Peninsula and Madagascar, suggests that these mites invaded Madagascar from the African continent.

Keywords: Endotrombicula, frogs, Madagascar

Molecular evidence for black flies (*Simulium* spp.) as vectors of an uncharacterized *Onchocerca* species

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¹Infectious Diseases/ University of Georgia/ United States, ²Global Health/ University of South Florida/ United States, ³Disease Surveillance/ San Gabriel Valley Mosquito & Vector Control District/ United States, ⁴Vector Control/ Greater Los Angeles County Vector Control District/ United States, ⁵Veterinary Public Health Program/ Los Angeles County Department of Public Health/ United States

Abstract Content

The role of different black fly (Diptera; Simuliidae) species as vectors of filarial nematodes of the genus *Onchocerca* (Nematoda; Onchocercidae) infecting wild ungulates in North America is scarcely known. To understand the vector species of *Onchocerca* in the Los Angeles County, southern California, USA, 1056 female black flies were collected from 39 sites using Carbon Dioxide-baited mosquito traps from March to November, 2015. Black flies were morphologically identified to species complex: *Simulium tescorum* (356), *Simulium vittatum* s.l. (683), and additional 17 specimens were not assessed morphologically. Specimens were individually processed for DNA extraction. A nested PCR targeting the cytochrome oxidase subunit 1 (COI) gene of filarial nematodes was performed. Products of PCR-positive samples were sequenced. Phylogenetic analyses were performed in MEGA 7. *Onchocerca* sequences were detected in 4.8% (n=17) *S. tescorum* samples, and only one *S. vittatum* (0.15%), from 6 sites in 3 cities (Azusa, Glendora and San Dimas). COI sequences were reciprocally monophyletic (99.5-100% pairwise identity; PI), and most similar to *Onchocerca gutturosa* (93.9-94.9% PI) and not to *Onchocerca cervipedis* (89.5-90.2% PI), as hypothesized. PCR targeting the cytochrome B of vertebrate species to assess from which hosts the black flies acquired a blood meal detected DNA of *Odocoileus* deer, natural host of *O. cervipedis* and of two other *Onchocerca* species in Eastern USA pending characterization. *Simulium tribulatum* from the same area is the putative vector of zoonotic *Onchocerca lupi*. This is the first evidence of *S. tescorum* and *S. vittatum* s.l. as putative vectors of an uncharacterized *Onchocerca* species.

Keywords: Onchocerca; vector; black fly; Simuliidae

Detection and quantification of *Toxoplasma gondii* in tissues of harvested wildlife in the Arctic

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Abstract Content

Toxoplasma gondii, a zoonotic apicomplexan protozoal parasite, infects mammals and birds worldwide. Infection is asymptomatic, though illnesses occur in immunocompromised people and fetuses of susceptible women. In Nunavik, Canada, 60% of Inuit are seropositive to *T. gondii* compared to 33% of the global population. Despite studies highlighting wildlife consumption as a risk factor, no information exists on the *T. gondii* infection status of wildlife tissues. Until recently, knowledge of tissue infection in animals has been scarce since small quantities of tissue (100 milligrams) were used to extract parasite DNA. Being able to detect and quantify *T. gondii* in animal tissues harvested for food is important to ensure food safety. A recently developed magnetic capture DNA extraction and Taqman real-time PCR protocol was optimised to detect *T. gondii* from large amounts of tissues (up to 100 grams) of 453 hunter-harvested animals: 166 ptarmigan, 156 geese, 61 ringed seals, 39 foxes and 31 caribou. DNA of the type II *T. gondii* strain was detected in 44% (CI: 28-60%) of foxes and 9% (CI: 3-15%) of geese, but was not detected in other wildlife including seropositive ringed seals and caribou. In positive geese, parasite DNA was present in brain, heart, muscle, liver and gizzard with parasite concentrations ranging between 150-2500 tachyzoites per 100 grams of tissue. This is the first account of *T. gondii* detection and quantification in tissues of hunter-harvested wildlife in the Arctic. A risk assessment of Inuit exposure to *T. gondii* from the consumption of geese will now be developed.

Keywords: Toxoplasma gondii, zoonosis, foodborne pathogen, wildlife

Abstract No: 4247

6 Sept 2017, 1700 – 1715

Gastrointestinal parasites of free-ranging Sperm Whales (*Physeter macrocephalus*) from the Mediterranean Sea

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Abstract Content

Sperm whales (*Physeter macrocephalus*) are the largest of the toothed whales and represent the only member of the family Physeteridae. With the present survey we provide the first report on the gastrointestinal helminth and protozoan parasite fauna of a free-living subpopulation of sperm whales inhabiting the Mediterranean Sea. A total of 25 individual sperm whale scat samples were collected from free-ranging animals during behavioral and ecological surveys in the summer of 2016. Samples were analyzed via classical parasitological diagnostic methods, such as the sodium acetate acetic formalin (SAF) method, carbol fuchsin-stained faecal smears and *Giardia/Cryptosporidium* coproantigen ELISAs. Additionally, an *Anisakis*-specific PCR was applied. Overall, 92% (23/25) of the sperm whales were infected with at least one parasite species. In total, six different gastrointestinal parasite species belonging to protozoan and metazoan taxa were detected. The most prevalent parasite was *Anisakis physeteris* (80%), followed by Diphylobothriidae gen. sp. (52%), *Zalophotrema* (40%), *Giardia* (16%), *Cystoisopora* (12%) and *Balantidium* (8%) species. In addition, spirurid-like eggs and *Cystoisopora*-like oocysts were identified. Thus, the current study provides first records on the occurrence of *Giardia* sp. and *Balantidium* sp. in sperm whales. Four of the detected parasite species bear an anthroponotic potential: *A. physeteris*, *Balantidium*, Diphylobothriidae gen. sp. and *Giardia* sp. PCR characterization confirmed the presence of *A. physeteris*. The current study delivers reference data for future monitoring surveys. Further studies on the impact of parasitic infections on the sperm whale subpopulation of the Mediterranean Sea are planned.

Keywords: Physeter macrocephalus; sperm whales; parasites

Lufenuron for the prevention and control of *Lepeophtheirus salmonis* and *Caligus elongatus* infesting farmed Atlantic salmon: efficacy in field studies (Canada) and against multi-resistant *L. salmonis* (Norway)

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¹Global R&D/ Elanco Australasia Pty Limited/ Australia, ²Clinical Studies/ AlcheraBio LLC/ United States, ³Veterinary Services/ Kelly Cove Salmon Limited/ Canada, ⁴Clinical Studies/ Midwest Veterinary Services/ United States, ⁵Clinical Services/ Endris Consulting/ United States, ⁶Global R&D/ Elanco Animal Health Inc./ United States, ⁷Statistics/ ATS Limited Inc./ United States, ⁸Global R&D/ Elanco France/ France, ⁹Global R&D/ Elanco UK/ United Kingdom, ¹⁰Emerging Markets, Aqua/ Elanco Norge/ Norway

Abstract Content

Sea lice are recognized as the main health challenge in salmonid farming with resistance to multiple classes of lousicides reported in most farming regions. Field studies at three commercial farms in Canada assessed the efficacy against sea lice of the benzoyl phenyl-urea, lufenuron formulated as a 10% pre-mix 'AH-2178'. Treatment of smolts was at fresh water hatcheries *via* medicated feed at a target dose rate of 5 mg/kg/day for 7 days. Approximately 625,000 Atlantic salmon smolt were administered either un-medicated feed; (n = 108,000) or AH-2178 medicated feed (n = 517,000). Sea transfer occurred shortly after treatment. While study design varied, efficacy was determined by comparing arithmetic mean louse counts on treated and untreated fish at regular intervals. In summary, oral administration of AH-2178 to Atlantic salmon pre-transfer to the three marine sites provided effective protection (greater than 90%; $P \leq 0.05$) against infestations of *L. salmonis* for 39.7, 36.5 and 35 weeks (approximately 9 months) and against *Caligus elongatus* for 25.3, 28.5 and 30.7 weeks (up to 7 months). These results are complemented by a Norwegian field study that demonstrated AH-2178 provided effective protection against *L. salmonis* with resistance and/or reduced treatment effects to deltamethrin, azamethiphos, hydrogen peroxide and emamectin benzoate. The same study also demonstrated reduced hatchability of eggs in multi-resistant *L. salmonis* that had fed on AH-2178 treated fish after loss of protection; hatching rates were 5x times less compared to strings collected from untreated fish. No drug related adverse events were reported from the studies.

Keywords: *Salmon*; *lufenuron*; *efficacy*; *Lepeophtheirus*; *salmonis*

Abstract No: 4254

6 Sept 2017, 1730 – 1745

Morphological phylogeny of fish crustacean parasites (Isopoda: Cymothoidae)

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Abstract Content

The family Cymothoidae consists of obligate fish crustacean parasites, with more than 380 species in 43 genera. Different genera display high host and site specificity (e.g. burrowed inside the flesh, branchial or buccal cavity). In the past 10 years, cymothoids have received taxonomic attention via reviews and redescrptions on generic and species levels. Conversely, morphological and molecular phylogeny remains unresolved due to poor character dataset and limited genetic analyses. This study aimed to investigate the morphological phylogeny of 30 ingroup taxa within the Cymothoidae by expanding 71 characters scored based on female specimens and literature. A data matrix was constructed in DELTA and generated for input into PAUP. Preliminary results revealed that 1) the family is monophyletic, 2) the analysis does not support the view of a linear evolutionary pathway based on site attachment, and 3) the analysis does not reveal host-specificity in more derived genera. The morphological cladistics resulted in two fairly distinct clades: 1) the predominantly buccal and gill-attaching cymothoid clade, and 2) the South American freshwater cymothoid clade. It is likely that cymothoid taxonomic classifications reflect convergence due to similar life styles (morphological adaptations).

Keywords: Cymothoidae; morphological phylogeny; fish crustacean parasites; isopods

Abstract No: 4279

6 Sept 2017, 1745 – 1800

Lead (Pb) bioaccumulation efficiency of *Acanthogyryus* sp. in freshwater fishes

Modesto Bandal, Jr.^{*1}; Leanne Jay Manceras¹; Vachel Gay Paller¹

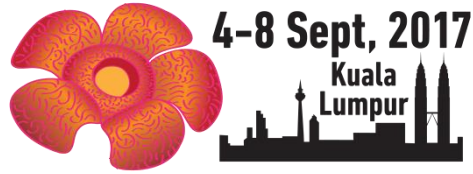
¹*Parasitology Research Laboratory, Animal Biology Division/ Institute of Biological Sciences, University of the Philippine Los Baños/ Philippines*

Abstract Content

Parasites have provided promising roles as pollution bioindicators over routinely used sentinel organisms such as bivalve mollusks. The potential use of acanthocephalan parasites in freshwater fishes as a lead (Pb) bioindicator in a semi-pristine oligotrophic setting of Yambo Lake, Laguna, Philippines was investigated. Wild Nile tilapia (*Oreochromis niloticus*), acanthocephalan parasites (*Acanthogyryus* sp.), Asian clam (*Corbicula fluminea*) and lake water were collected and Pb concentrations were determined and compared. Results of heavy metal analysis reveal the mean concentration levels of Pb accumulated in *Acanthogyryus* sp. (10.13 mg kg^{-1}), followed by the fish host tissues: liver (6.19 mg kg^{-1}), intestine (2.80 mg kg^{-1}), and muscle (0.75 mg kg^{-1}). *C. fluminea* accumulated only an average of 0.16 mg kg^{-1} Pb in its soft tissues. The results of heavy metal analysis for lake water samples showed a relatively low level of Pb concentration (0.019 mg L^{-1}). The bioaccumulation capacity of *Acanthogyryus* sp. against the fish host, and water samples were presented respectively: 35 times higher than the liver, 190 times than the intestine and 211 times than the muscle. The parasite was also found to accumulate 3,015 times higher Pb concentration than *C. fluminea* in ambient lake water. Thus, *Acanthogyryus* sp. showed a higher accumulation capacity among all samples analyzed. Acanthocephalans can offer advantages over existing biological indicators. The abovementioned findings provide support to acanthocephalans as potential sentinels, and that they can be utilized in detecting and monitoring the occurrence of heavy metals in seemingly pristine aquatic environments.

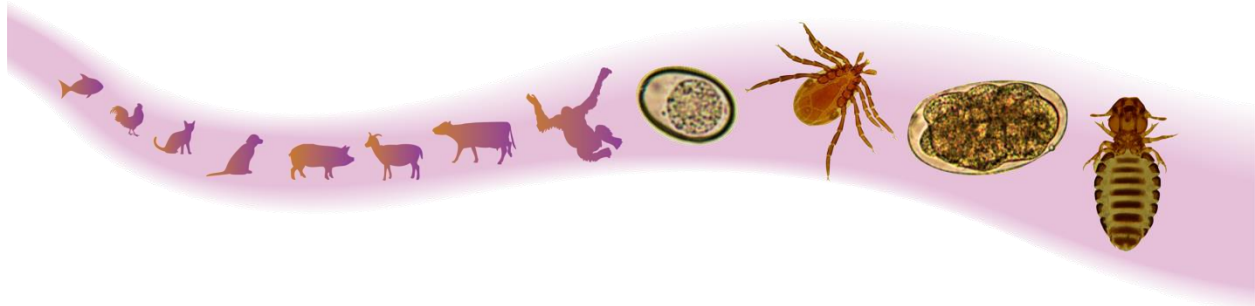
Keywords: Acanthogyryus sp.; Bioindicator; Corbicula fluminea; Philippines

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The impact of a diet with fructan-rich chicory roots on *Oesophagostomum dentatum* worm population dynamics and host immune responses in pigs

Stig Milan Thamsborg¹; Helena Mejer¹; Kerstin Skovgaard¹; Mita E. Sengupta¹; Helene Kringel¹; Heidi H. Petersen¹; Bent Borg Jensen²; Annette Andreasen¹

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Abstract Content

Oesophagostomum infections in pigs persist for months. We hypothesized that feeding fructans (dried chicory roots) may improve immunity and facilitate worm expulsion. We therefore examined the effects of long-term chicory on *O. dentatum* population dynamics and host immune responses. Methods: Seventy-two pigs were allocated to four groups in a 2-factorial design. Group O was fed regular feed and trickle inoculated with 15 *O. dentatum* L₃/kg/day 0-12 weeks post-infection (pi.) start. Group OC was also trickle inoculated but switched to a chicory-rich diet (12% inulin in DM) weeks 3-12 pi. Group C was uninfected but switched to chicory diet while Group Ctr remained uninfected on regular feed. Six pigs per group were necropsied 5, 9 and 12 weeks pi. for worm counts and qRT-PCR for gene expression in the gut. Faecal egg counts (FEC) and specific antibody levels were assessed regularly. Results: When group OC switched to chicory diet, FECs dropped within 3-4 days and remained very low. Worm counts were reduced 50-65% by chicory feeding (Group OC versus O; p<0.001) and was accompanied by a 2-fold higher *O. dentatum*-specific IgG1 response. In group O, a build-up of a typical Th2-type immune response was seen but leveled out later and worm counts remained stable. Group C had a down-regulated Th1-type response and thus an anti-inflammatory effect in colon. Conclusions: We found little evidence that chicory feeding improved host protective immunity against *Oesophagostomum*. It seems more likely, as previously suggested, that physico-chemical changes in caeco-colon are responsible for the observed anthelmintic effects.

Keywords: swine; oesophagostomum; chicory; prebiotics; immune response

Abstract No: 4119

6 Sept 2017, 1615 – 1630

Developing decision support tools for sustainable parasite control in livestock

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Abstract Content

Climate change, unpredictable weather and anthelmintic resistance are challenging the *status quo* in livestock parasite control worldwide and new tools are needed to help farmers determine the optimal strategy for sustainable parasite management. Mathematical models have been developed to explore the climate change impacts on parasites and parasitic disease, and to evaluate competing control strategies. Although these models were primarily developed as research tools there is potential to extend them to help farmers manage parasite risk. Furthermore, technological developments in the livestock sector such as EID (electronic identification) and existing simple hazard-based DSS (decision support systems) may prime farmers for more complex DSS. Here we present an example hazard-based DSS for the control of *Nematodirus battus* in lambs in the UK, developed in collaboration with SCOPS (Sustainable Control of Parasites in Sheep). The model predicts the date of hatch of *N. battus* eggs on pasture using real-time meteorological data, which is displayed on a map on the SCOPS website with guidance on risk assessment and control options. A feedback survey of users of the *Nematodirus* DSS indicates an improvement in their ability to control nematodirosis, with users reporting fewer lamb deaths, less scouring and improved weight gain. A similar DSS is under development for haemonchosis as part of the BBSRC project 'BUG: Building Upon the Genome'.

Keywords: decision support systems; Haemonchus; Nematodirus; model; weather

Abstract No: 4025

6 Sept 2017, 1630 – 1645

WormLoad: a pasture infectivity risk model of four nematode species in Australia

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¹*Animal Science/ University of New England/ Australia*

Abstract Content

Gastrointestinal parasites cost the Australian sheep industry AU\$436 million annually. Early warning of impending worm risk may reduce this cost by providing producers sufficient time to implement control strategies. The provision of 90 day weather forecast data at a 6km grid resolution across Australia (Australian Bureau of Meteorology) has enabled the development of a mathematical model to predict the risk arising from nematode pasture infectivity for inclusion in the Sheep CRC's 'AskBill' application. A biophysical modelling approach was used to simulate the on-pasture lifecycle stages of 4 nematode species (*Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus colubriformis* and *Trichostrongylus vitrinus*). Mortality and development/migration rates of each lifecycle stage were described using modified β -distribution functions to account for the impact of temperature and water availability. The model was parameterised against point estimates from available literature and experimental data for the 4 species (*H.contortus*: $R^2 = 0.88$, $n = 1409$; *T.circumcincta*: $R^2 = 0.56$, $n = 243$; *T.colubriformis*: $R^2 = 0.61$, $n = 355$; *T.vitrinus*: $R^2 = 0.66$, $n = 147$). In the absence of a model predicting the quantity of eggs deposited, a probabilistic approach was used assuming 1 egg species⁻¹ sheep⁻¹ day⁻¹ (thereby accounting for stocking rate). The impact of anthelmintic treatments were accounted for by assuming that no eggs were deposited for the duration of claimed efficacy. Risk was calculated by summing the proportion of infective larvae available for ingestion for each nematode species across each day of prior egg deposition and adjusting for herbage availability, species fecundity and productive impact.

Keywords: Sheep; Nematodes; Infectivity; Animal Welfare; Mathematical Modelling

Abstract No: 5186

6 Sept 2017, 1645 – 1700

The messy pragmatism of trying to share real time veterinary laboratory data on a country-wide or regional scale

Joseph Bove^{*1}

¹*Software/ Advanced Technology Corp/ United States*

Abstract Content

At present, there is a growing desire to attain consolidated veterinary laboratory results on both country wide and region wide bases. The theoretical value of such data is amazing in terms of analyzing outbreaks; evaluating healthcare trends; securing food safety; and ultimately transitioning from reactive testing to proactive policies. However, for as great as the perceived value of having access to this data on a macro scale, a myriad of issues continue to frustrate these efforts. In this presentation, we will examine a number of common challenges and outline specific principles that can increase the probability of a successful outcome. Among the topics to be presented are as follows:

- Understanding that data is contextual
- Matching technical capabilities and resources with proposed solutions
- Ensuring consistency of data capture and analysis over the long term
- Determining whether or not solutions are self-sustaining beyond the life of the grant
- Resisting the temptation to overinterpret what information databases actually contain

Keywords: LIMS epidemiological trends data analysis

Abstract No: 3995

6 Sept 2017, 1700 – 1715

Weight-based targeted selective anthelmintic treatment (TST) on hill and upland sheep flocks

Fiona Kenyon^{*2}; Claire Morgan-Davies¹; Nicola Lambe¹; Harriet Wishart¹; Anthony Waterhouse¹; David McBean^{2 1}; Davy McCracken^{2 1}

²*Disease Control/ Moredun Research Institute/ United Kingdom* ¹*Hill and Mountain Research Centre/ Scotland's Rural College/ United Kingdom*

Abstract Content

Weight-based targeted selective treatment (TST) has been shown to reduce wormer treatments with no negative effects on lamb growth in lowland sheep farms. However, this system has not yet been tested on upland or hill farms. A three-year study (June 2013 – October 2015) was conducted on a mountain research farm in the Scottish Highlands, to compare conventional (CON) and precision livestock farming (PLF) approaches to worm control. The flock was divided between CON and PLF (n=435 and 467 mean ewes/year, respectively). Treatment groups co-grazed and animals were grouped in batches that moved between upland and hill pastures. Lambs were individually tagged with electronic ear tags with weights and anthelmintic treatment decisions made monthly from June to September. PLF lambs were wormed only if they did not meet individual weight gain targets, calculated using the Happy Factor decision support system, whereas CON lambs were wormed if pooled faecal egg count was > 500 eggs/gram. Overall there was no statistically significant difference between the two systems for lamb weight post-weaning. However, the number of lambs that required worming was 40% lower (χ^2 test, $P < 0.001$) in the PLF than the CON groups, across all possible worming events and years. This reduction in the number of lambs wormed also resulted in reduction of labour required; an important consideration when seeking to encourage uptake among farmers. The implementation of the TST worming approach resulted in a sustainable worm control by reducing wormer usage and labour required with no negative effects on final lamb weights.

Keywords: Gastrointestinal nematodes; targeted selective treatment; sustainable worm control;

Abstract No: 4401

6 Sept 2017, 1715 – 1730

Duration of protection provided by *Barbervax*® in merino ewe hoggets

Sarah Baker^{*1}; Stephen Walkden-Brown¹; David Smith²; Lewis Kahn¹; Michael Raue¹; Madeleine Broomfield¹

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Abstract Content

The *Barbervax*® vaccine directed at hidden antigens of *Haemonchus contortus* is an important development in gastrointestinal nematode control in sheep. We investigated the duration and level of protection against *H. contortus* in hoggets following a full *Barbervax*® course in the 2nd year of life. The effects of vaccination on haematological variables and the association between antibody levels, in response to vaccination, and faecal worm egg counts (WEC) are also reported. A 2 x 3 factorial design was used to test Year 2 vaccination (vaccinated vs control) on the duration of protection following artificial challenge at 4, 8 and 12 weeks post final vaccination with a bolus dose of 5000 *H. contortus* larvae per ewe. Vaccinated animals received four Year 2 vaccinations 4-5 weeks apart between December 2015 and March 2016, controls only received the first vaccination. 120 Merino ewes approximately 16 months old were used with 20 animals per group. Vaccination significantly reduced WEC following challenge at 8 weeks but not at 4 or 12 weeks. Vaccination had no effect on red and white blood cell parameters. Vaccine-specific antibody levels were higher in vaccinates than controls and declined with time after vaccination. No association between individual antibody titre and WEC was found. The results of the experiment demonstrated that *Barbervax*® protection may persist for up to 8 weeks after the final vaccination in year 2 but suggest that antibody titre is a poor indicator of protection following artificial challenge.

Keywords: Haemonchus contortus; Barbervax; sheep; vaccine; nematode infection

Vaccination with a recombinant *Teladorsagia circumcincta* prototype in two sheep breeds native to the Canary Islands

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Abstract Content

Gastrointestinal nematodes (GINs) cause severe production losses worldwide. This situation is aggravated by increasing drug resistance. Therefore, alternative control methods to anthelmintics are needed. In this sense, researchers from Moredun Research Institute (UK) have developed a successful prototype of recombinant vaccine against *Teladorsagia circumcincta*. On the other hand, it has been demonstrated that two native sheep breeds from the Canary Islands, Canaria (CS) and Canaria Hair Breed (CHB) present differences in susceptibility in experimental inoculations against *Haemonchus contortus* and *T. circumcincta* in natural infections. The objectives of this study were comparing the efficacy of this vaccine in these two breeds experimentally infected with *T. circumcincta* and to confirm breed differences in resistance to this nematode specie. The vaccine regulated worm length and fecundity in CS and reduced, although not significantly, the worm burden in CHB, suggesting a different vaccination-response between breeds. The comparison of control groups showed lower fecal egg counts, delay in development of worms and reduction in the length and fecundity of adult worms in CHB compared to CS, confirming differences in resistance between breeds. Finally, significant differences in worm burdens in CHB-vaccinated group was observed with respect to both CS groups, suggesting a synergistic effect of genetic resistance and vaccination. Acknowledgements: European Union's Horizon 2020 research and innovation programme under the grant agreement No. 635408 (PARAGONE).

Keywords: recombinant vaccine, gastrointestinal nematodes, sheep, Teladorsagia circumcincta

Abstract No: 4463

6 Sept 2017, 1745 – 1800

Dietary inulin influences gut health and immune response in helminth-infected pigs

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Abstract Content

Dietary fibres, such as prebiotic chicory-derived inulin, have been shown to influence host immunity and gut health, and may have anti-parasitic activity against gastrointestinal helminths. Infections with helminths such as *Trichuris suis* lead to a polarised Th2 immune response, resulting in release of Th2 cytokines and increased antibody secretion in the host. This host immune response also modifies the gut environment by increasing mucin secretion and epithelial cell proliferation, eventually resulting in expulsion of the parasite. Our group have utilised a *T. suis*-infected pig model to study the interactions between a prebiotic-based diet, gut health and host immune responses during helminth infection. Intestinal tissue samples were collected to measure local immune related parameters, utilising techniques such as qPCR, histology and ELISA. Preliminary findings indicate that diets containing prebiotic inulin can have positive effects on the gut health of helminth-infected pigs by increasing expression of intestinal epithelial barrier-related genes such as trefoil factor 3 (TFF3) and sodium/glucose co-transporters, and down-regulating proinflammatory immune genes. In *T. suis*-infected pigs fed inulin, co-operative suppression of proinflammatory Th1-type genes and enhancement of genes encoding TFF3 was observed, suggesting that the host response to prebiotic inulin may enhance the host-protective Th2 immune response usually observed during helminth infections. Thus, our results indicate a profound effect of diet on immune function in helminth-infected pigs that may be exploited to improve gut health.

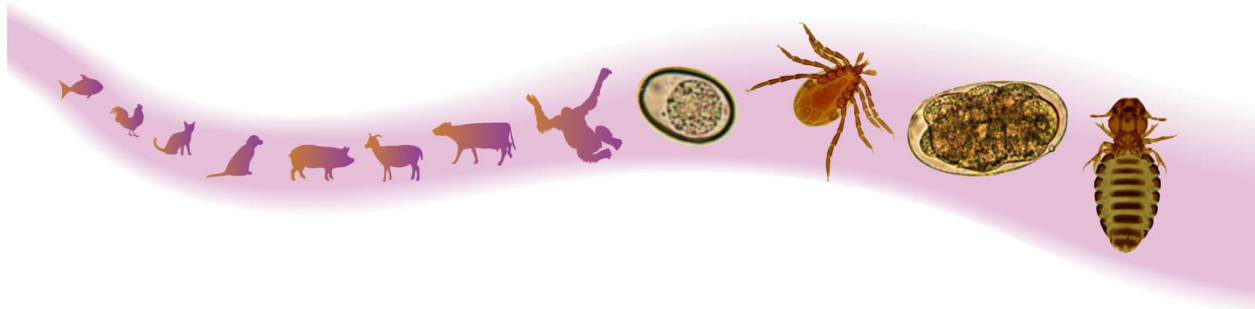
Keywords: Prebiotics; helminths; gut health; immunology; pig

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Oral Presentation – 6 Sept 2017: Zoonotic Primate Malaria



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Abstract No: 5597

6 Sept 2017, 1600 – 1630

***Plasmodium knowlesi*: History and Epidemiology**

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Abstract Content

Until recently, malaria in humans was thought to be caused by four species of *Plasmodium*: *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. Naturally acquired human infections with simian malaria parasites were considered to be extremely rare, until a large focus of human *P. knowlesi* infections was reported in 2004 in the Kapit Division of Sarawak, Malaysian Borneo. Human knowlesi malaria cases have since been described in other parts of Malaysia and in Thailand, Myanmar, Singapore, Brunei, Vietnam, Cambodia, Philippines, Indonesia and India, resulting in the recognition of *P. knowlesi* as the fifth species of *Plasmodium* causing human malaria. The talk will begin with a description of the studies of Knowles and Das Gupta in India following their isolation of *P. knowlesi* from a long-tailed macaque (*Macaca fascicularis*) in 1931, and of other early studies leading to the discovery of the large focus of human infections in Sarawak. More recent epidemiological data will be presented, together with molecular data of *P. knowlesi* isolates derived from humans and macaques in Malaysia, which indicate that knowlesi malaria is primarily a zoonosis. Furthermore, there are three subpopulations of *P. knowlesi*; two found in Malaysian Borneo and each associated with long-tailed and pig-tailed (*M. nemestrina*) macaques, with the third subpopulation found in Peninsular Malaysia. Whether it continues as a zoonotic infection or whether ecological changes due to deforestation, with an associated increase in the human population, result in *P. knowlesi* switching to humans as the preferred host, remains to be seen.

Keywords: Plasmodium knowlesi; macaques; zoonosis

Abstract No: 4642

6 Sept 2017, 1630 – 1700

***Plasmodium knowlesi* in humans: Vector control dilemma in public health**

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Abstract Content

Plasmodium knowlesi, a life-threatening malaria parasite in humans, is the predominant simian malaria species affecting humans in Malaysia, especially in East Malaysia. The Leucosphyrus group of *Anopheles* mosquitoes are bridge vectors between humans and macaques. Deforestation and changes in land-use have contributed to the current situation during the time when malaria elimination program is in progress. The incriminated vectors such as *An. balabacensis* demonstrated early biting activity and predominantly feeding outdoors, which suggest that current vector control tools such as indoor residual spraying and insecticide treated bed-nets may not be appropriate. But models suggest that they are sufficient to reduce the risk of transmission to humans. Currently available data shows that *An. balabacensis* is the most efficient vector with high sporozoite rates. This vector has been found positive not only with *P. knowlesi* but with multiple species of simian malaria parasites. Presently *P. knowlesi* appears to be a zoonotic disease but when human malarias are eliminated perhaps it may become anthroponotic. Thus, more in depth studies are required on the dynamics of the vectors in association with the macaques and humans. The transmission dynamics of the current vectors and the way forward to prevent this zoonotic infection will be discussed.

Keywords: Plasmodium knowlesi, simian malaria, vector

Molecular epidemiology of *Plasmodium knowlesi* in the natural reservoir host (*Macaca fascicularis*)

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Abstract Content

The primate malaria parasite *Plasmodium knowlesi* is a fatal zoonotic pathogen in Southeast Asia, being transmitted from macaques to humans via *Anopheles* mosquitoes. The present study highlights aspects of this emerging zoonosis with regards to the spatial distribution patterns, molecular epidemiology and genetic diversity of *P. knowlesi* infecting Long-tailed Macaques (*Macaca fascicularis*) on the west coast of Peninsular Malaysia. Screening for *P. knowlesi* and other simian malaria parasite infections was conducted using nested PCR targeting the 18S SSU rRNA, while genotyping was based on the Circumsporozoite Protein (CSP) gene. The prevalence of *P. knowlesi* infection among the macaques was 13.6%. The highest prevalence was recorded for *P. inui* (26.4%), followed by *P. cynomolgi* (17.7%), *P. coatneyi* (12.8%) and *P. fieldi* (11.8%). Macaques inhabiting plantations/orchards showed the highest prevalence rate of *P. knowlesi* infection (18.2%), followed by sub-urban areas and secondary forest. All putative risk factors except gender, posed a significant risk for infection among the macaques. Genotyping of 192 *P. knowlesi* *csp* gene sequences revealed 25 haplotypes with 14 polymorphic sites. The nucleotide and haplotype diversities were high, but low geographic differentiation was observed. Three dominant haplotypes were identified, with a wide distribution across the sampling locations. With the increasing destruction of forest habitats for human activities, wild macaques are driven closer to human habitations, thus narrowing the disease transmission interface. It is important to determine the epidemiology of zoonotic primate malaria in order to facilitate the implementation of better prevention and control measures in the country.

Abstract No: 5318

6 Sept 2017, 1715 – 1730

Rapid detection of *Plasmodium knowlesi* by isothermal recombinase polymerase amplification assay (RPA)

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Abstract Content

In this study, we developed a RPA assay for specific diagnosis of *Plasmodium knowlesi* (*P. knowlesi*). With incubation at 37°C, the samples were successfully amplified within 20 min. The end product of RPA was further examined by loading onto agarose gel and a specific band was observed with a size of 128 bp. The RPA assay exhibited high sensitivity with limits of detection down to one copy of the plasmid. From the specificity experiments, it was demonstrated that all *P. knowlesi* samples (n = 45) were positive while other *Plasmodium* spp. (n = 42) and negative samples (n = 6) were negative. Therefore, the RPA assay is a highly recommended approach for the diagnosis of *P. knowlesi* infection. This system is a rapid and cheap means to carry out nucleic acid amplification reactions, making it suitable for use in resource-limited settings.

Keywords: zoonosis malaria, rapid diagnosis

Development of PCR assays for identification of *Plasmodium knowlesi* subpopulations and assessment of temporal variation in frequency of subpopulations

Ting Huey Hu^{*1}; Khamisah Abdul Kadir¹; Paul C.S Divis¹; Dayang Shuaisah Awang Mohamad¹; Cyrus Daneshvar²; King Ching Hii³; David J. Conway^{1,4}; Balbir Singh¹

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Abstract Content

Plasmodium knowlesi is a zoonotic parasite of macaques that can cause serious infections in humans and is currently the major contributor of malaria cases in Malaysian Borneo. Population genetic studies on *P. knowlesi* infections in macaques and humans from the Kapit Division in Sarawak, Malaysian Borneo, identified two divergent subpopulations, associated respectively with long-tailed (termed Cluster 1) and pig-tailed macaques (Cluster 2). In order to determine whether there were temporal variations in the composition of the two subpopulations in human infections, the prevalence of each subpopulation was determined for isolates in the Kapit Division - between 2006 to 2008 (n=171) and between 2013 to 2016 (n=527). Using allele-specific genotyping assays for discriminating the two subpopulations, we found that the proportion of Cluster 1: Cluster 2 was 70:30 for infections between 2006-2008, 79:21 for infections in 2013, 63:37 for infections in 2014, 73:27 for infections in 2015 and 65:35 for infections in 2016. Overall, Cluster 1 was the dominant subpopulation accounting for 68.8% (95% CI 65.2-72.1) of infections. The proportions across these five different periods showed significant variation (P = 0.039, chi-square test with 4 degrees of freedom). Testing for pairwise differences between consecutive periods showed only one of these to be significant, 2013 versus 2014 (unadjusted P = 0.006, after Bonferroni adjustment P = 0.024). These temporal variations may be due to varying frequency of human encroachment into the deeper forested areas where pig-tailed macaques live, and/or other changes affecting vector and macaque distributions.

Keywords: Plasmodium knowlesi; Malaria; Subpopulations; Macaques

New vectors in northern Sarawak, Malaysian Borneo for the zoonotic malaria parasite *Plasmodium knowlesi*

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Abstract Content

The vectors for *Plasmodium knowlesi*, a significant cause of human malaria in Southeast Asia, identified previously in nature in Malaysia and Vietnam all belong to the Anopheles Leucosphyrus Group. In the Kapit District of Sarawak, *Anopheles latens* was incriminated as the vector of *P. knowlesi*. This study was undertaken to identify malaria vectors in another location in Sarawak. Mosquitoes landing on humans and resting on leaves in the Lawas District of northern Sarawak were collected and identified. DNA samples from dissected salivary glands of anophelines were subjected to nested PCR malaria-detection assays. The small sub-unit ribosomal RNA (SSUrRNA) genes of *Plasmodium*, and the internal transcribed spacer 2 (ITS2) and mitochondrial cytochrome c oxidase subunit 1 (CO1) sequences of the mosquitoes were derived from the *Plasmodium*-positive samples. Sixty five anophelines and 127 culicines were collected over a 4-day period. PCR and phylogenetic analyses of the SSUrRNA genes identified 3 *An. barbirostris* and 3 *An. balabacensis* as having single *P. knowlesi* infections, while 3 *An. balabacensis* were infected with two or more *Plasmodium* species (*P. inui*, *P. knowlesi*, *P. cynomolgi* and other novel *Plasmodium* species). Phylogenies inferred from the ITS2 and CO1 sequences of *An. balabacensis* and *An. barbirostris* indicate that the former is genetically indistinguishable from *An. balabacensis* in Borneo while the latter is a novel sibling species belonging to the Barbirostris Subgroup. Two new vectors for *P. knowlesi* in Sarawak were identified, including a species (*An. barbirostris*) that does not belong to the *An. leucosphyrus* group.

Keywords: zoonosis, Plasmodium knowlesi, vectors, malaria

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***Alaria alata* mesocercariae in wild boar meat: a public health issue?**

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¹*Biological Safety/ Federal Institute for Risk Assessment/ Germany*

Abstract Content

Adult trematodes of the genus *Alaria* (*A.*) parasitize in the intestine of carnivores. The life cycle comprises water snails and frogs as first and second intermediate hosts, respectively. Humans can be infected through ingestion of the second larval stage (mesocercaria) which may occur in meat of carnivorous or omnivorous 'paratenic hosts'. Alariosis cases caused by *A. americana* or *A. marciana*e have been reported in North America after consumption of frog legs and meat from raccoon or wild goose.

As *A. alata* is known to be autochthonous in Europe and coincidental findings of mesocercariae during *Trichinella* meat inspection are occasionally reported in some regions, the prevalence of this parasite was monitored in wild boars as paratenic hosts in Germany in 2015. This study comprised a total of 951 randomly tested wild boars from Schleswig Holstein (n=95), Brandenburg (n=166), North Rhine-Westphalia (n=141), Saxony-Anhalt (n=186), Saarland (n=17), Bavaria (n=121), and Baden-Württemberg (n=225). From each specimen a 30 g sample (diaphragm pillar as well as pharyngeal connective, fat and muscle tissue) was examined by larval migration technique. Mesocercariae were found in 44 out of 951 wild boars resulting in an average prevalence of 4.6 %.

As *A. alata* mesocercariae may be present in wild boar meat in Germany, further studies are required to better assess the exposure risk for consumer. Such studies should consider aspects of mesocercarial infection density in the tissue, tenacity and inactivation of mesocercariae in meat products as well as application of appropriate serological methods for human diagnosis.

Keywords: Alaria alata; wild boar meat; Germany

Abstract No: 4190

6 Sept 2017, 1615 – 1630

Estimating values for prevalence and diagnostic test characteristics of bovine cysticercosis in Belgium using a Bayesian approach

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Abstract Content

Introduction: Diagnosis of bovine cysticercosis (cc), caused by the cestode *Taenia saginata*, is currently based on routine meat inspection (MI) at the slaughter line, with a known low sensitivity. Prevalence in Belgium is estimated at 0.3% by official MI results.

Materials and Methods: We conducted a 3-year study on post-mortem detection techniques for cc in Belgium. Blood samples and predilection sites (PP) (tongue, masseters, oesophagus, diaphragm and heart) were collected from 614 bovine carcasses that were classified as cysticercosis-free at MI. Results of four imperfect post-mortem detection methods, i.e. dissection of the PP, MI with additional incisions in the heart, B158/B60 monoclonal antibody-based antigen detection (Ag-ELISA) and excretion/secretion (ES) Ab-ELISA are combined and analysed using a Bayesian approach to estimate prevalence and diagnostic test characteristics (sensitivity (Se) and specificity (Sp)), analogous with previous work done on *Taenia solium*. The model is based on a multinomial distribution and includes all possible interactions between the different tests. Only 15 parameters are allowed to be estimated but the model requires estimation of 31 parameters. Inclusion of prior expert opinion information on test characteristics and (in-) dependence of the different tests is necessary to reduce the effective number of parameters to be estimated and allow estimation.

Results/Discussion: Inconsistencies were discovered between current expert prior information and the data at hand. A revision of the expert opinion is necessary to achieve a well fitted model.

Keywords: *Bovine cysticercosis; test characteristics; prevalence; Bayesian analyses*

Abstract No: 4205

6 Sept 2017, 1630 – 1645

The effectiveness of freezing to kill anisakid nematodes: experimental evaluation of the time-temperature conditions

Magdalena Podolska^{*1}; Katarzyna Nadolna-Ałtyn¹; Joanna Pawlak¹; Bogusław Pawlikowski¹; Katarzyna Komar-Szymczak¹

¹*Department of Fisheries Resources/ National Marine Fisheries Research Institute/ Poland*

Abstract Content

The consumption of raw or inadequately processed marine fish may lead to several disorders caused by the ingestion of viable anisakid nematodes. Anisakid larvae may survive in subzero temperatures, therefore adequate freezing is the most important measure to control this potential health hazard. The aim of the study was to experimentally evaluate the time-temperature conditions, necessary to kill anisakid larvae (*Anisakis simplex* and *Pseudoterranova* sp.). The effectiveness of freezing process was tested on two species of fish, that are known to be naturally infected with anisakids: cod *Gadus morhua* from the North Atlantic and herring *Clupea harengus* from the southern Baltic Sea. Samples comprised of fillets of cod (n = 40) with visible parasites and whole herring (n = 240) were separately placed into plastic bags and exposed to temperature of -15°C, -18°C, -20°C and -25°C for 24 h. During the entire freezing process, internal temperature of samples was recorded with wireless temperature sensor Track Sense Pro. After thawing, nematodes were carefully removed from fillets and fish. Fillets of cod contained 990 individuals of *Pseudoterranova* sp. A total number of 955 *A. simplex* were found in the body cavity of herrings. All *Pseudoterranova* sp. larvae in fillets of cod died at temperature -15°C and lower. Freezing did not kill all the *A. simplex* larvae in herring. Spontaneous movement of parasites was observed in samples frozen at -15°C and -18°C. This research was supported by The National Centre for Research and Development under the Strategic Program Biostrateg (grant no. 296211/4/NCBR/2016).

Keywords: Anisakis, Pseudoterranova, anisakid, freezing, survival

Abstract No: 4935

6 Sept 2017, 1645 – 1700

Validation and comparison of the PrioCHECK *Trichinella* AAD Kit for the detection of larvae in pork, horse meat and wildlife tissue

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Abstract Content

A new artificial digestion assay was recently developed and commercialized for the detection of *Trichinella* larvae in the muscle of infected animals. The PrioCHECK™ *Trichinella* AAD Kit uses an alternative enzyme, serine protease, and no hazardous substances such as HCl or pepsin. Activation of the enzyme requires an elevated digestion temperature of 60°C which kills the parasite and reduces the risk of contaminating the environment with *Trichinella*. Compared to the pepsin/HCl method, digestion using the PrioCHECK *Trichinella* AAD Kit is significantly faster. To assess the Kit's suitability for *Trichinella* testing, and to validate its performance relative to the conventional pepsin/HCl digestion method, several comparative studies were conducted using meat from domestic food animals and wildlife species. Multiple muscle samples were collected from diaphragm, tongue, masseter, loin or foreleg of adult pigs, horses, wild boars, bears and wolves. Samples were naturally infected or spiked with 3, 4, 5, or 25 *Trichinella spiralis* larvae. A total of 638 100 g meat samples were used to validate and compare the diagnostic proficiency of the Kit with the pepsin/HCl digestion method. Analysis of the data produced from these studies showed that both methods are capable of consistently detecting *Trichinella* in 100 g samples which contained as few as 3 larvae. Overall, the PrioCHECK *Trichinella* AAD Kit performed satisfactorily according to various international guidelines for the detection of *Trichinella* infection in all of the various types of meat samples tested.

Keywords: Trichinella, diagnostic kit, pork, horse meat, wildlife

Impact of pH on the viability and morphology of *Blastocystis* isolates

Siti Nursheena Mohd Zain^{**1}; Farah Haziqah Meor Termizi¹; Chandrawathani Panchadcharam; Douadi Benacer¹; Suresh Kumar Govind; John-James Wilson; Mohd Khairul Nizam Mohd Khalid
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Abstract Content

Blastocystis sp. is ubiquitous in a wide range of animals including human and optimally propagates in neutral or slightly alkaline pH. However, presence of this enteric protozoan in carnivores is of interest as worldwide prevalence of *Blastocystis* in feline and canine animals is low (0-24%). We performed *in vitro* cultivation method on 130 *Felis catus* and 71 *Canis lupus* and failed to detect any positive isolates. Next, we investigated the impact of pH on viability and morphology of *Blastocystis* sp. using isolates cultivated previously from both available human and avian samples. Trypan blue marked increase number of viable cells as the medium pH moved towards neutral however, development for both isolates were suppressed in medium less than pH 5. Avian isolate showed sensitivity towards slightly acidic pH with absence of viable cells when pH of medium is less than 4. Morphologically, the avian isolate appeared less rounded and wrinkled or shrunken surface. In contrast, the human isolate remained viable with typical morphology at the same pH (pH 4). However, at pH lower than 3, no viable human isolate was visible. Our findings suggest that intestinal pH is an important determinant of *Blastocystis* viability and consequently prevalence in a host.

Keywords: Blastocystis sp.; canine; feline; pH

Abstract No: 3036

6 Sept 2017, 1715 – 1730

Differences in cryptosporidiosis amongst Aboriginal and non-Aboriginal humans in Western Australia

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¹*School of Veterinary and Life Sciences/ Murdoch University/ Australia*

Abstract Content

Cryptosporidiosis is a diarrhoeal illness caused by the waterborne parasite *Cryptosporidium*. In Australia, very little is known about the epidemiology of cryptosporidiosis in Aboriginal populations. The present study analysed long-term cryptosporidiosis patterns across Western Australia (2001–2012), combined with genotyping and subtyping data at the 18S and glycoprotein 60 (gp60) loci respectively. Comparison of cryptosporidiosis notifications between Aboriginals and non-Aboriginals in WA revealed that notification rates among Aboriginals were up to 50 times higher compared to non-Aboriginals, highlighting the burden of the disease in this population. Among Aboriginals, children aged 0-4 years consisted of >90% of notifications and were 20 times more at risk (RR20.5:1) of being infected when compared to non-Aboriginals children in the same age group. *Cryptosporidium hominis* was the predominant species infecting both Aboriginal and non-Aboriginal populations, however, Aboriginals were mainly infected with the *C. hominis* IdA15G1 subtype whereas non-Aboriginals were predominantly infected with the IbA10G2 subtype. To control cryptosporidiosis in Aboriginal populations in Australia, effective health interventions/promotion need to be a priority for public health research and action.

Keywords: Cryptosporidium; Waterborne; Aboriginal; Non-Aboriginal; Western Australia; gp60

***Cryptosporidium* and *Giardia* in different water catchments within a high dense farming area in Greece**

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¹Laboratory of Parasitology/ Faculty of Veterinary Medicine, Ghent University/ Belgium, ²Laboratory of Infectious and Parasitic Diseases/ Veterinary Research Institute, HAO-DEMETER/ Greece

Abstract Content

Surface water is a potential source of *Cryptosporidium* and *Giardia* infections, especially when used for drinking water production. Our objective was to monitor the presence of both parasites in a high dense farming area intersected by four rivers (Gallikos, Axios, Loudias and Aliakmonas) in Northern Greece. Water samples were collected monthly from 12 sampling points from river banks, irrigation canals and a water production site for two consequent years. USEPA method 1623.1 was used for the detection of *Cryptosporidium* and *Giardia* in water samples. Moreover, 254 faecal samples from young animals were collected from 15 cattle and 12 sheep farms located in close proximity to the above sampling points and screened with immunofluorescence for the presence of both parasites, in order to estimate their contribution to water contamination. Water samples collected from river banks were frequently contaminated with *Giardia* (65%) and *Cryptosporidium* (51%). Peak values up to 48.6 *Giardia* cysts/l and 13.5 *Cryptosporidium* oocysts/l were obtained during winter in the Axios and Loudias rivers. From January to May 2016, (oo)cysts were recovered from drinking water samples (<1/liter), suggesting a potential risk for public health. All farms were infected by both parasites, with 41.3% of the calves and 43.1% of the lambs infected with *Giardia* and 16.7% of the calves and 17.2% of the lambs with *Cryptosporidium*. DNA was collected from positive water and faecal samples for genotyping purposes, in order to investigate whether farming activity of the area may contribute to water contamination.

Keywords: Cryptosporidium; Giardia; transmission patterns; water; livestock

Abstract No: 4033

6 Sept 2017, 1745 – 1800

The impact on water quality and cattle health by implementing management solutions based on the results of a *Cryptosporidium* study in a catchment with a history of public water supply contamination.

Beth Wells^{*1} ; Hannah Shaw¹ ; Emily Hotchkiss¹ ; Janice Gilray¹ ; Remedios Ayton¹ ; James Green¹ ; Frank Katzer¹ ; Andrew Wells¹ ; Elisabeth Innes¹

¹*Protozoology/ Moredun Research Institute/ United Kingdom*

Abstract Content

Cryptosporidium, in particular *C. parvum*, is an important zoonotic parasite which represents a threat to livestock health, water quality and public health. The main reservoirs of *C. parvum* are known to be farm livestock but the contribution from wildlife in water catchments is unclear. The aim of the study was to establish *Cryptosporidium* prevalence, species and genotypes present in livestock, wild deer and water samples from a water catchment with a history of *Cryptosporidium* contamination in the public water supply and record the impact on water quality of management improvements to the catchment as a result of the study. Nested species specific multiplex PCR, targeting the 18S rRNA gene was used to detect and speciate *Cryptosporidium*. A multilocus fragment typing (MLFT) tool and GP60 sequencing was used to genotype *C. parvum* positive samples. Results indicated a very high prevalence of *Cryptosporidium* with a predominance of *C. parvum* in livestock, deer and water samples. Four GP60 subtypes were detected with the majority IlaA15G2R1 found in all host species and on all farms. MLFT further differentiated these into 6 highly related multilocus genotypes. The predominance of *C. parvum* in livestock and deer suggested that they represented a significant risk to water quality and public health, with genotyping results suggesting that all animal species had a role to play in contamination of the water sources. Management solutions to reduce *Cryptosporidium* on farms and in the public water supply have been implemented and shown to improve both animal health and water quality.

Keywords: Cryptosporidium, zoonotic, livestock, wildlife, water quality.

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Abstract No: 4102

6 Sept 2017, 1700 – 1715

Contemporary status of equine piroplasmosis in Punjab, India

Lachhman Singla^{*1}

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Abstract Content

Equine piroplasmosis (EP) caused by apicomplexan, obligate, eukaryotic, intracellular haemo-protozoan parasites *Babesia caballi* and *Theileria equi* is an unsystematically reported disease of equids in India. The dearth of information regarding the epidemiological status, analysis of tick transmission status, risk-factors and *in-vivo* drug efficacy studies of EP in Punjab (India) prompted to undertake this pioneer comprehensive epidemiological survey of EP under University Grants Commission, Government of India funded project over a period of four years using microscopy, conventional-PCR, nested-PCR, multiplex-PCR, competitive-ELISA and Indirect-ELISA to determine the current status and the exposure level casing all agro-climatic zones of Punjab State. The investigation revealed the endemicity of EP in Punjab state with an increasing trend in geographical distribution from north-east to south-west. Multiplex-PCR and cELISA used to determine the prevalence, agreement between diagnostic tests and associated risk factors of *T. equi* and *B. caballi* infection depicted the possible absence of *B. caballi* in both conducive and non-conducive areas of Punjab and projecting *T. equi* as the potential agent of EP in Punjab. Prevalence trend of *T. equi* was resiliently correlated with temperature, evapo-transpiration but inversely correlated with precipitation and cloud cover. Presence of tick vectors was the most influential factor with *T. equi* infection. Studies revealed that Western Plain Zone of Punjab is more prone to *T. equi* infection. A systematic report on *in-vivo* drug efficacy testing for natural infection of EP indicated butalex to be an efficacious drug in clearing *T. equi* while berenil proved to have low efficacy.

Keywords: equine piroplasmosis; epidemiology; current status; drug efficacy; India

Abstract No: 4402

6 Sept 2017, 1715 – 1730

Evaluation of the novel FECPAKG2 online FEC platform for horses

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Abstract Content

Faecal egg counts (FECs) are the gold standard method for diagnosing the level of parasitic worm infection in horses and other grazing animals. Testing prior to, and regularly after, treatment is the key factor in slowing development of anthelmintic resistance (AR) in nematode parasites of horses. FECPAKG2 allows owners to perform FECs on their own animals without microscopy or specialist knowledge of helminths. Samples can be prepared in the field/clinic, where a digital image of the sample is captured and submitted online for remote analysis. This study compared the FECPAKG2 (G2) method with an equine industry standard FEC method (FECPAKG1) using faecal samples from 17 horses in Wales and 22 horses in New Zealand. There was no significant difference between the FECs obtained using the two methods (rmANOVA: $F_{1,37} = 0.052$, $p = 0.821$, $\eta^2p = 0.001$), and no effect of the country of origin of the data (rmANOVA: $F_{1,37} = 2.084$, $p = 0.157$, $\eta^2p = 0.053$). Accuracy of the G2 method remained high and was not affected by FEC level ($r = -0.251$ (CI: 0.030, -0.472) $p = 0.124$ $n = 39$) yet repeatability was improved in the G2 method. We concluded that the FECPAKG2 method is a highly acceptable method for performing FECs in horses. It is envisaged following this validation that the low user input of the new G2 method will encourage the uptake of FECs amongst horse owners, either by direct use of the technology or through their veterinary practice, thereby slowing the development of AR.

Keywords: FEC; FECPAKG2; evaluation; horses; parasites

Abstract No: 4490

6 Sept 2017, 1730 – 1745

Equine piroplasmosis: Assessing the threat to the UK and Ireland

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Abstract Content

Equine piroplasmosis (EP) has an almost global prevalence, with an estimated 90% of the world's equine population living in EP endemic regions. The UK and Ireland have remained free from endemic disease, largely due to the absence of competent tick vectors. However, recent times have seen the incursion of competent tick species such as *Dermacentor reticularis*, which combined the introduction of trade agreements relaxing international transport restrictions; make introduction and establishment of EP in the UK a genuine concern. The present study utilised an 18S SSU rRNA gene PCR approach to screen over 1,000 samples submitted to UK and Irish agencies for EP serological testing. This targeted screening approach revealed prevalence of *Theileria equi* in up to 1.4% of submitted samples, as well as the identification of a pathogen-positive animal with clinical signs of piroplasmosis. Whilst *T. equi* has a high degree of polymorphism of the 18S SSU rRNA gene in the parasite population, in contrast to UK isolates, pathogen-positive Irish samples demonstrated a remarkably high degree of genetic uniformity at this locus, suggesting a point source infection. This present study is investigating these findings using high-resolution molecular tools, including microsatellites. Our study demonstrates the presence of animals in the UK infected with viable parasites. This finding combined with the increasing spread of competent tick vectors and current lack of EP biosecurity controls, makes the establishment of endemic disease a clear possibility.

Keywords: Equine; Piroplasmosis; UK; Ireland

Abstract No: 4321

6 Sept 2017, 1745 – 1800

Comprehensive worm control in a horse farm based in holistic management in central Spain

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Abstract Content

Worm control in Spain is mainly based in strategic programmes for periodic deworming (two to four times a year). Since 2015, a horse farm in central Spain started a holistic management programme which included selective treatment for worm control: animals younger than two years old are dewormed strategically every three to six months with different drugs according to the veterinary criteria and using different drugs to avoid worm resistance (fenbendazole, ivermectine, pyrantel); older animals (>2 years old) go through coprological examination every three months and only those with egg loads over 200 epg (eggs per gram) are treated. The holistic management programme includes systematic pasture rotation: animals are confined in small areas (range 25-140 animals/ha) and stay no more than three days. During spring or autumn, when the pasture grows fast, the same area is used every 45 days; in summer or winter with no pasture growth, the same area is used every 90 days. Data recovered from 20 months of holistic pasture rotation showed a total absence of colic nor diarrhoea from parasitic origin, while only cyathostomine larvae were obtained from coprocultures. No eggs of any of the following were detected in adult animals: *Oxyuris equi*, *Parascaris spp.*, *Strongylus spp.*; neither other parasites as *Gasterophilus spp.*, *Strongyloides spp.* This control strategy permits a reduction on drug use and soil contamination as only those animals who need the treatment receive it: only 14% of animals at potential risk were treated. A beneficial effect on dung beetles has been observed.

Keywords: holistic, control, selective-treatment

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Abstract No: 4379

7 Sept 2017, 0900 – 0915

Pathway of oxfendazole from host animal to *Trichuris suis*

Tina Vicky Alstrup Hansen¹; Andrew R. Williams¹; Peter Nejsum¹; Stig M. Thamsborg¹; Christian Friis
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Abstract Content

It is well-known that the efficacy of a single oral dose of benzimidazole against *Trichuris* spp. infections in humans and animals is unsatisfactory. Still, it is currently used in control programmes against human trichuriasis. However, the delivery route of the benzimidazoles from the host to *Trichuris* is still unknown. As adult *Trichuris* in caecum are situated partly intracellular, they are exposed to anthelmintic drugs in the caecal content and the mucosa. In this study, the pathway of oxfendazole (OFZ) and metabolites was explored in *T. suis* infected pigs, by measuring drug concentrations within the worms, plasma, caecal content, caecal tissue and caecal mucosa over time after an oral dose of 5 mg/kg. OFZ and metabolites were measured in *T. suis* and media after *in vitro* incubation. All drug measurements were performed using HPLC. Multiple linear regression analysis showed the drug levels of OFZ within the plasma and the caecal tissue of the pig to have a significant effect on the OFZ level within *T. suis* ($F_{(1,15)}=23.5$, $p<0.001$) whereas OFZ concentrations in the caecal content did not. The fenbendazole sulfone (FBZSO₂) concentrations in plasma and content had a significant effect on the FBZSO₂ levels within *T. suis* ($F_{(2,15)}=17.51$, $p=0.003$), as did the FBZSO₂ concentration within the caecal tissue ($F_{(2,15)}=18.84$, $p=0.003$). Our result strongly suggests that OFZ reaches *T. suis* after absorption from the gastrointestinal tract and is distributed to the worms by the systemic circulation-enterocyte-pathway, and that FBZSO₂ may reach the worm in a similar manner or through the enterohepatic circulation.

Keywords: Trichuris spp.; benzimidazole; drug efficacy; drug pathway

Survival and infectivity of chicken ascarid eggs in soil after exposure to an egg-degrading microfungus

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Abstract Content

The microfungus *Pochonia chlamydosporia* has been shown to kill high numbers of chicken ascarid (*Ascaridia galli* and *Heterakis* spp.) eggs *in vitro* but it is not known if surviving eggs may be infective. Unembryonated ascarid eggs (predominantly *A. galli*) were therefore isolated from faeces and added to sterilised (S) or non-sterilised (N) soil in Petri dishes that were either treated with *P. chlamydosporia* (F) or left untreated (C) during incubation at 22°C for 35 days. Egg recovery was estimated before (day 0) and after (day 35) treatment. Thereafter, each of four groups of parasite-free egg-laying hens was exposed to the soil from one of the four treatments in the feed over 12 days. The hens were necropsied day 42 post first exposure. The number of surviving eggs was most substantially reduced in SF soil and SF hens had statistically lower worm burdens (both parasites) compared to SC, NC and NF hens. However, adult *A. galli* were primarily found in SF hens while the other groups mainly harboured immature *A. galli*. Accordingly, SF hens also had the highest ascarid faecal egg counts and lowest serum *A. galli* IgY titre. Overall, *A. galli* recovery increased with increasing exposure, but contrastingly resulted in a reduced risk of egg-producing adult worms, at least short-term. Eggs not destroyed by *P. chlamydosporia* were clearly infective to the hens. The fungus did not appear to be sufficiently effective as a biocontrol agent of ascarid eggs in natural (i.e. non-sterilised) soil unless its effect can be further optimised.

Keywords: Ascaridia galli; Pochonia chlamydosporia; hens; soil

Strategies to optimise management of pre-weaning Barbervax® vaccination in Merino lambs

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Abstract Content

The Barbervax® vaccine is a recent major advance in the control of *Haemonchus contortus* in sheep. The vaccination schedule for lambs requires 3 priming vaccinations at 4-weekly intervals between lamb marking (M, approx. 6 weeks after lambing) and weaning (W, approx. 14 weeks after lambing). Marking and weaning are routine husbandry events, but the second primer (V2) requires an inconvenient extra muster of ewes with lambs at foot so we investigated strategies to remove this muster. Fine-wool merino lambs (175) running with their dams in a single mob were allocated to 5 treatments: UV (unvaccinated); VP (vaccinated as per protocol, M+V2+W), MddW (M double dose+W); MWdd (M+Wdd) and MddWdd (Mdd+Wdd). The marking to weaning interval was shortened to 6 weeks and at weaning all lambs were treated with short-acting anthelmintic. Booster vaccinations were given at 5 and 10 weeks post-weaning. Faecal samples were collected at frequent intervals to assess faecal worm egg count (WEC). As expected there was no difference in WEC between groups at weaning, but at 5 weeks post weaning the VP group with 3 vaccinations had significantly ($P=0.02$) lower WECs than the UV group, but not the other vaccinated groups, which only had significantly lower WEC when contrasted with UV ($P=0.01$). Following the first and second boosters all vaccinated treatments had significantly reduced WEC relative to the controls ($P<0.001$, WEC approximately halved) with no differences between them. Removal of V2 delayed maximal protection until the first post-weaning vaccination however the treatments tested provided an effective alternative.

Keywords: Barbervax®; Haemonchus contortus; lambs; nematode infection; vaccination

Abstract No: 4371

7 Sept 2017, 0945 – 1000

The potential of nitrogen based fertilisers to interrupt nematode development

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Abstract Content

Targeting gastro-intestinal parasites at life stages outside the host may provide a tool for aiding parasite control. Topical application of urea onto faeces has recently been shown inhibit larval recovery by 97%. This series of experiments investigated the potential of a variety of nitrogenous fertilisers on their ability to affect gastrointestinal nematode development. Hatching of *Trichostrongylus colubriformis* eggs when immersed in solution showed a fertiliser type x concentration interaction with all fertilisers at concentrations greater than 10% w:v inhibiting greater than 94% of eggs from hatching ($P < 0.001$) in comparison with 10% of eggs failing to hatch in the control (water). Regression analysis credited the variation in percentage of eggs hatched could be explained by both nitrogen percentage and electrical conductivity ($P < 0.05$ for both), but not phosphorous, potassium, sulphur levels or pH ($P > 0.05$ for all). These effects appeared to be nematocidal rather than nematostatic as unhatched eggs failed to hatch following washing and immersion in water. Further, the effects appeared to directly influence the hatching process *per se* as larval development in the unhatched eggs was clearly apparent while there were limited effects on L3 larvae. Current studies are examining the suitability of targeted application of nitrogen based fertilisers on parasite species from a range of hosts, including cattle and deer and will be reported. Overall, although some practical and environmental limitations exist on the suitability of N based fertilisers, with further refinement this approach has the potential to provide a novel tool to assist with parasite control.

Keywords: Egg hatching; fertiliser; development; nematocidal

**Efficacy of a novel Neem oil formulation (RP03™) to control the poultry red mite
*Dermanyssus gallinae***

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Superiore di Sanità/ Italy

Abstract Content

Poultry red mite (PRM) *Dermanyssus gallinae* is of major concern for the poultry industry. Several chemicals are effective against PRM, but acaricide resistance, the limited number of active ingredients, and the risk of residues create a demand for alternative products, such as plant-derived acaricides. We investigated the efficacy of neem oil against PRM on a laying egg farm with a high infestation level. A formulation of 20% neem oil dilution from a 2,400 ppm azadirachtin-concentrated stock (RP03™) was sprayed three times, at intervals of three days. Using corrugated cardboard traps, PRM-density was monitored before, during and after treatment. Results were analyzed by multi-factorial ANOVA. Following each treatment, PRM populations in the outer row of cages (treated) dropped by 94.65%, 99.64%, and 99.80% from the initial density, respectively; by 59.93%, 75.68% and 83.68% in an untreated middle row (buffer), and by 63.24%, 80.02% and 82.27% in the furthest untreated row (control). After 11 weeks post-treatment, population densities were 0.36% of initial density in the treated, 2.82% in the buffer, and 5.98% in the control rows. The treatment was most effective in the 10 days following the first application, and its effects persisted for over two months. Forced ventilation may have spread the product, affecting mite density in the buffer and control rows. The neem-based product had a strong and long-lasting bioactive effect against PRM. Further studies will aim to reduce the treatment schedule and neem concentration to overcome unwanted effects recorded on equipment and eggs.

*Thanks to COST-Action FA1404-COREMI and Farmaneem-SRL

Keywords: Dermanyssus gallinae, Field trial, laying hens, Neem oil, Control

Abstract No: 4563

7 Sept 2017, 1015 -1030

Plant-based therapy of the common parasites of livestock and poultry

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Abstract Content

The objective of the current study was to evaluate the anti-tick, anthelmintic and anticoccidial activities of a herbal formulation (HF) based on water decoction of leaves of *Azadirachta indica* and *Nicotiana tabacum*, flowers of *Calotropis procera* and seeds of *Trachyspermum ammi*. The HF demonstrated anti-tick activity against *Rhipicephalus microplus* by inhibiting the egg laying, larval mortality and reduced tick intensity/infestation on animals. Anthelmintic activity of HF was evident from *in vitro* mortality of *Haemonchus contortus*, ovicidal effects on egg hatch test and fecal egg count reduction in sheep naturally parasitized with gastrointestinal nematodes. Anticoccidial effects of HF were confirmed by reduction in the oocyst counts in droppings, oocyst scores, bloody diarrhea and FCR in chicks treated with HF compared with infected unmedicated chicks. The survival rate and weight gain was higher in chicks treated with HF compared with infected unmedicated chicks. Based on these results, the HF is promising solution 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. It is anticipated that the HF will gain a significant rank of integrated parasite management control. Large scale controlled studies are, however, recommended for standardization of the doses and applications of the product. Studies on fraction based activity of formulation will also be useful in identifying the active principles leading to development of a refined product.

Keywords: Rhipicephalus microplus, Haemonchus contortus, Eimeria tenella, Herbs

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Abstract No: 3925

7 Sept 2017, 0900 – 0915

The effect of anthelmintics in horse strongyles in Ireland

Nagwa Elgryani^{*1}; Theo De waal¹; Amanda Lawlor¹; John Browne
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Abstract Content

Management of equine strongyles has always relied on the intensive use of anthelmintics, however, resistance has started to develop against many of the commonly used anthelmintic drugs. Today ivermectin (IVM) and moxidectin (MOX) have become the most commonly used drugs, but evidence of emerging resistance (i.e. shortened egg reappearance period (ERP)) has been identified in many countries. This project was to determine the efficacy of anthelmintic drugs used in horses and donkeys in Ireland. The anthelmintic efficacy was determined by the faecal egg count reduction test (FECRT). In addition the ERP was determined for benzimidazole (BZ), IVM and MOX. The larval development assay (LDA) was used to detect resistance to BZ, pyrantel (PYR) while the larval migration inhibition assay (LMIA) was performed to measure the sensitivity to IVM and MOX. The results of FECRT indicate BZ-resistance on two groups (61% and 86%); an ERP of only two weeks. The effective concentrations (EC₅₀) for 13 groups with BZ and PYR in the LDA range from 0.003-0.8 µg/ml and 0.04-16.11 µg/ml, respectively. While MLs were still effective in all cases (FECR >95%), the ERP ranged from only 4 to 10 weeks and the EC₅₀ for IVM and MOX in the LMIA ranged from 0.013-1.488 µg/ml and 0.001-1.054 µg/ml respectively. Overall the results indicate that BZ was ineffective and also the reduced ERP results for the MLs would suggest that these products are less effective compared to label claims - a shortened ERP is believed to be an early indicator of resistance.

Keywords: Anthelmintic; resistance; horse; Ireland

Abstract No: 4209

7 Sept 2017, 0915 – 0930

The clinical importance of *Fasciola hepatica* infection in horses

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¹*Infection Biology/ University Of Liverpool/ United Kingdom*

Abstract Content

Fasciola hepatica is recognised as a parasite affecting grazing animals, and reports of clinically affected horses appear in the literature. Anecdotal information suggests that although there is awareness of the parasite amongst equine veterinary surgeons, affected horses are not always diagnosed or treated due to a lack of information about prevalence and the typical clinical picture associated with *F. hepatica* in horses. Our study aimed to establish the importance of equine fasciolosis in the UK, to assist with diagnosis and ultimately to improve the welfare of horses. The work was made possible by a recently developed *F. hepatica* antibody ELISA. Firstly, we undertook a prevalence survey of UK horse abattoir population, with *F. hepatica* infection status determined by both liver inspection and ELISA. Secondly, we recruited horses with and without liver disease from the UK horse population, tested them for *F. hepatica*, and collated clinical, diagnostic and management information from their veterinarians and owners. This information was used to determine the strength of the association between *F. hepatica* and liver disease, and to establish the clinical features most commonly associated with infection.

Keywords: fasciola; equine; ELISA

Abstract No: 4133

7 Sept 2017, 0930 – 0945

Prevalence of *Strongylus vulgaris* after 10 years of target selective treatment in Sweden

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Institute/ Sweden

Abstract Content

Introduction *Strongylus vulgaris* is the most pathogenic equine parasite. Frequent deworming programs since the 1970s have reduced the prevalence of *S. vulgaris* to low levels but simultaneously anthelmintic resistance has developed among small strongyles (Cyathostominae). To slow down the progression of anthelmintic resistance, targeted selective treatment was introduced in Sweden approximately ten years ago. The aim of this study was to investigate the prevalence of *S. vulgaris* and obtain knowledge of current deworming routines in Swedish horse farms. **Materials and methods** From March to May 2016, faecal samples were collected from horses in three geographically different regions: southern, central and northern Sweden. Five samples from each of 58 farms were analysed by individual larval cultures and McMaster of 3 g samples to detect *S. vulgaris* and determine number of eggs per gram faeces, respectively. **Results** A prevalence of *S. vulgaris* of 66% on farm level and 26% on individual level was detected, and a significantly higher proportion of *S. vulgaris* positive horses was found in southern Sweden. The highest prevalence (25%) of *S. vulgaris* was seen in horses that had not been dewormed for at least 24 months prior to sampling. **Conclusion** The prevalence of *S. vulgaris* has increased in Sweden. It is vital not to leave horses with low faecal egg counts untreated without analysing for *S. vulgaris*. This important message should be communicated to horse owners and veterinarians. The study will be repeated during spring 2017 and compared to similar ongoing studies in Norway.

Keywords: Strongylus vulgaris; prevalence; target selective treatment

Abstract No: 3609

7 Sept 2017, 0945 – 1000

First detection of *Besnoitia bennetti* (Protozoa: Apicomplexa) in donkey (*Equus asinus*) in Europe.

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Abstract Content

A two year old male donkey was purchased in May 2016 in poor body condition (cachexia, alopecic areas, pruritus mainly on neck and head) by the present owner in Le Roeulx (Belgium) from a donkey farm aimed at milk production in Frasnés-lez-Buissenal (Belgium). Shortly after its purchase, the donkey was shorn and showed crusts, and hyperkeratosis (both flanks and neck) with no other clinical signs except anorexia and cachexia. A treatment with phoxim was given without improvement; a cutaneous biopsy of hyperkeratotic skin was performed in July and showed a perivascular eosinophilic infiltrate with a large thick walled cyst located in the dermis. The cyst was filled with numerous bradyzoites. This was highly suggestive of besnoitiosis. A daily treatment based on sulfamethaxazole and trimethoprim (Emdotrim 60% Mix®, 30 mg/kg) was given orally and some improvement was noticed. Further clinical examination performed on August highlighted scleral pin-head sized cysts (pearl) in right eye and between nares. Several skin biopsy samples were obtained for qPCR analysis and confirmed the presence of *Besnoitia* spp.'s DNA. Further laboratory diagnosis tests were realized (Western Blot and rDNA sequencing) and confirm *Besnoitia bennetti* aetiology. Another ten year old female donkey purchased in France and sharing the same accommodation showed a good clinical condition but a deepest clinical examination showed the presence of numerous cysts on the inner face of upper labial mucosa. The punch-biopsy, haematology and qPCR were negatives but the Westernblot showed the presence of antibodies directed to *Besnoitia* spp.

Keywords: Scleral and labial cysts; Besnoitia bennetti, Besnoitiosis, donkey

Abstract No: 4571

7 Sept 2017, 1000 – 1015

An ivermectin-sensitive glutamate-gated chloride channel from the horse parasite *Parascaris equorum*

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Abstract Content

Parascaris equorum is the largest nematode parasite of equids that is responsible of colic and death in foals through intestinal obstruction. Parasite control largely relies on the use of macrocyclic lactone anthelmintics including ivermectin that acts on glutamate-gated chloride channels (GluCl). However, *P. equorum* resistance to avermectins is a growing issue over the recent years throughout the world. Here we have cloned the cDNA of *glc-5* encoding a *P. equorum* GluCl subunit and expressed it in *Xenopus laevis* oocytes, demonstrating that it is an important molecular target for ivermectin *in vivo*.

Two-electrode voltage clamp recordings were made from *Xenopus* oocytes injected with *glc-5* subunit cRNAs to explore the GluCl pharmacology. The GLC-5 subunit formed a functional homopentameric channel that was gated by L-glutamate. Moreover, ivermectin was found to be more potent agonist than glutamate in activating the *P. equorum* GluCl. Interestingly, the glutamate and ivermectin EC₅₀ values were consistent with the pharmacology observed for the *Haemonchus contortus* GluCl encoded by the previously identified GLC-5 homologue.

Here we report the functional characterisation of the first ivermectin-sensitive GluCl of *P. equorum*, thus opening the way for better understanding the mode of action of macrocyclic lactones in equine parasites.

Keywords: *glutamate-gated chloride channel, Parascaris equorum, GLC-5, Xenopus oocytes, macrocyclic lactones, ivermectin*

Efficacy of Pyrantel against *Parascaris Spp.* Infection in Foals in Sweden.

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Abstract Content

Introduction: *Parascaris spp* infection in foals is a common problem around the world. Resistance against the anthelmintic ivermectin is well established in the worldwide parascaris population. Also multi-resistance to ivermectin and pyrantel has been reported in North America and Australia. The aim of this study was to investigate the efficacy of pyrantel on stud farms in Sweden. Previous Swedish studies from 2005 showed resistance to ivermectin on 5 out of 6 investigated farms, but that pyrantel and fenbendazol were still effective. **Material and methods:** A Faecal Egg Count Reduction Test (FECRT) was performed on a total of 106 foals on 5 stud farms in Sweden from September 2016 to March 2017. Horses with a minimum of 150 eggs per gram feces were included in the study. Faecal samples were collected before treatment with pyrantel, and 14 days post-treatment. **Results:** On two of the five farms that met the inclusion criteria, the FECR after pyrantel treatment was 73% and 82% (lower confidence limits 55% and 62%) respectively, which indicates resistance and suspected resistance. On the remaining three farms the efficacy was in the range between 91% and 98%, which indicates that the population is susceptible to pyrantel. **Conclusion:** These preliminary results show that there is reduced efficacy of pyrantel against *Parascaris spp* on some Swedish farms. This is the first time evidence for pyrantel resistance in *Parascaris spp* have been found in Sweden.

Keywords: Parascaris spp; Horse; Pyrantel; Resistance; Faecal egg count

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Abstract No: 3873

7 Sept 2017, 0900 – 0915

Intestinal Parasite Diagnostics - Advances in Coproantigen Detection

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Abstract Content

Three independent antigen detection assays have been developed for the detection of coproantigens for *Ancylostoma caninum*, *Toxocara canis*, and *Trichuris vulpis*. The antigen assays detect excreted or secreted proteins from the young adult to adult stages. The protein markers are not associated with reproduction or with eggs and thus provide a different marker for nematode infection. Experimental infection studies demonstrate earlier detection by antigen ranging from 7 days for *T. canis*, 14 days for *A. caninum*, and 46 days for *T. vulpis*. These assays were applied to a 1000-member field population to assess performance compared to egg flotation results. The majority of samples agreed with either the egg positive or egg negative flotation result. Discrepant results were observed for each nematode. These discrepant results were investigated in detail for the *T. canis* samples. Four of the five *T. canis* egg positive, ELISA negative samples most likely were spurious eggs. One of the five was confirmed to be *T. canis* egg positive and the ELISA signal was elevated but below cut-off. Seven samples were egg negative, ELISA positive. ELISA signal was confirmed to be specific to *Toxocara* antigen. Flotation and ELISA data was collected for over 54 thousand tests. The overall pattern of detection was similar to that seen in the smaller 1000-member population. Together the data supports that the combination of the traditional flotation assay and the new antigen detection technology will allow identification of more infected pets.

Abstract No: 4406

7 Sept 2017, 0915 – 0930

Laboratory diagnostics for *Fasciola hepatica* in Australian livestock

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Abstract Content

In Australia, *Fasciola hepatica* causes fasciolosis in ruminants, with a recent estimate of the annual cost to the national sheep industry alone of approximately 25 million dollars. Detection of *F. hepatica* in live animals can be attempted using a variety of laboratory tests - egg sedimentation from faeces, blood or milk antibody ELISA, faecal antigen ELISA and LAMP assays. This paper will summarise various pieces of work investigating the practical application of several of these tests for Australian sheep and cattle. Test sensitivity and specificity and the usefulness of testing pooled, or composite, faecal samples will be investigated. The importance of sub-sampling methodology prior to testing will also be discussed.

Keywords: Fasciola hepatica ELISA LAMP sedimentation

Use of four commercially available ELISAs for detection of *Fasciola hepatica* infection in Irish beef and dairy cattle

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Abstract Content

Fasciola hepatica is a liver parasite of mammals. It has a worldwide distribution and results in poor welfare outcomes and economic losses in ruminants. While faecal egg count (FEC) is the gold standard test for diagnosis, it does not indicate presence of migrating immature stages. Serological techniques may, therefore, increase sensitivity for detecting all stages of liver fluke infection. To further investigate this hypothesis, we tested three sample types; (i) known negative and positive sera from an experimental infection study, (ii) sera from pre- and post-triclabendazole-treated beef cattle, and (iii) bulk tank milk (BTM) samples from dairy herds sampled during high and low *F. hepatica* risk periods. Samples were tested using ELISA kits supplied by four manufacturers (Ildana Biotech, IDEXX, Svanova, and BIOX). Samples were analysed simultaneously and in duplicate. All tests investigated detected *F. hepatica* antibodies four weeks prior to positive FEC following experimental infection. In flukicide-treated beef cattle, all tests highlighted decreasing S/P% values but to varying degrees. Finally, BTM results indicated a significant decrease in S/P% between low and high risk fluke periods ($P \leq 0.0007$). The present study highlights that earlier detection of fluke is possible through use of commercially available ELISAs compared with standard FEC techniques, and that to a greater or lesser degree, the tests are suitable for detecting the success or failure of a triclabendazole-based flukicide treatment. Finally, the use of BTM samples for herd status monitoring is feasible, as the four tests showed expected seasonal variations, albeit some kits performing more optimally than others.

Keywords: Fasciola hepatica; ELISA; diagnostic; dairy; beef

Scrambled eggs: a highly sensitive molecular diagnostic workflow for *Fasciola* species specific detection from faecal samples

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Abstract Content

Fasciolosis is a re-emerging zoonotic disease of worldwide importance. Ante-mortem diagnosis of human and animal infections commonly occurs through traditional sedimentation faecal egg-counting (FEC). This technique is time-consuming and prone to sensitivity errors when a large number of samples are processed or if the operator lacks sufficient experience. Additionally, diagnosis can only be made after the 12-week pre-patent period (PPP). Recently a commercially available coprological antigen ELISA has enabled detection prior to the completion of the PPP. While this method allows for earlier detection and increased throughput, species differentiation is not possible. Real-time PCR offers the combined benefits of highly sensitive species differentiation for a large numbers of samples. We have optimised a molecular diagnostics workflow for the highly sensitive detection and quantification of *Fasciola* spp. in faecal samples. The method involves sedimenting and pelleting the samples prior to DNA isolation to allow for the concentration of eggs, followed by disruption in a bead-bashing tube to ensure all eggs were broken. While both the qPCR and FEC were 100% specific, the sensitivity of the qPCR was higher (100% compared to 90%). Good correlation ($R^2 = 0.74$) was observed between the qPCR values and the FEC. Species-specific assays allow for sensitive ante-mortem diagnosis in both human and animal settings, including Southeast Asia where there are potentially many undocumented human cases and where post-mortem examination of production animals is often not possible. The optimised workflow supercedes the FEC and provides a sensitive diagnostic tool for rapid testing of large numbers of samples.

Keywords: Fasciolosis; molecular diagnostics; qPCR; sedimentation; zoonosis

Abstract No: 4138

7 Sept 2017, 1000 – 1015

Development of a pen-side diagnostic test for liver fluke infection in cattle and sheep

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Abstract Content

Fasciola hepatica (liver fluke) is a common trematode parasite of cattle and sheep worldwide. It is highly pathogenic and therefore raises health and welfare concerns for infected individuals. Over recent years, the prevalence of liver fluke has increased significantly within the UK, which is linked to many factors such as climate change and increased evidence of resistance to commonly used drugs. Current diagnostics are primarily based around faecal egg counts and ELISA based assays for antibody detection in serum and milk; however these tests can be slow, insensitive, and require samples to be sent to the laboratory for testing, which adds time and cost to the diagnosis. The aim of this project is to develop a novel lateral flow pen-side diagnostic test to allow farmers to quickly identify infected individuals to target treatment, thereby reducing the reliance on repeated whole herd treatments. A recombinant Cathepsin L1 (CL1), the immunodominant fluke antigen, has been produced in the yeast *Pichia pastoris*. Western blotting and ELISA showed that the recombinant antigen was more readily recognised by animals experimentally infected with *F.hepatica*. This was confirmed by peptide arrays, which identified 4 key immunogenic regions recognised by antibodies from animals experimentally infected. Preliminary data from proteomic identification of 2D gel-electrophoresis spots of fluke excretory-secretory (ES) antigen showed that bovine anti-fluke antibodies recognise multiple targets within ES. The results indicate that whole fluke ES provides a much better antibody target for use in a prototype lateral flow test for diagnosis of infection in cattle and sheep.

Keywords: Fasciola hepatica; liver fluke; diagnostics

Proteins of *Echinococcus granulosus* of dogs as diagnostic antigens in Immunoreactive Enzyme Immuno-transfer Blot (EITB) Assay

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Abstract Content

Echinococcosis, a cyclozoonotic helminthosis caused by dwarf dog tapeworm *E.granulosus* is highly endemic and considered as one of the most important parasitic diseases. The economic losses in animal production and health hazard along with treatment costs of cystic echinococcosis in man are enormous. The present study was undertaken to identify the proteins of diagnostic importance in *E.granulosus* infection in dogs by EITB. The antigenic characterization of three different antigens viz., somatic, excretory secretory (ES) and faecal supernatant (FS) antigens of *E.granulosus* were studied by SDS-PAGE with 10% resolving and 4.5% stacking gel concentration. The protein profile of somatic antigen of *E.granulosus* revealed 11 polypeptides of 114, 94, 84, 74, 68, 66, 56, 45, 34, 24 and 16 kDa. The excretory/secretory antigen revealed nine polypeptides of 82, 78, 66, 45, 38, 34, 30, 24 and 14 kDa whereas eight polypeptides were noticed in faecal supernatant antigen 76, 66, 54, 45, 37, 34, 24 and 17 kDa. The immune-reactive proteins were detected by EITB revealed 84, 66, 45 and 18 kDa with somatic antigen of *E.granulosus*, using homologous positive serum. The excretory secretory (ES) antigens of *E.granulosus* showed six immune-reactive proteins of 98, 84, 66, 45, 34 and 24 kDa on blots. Further, a total of 66, 45, 34 and 18 kDa immune-reactive proteins were identified on western blot with faecal supernatant (FS) antigen of *E.granulosus*. The immune-reactive proteins of 66 and 45 kDa were found common among the three different antigens of *E.granulosus*.

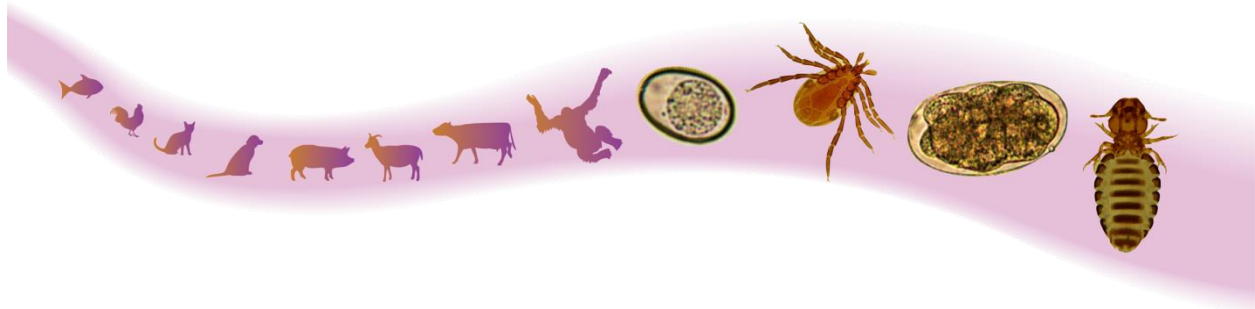
Keywords: Dog; Echinococcus granulosus; somatic Ag; ES & FS Ag; EITB

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Prevalence and risk factors of trematode infection of Swamp Buffalo (*Bubalus bubalis*)
in Community Farms (SPR) Banten Province, Indonesia

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Abstract Content

Trematodes infection or trematodosis causes chronic infection which directly affect health and production performance in large ruminant including swamp buffaloes (*Bubalus bubalis*). A cross-sectional study was conducted to determine the prevalence and risk factors of trematodosis in swamp buffaloes located on Banten Province, Indonesia. A total of 300 fecal samples, randomly collected from swamp buffaloes in community farms (SPR) of Serang and Lebak Regencies, Banten Province Indonesia, were examined for trematodes eggs by a filtration (DBL) method. Prevalence of trematodosis in the buffaloes was very high (71.67%) which consist two major parasite genera, i.e. *Fasciola* (21.00%, mean of EPG 13.42±66.920) and *Paramphistome* (69.33%, mean of EPG 209.69±694.108). Higher prevalence was found in female (73.91%) than male (59.57%). Infection due to age categories can be summarized as >18-32 months (50.00%), >8-18 months (60.00%), 0-8 months (60.61%) and >32 months (76.71%), respectively. Only feeding animals with rice straw was being factor related to infection (Chi-square analysis with *p* value was 0.047). Multivariate analysis summarized the significancy values of rice straw (0.02), absence of anthelmintic administration (0.01), and combination of grazing locations (0.036) to the infection. Odd Ratio (OR) numbers of feeding animals with rice straw, absence of anthelmintic administration, and combination of grazing locations were 14.475, 3.628 and 0.106, respectively. However, the infected animals did not show any typical symptoms. It could be concluded that trematodosis still being unrevealed problem in swamp buffaloes' farming and need comprehensive study for future control.

Keywords: trematode, buffalo, trematodosis, prevalence, helminthiasis

Abstract No: 4481

7 Sept 2017, 0915 – 0930

Prevalence and control of gastrointestinal flukes in an organic deer farm in Phetchaburi, Thailand

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Abstract Content

Organic deer farms have been dramatically increased in Thailand for the last few decades. The deer farming is more profitable than the traditional livestock farming because of fewer feedstuffs consuming, less pastures damaging, fast-growing, and high reproductivity of deer. Generally, organic animal farms need a proper control program for parasitic diseases. In this study, we examined the prevalence of gastrointestinal flukes and evaluated Closantel administration in an organic deer farm, where Ivermectin (50 mg/kg) has been administered tri-annually. In June 2016, 320 feces were collected randomly from 4 different species of deer (Chital, Rusa, Samba and Hog deer). All deer were given Closantel (5 ml/kg) at early of July, and thereafter, 241 faecal samples were collected likewise in October 2016. A sedimentation technique was conducted and eggs of trematodes were observed under a light microscope. For 320 samples collected in June, 230 were positive with the eggs of trematodes: 66.6% were infected with only *Paramphistomum* sp. and 5.3% were infected with both *Paramphistomum* sp. and *Fasciola* sp. For 241 samples collected after Closantel administration, 50 were positive for the eggs of trematodes, 19.9% were infected with only *Paramphistomum* sp. and 0.8% was infected with both *Paramphistomum* sp. and *Fasciola* sp. A high prevalence of flukes was seen in the deer farm studied, and after Closantel administration the prevalence of the eggs of flukes was significantly decreased. The results suggested that a proper management program for control trematodes infection is recommended in organic deer farms.

Keywords: Gastrointestinal trematodes; fluke; prevalence; organic farm; deer

Farm specific transmission patterns of *Fasciola hepatica* in Danish dairy cattle based on different diagnostic methods and monitoring of grazing management

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Abstract Content

A recent survey based on meat inspection data showed that approximately 30% of Danish cattle farms were infected with liver flukes, leading to significant economic losses. Despite the widespread problem, up-to-date knowledge on transmission patterns, diagnostic methods and practical measures for control is still lacking. We therefore initiated a longitudinal, observational study in a few infected dairy farms to elucidate farm specific transmission patterns based on different diagnostic methods and grazing management. Two organic and two conventional dairy farms with high *F. hepatica* antibody levels in bulk tank milk were selected. From each farm a cohort of 40 animals from different age groups (calves, heifers, primiparous and multiparous cows) were sampled 7 times between April 2015 and January 2017. Diagnostic methods included faecal egg count by sedimentation, serum ELISA and coproantigen ELISA. Additionally, monthly bulk tank milk samples were analyzed by ELISA. The analyses are ongoing, but preliminary results indicate that *F. hepatica* is mainly transmitted via summer infection of snails as most animals seroconvert in late autumn without shedding of eggs. However, infection early in the grazing season due to overwintered snails has also been observed. One farm where cows are stabled have had some older cows continuing to shed *F. hepatica* eggs, suggesting long life span of *F. hepatica*, although other routes of infection cannot be ruled out. The final results will provide novel and practical information about different diagnostic tests and transmission patterns related to grazing management on farm-level.

Keywords: Fasciola hepatica; cattle; ELISA; bulk tank milk; epidemiology

Abstract No: 4199

7 Sept 2017, 0945 – 1000

Identification of farm-level risk factors for *Fasciola hepatica* infection in UK beef and dairy cattle

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Abstract Content

The liver fluke (*Fasciola hepatica*) is a zoonotic parasite of worldwide importance, causing economic losses, morbidity and mortality in livestock. In the UK, emergence of anthelmintic resistance and increasing disease prevalence pose a major problem for livestock producers. Predictive models based on climate data are useful to identify high risk periods for infection but lack sufficient accuracy to resolve beyond a regional level. In this study, we set out to investigate and identify important farm-level risk factors, including presence and distribution of the snail intermediate host, *Galba truncatula*. Beef and dairy farms (n=195) were recruited from Shropshire, UK. Questionnaire data on herd characteristics, farm management and environmental conditions were collected and compared to fluke infection status (determined through faecal egg counts and bulk milk tank antibody ELISA) using multivariable logistic regression. Forty farms were re-visited to collect data on intermediate host species, habitat and population density with specimens collected and tested for *F. hepatica* infection by PCR. Evidence of *F. hepatica* infection was found in cattle on 125 farms. Logistic regression analysis identified an increasing percentage of grass in the diet, buying in cattle, co-grazing of sheep and presence of rabbits as statistically significant (P<0.05) variables associated with infection. Additionally, *G. truncatula* was identified on 25 re-visited farms, with evidence of *F. hepatica* infection in 19 snails collected from 6 farms. This study identifies several farm-level risk factors associated with bovine fasciolosis, informing sustainable disease control programmes. Further analysis incorporating snail habitat and infection data into these models is ongoing.

Keywords: Fasciola hepatica, cattle, Galba truncatula, epidemiology, control

Prevalence of *Fasciola hepatica* in dairy cattle in the state of Paraná, Brazil

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University Belfast/ United Kingdom

Abstract Content

Fasciola hepatica (liver fluke) is a parasitic helminth responsible for the disease called fasciolosis, which affects ruminants and other animals. This disease has a major economic impact on the health of cattle herds, and is an important zoonosis worldwide. The main clinical signs on infected animals are: weight loss, weakness, anemia, hypoproteinemia and submandibular edema. The objective of our work was to determine the prevalence of *F. hepatica* in dairy cattle in Parana, South of Brazil. Milk samples (n=1802) were analyzed from 10 mesoregions of the state for the detection of anti-*Fasciola hepatica* antibodies, employing a recombinant form of cathepsin L1, FhCL1 in an ELISA that exhibits no cross reactivity with other helminths. The sample size corresponds to the total amount of Dairy Industries in the State (122) and the monthly number of milk analysis (SCC, protein, fat) (120 thousand). We found a prevalence of 9.8% (176/1802), distributed in the: Metropolitan area of Curitiba (26.70%); West (18.75%); Northwest (14.77%); Center-South (13.63%); Center-East (6.25%); Southwest (5.68%); North (4.54%); Center-West (3.40%); Center-North (3.40%) and Southeast (2.84%). This is the first study focusing on *F. hepatica* distribution in dairy cattle covering the entire state of Parana. The data revealed a much different distribution when compared to the diagnosis of *F. hepatica* in slaughtered beef cattle (0.71%). This information will be used to map hot-spots of fasciolosis, creating remote sensing GIS Maps to help producers and the local authorities to monitor and to control associated economic losses, as well as to improve animal welfare.

Keywords: Fascioliasis; Serological Diagnosis; Geospatial Remote Sensing; Parasite

Seroepidemiology of *Fasciola gigantica* in the goat population of District Sargodha, Punjab, Pakistan

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Abstract Content

Fascioliasis is an economically important helminth infection which causes significant losses worldwide. Early diagnosis of the disease is helpful for its control and treatment. The present study is planned to procure excretory/secretory antigens (ES Ag) from indigenous strains of *Fasciola gigantica* for standardization and application of enzyme-linked immuno sorbent assay (ELISA) for sero-epidemiology in goat population. Livers were examined from slaughter house for adult *Fasciola*. The procured adult flukes were used for preparation of ES Ag for the development of indigenous ELISA. In the second phase, using stratified random sampling a total of 3504 blood samples were collected from district Sargodha for separation of sera. The serum samples were screened for anti-*Fasciola* Ab using ES Ag of indigenous strains of *Fasciola* spp. through BioRad Microplate Reader at 450 nm wavelength. Out of 3504 sera, 1496 (42.69%) were found positive for Ab against *Fasciola* spp. Monthly distribution of Ab was highest in January (55.47%) and lowest in July (30.82%). The highest prevalence was found in tehsil Bhalwal (50.32%) followed in order by Shahpur (49.58%), Kot Momin (49.27%), Sahiwal (43.70%), Sillanwali (33.90%) and Sargodha (32.24%). The prevalence of disease was significantly higher in female (48.03%) and adult (48.93%) goats as compared to male (36.34%) and young (36.39%). However, breeds of goat were not found having any statistical association with the sero-positivity. The development of ELISA using indigenous ES Ag and its application over a wider spectrum of host will help in screening sub-clinical infection at a cheaper cost in Pakistan.

Keywords: Epidemiology; Excretory/Secretory Antigens; Indigenous ELISA; Goat; Fascioliasis; Sargodha

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Azithromycin, a candidate orally administrable drug for African trypanosomosis

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Abstract Content

Animal trypanosomosis is a parasitic disease resulting in economic losses of livestock production from anaemia, paralysis and emaciation, untreated cases are mostly fatal. The infection is categorized into nagana (animal African trypanosomosis), surra and dourine. The treatment is based solely on few compounds discovered decades ago which are either toxic or are no longer effective due to pathogen resistance. Therefore, new, effective and less toxic drugs are urgently needed. The objective of this study is to determine the efficacy of various compounds *in vitro* and *in vivo* on animal trypanosomes. Eight compounds were selected according to the inhibitory pathways exhibited in protozoa and were tested *in vitro* for effectivity. Four compounds, triclosan, azithromycin, curcumin and nerolidol possessed trypanocidal effects *in vitro*, with less cytotoxicity effects on the MDBK and NIH 3T3 cells. *In vivo*, Balb/C mice infected with *T. congolense* were treated with azithromycin for 7 and 28 days as short-term and the long term-treatment, respectively. Azithromycin possessed effectivity on *T. congolense* orally, as compared to intraperitoneal administration, with the survival rate of 30%, 30% and 10% survivals at 200, 300 and 400 mg/kg, respectively in the short-term treatment. Parasitemia was suppressed with the clearance of parasites from the peripheral circulation; although, relapse was later observed. The long-term treatment yielded 90% and 100% survival rate at 300 and 400 mg/kg, respectively without any relapse in the surviving mice. In conclusion, azithromycin possesses trypanocidal effects on *T. congolense* both *in vitro*, and *in vivo* when administered orally.

Keywords: African trypanosomosis; Azithromycin; Oral administration; Trypanosoma congolense

Abstract No: 5116

8 Sept 2017, 0915 – 0930

In vitro efficacy of three approved drugs and their synergistic interaction against *Leishmania infantum*

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Abstract Content

Leishmaniasis is one of the most neglected tropical diseases. Unfortunately, the available drugs have many side effects and drug resistance. In the current work the leishmanicidal effect of three approved drugs, auranofin, aluvia and sorafenib, acting on three different enzymes had been done. Their synergistic, additive or antagonistic effects against *L.infantum* promastigotes were investigated. The ultrastructural changes of the treated parasite demonstrated evidences that auranofin, aluvia and sorafeninb had significant anti-leishmanial effect against the promastigotes of *L. infantum* and auranofin showed the highest effect. The combined administration of the drugs in two way combinations led to additive interactions. Whereas, combination of the three drugs had shown synergistic action. The electron microscopic study revealed that the three drugs exerted their leishmanicidal action by inducing apoptosis while alluvia leads also to autophagy.

Keywords: Leishmania infantum, synergism, additive, apoptosis, autophagy.

Abstract No: 4114

8 Sept 2017, 0930 – 0945

Application of probucol as a prophylactic strategy in murine malaria

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Abstract Content

The leading preventive measures against malarial infection seem to be protection from mosquito bites and chemoprophylaxis by antimalarial drugs. However, administration of antimalarial drugs is frequently associated with adverse events and emergence of drug resistant parasites. The results of the present study show a novel preventive and therapeutic strategy against murine malaria based on probucol, an anti-hyperlipidemia drug that modifies the alpha-tocopherol concentration in host plasma. When C57BL/6J mice were fed with 0.5% (w/w) probucol mixed diet for 2 weeks before infection with *Plasmodium yoelii* XL-17 and for 1 week after the infection, they showed remarkably increased survival rate and decreased parasitemia, as compared with the infected mice without probucol treatment. The amount of 8-OHdG, a biomarker of oxidized DNA, detected by immunofluorescence in infected red blood cells from the probucol treated mice infected with parasites was significantly larger than that from the control mice. Alpha-tocopherol supplementation (500 mg/kg in diet) attenuated the effect of probucol on the plasma alpha-tocopherol concentration and eliminated the positive effects of probucol on the *P. yoelii* XL-17 infection. These results indicate that probucol has some impacts on malaria parasites by oxidative stress through the induction of vitamin E deficiency in plasma. The alpha-tocopherol reducing drug such as probucol might be candidates for prophylactic drug against malaria for travelers from malaria-free area.

Keywords: Malaria, Probuco, Vitamin E, C57BL/6J mice, Oxidative stress

The effect of nitidine chloride and camptothecin on the in vitro growth of *Babesia* and *Theileria*

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Abstract Content

Babesia and *Theileria* are tick-transmitted parasites that cause piroplasmosis. Current remedies against piroplasmosis include diminazene aceturate (DA) and imidocarb propionate. Circumstantial evidence, however, has reported emerging resistance against the current drugs necessitating the discovery of new drug compounds and targets. Nitidine chloride (NC) is a benzophenanthridine alkaloid that was previously found as the main active ingredient of a herbal extract used against malaria and influenza in parts of Africa and camptothecin (C) is a quinolone alkaloid whose synthetic analogues, topotecan and irinotecan are readily available anticancer drugs. Previous studies have documented the mode of action of NC and C as topoisomerase inhibitors. In the current study, we tested the efficacy of NC and C against the *in vitro* growth of *Babesia* and *Theileria* parasites. NC and C were effective against *B. bovis*, *B. bigemina*, *B. caballi* and *T. equi* with the half maximal inhibitory concentration (IC₅₀) values of 1.01 ± 0.2, 5.34 ± 1.0, 0.11 ± 0.03, 2.05 ± 0.4 µM and 11.67 ± 1.6, 4.00 ± 1.0, 2.07 ± 0.6, 0.33 ± 0.02 µM respectively. Post-treatment parasite recovery was evaluated by microscopy and both compounds showed similar growth inhibition pattern; NC and C completely cleared *B. bovis*, *B. caballi* and *B. bigemina* at 4x, 4x and 2x of IC₅₀, respectively, with exception of *T. equi*, which regenerated even at the highest concentration 8x of IC₅₀. These results showed the promising efficacy of the NC and C against *Babesia* and *Theileria in vitro*.

Keywords: *Camptothecin; Nitidine chloride; Piroplasmosis; Topoisomerase inhibitors*

Abstract No: 4176

8 Sept 2017, 1000 – 1015

Concurrent proteomic profiling and molecular characterization of cyathostomins

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Abstract Content

With global prevalence up to 100%, potentially fatal infections, and increased reports of anthelmintic resistance, cyathostomins are considered as the most important equine parasites. Currently more than 50 species are described, and typically, mixed infections with 10–15 species occur. Despite their importance, but due to insufficient diagnostic methods, little is known about species-specific characteristics of cyathostomins such as length of life-cycles and pathogenicity. MALDI-TOF MS is a method already established as a routine diagnostic tool for microorganisms and can also be applied to arthropods. Few reports on the application of MALDI-TOF MS on parasitic plant nematodes and larvae of *Trichinella* are published. The two cyathostomin species *Cylicocyclus ashworthi* and *C. insigne* were analyzed. A protein extraction protocol using formic-acid/acetonitril extraction following an ultrasound-mediated tissue disruption was applied, and the soluble material was submitted to MALDI-TOF MS. The insoluble material was used for DNA isolation after buffering to neutral pH. The DNA was used for PCR targeting the internal-transcribed spacer-2 and cytochrome oxidase 1. This protocol allows to record MALDI-TOF MS spectra and PCR-derived sequence data from the same individual nematode. Accordingly, this facilitates the assignment of the spectra unequivocally to morphologically and genetically defined species. MALDI-TOF MS spectra could be generated for adult cyathostomins of both sexes. The established protocol will now be used to generate a comprehensive MALDI-TOF MS master spectra library for adult cyathostomins. Species-specific life-cycle independent peaks that would allow diagnosis of non-adult specimens remain to be identified. Both will be important steps towards species-specific research on Cyathostominae.

Keywords: Cyathostomins; MALDI-TOF MS;

***Fasciola gigantica* control in smallholder larger ruminants by use of anthelmintic-mediated molasses blocks**

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Abstract Content

This study aimed to determine whether triclabendazole-mediated molasses blocks were potentially efficacious for the control of *Fasciola gigantica* in large ruminant smallholder production in Laos. The study involved 241 cattle, allocated into three groups: (1) medicated molasses block(MMB); (2) unmedicated molasses block(UMB); and (3) a control group. On commencement of the study, faecal samples were collected and examined by the sedimentation technique for trematode eggs, and relevant animal details were obtained from 4 data collections(weeks 1,4,8&12). Data analysis was performed in the Genstat statistical package. Reductions in faecal egg counts (FECR) ranged between 45.24% and 90.48% in the MMB group 4 and 12 weeks post-treatment, respectively. There were significant differences in live weights within the MMB, with the mean live weights of 174.60(±3.35)kg and 191.50(±3.69)kg in weeks1 and week12($p=001$), with average daily gain of 201g/day. This study suggests that the use of nutritional supplements via molasses blocks may have a positive impact on the susceptibility of the animal to this important trematode parasite, possibly due to the increased feed conversion efficiency from additional nutrition provided to the cattle by UMB. The positive impacts of supplementation are enhanced if triclabendazole is added in compounding of the block and able to deliver parasite suppressive or potentially therapeutic doses on feeding MMB's. The innovation control of *F.gigantica* infection via MMB may offer a more sustainable parasite management strategy for Lao farmers where farmer knowledge of parasites is very poor and availability of restraint facilities for administration of therapeutics is largely non-existent.

Abstract No: 3090

8 Sept 2017, 1400 – 1415

Development of the double-stranded RNAs as a novel anti-tick biological agent

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Abstract Content

Control of ticks has been attempted primarily by application of acaricides, a method accompanied by dual drawbacks of environmental contamination and by selection of pesticide-resistant ticks. Vaccines administered to host animals have shown promise as a tick control method, but their use and efficacy have been limited. All of these issues reinforce the need for alternative approaches to control tick infestations. RNA interference, the sequence-specific degradation of mRNA mediated by homologous double-stranded (dsRNA), has become a valuable tool in gene knockdown in eukaryotes and in medicine. The sequence specificity of dsRNA coupled with its ability to suppress genes critical for tick survival suggests that dsRNAs could be developed as tailor-made pesticides. In this study, the dsRNA of P0 gene and Rhipilin-1 gene from the tick *Rhipicephalus hemaephsalidis* were used to evaluate the potential as the anti-tick agent. Effects of the dsRNAs treatment on ticks using different dsRNA delivery methods were tested by Quantitative RT-PCR and tick bioassays for the survival, feeding and reproductions. Results showed P0 dsRNAs could be effectively delivered into tick body and silenced by soaking with liposomes. Soaking time was found to be the most important factor in dsRNA delivery and gene silencing compared with liposome types and dsRNA concentration. Effects of dsRNA of Rhipilin-1 and P0 treatment on ticks were tested and found the significant anti-tick role in either blood feeding or molting or reproductions. We concluded that anti-tick agents based on dsRNAs might have huge potential and researches should be explored further.

Keywords: tick; double-stranded RNA; biological agent

Abstract No: 2643

8 Sept 2017, 1415 – 1430

Immunoprotective efficacy of purified midgut antigens of *Hyalomma anatolicum anatolicum* in Egypt.

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Abstract Content

H. a. anatolicum was collected from naturally infested cattle in Baharia, Al Kharga Oases and Siwa (Egypt). Midgut antigens were purified into three peaks (Peak I, peak II and peak III) by gel filtration chromatography for immunization of rabbits against tick infestation. Purified midgut peak III showed a significant reduction in the biological parameters of tick and effect in feeding and reproduction performance. Serum globulins cleared a significant ($P < 0.05$) in rabbits immunized with peak II then peak III of purified midgut. Antibody levels were detected by ELISA with a significant ($P < 0.05$) increase in rabbits with peak III. Detection of polypeptide bands was done by using SDS-PAGE. By using western blot, identified a 59, 51, 33 and 20 KDa protein might be diagnostic specific in purified midgut (peak II & peak III) of *H. a. anatolicum* ticks while the polypeptide band with molecular weight 66 KDa was the most immunogenic epitopes which might be play a role in immunization and protection against *H. a. anatolicum* in Egypt.

Keywords: purified midgut antigens; *Hyalomma anatolicum anatolicum*; gel filtration chromatography; SDS-PAGE; Western blot.

Abstract No: 4007

8 Sept 2017, 1430 – 1445

A recombinant subunit vaccine for the control of ovine psoroptic mange (sheep scab)

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Abstract Content

Sheep scab, caused by infestation with *Psoroptes ovis*, is highly contagious, causing intense pruritus and represents a welfare and economic concern. Disease control relies on chemotherapy, however, sustainability is questionable due to chemical residues, eco-toxicity and acaricide resistance. Control by vaccination is supported by demonstration of protective immunity in previously infested sheep. We identified vaccine candidates for *P. ovis* based on: (1) antigens selected by their proposed interaction with host signalling pathways and the host immune-response; and (2) those shown to be either immunogenic during natural infestations or involved in mite feeding. This resulted in the development and validation, in repeated immunisation and challenge trials, of a seven recombinant protein sub-unit cocktail vaccine. Sheep were immunised on three occasions, 2 weeks apart, with QuilA. Immunisation resulted in highly significant reductions in lesion size (up to 63%) and mite numbers (up to 56%) following parasite challenge. Mean lesion size in vaccinates was significantly smaller than controls from 1 week post infestation (wpi) until the end of the experiment at 6 wpi ($p=0.001$). All antigens elicited serum IgG responses following immunisation, whereas control sheep, which were immunised only with adjuvant, did not produce antigen-specific IgG during the pre-infestation period. Vaccinated animals showed an amnestic response, with levels of antigen-specific IgG against muGST, Pso o 1 and Pso o 2 increasing following infestation. The effect of this vaccine represents the greatest reduction in lesion size to date with a sheep scab vaccine, providing encouragement for future production of a commercially-viable means of immunoprophylaxis.

Keywords: Vaccine, ectoparasite, control, recombinant, sheep scab

Kapok (*Ceiba pentrand*) fiber mite trap as a non-chemical mite control in tropical countries

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Abstract Content

Red poultry mites (*Dermanyssus gallinae*) infestation can cause high economic losses, mainly in layer chicken. Conventional control of the parasite has been hampered with the wide spread of insecticide resistant. A new non-chemical mite control method using a polyurethane fiber trapping device (i-Trap, Kondo Electronics) has been developed in Japan but still not used in tropical countries due to cost and ignorance towards poultry parasite problem. This study was designed to evaluate the effectivity of low-cost Kapok (*Ceiba pentrand*) fiber in trapping *Dermanyssus gallinae*. Three trap types were made in this research, the standard (polyurethane) i-Trap as control, the hybrid (polyurethane – kapok) trap, and kapok trap respectively. The traps were exposed to natural red poultry mites infestation in a research farm. The electrostatic mapping showed that control trap has the highest electrostatic charge followed by hybrid and the kapok trap. The average total mite number counting showed that the control trap captured highest amount of mites (5184.5) followed by hybrid (4598) and the kapok trap (1898.5). This result showed that there is a connection between electrostatic charge and trap ability to attract mites despite it is not the only variable that influences this ability. The material characteristic, such as fiber size and pattern also influence the result as showed in the microscopic examination result where Kapok fiber could effectively stop mite movement. Therefore, Kapok fiber is still applicable as mite trap filling material especially for tropical areas such as Indonesia and could induce paradigm shift towards non-chemical parasite control development.

Keywords: Kapok fiber; Mite trap; Non-chemical control; Electrostatic; Poultry mite

Abstract No: 4921

8 Sept 2017, 1500 – 1515

Seroprevalence of *Toxoplasma gondii* infection among ruminants in Bangladesh

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Abstract Content

Toxoplasmosis is a zoonotic protozoan disease. The objective of the study was to estimate the seroprevalence of toxoplasmosis along with associated factors in sheep, goats and cattle in Dhaka, Mymensingh, Sirajganj and Chittagong districts in Bangladesh. A total of 1104 sera sample (552 sheep, 300 goats and 252 cattle) from study areas were randomly selected and tested by iELISA. The overall seroprevalence of toxoplasmosis was 12.23%. The seroprevalence of toxoplasmosis was significantly ($p=0.008$) higher in goats (16.0%) compared to cattle (8.33%). The odds of toxoplasmosis was 2.09 times higher in goats than cattle (Odds ratio (OR): 2.09, 95% Confidence Interval (CI): 1.23-3.67). In sheep, significant variation of seroprevalence in herd types, districts and pregnancy status were observed. The odds of toxoplasmosis was 13.96 times higher in pregnant sheep (OR: 13.96, 95% CI: 6.02-38.31) compared with young and male. Significant variation of toxoplasmosis was observed in different cattle breeds and districts. The odds of toxoplasmosis was 5.79 times higher in Holstein Friesian cross cattle than indigenous cattle (OR: 5.79, 95% CI: 1.13-24.62). Our study indicates that exposure of sheep, goats and cattle to oocysts of *T. gondii* are widespread and suggest that consumption of raw and undercooked meat of these animals can be a probable source of human toxoplasmosis. It is necessary to explore the nation-wide epidemiology of toxoplasmosis and the transmission dynamic to understand the socio-economic impact.

Keywords: Toxoplasmosis, Zoonotic, Seroprevalence, Food Animals, Bangladesh

Abstract No: 4503

8 Sept 2017, 1515 – 1530

Evaluation of maggot therapy in healing of dermal wound in Wistar rats with diabetes mellitus

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Abstract Content

The present study was aimed to evaluate the effectiveness of maggot therapy in healing of chronic wound in Streptozotocin induced diabetic Wistar rat. The sterilized eggs of *Lucilia sericata* were obtained from colonies established in laboratory. A total of 48 numbers of male Wistar rat were taken for the study. Wounds were prepared in all and induced with mixed colonies of bacteria like *Staphylococcus aureus*, *E.coli* and *Pseudomonas aeruginosa*. Whole animals further divided in 4 groups with 12 rats each being presented as control, antibiotic, maggot and maggot and antibiotic in combination treated. All treatments were applied once and held for 24 hours. Different wound kinetics in maggot treated wounds revealed significant reduction in wound area with maximum contraction was found (>95%) led closure of wound in maggot treated when compared to antibiotic treated (79%) and control (72%) respectively and early elimination of bacterial bioburden (7.88 ± 0.03)log CFU/ml to (1.12 ± 0.65)log CFU/ml and (7.86 ± 0.04)log CFU/ml to (1.54 ± 0.52)log CFU/ml in maggot as well as maggot and antibiotic in combination respectively in three weeks of time. The histopathological examination of wounded tissue of maggot treated groups showed early and better epithelialization, collagenation and neovascularization with complete healing of wound in three weeks in comparison to antibiotic and control respectively. The effect of maggot and maggot with antibiotic combination used in the present study didn't show any difference in healing of wound.

Keywords: Chronic wound; Diabetes; Healing; Lucilia sericata; Maggot therapy; Wistar rat

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Changing epidemiology of *Nematodirus battus* in the UK: Alternative hatching patterns and investigation of drivers

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Abstract Content

Nematodirus battus, a gastrointestinal nematode associated with disease in lambs, appears to be demonstrating an emerging trend for hatching outside the expected spring window, thus lengthening its seasonality. Literature suggests that hatching of *N. battus* eggs is largely governed by temperature and water availability, with eggs requiring a chill stimulus followed by at least 10 days within the optimum temperature range. Recent reports of *N. battus* in late summer and autumn suggest that hatching may be occurring without a period of chilling as previously documented. The hatching experiments conducted, assessed the ability of eggs from 90 UK *N. battus* populations to hatch *in vitro* with and without a chill stimulus. Preliminary analyses indicate that the proportion of eggs successfully hatched without a chill stimulus is $13 \pm 2\%$ (mean \pm SEM) ranging between 0-81% with almost a quarter of isolates (24%) exhibiting a hatch over 20%. The data indicate a trend towards higher proportional hatch in the North in the absence of a chill stimulus (estimate 0.49; 95% CI 0.46 - 0.53, $p < 0.001$). We believe that the control and alteration of hatching patterns in *N. battus* is governed by a combination of drivers including several environmental and climatic factors in addition to animal management and selection pressures. Modelling of these factors will allow for detailed investigation of the impact and interactions of these drivers on the ability of eggs to hatch in the absence of a chill stimulus in different populations of *N. battus*. This could lead to future management advice.

Keywords: Nematodirus battus; Gastrointestinal nematode; epidemiology; hatching patterns; risk factors

Comparison of faecal egg counts distribution in Boer goats between natural infection in England and deliberate infection in Malaysia

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Abstract Content

Faecal egg counts (FEC) are widely used as an indicator for nematode resistance in breeding small ruminants. However, less is known about FEC distribution in goat populations than sheep. This study compared FEC distribution in Boer goats following natural infection in England and deliberate infection in Malaysia. A total of 158 goats were sampled from a farm in Lancaster, England between August and October 2014 and 30 goats were sampled from a farm in Selangor, Malaysia between July and September 2015. In England, the goats were grazed on pasture. In Malaysia, all goats were kept indoors where 25 goats were each infected with 2400 L3 of *Haemonchus contortus*, *Trichostrongylus* and *Oesophagostomum* at 6:1:1 ratio. Faecal samples were collected per-rectum at four weeks intervals in England and twice a week for eight weeks post-infection in Malaysia. Faecal samples were counted by modified McMaster technique and analysed by SAS 9.4. FEC varied from 0 to 13850 epg in England and 0 to 2325 epg in Malaysia. In both locations, FEC variances were greater than means. FEC distributions were typically right-skewed and highly overdispersed while k ranged from 1 to 3 ($p < 0.0001$) in England and 1 to 2 ($p < 0.0001$) in Malaysia. *Teladorsagia circumcincta* and *H. contortus* were the predominant species harvested from faecal culture in England and Malaysia, respectively. Most Boer goats had low FEC while only a few had high FEC regardless of location, climate, infecting parasite and infection types. This suggests opportunities for genetic selection of Boer goats for nematode resistance.

Keywords: faecal egg counts; resistance; distribution; Teladorsagia circumcincta; Haemonchus contortus

Abstract No: 4517

8 Sept 2017, 0930 – 0945

Warble fly infestation in goats of Jammu region, North India - Present status and future strategies

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Abstract Content

Goat warble fly infestation (GWFI) is a myiasis caused by larvae of *Przhevalskiana silenus* Brauer, 1858, an insect belonging to Order *Diptera*, Family *Oestridae*, Subfamily *Hypoderminae*. This myiasis is characterized by presence of subcutaneous warbles on the dorsal and lumbar region of goats. Although, the prevalence of bovine hypodermosis has been estimated throughout the world, because of limited geographical distribution, not much reports have been documented for *P. silenus* infestation. The present study was carried on 360 slaughtered goats at the Jammu Municipal Committee abattoir, Jammu and Kashmir state, North India. An overall 172 (47.77%) goats were found positive for *P. silenus* infestation. Trimming and discard of larvae infected edible meat and deprecation of hide because of holes in the skin resulted in economic losses. Presently, the farmers follow manual extraction of larvae from affected goats as the mean of the treatment. Use of chemicals as prophylaxis/treatment of the infestation is very rarely followed. Considering the economic significance of the disease, future strategies has been devised wherein immunosurveillance of GWFI in goats of Jammu and Kashmir state using ELISA will be carried out along with prophylaxis campaign using cost effective minidose ivermectin/eprinomectin application.

Keywords: Goat Warble Fly infestation, Przhevalskiana silenus, Goat, India

Abstract No: 3376

8 Sept 2017, 0945 – 1000

Prevalence of *Sarcocystis* in goat carcasses slaughtered at the industrial Abattoir of Urmia, Iran by Digestive method

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Abstract Content

Sarcocystis is an obligatory intracellular protozoan parasite which can cause digestive disturbances in infected patients. Also, can cause important economic loss and disease in livestock. This study aimed to determine the incidence of *sarcocystis* sp. Infection in slaughtered goats at the abattoir of Urmia, Iran by digestive method a. During april 2015 to march 2016, 288 slaughtered goats in urmia industrial abattoir (urmia, iran), were investigated for the presence of macroscopic *sarcocystis* by direct observation. Each of the investigated goat was classified into groups according to the age, sex and infected muscle tissue. All tissue samples were sectioned to 2-3 mm slices and observed carefully for probable macroscopic cysts. The sections were then examined by the peptic digestion method. Our study showed that the digestion method is useful to identify infected samples. According to the results, 12 out of the 288 goats (4.17%) were diagnosed as being infested with macroscopic cysts and high frequency of microscopic *sarcocystis* infection in goat slaughtered in this area was seen. About 154 goats (53.47%) was diagnosed as positive infasted *sarcocystis* species by the digestion method. The data analysis indicated that there is a statistically significant difference between age groups, the infection rate increased with age ($p < 0.05$). the infection rate was independent of sex being (74/138, 53.62%) in males and (80/150, 53.33%) in females, and this difference was not significant ($p > 0.05$). Also, there was a significant difference between prevalence of *sarcocystis* infection in different examined muscles and most microcysts were diagnosed in skeletal ($p < 0.05$).

Keywords: Sarcocystosis; Digestive method; Urmia; Small ruminants; goat

Plant strata and not plant species help to explain gastrointestinal nematode worm burden in sheep and goats browsing the tropical forest

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Abstract Content

This study linked the feeding behavior of sheep and goats browsing a tropical forest (TF) during rainy season with their gastrointestinal nematodes (GIN) burden. The study lasted 12 weeks (August to November) in Yucatán, México. Six pairs of worm-free tracers kids (19.5 ± 2.5 kg) and lambs (22.4 ± 4 kg) were exposed to infection. Respective pairs of tracer kids and lambs browsed daily for 4 h over a 3-week period. Direct observation of bites was used to estimate dry matter intake (DMI) from grasses, vines, herbs and shrubs. Height of foliage strata consumed was recorded as low (< 25 cm), medium (25-50 cm) and high (>50 cm). Tracers were recovered after exposure to the TF, were isolated for 28 days and were humanly sacrificed to retrieve GIN burdens. Median GIN burdens were 582 (40-1890) and 495 (100-3680) for kids and lambs ($P>0.05$). Higher GIN burdens on weeks 7 to 12 ($P<0.05$) indicated a gradual infectivity build-up in the TF. Kids and lambs consumed 60 and 63% of DMI from grasses, 2 and 1% from vines, 0.2 and 0.3% from herbs and 38 and 36% from shrubs. Kids and lambs consumed 15.1 and 12.3% of DMI from high strata, 21.4 and 19.4% from mid and 63.4 and 68.3% from low strata. No significant correlation was found between the group of plant consumed and GIN burden, but high strata DMI was negatively correlated with GIN burden in goats and sheep (-0.58 and -0.65) ($P<0.05$). Funded by CONACYT CB-2013-01 221608.

Keywords: Infectivity, gastrointestinal nematodes, goats, sheep, feeding behavior

Abstract No: 4328

8 Sept 2017, 1015 – 1030

Antibody Concentrations against Gastrointestinal Nematodes in Adult Beef Cows From the Prairie Provinces of Western Canada

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Abstract Content

It was the objective of this study to describe the seroprevalence and quantity of serum anti-*Ostertagia* antibodies of adult beef cows from the western Canadian Prairie Provinces, using the SVANOVIR *Ostertagia ostertagi*-antibody enzyme-linked immunosorbent assay (ELISA). Serum from 2,064 adult beef cows from 106 herds from Alberta, Saskatchewan, and Manitoba, were collected in the fall of 2014. Frozen (-80°C) serum samples were analyzed in duplicate for the presence of anti-*Ostertagia* antibodies using the SVANOVIR *Ostertagia ostertagi*-Ab ELISA. Optical density values were standardized as an optical density ratio (ODR) using duplicate negative and positive control sera on each plate. Mean ODR were calculated for each cow. A cut-off point of 0.5 ODR was used to identify cows with high amounts of anti-*Ostertagia* antibodies. The mean cow ODR was 0.7 (Standard Deviation 0.3). Seventy-four percent (95% Confidence Interval 72-76) of cows had an ODR above the 0.5 ODR cut-off point suggesting a high amount of antibody. This is the first study demonstrating the use of serology for determining anti-*Ostertagia* antibodies in adult beef cattle. Mean ODR in this study were similar to those seen in mature dairy cattle, but higher than mean ODR from young beef stock when compared to published literature. In this study, 74% of ODR were above 0.5, a value that correlated with reduced milk production in dairy cattle. The relationship between adult beef cow ODR and production indicators should be explored, for this test to be considered as a potential diagnostic tool.

Keywords: beef cows, seroprevalence, anti-Ostertagia antibodies, western Canada

Abstract No: 4357

8 Sept 2017, 1400 – 1415

100 important research questions on helminths of livestock

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Abstract Content

The Livestock Helminth Research Alliance (LiHRA, www.lihra.eu) was founded in 2014 to promote collaboration among researchers in this field, initially in Europe. Following initiatives in other subject areas, an exercise was conducted to identify the 100 most pressing or interesting questions in livestock helminthology. LiHRA members were asked to provide between three and five questions each, independently of each other, and these were combined and analysed for repetition, before being redistributed and scored. A prioritised list ensued, which was organised into sub-areas. Here we present the draft list and seek inputs from other areas of the world, to achieve a more globally representative perspective. The aim is not to judge the relative importance of different areas or to provide an authoritative research agenda, but rather to take stock of the state of the art, and stimulate research at a range of scales and complexity, to address the most exciting opportunities and challenges.

Keywords: helminths, ruminant, livestock, research priorities

Abstract No: 4580

8 Sept 2017, 1415 – 1430

The health seeking behaviours and usage of anthelmintics for schistosomiasis in animals by farmers and veterinarians in rural Senegal.

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Abstract Content

Background; Molecular genetic studies of parasites isolated from children in West Africa have identified viable hybrids of human *Schistosoma haematobium* with livestock *S. bovis* and/or *S. curassoni*, demonstrating a zoonotic component. Yet, little work exists on anthelmintic usage in animals. The potential emergence and establishment of drug resistance is a concern for current mass drug administration of human populations, using the only available drug, Praziquantel, also for future treatment regimes for livestock. Aim; to establish access and use of Praziquantel in rural Senegal. Methods; In-depth key informant interviews (n = 12) were performed with veterinarians and veterinary wholesalers to establish access to pharmaceuticals in Senegal. Focus groups (n = 30) discussed health seeking behaviours, access to pharmaceuticals and treatment practices with farmers in four Senegalese regions (Barkadji, Richard Toll, Dakar and Koungheul). Interviews were recorded and transcribed, NVivo qualitative data analysis Software, QSR International Pty. Version 11, 2016, was used for thematic analysis according to the NIH framework. Provisional Results; Praziquantel was the main medication used to treat schistosomiasis in livestock. Farmers purchased medication from their veterinarian or pharmacy however most used the local market. Farmers felt that there was good efficacy of both the market bought and prescribed Praziquantel. Livestock death following treatment in high infection intensity cases was noted. Human and animal formulae were being used for treatment of animals. Dosages used ranged from 4mg/kg – 50mg/kg. Impact; The appropriate availability and usage of praziquantel for livestock schistosomiasis needs further investigation into dosages, resistance risk and standardised treatment.

Keywords: Schistosomiasis, Resistance, Praziquantel, Livestock, Zoonotic, Hybrids, Anthelmintic

Abstract No: 4475

8 Sept 2017, 1430 – 1445

B cell epitope mapping of cathepsin L1 in *Fasciola hepatica*-infected and vaccinated cattle

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Abstract Content

The worm parasite *Fasciola hepatica* exists on every continent of the world causing important economic losses in livestock, as well as zoonotic infection. In the Republic of Ireland, *F. hepatica* infection has an 85% prevalence in cattle. Due to the increase of anti-helminthic resistance, a vaccine-based approach to control of Fasciolosis is urgently needed. A recombinant version of cathepsin-L 1 (rmFhCL1) from *F. hepatica* has been a vaccine candidate for many years, and is capable of delivering partial protection against infection in cattle. Here we show for first time the linear B-cell epitopes recognised in the FhCL1 protein by serum from *Fasciola hepatica*-infected and vaccinated cattle. B-cell epitope mapping assays were carried out by ELISA using 9 mer-overlapping peptides from FhCL1 with an overlap of 7 mers. One of the regions in the protein (aa 19-31) is consistently and highly recognised in all infected and vaccinated animals. In the 3D structure of FhCL1 this region appears exposed in the periphery of the protein. Other commonly recognised epitopes overlap with the active site of the protein. We observed differences in the recognition pattern by vaccinated vs infected animals. Epitope mapping studies are an important tool in the development and refinement of vaccines capable of protecting against *F. hepatica* infection, as well as in the advancement for understanding *F. hepatica* infection dynamics.

Keywords: Fasciola hepatica; cattle; epitope mapping; Cathepsin L1

Abstract No: 3893

8 Sept 2017, 1445 – 1500

Serological evidence of *Neospora caninum* in smallholder cattle and buffalo in central and northern Lao PDR

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Abstract Content

An enzyme-linked immunosorbent assay (ELISA) was used to screen for antibodies to the protozoan parasite *Neospora caninum* in serum collected and stored from large ruminant females on smallholder farms in Laos. Serum from 61 buffalo and 90 cattle were selected from the National Animal Health Laboratory in Vientiane, Laos, collected between 2013 and 2016, with proportional random selection from six agriculturally significant provinces. Animal age, season and year of collection were recorded. Buffalo samples demonstrated a seroprevalence of 68.9% (95% CI \pm 17.2) which was significantly higher than the cattle seroprevalence of 7.8% (95% CI \pm 1.5) ($P < 0.001$). There were significant differences in seroprevalence among the six provinces ($P = 0.007$) indicating the potential for regional risk factors conducive to increased pathogen transmission rates in some areas. A negative correlation was also observed between *N. caninum* and bovine viral diarrhoea virus (BVDV) seropositivity, reflected in univariable modeling where increasing BVDV titres were associated with decreased probability of *N. caninum* seropositivity in buffalo ($P = 0.018$) and cattle ($P = 0.003$). The investigation of this abortifacient parasite which has also been noted on serological examinations in neighbouring South-East Asian countries is of potential importance in understanding reproductive constraints in large ruminant populations that currently inhibits the development of market-driven beef supply from smallholder farmers. These findings justify further research to determine potential productivity impacts of *N. caninum* and disease risk factors, particularly in buffalo.

Keywords: Buffalo, cattle, Laos, N.caninum, reproduction, smallholders

Abstract No: 4228

8 Sept 2017, 1500 – 1515

Sheep worms in young cattle

Tania Waghorn^{*1}

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Abstract Content

The practise of mixed or rotational grazing, cattle following or co-grazing with sheep is common on a lot of New Zealand sheep and beef farms. These practises are recommended as clean-up measures for parasites of both cattle and sheep, with the thinking that few parasite species cross infect and when they do, they do poorly in the non-preferred host. Over two seasons we ran a number of trials looking at the prevalence, generic composition and possible impact of sheep gastro intestinal parasites in young beef calves. In the first season we followed three groups of calves for approximately a year, collecting and culturing faecal samples to determine which species were present when. Results from the first season showed that a number of sheep worm species were commonly present in calves. On some farms, the sheep worms *Cooperia curticei* and *Haemonchus contortus* were present in all the samples collected from calves through until March (autumn), and in quite high numbers. Two studies were subsequently undertaken to further assess the prevalence and impact of *H. contortus* in calves. As an adjunct to this a slaughter study was undertaken to look at the ability of a field strain of *H. contortus* to cross infect sheep and calves. *H. contortus* was consistently found in calves cross grazing with sheep and was able to establish viable infections in both species but had no impact on the growth of the calves. The parasites were smaller and fewer in number but seemed to be fitter from calves.

Keywords: Cattle; Haemonchus contortus; co-grazing; sheep parasites

Gastrointestinal nematode prevalence and species composition in breeding-age heifers on Canadian dairy farms

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Abstract Content

Gastrointestinal nematodes (GIN) are a threat to the health and welfare of cattle worldwide and have substantial detrimental effects on cattle productivity. Furthermore, evidence of anthelmintic drug resistance is increasing worldwide. There is a scarcity of information regarding the GIN burden in Canadian dairy heifers. Additionally, there is no information on the predominant nematode species present in these heifers on which to make evidence-based recommendations for sustainable control. Therefore, fecal samples (n=2,369) were collected from breeding-age heifers on 306 dairy farms from all 10 Canadian provinces. Eggs per gram of feces (EPG) were determined using the Modified Wisconsin Sugar Flotation Technique. Predominant GIN species at the farm-level were identified by deep amplicon nemabiome sequencing of the ITS-2 rDNA locus of nematode larvae. Population-averaged prevalence and overall EPG, accounting for clustering on farms, were 20.9% (95% CI: 17.2 – 24.6%) and 1.10 EPG (95% CI: 0.64 – 1.55 EPG), respectively. Individual heifer egg counts ranged from 0 – 141 EPG (median: 0 EPG; range: 0 – 70.6 EPG). The predominant species were *Cooperia oncophora* and *Ostertagia ostertagi*. Although the results of this study are consistent with the literature for young cattle in temperate climates, they provide much needed epidemiologic data on GIN in Canadian dairy heifers. The use of the high-throughput deep-sequencing assay to determine nematode species, in combination with traditional egg count methods, provides improved opportunity to apply evidence-based, sustainable control strategies and to better investigate treatment efficacy in the future.

Keywords: Gastrointestinal nematode; prevalence; dairy; heifer; Canada

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Abstract No: 3934

8 Sept 2017, 0900 – 0915

Repellency effect of an imidacloprid/ flumethrin (Seresto®, Bayer) controlled release polymer matrix collar against the Australian paralysis tick (*Ixodes holocyclus*) in dogs.

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¹Animal Health/ Bayer Australia Ltd./ Australia, ²Wongaburra Research Centre/ Invetus Pty. Ltd./ Australia

Abstract Content

Repellency is a highly desirable attribute for an acaricide that protects dogs against the Australian paralysis tick (*Ixodes holocyclus*). A bite from a single tick of this species can be fatal and carries the risk of transmission of vector-borne diseases. A randomised, controlled study was conducted to confirm the repellency *sensu stricto* and *sensu lato* (expellency) of a 10 % imidacloprid/4.5 % flumethrin collar (Seresto®, Bayer) on dogs, against artificial infestations of *I. holocyclus*. Based on a novel protocol, dogs treated with Seresto® or a placebo collar, were sedated and placed in infestation chambers for 1 h at varying time points up to 6 months after treatment. Ticks were released alongside the dogs, and allowed to approach and/or attach. Treating dogs with a Seresto® collar resulted in significantly ($p < 0.001$) more residual ticks being found in the infestation chambers (repellency *sensu stricto*) compared to the placebo treated dogs for the 168-day duration of the study (range 67.5% to 90.2 %). Repellency *sensu lato* was reflected in significantly ($p < 0.002$) fewer ticks being found attached to treated dogs throughout the study. Comparative decrease in ticks attached exceeded 95 % for 84 days, when assessed after 6 h. Efficacy (killing effect) of the Seresto® collar based on total live tick counts was $> 95 \%$ 6 h after tick challenge throughout the 6 month study period. This study demonstrated the excellent repellency effect of the Seresto® collar against *I. holocyclus* in dogs.

Keywords: Seresto®; Australian paralysis tick; *Ixodes holocyclus*; repellency; dog

Abstract No: 4498

8 Sept 2017, 0915 – 0930

Assessment of the onset of lotilaner (Credelio™) speed of kill of fleas on dogs

Daniela Cavalleri¹; Martin Murphy¹; Wolfgang Seewald¹; Jason Drake²; Steve Nanchen¹
¹R&D/ Elanco Animal Health/ Switzerland (Schweiz), ²Global Marketing/ Elanco/ United States

Abstract Content

Introduction: Lotilaner (Credelio™, Elanco, Greenfield, USA) is the newest member of the novel isoxazoline class; administered orally, it is rapidly absorbed with peak blood levels within two hours post-treatment. A study was undertaken to determine the earliest onset of lotilaner's efficacy against existing flea (*Ctenocephalides felis*) infestations. **Materials/Method:** 64 Beagles were randomly allocated to eight groups: Groups 1 to 4 were administered lotilaner, at as close as possible to the minimum dose rate of 20 mg/kg within 30 (\pm 5) minutes after feeding; Groups 5 to 8 were untreated controls. All dogs were infested with 100 ± 5 fleas on Day -2, and flea counts completed at 30 minutes and one, two and eight hours after treatment on Day 0. **Results:** At 30 minutes and one hour post-treatment, there were no significant reductions in mean flea counts in lotilaner-treated dogs, when compared with the control groups, although moribund fleas were evident at one hour post-treatment on the treated dogs. At two hours after treatment, the geometric mean flea count reduction in the lotilaner group was 64.0% ($P = 0.0242$). At eight hours after treatment, lotilaner efficacy was 99.6%. There were no treatment-related adverse events. **Conclusions:** This study demonstrates that lotilaner flavored chewable tablets are well tolerated and begin to kill fleas within two hours of treatment, achieving 99.6% efficacy within eight hours.

Keywords: lotilaner; Credelio; dogs; fleas; efficacy

Abstract No: 4454

8 Sept 2017, 0930 – 0945

Assessment of the speed of flea kill of lotilaner (Credelio™) throughout the month following oral administration to dogs

Daniela Cavalleri^{*1}; Martin Murphy¹; Wolfgang Seewald¹; Jason Drake¹; Steve Nanchen¹
¹R&D/ Elanco Animal Health/ Switzerland ¹Global Marketing/ Elanco Animal Health/ United States

Abstract Content

Introduction: Lotilaner (Credelio™, Elanco, Greenfield, IN, USA), a novel isoxazoline, is a systemic ectoparasiticide rapidly absorbed following oral administration to dogs, with a half-life of 30 days. Studies were undertaken to investigate its initial and sustained efficacy and speed of kill against fleas. **Materials/Methods:** Lotilaner speed of flea knockdown and sustained speed of flea kill (SOK) up to 35 days post-treatment were evaluated in four studies, assessing one or two specific time points (4, 6, 8 and 12 hours) post-treatment and following weekly re-infestations. Dogs were randomized to lotilaner or untreated groups, and before treatment infested with adult *Ctenocephalides felis*. Lotilaner dogs received one treatment on Day 0, close to the minimum recommended dose rate of 20 mg/kg, after feeding. Efficacy was calculated using geometric and arithmetic mean flea counts in treated versus untreated groups. **Results:** On Day 0, lotilaner efficacy was 89.9% at four hours, 99.2% at six hours, 99.9% at eight hours, and 100% at 12 hours post-treatment. At weekly assessments, lotilaner efficacy at four hours was > 97%, at eight hours > 99%, and at 12 hours 100% through Day 35. There were no treatment-related adverse events. **Conclusion:** Lotilaner consistently showed rapid flea knockdown and sustained SOK through 35 days post-treatment, thus showing the capability to disrupt the flea life cycle in a contaminated environment, and to prevent resurgence in flea burdens with the potential consequence of flares in flea-bite hypersensitivity.

Keywords: Lotilaner; Isoxazoline; Dog; Flea; Speed of kill

Abstract No: 4459

8 Sept 2017, 0945 – 1000

Laboratory evaluation of the speed of kill of lotilaner (Credelio™) against *Ixodes ricinus* ticks on dogs

Martin Murphy^{*1}; Daniela Cavalleri¹; Wolfgang Seewald¹; Jason Drake; Steve Nanchen¹
¹Research and Development/ Elanco/ Switzerland (Schweiz)

Abstract Content

Introduction: Lotilaner, a novel isoxazoline, is rapidly absorbed following administration of a chewable tablet formulation (Credelio™, Elanco, Greenfield, IN, USA) to dogs, providing at least 98% efficacy for a minimum of one month against *Ixodes ricinus*. A study was conducted to determine its speed of kill against ticks. **Materials/Methods:** 32 dogs were randomized to four groups: two received lotilaner tablets at a minimum dose rate of 20 mg/kg and two were left untreated. Infestations with *I. ricinus* were performed on Days -2, 7, 14, 21, 28 and 35. Counts were completed 4 and 8 hours on Day 0, and 8 and 12 hours following subsequent infestations. All live ticks were incubated for 24 hours following removal. **Results:** At 4 hours post-treatment, there was 69.8% reduction in geometric mean live tick counts in treated dogs compared to controls. After incubation, reduction increased to 97.2%. At 8 hours after treatment, pre- and post-incubation reductions were 99.2% and 100%, respectively. Following post-treatment challenges, post-incubation efficacy through Day 28 at 8 and 12 hours was at least 94.3% and 98.0%, respectively, and 85.7% and 94.2% at 8 and 12 hours after the Day 35 challenge. There were no treatment-related adverse events. **Conclusions:** Lotilaner at a minimum dose rate of 20 mg/kg was well tolerated and began to kill ticks on dogs within four hours of treatment and efficacy was 100% within 8 hours and sustained a rapid kill of *I. ricinus* ticks through 35 days.

Keywords: Lotilaner; *Ixodes ricinus*; Speed of kill; Dog; Isoxazoline;

Abstract No: 4439

8 Sept 2017, 1000 – 1015

A randomized, blinded, controlled field study to assess the efficacy and safety of lotilaner tablets (Credelio™) in controlling fleas in client-owned dogs in European countries.

Daniela Cavalleri¹; Martin Murphy¹; Wolfgang Seewald¹; Jason Drake²; Steve Nanchen¹
¹R&D/ Elanco Animal Health/ Switzerland (Schweiz), ²Global Marketing/ Elanco Animal Health/ United States

Abstract Content

Introduction: Lotilaner, a novel isoxazoline, administered orally to dogs, was shown to be safe and produce rapid flea and tick knockdown, with sustained speed of kill for at least 1 month. A study was undertaken to demonstrate efficacy, safety and palatability of lotilaner chewable tablets (Credelio™, Elanco, Greenfield, IN, USA), under field conditions. Materials/Methods: 192 dogs with at least 5 fleas, from 17 veterinary clinics across Germany, Hungary and Portugal were randomized 2:1 to lotilaner (minimum dose rate 20 mg/kg) or Frontline® Spot-on (Merial GmbH, Hallbergmoos, Germany) according to label, administered on Days 0, 28 and 56; supplementary household dogs received the same treatment as primary dogs. Flea counts and flea allergy dermatitis (FAD) assessments were made on primary dogs on Days 0, 14, 28, 56, 84. Safety and palatability of lotilaner tablets were assessed in all dogs. Results: Lotilaner efficacy was 99.1%, 99.5%, 99.9% and 99.8% on Days 14, 28, 56 and 84, respectively. Corresponding reductions for fipronil were 93.4%, 91.2%, 94.4% and 97.0%. Lotilaner was superior to fipronil at all assessments ($t_{(186)} \geq 3.43$, $P \leq 0.0007$). At every assessment, at least 90% of lotilaner-treated dogs (98.4% on Day 84) and fewer than 90% of fipronil dogs were flea-free. Credelio™ was well tolerated, palatable, and alleviated or eliminated FAD signs, including pruritus. Conclusions: Credelio™ flavoured chewable tablets were palatable, safe and provided superior flea control to fipronil. Efficacy was greater than 99% at the first post-treatment assessment and was maintained through Day 84, with corresponding improvements in FAD.

Keywords: Lotilaner; Isoxazoline; Flea; Dog; Field efficacy

Abstract No: 4423

8 Sept 2017, 1015 – 1030

A study on the long term efficacy of Seresto® collars in preventing *Babesia canis* transmission to dogs by infected *Dermacentor reticulatus* ticks

Josephus Fourie^{*1}; Julian Liebenberg¹; Katrin Deuster²; Matthias Pollmeier²; Bettina Schunack²
¹Scientific operations/ Clinvet International (Pty) Ltd/ South Africa, ²GmbH/ Bayer Animal Health/
Germany

Abstract Content

Introduction: Seresto® collars were previously shown to prevent infection with *Babesia canis*, transmitted by *Dermacentor reticulatus*, in dogs, for up to one month after application. The current study evaluated prevention of transmission over an extended period. **Methods:** Eight animals were randomly included in groups 1 (negative control) and 2 (Seresto® collar), respectively. Animals in group 2 received the collar on Day 0. Assessment criteria included *in situ* tick counts (last challenge on Day 224), polymerase chain reaction (PCR) analyses and immunofluorescence assays (IFA)(last assessments on Day 252). Whenever dogs were diagnosed with babesiosis they were "rescue treated", excluded and replaced. Twenty-four replacement animals were introduced at various times throughout the study in the control group. **Results:** No *B. canis* DNA or antibodies were detected in any Seresto® treated dog at any time. *Babesia canis* DNA and antibodies were detected in 26 out of the 32 control dogs confirming the validity of the challenge. Acaricidal efficacy was 90% on Day 30, and ranged from 95% to 100% thereafter. **Conclusion:** The Seresto® collar fully protected dogs against *B. canis* infection, transmitted by *D. reticulatus* ticks, for up to 8 months.

Keywords: Seresto®; Babesia canis; Dermacentor reticulatus; transmission blocking; imidacloprid

Abstract No: 4505

8 Sept 2017, 1400 – 1415

**Evaluation of the persistent preventive efficacy of 2.5 % Moxidectin / 10 %
Imidacloprid spot-on (Advocate®, Bayer) in dogs experimentally infected with
*Angiostrongylus vasorum***

Claudia Böhm^{*1} ; Gabriele Petry¹ ; Franziska Barthel¹ ; Roland Schaper¹ ; Holger Schmidt¹ ; Katharina Raue

¹*Drug Discovery Parasitocides/ Bayer Animal Health GmbH/ Germany* ¹*BioMedVet Research GmbH/ BioMedVet Research GmbH/ Germany*

Abstract Content

A controlled, randomized, blinded dose confirmation study was conducted to assess the persistent preventive efficacy of a treatment with Advocate® spot-on in dogs 4 weeks before inoculation of third stage larvae of *Angiostrongylus vasorum*. Twenty four adult dogs were randomly allocated to 3 treatment groups. Dogs in group 1 were treated with Advocate® spot-on at the minimum recommended dose of 0.1 ml per kg BW at day 84 and inoculated with approx. 250 third stage larvae of *A. vasorum* at day 112. Dogs of group 2 were treated monthly with Advocate® spot-on at days 0, 28, 56 and 84 at the minimum recommended dose of 0.1 ml per kg BW and inoculated with approx. 250 third stage larvae at day 112, i.e. 28 days after the last treatment. Dogs of the third group served as inoculated but untreated control. All dogs were euthanized and necropsied 70-72 days after experimental inoculation. In no dog of group 1 and 2 any adult *A. vasorum* worm was detected at the post mortem examination, whereas in all dogs of the untreated control group adult worms were recovered. The study results prove that already a single Advocate® administration has a 100% persistent efficacy for one month and protects dogs against infection with *A. vasorum* and thus can prevent lung damages which are caused by immature development stages.

Keywords: Angiostrongylus vasorum, persistent efficacy, moxidectin

Abstract No: 4302

8 Sept 2017, 1415 – 1430

Development of *Angiostrongylus vasorum* infection in slug intermediate hosts

Nor Azlina Abdul Aziz^{1 2} ; Simon Allen^{3 2} ; Ben Rowson⁴ ; Carolyn Greig³ ; Dan Forman³ ; Eric R Morgan²
¹School of Animal Sciences, Faculty of Bioresources and Food Industry/ Universiti Sultan Zainal Abidin, / Malaysia, ²Veterinary Parasitology and Ecology, School of Biological Sciences/ University of Bristol/ United Kingdom, ³Swansea Ecology Team, Dept. Of Biosciences/ Swansea University, / United Kingdom, ⁴Dept. Natural Sciences, / National Museum of Wales, / United Kingdom

Abstract Content

Increasing geographical distribution of *Angiostrongylus vasorum* could be related to climate change, which can favour activity of gastropod hosts. The study aimed to investigate use of real-time PCR as a tool to study development of *A. vasorum* larvae in the intermediate host, and to survey prevalence. Eleven groups of *Arion owenii* and *Deroceras reticulatum* slugs were infected with 21000-52500 L1 of *A. vasorum* for eight weeks. Three slugs were selected each week, subjected to Baermann detection of larvae, general and tissue specific PCR, and histology to investigate localization of larvae in tissues. Finally, gastropods were submerged in tap water for 24 hours and the sediment was observed for larvae. After 5 days, dead slugs were examined for the presence of larvae in tissues. Various tissues tested positive for larval and DNA presence, with no obvious tissue preference from real-time PCR. Larvae were observed in fibromuscular tissue (27 larvae) and smooth muscle of the intestinal wall (9 larvae) by histology. Larvae only detected by Baermann extraction after week 6 post infection, indicating presence of L3, with earlier stages not migrating out of tissue. Higher numbers of larvae observed in tissues 5 days after death of slugs (6 to 11 larvae per slug) in comparison with after 24 hours of submersion in water (1 to 4 larvae). Results indicate that *A. vasorum* DNA is widely distributed in slugs, while larvae mainly observed histologically in foot tissue. It also highlights potentially important alternative transmission pathway of larvae through water following emergence from dead slugs.

Keywords: Angiostrongylus vasorum, gastropod, real-time PCR, slug

The prevalence and distribution of *Borrelia* and *Babesia* pathogens in ticks infesting domestic dogs in the UK

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Abstract Content

To assess the prevalence and distribution of *Borrelia* and *Babesia* pathogens in ticks infesting domestic dogs in the UK a large scale survey was undertaken. 1094 veterinary practices were recruited and each randomly examined 5 dogs each week and sent a clinical history and any ticks to the investigators. A total 12,096 dogs were examined. The ticks were identified to species. The overall prevalence of tick attachment was 30%. For pathogen analysis DNA was extracted from 4,750 ticks and subjected to PCR and sequence analysis to identify *B. burgdorferi* (s.l.) and *Babesia* species. From 4,737 ticks, *B. burgdorferi* (s.l.) was detected in 94 (2.0%). Four *Borrelia* genospecies were identified: *Borrelia garinii* (41.5%), *Borrelia afzelli* (31.9%), *Borrelia burgdorferi* (s.s.) (25.5%) and *Borrelia spielmanii* (1.1%). One *Rhipicephalus sanguineus*, from a dog with a travel history outside the UK, was positive for *B. garinii*. Seventy ticks (1.5%) were positive for *Babesia* spp.: 84.3% were *Babesia venatorum*, 10.0% were *Babesia vulpes* sp. nov., 2.9% were *Babesia divergens/capreoli* and 1.4% were *Babesia microti*. One isolate of *Babesia canis* was detected in a *D. reticulatus* tick from a dog that had recently travelled to France. The prevalence of *B. burgdorferi* (s.l.) and *Babesia* spp. did not differ significantly between different regions of the UK. The results map the widespread distribution of *B. burgdorferi* (s.l.) and *Babesia* spp. in ticks in the UK and highlight the potential for the introduction and establishment of exotic ticks and tick borne pathogens.

Keywords: Ixodes, Dermacentor, Rhipicephalus sanguineus, Introduction, PCR

Abstract No: 4232

8 Sept 2017, 1445 – 1500

Migration behavior and a long-term maintenance of infectivity of *Toxocara cati* larvae in mic

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Abstract Content

Small rodents are known to serve as important paratenic host of *Toxocara cati* but the migration behavior and the infectivity of the larvae have not been clarified. In this study, mice were inoculated with *T. cati* eggs, and the migration and infectivity of larvae were studied until 360 days post-inoculation (dpi). Two strains of mice (C57BL/6 and C3H, n=8/group) were inoculated with 1,000 eggs (/head) of *T. cati*, and necropsied at 7, 14, 30, 90, 180 or 360 dpi. At necropsy, carcass, visceral organs, brain and eyes were separated, digested or pressed between slide-glasses, and larvae were counted. The larvae from 180 or 360 dpi mice were inoculated in the other ICR mice, necropsied at 19-36 dpi and digested for larval recovery. The mean total number of larvae recovered was 262.8 (range 40–519) but there was no significant difference among the mean number of larvae recovered on various necropsy date nor between the 2 strains of mice. Most of the larvae (88.9%) were recovered from the carcass, and some from the visceral organs (10.2%) and the brain (1%). The recovery percentage of larvae from mice given 180 or 360 days old larvae were 30.0% or 36.4-42.9%, respectively. Most of *T. cati* larvae migrated into muscles of carcass and some larvae migrated into the visceral organs and the brain of mice by 7 dpi. These larvae remained there for at least 1 year. Thus, high-infectivity of larvae that have parasitized the mice for 1 year was demonstrated in this study.

Keywords: Toxocara cati; Mice; Paratenic hosts; larva migrans; Experimental infection

Abstract No: 4251

8 Sept 2017, 1500 – 1515

Rickettsia felis and *Bartonella* species in cat fleas in Hong Kong

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Abstract Content

Fleas are commonly recorded on stray as well as domestic dogs and cats in Hong Kong. Fleas can be a major cause of pruritus in dogs and cats and also vectors of potentially zoonotic bacteria in the genera *Rickettsia* and *Bartonella*. Morphological examination of 174 fleas from dogs and cats living in Hong Kong revealed only cat fleas (*Ctenocephalides felis*). Cytochrome oxidase 1 gene (cox1) genotyping of twenty randomly selected representatives, revealed three cox1 haplotypes (HK-h1 to HK-h3). The most common haplotype was HK-h1 with 17 representatives (17/20, 85%). HK-h1 was identical to cox1 sequences of fleas in Thailand and Fiji. HK-h1 to HK-h3 form a distinct cat flea cox1 clade previously recognized as the Clade 3. A multiplex *Bartonella* and *Rickettsia* real-time PCR of 20 *C. felis* DNA found *Bartonella* and *Rickettsia* DNA in three (15%; Ct-value range 24.44 – 26.80) and ten (50%; Ct-range 21.34 – 32.89) *C. felis*, respectively. DNA sequencing confirmed the presence of *R. felis*, *B. clarridgeiae* and *Bartonella henselae*. This is the first reported study of that kind in Hong Kong, and further work is required to expand the survey of companion animals in the geographical region. The sampling of fleas on domestic small animals in Hong Kong revealed them to be exclusively infested by the cat flea and to be harbouring pathogens of zoonotic potential.

Keywords: Ctenocephalides felis; pathogens; Rickettsia; Bartonella; real-time PCR; sequencing

Abstract No: 4418

8 Sept 2017, 1515 – 1530

***Ctenocephalides canis* as the dominant flea species infesting dogs from Korea**

SungShik Shin^{*1}; Kyu-Sung Ahn¹; Ha-Jung Kim²; Guk-Hyun Suh²

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Abstract Content

Although the most common flea species found on dogs worldwide is the cat flea (*Ctenocephalides felis felis*), the dog flea (*C. canis*) is sometimes the dominant species of fleas on dogs from countries such as Argentina, New Zealand and Greece. We investigated the incidence of flea infestation among dogs from rural areas of the Republic of Korea. From April 2016 to March 2017, adult fleas were collected from domestic dogs from five areas of southwestern region of Korea: Damyang, Hampyung, Jindo, Jangsung and Gwangju. All the hosts were healthy outdoor dogs that lived in rural areas. Skin of a total of 67 dogs were thoroughly examined and at least two adult fleas were collected per animal. Adult fleas were found from the skin of 13 dogs (19.4%). *Ctenocephalides canis* was the only flea species found on the dogs from all five areas based on the morphological characteristics: a bluntly rounded head, a short stout dorsal incrustation, the first spine of the genal ctenidia being equal to or less than half the length of the second spine and the dorso-posterior margin of the hind tibia bearing two notches with stout setae between the postmedian and apical setae. Considering that *C. canis* has been reported to be more prevalent among rural outdoor dogs and wild canids whereas *C. felis felis* are more prevalent among urban dogs, an exclusively rural and outdoor population of dogs investigated in this study might have influenced the biased infestation status of flea species in dogs from Korea.

Keywords: Ctenocephalides canis; dog flea; survey; South Korea

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Abstract No: 3316

8 Sept 2017, 0900 – 0915

Assessing the performance of multiplexed tandem PCR for the diagnosis of pathogenic genotypes of *Theileria orientalis* using pooled blood samples from cattle

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Abstract Content

Oriental theileriosis caused by multiple genotypes of *Theileria orientalis* is an important tick-borne disease of bovines. This study assessed the performance of multiplexed tandem PCR (MT-PCR) for the diagnosis of *Theileria orientalis* infection in cattle using pooled blood samples. A total of 265 blood samples from cattle representing group 1 (high prevalence area for *T. orientalis*, $n = 155$) and group 2 (low prevalence area, $n = 110$) were tested individually or as pools of five or ten blood samples using MT-PCR. Prevalence, diagnostic specificity and sensitivity were estimated based on results for individual blood samples. For group 1, the prevalence estimated using the five- (97%) and ten-pooled blood samples (100%) were significantly higher than for individual samples (75%). For group 2, a higher prevalence was also recorded using the five- (9%) and ten-pooled blood samples (36%) than for individual samples (7%). Overall, less DNA copies were measured in pooled than individual samples. The estimated diagnostic specificity of MT-PCR was 95%, 94% and 94%, for pools of five, ten and individual samples, respectively. The estimated diagnostic sensitivity of this assay (98%) was same when individual and pooled blood samples were tested. This study shows that the testing of pooled blood samples is a cost-effective alternative approach for the diagnosis of *T. orientalis* infection.

Keywords: Theileria orientalis; Pooled blood sample; Diagnosis, Cattle; Multiplex-tandem PCR

Molecular and serological detection of bovine babesiosis in Indonesia

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Abstract Content

Bovine babesiosis, mainly caused by *Babesia bovis* and *B. bigemina*, has a huge threat in livestock industries. In Indonesia, the current distribution of the disease is unknown due to the lack of scientific study. In the present study, 487 blood samples were collected from cattle in the broad geographic area across the archipelago. The presence of antibody and the current infections of *B. bovis* and *B. bigemina* were determined using enzyme-linked immunosorbent assay (ELISA), immunochromatographic test (ICT), and nested PCR (nPCR) targeting *B. bovis* sbp4 and *B. bigemina* rap-1a gene. Among 487 samples, ELISA, single-ICT, dual-ICT, and nPCR detected *B. bovis* positive in 340 (69.8%), 317 (65.1%), 307 (63.0%), and 247 (50.7%) of the total sample, respectively. For *B. bigemina*, the positive sample was detected in 134 (27.5%), 130 (26.7%), 127 (26.1%), and 93 (19.1%), respectively. Furthermore, the mixed infections were found in 125 (25.7%), 113 (23.2%), 109 (22.4%), and 52 (10.7%) samples, respectively. The obtained nucleotide sequences of *B. bovis* sbp4 and *B. bigemina* rap-1a genes in this study showed a high homology with other isolates from many countries, and the identity among the Indonesian isolates were 94-100%. These data revealed the current distribution of the *B. bovis* and *B. bigemina* infection in cattle in Indonesia, and we found that the positive rate of the disease is high in most sampling locations. Lastly, the data present in this study is important to develop the effective control strategy of the disease in the country.

Keywords: bovine babesiosis; serological; molecular; Indonesia

An automated, multiplex-tandem PCR platform for the diagnosis of gastrointestinal nematode infections in cattle: An Australian-European validation study

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Abstract Content

Parasites of livestock cause diseases that have a major economic impact, costing billions of dollars in losses, worldwide. In particular for gastrointestinal parasites, the accurate diagnosis of infections and the detection of drug resistance are central to their effective control, but can be challenging by routine diagnostic methods. Current methods used for the *ante mortem* diagnosis in cattle, including faecal egg counts and larval culture, are time-consuming and laborious to perform, and are of limited sensitivity and specificity. To overcome these problems, our aim was to develop an advanced molecular method for the specific diagnosis of key strongylid infections in cattle and apply it to cattle in Australia and Europe. This novel molecular assay is based on the isolation of nematode eggs from faecal samples, the purification of genomic DNA, followed by multiplexed-tandem PCR of specific genetic markers and melting-curve analysis. Using this assay, specific and semi-quantitative amplification is consistently achieved from picogram amounts of genomic DNA from eggs of *Haemonchus placei*, *Ostertagia ostertagi*, *Trichostrongylus* spp., *Cooperia oncophora* and *Oesophagostomum radiatum*. The newly developed MT-PCR assay for cattle parasites achieves a superior performance compared to routine diagnostic methods in that it provides higher sensitivity, specificity and only requires a fraction of the time compared to routinely employed techniques for species differentiation. The assay, which is user-friendly and rapid because of largely automated procedure, can replace antiquated methods for species- or genus-specific diagnosis in live ruminants and is ready for commercial application in service laboratory settings.

Keywords: livestock; cattle; helminths; diagnosis; PCR

Abstract No: 4346

8 Sept 2017, 0945 – 1000

Enhancing the diagnosis of low-level *Dictyocaulus viviparus* infections through understanding the herd dynamics on dairy farms (MILC2 study)

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Abstract Content

Current diagnostic options to detect low-level presence of *Dictyocaulus viviparus* in dairy herds are limited. Bulk tank milk ELISA positivity relies on a minimum within-herd prevalence of 20%. We extend upon earlier work which suggests that testing a subsample of milking heifers provides more reliable in coughing herds. We identify further factors which influence an animal's antibody titre and investigate whether a pooled-milk sample test, created from the random selection of at-risk animals, can function at low herd prevalences. A longitudinal study provided 979 individual milk samples from four farms in England from July to October 2016. Antibody levels were measured using the MSP-ELISA (SVANOVA®). ODR values were significantly higher in heifers than in multiparous cows ($p < 0.001$). Results from a mixed effects model found that heifer vs. cow, days in milk and fat percentage were all negative prognosticators of an animal's lungworm titre. There was a strong, random effect of farm. Month of the year was not significant. Bootstrap analysis suggested that a test on pooled-milk samples from a limited number of heifers would reliably detect a herd-level prevalence below 20%. BTM ELISAs are likely to be unreliable indicators of the presence of lungworm on dairy farms when clinical signs are absent. During the grazing period, the pooling of a limited number of heifer samples is likely to facilitate the binary (positive or negative) classification for lungworm. Use of a pooled-milk sample test provides a reliable option for detecting *D. viviparus* herd-level prevalences below 20%.

Keywords: lungworm; prevalence; diagnosis; ELISA; presence-absence

Abstract No: 4898

8 Sept 2017, 1000 – 1015

Detection and absolute quantification of major gastrointestinal nematodes of sheep by ddPCR

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Abstract Content

In this study we report for the first time how droplet digital PCR (ddPCR) can be used for detection and absolute quantification of the most important gastrointestinal nematode (GIN) pathogens of sheep. Four combinations of primers and probes targeting the internal transcribed spacer region 2 (ITS2) of the ribosomal RNA gene array were developed by the use of the Primer3 software following *in silico* analysis of nucleotide sequences downloaded from common databases and viewed in Mesquite. The regions covered were both for universal detection of strongylid parasites, plus the three most important GIN, eg. *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus* spp. Analysis of samples containing serial dilution of DNA from adult worms proved to be useful in the assessment and selection of different threshold settings, which was based on QuantaSoft analysis. The interpretations of the ddPCR results were straightforward, and different analysis approaches seemed to have had little influence on the final results and the concentrations determined. Our data confirm the suitability of ddPCR for routine detection and quantification of these sheep pathogens. Results are still pending, but it seems like this assay can replace traditional egg counting techniques followed by larval differentials. The sensitivity and linear range were similar to those for real-time PCR (qPCR), for the analysis of DNA from serial dilution of adult worms, making ddPCR a viable choice when both detection and quantification are desired in complex samples such as from larval cultures.

Abstract No: 3996

8 Sept 2017, 1015 – 1030

Detection of Cat Whipworm Infections by Antigen ELISA

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Abstract Content

An antigen detection assay for whipworm has been developed based on secreted proteins from *Trichuris vulpis*. Cross-reaction with other *Trichuris* species was unknown. Feral cat fecal samples (n=35) were collected from the Federation of Saint Kitts and Nevis. Fecal flotation identified 28 feline *Trichuris* egg positive samples (*T. serrata* or *T. campanula*). Of these 28 egg positive samples, 23 samples (82%) were also antigen positive demonstrating the ability of the whipworm assay to detect feline whipworm. Antigen was also detected in 2 of the 7 egg negative samples highlighting the ability of the antigen test to detect occult infections. The study was extended by collecting 65 feral cat samples from southern Florida, USA. Twenty-five of these samples were egg positive of which 17 (68%) were also whipworm antigen positive. Of the 40 egg negative samples, 8 tested antigen positive. Together these studies of feral cat populations demonstrate that the whipworm antigen test is able to detect antigen produced by whipworms that infect cats. It is unknown which species of *Trichuris*, *serrata* or *campanula*, infected these cats but future PCR analysis hopes to elucidate this question.

Keywords: cat; whipworm, antigen, ELISA

Abstract No: 4295

8 Sept 2017, 1400 – 1415

Cost-Effective, High Throughput Qpcr Screening of Tick Transmitted Diseases in Cattle

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Abstract Content

Pathogen surveillance studies are usually hampered by sampling related issues (handling, preservation and shipping of samples) as well as rapid, reproducible and cost effective processing of the large sample numbers. Using FTA paper as a support matrix for blood samples resulted in efficient sampling, handling, preservation, storage and shipping of field samples. High throughput DNA isolation using a commercial kit and Kingfisher 96-well instrument resulted in the rapid isolation of high molecular weight DNA from blood impregnated FTA paper. Isolated DNA was subjected to optimized multiplex hydrolysis probe qPCR assays specific for *Anaplasma marginale*, *Anaplasma centrale*, *Babesia bovis*, *Babesia bigemina*, *Ehrlichia ruminantium* and *Theileria parva* including an internal amplification control. All liquid handling for qPCR assays was performed using a Hamilton Nimbus liquid handling robot and qPCR setup was performed in 384-well plates. The high throughput screening pipeline allowed for an end-to-end sample processing throughput of approx. 1200 samples per 8 hour shift, with low inter-run variability for both DNA isolation and qPCR analysis. Combining automated DNA isolation and liquid handling with sensitive (detection limit of ≥ 5 copies per multiplex qPCR) and specific multiplex qPCR detection assays allowed for cost-effective, high throughput screening of tick transmitted diseases using blood impregnated FTA samples as starting material.

Keywords: qPCR, tick transmitted diseases, screening

Abstract No: 4112

8 Sept 2017, 1415 – 1430

Potential of Cell-Free DNA as A Novel Diagnostic Biomarker for Parasite Infections in Dog

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Abstract Content

Cell-free DNA (cfDNA) comprises fragments of DNA found extracellularly and mainly in the blood circulation. cfDNA is relatively a new analyte and being applied in the current armamentarium of diagnostics. The present study was designed to assess the potential of cfDNA as a novel diagnostic biomarker for parasite infections. We employed 14 serum samples that were collected from stray dogs captured in visceral leishmaniasis-endemic areas of Bangladesh. Our previous studies showed that some of the dogs were infected with several pathogens such as *Leishmania donovani*, *Babesia gibsoni*, and *Anaplasma* sp. using conventional PCR. As negative control, dog sera obtained from 4 laboratory dogs were also included. cfDNA was extracted from 0.6 to 1.7 mL of sera using MagMAX™ Cell-Free DNA Isolation kit and the presence of cfDNA with the size of ~150 bp was confirmed by Agilent Bioanalyzer 2100. cfDNA libraries were prepared using the Illumina TruSeq Nano DNA Sample Prep kit and analyzed on Illumina MiSeq platform. After removing low-quality sequences, the resulting reads were subjected to BLAST search analysis. We obtained approximately 46 million reads for 18 dog samples from two independent MiSeq runs. Preliminary analysis showed that most of the reads (>98%) had the highest identities with dog genome sequences. However, some of the reads showed association with parasite sequences (e.g. *L. donovani*, *Dirofilaria* sp., and *Anaplasma* sp.). In conclusion, the analysis of cfDNA could be a novel diagnostic approach to produce an inventory of parasites carried by dogs in a high throughput manner.

Keywords: Cell-free DNA; Next-generation sequencer; Parasites; Dogs

Abstract No: 4374

8 Sept 2017, 1430 – 1445

Comparison of two commercially available serological rapid tests with the official screening test used to detect *Leishmania* seropositive dogs in Brazil

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Abstract Content

Visceral leishmaniasis is a major public health problem in Brazil, where seropositive dogs are usually culled as part of the control program. Currently, a rapid immunochromatographic test (DPP® Leishmaniose Visceral Canina) is used as the official screening test. However, the production of this test has not been sufficient to attend the national demand, particularly in private laboratories. In this study, we compared the level of agreement between results obtained with the official screening test with two commercial rapid tests (i.e., SNAP® Leishmania Test and Alere™ Leishmaniose Ac Test). By testing 95 serum samples of dogs from a visceral leishmaniasis-endemic area, we found a substantial agreement (Kappa = 0.77; P < 0.0001) between the official rapid test and SNAP® Leishmania Test and a fair agreement (Kappa = 0.26; P < 0.0001) between the official rapid test and Alere™ Leishmaniose Ac Test. In conclusion, SNAP® Leishmania Test should be considered as an equally reliable alternative to DPP® Leishmaniose Visceral Canina. The second commercial test, Alere™ Leishmaniose Ac Test, seems less reliable and could lead to a significant underestimation of the actual number of positive dogs during serological screenings in the framework of the Brazilian visceral leishmaniasis control program. Indeed, 16 dogs that were positive at both DPP® Leishmaniose Visceral Canina and SNAP® Leishmania Test were negative at Alere™ Leishmaniose Ac Test.

Keywords: Leishmania, diagnosis, serology, zoonosis, public health

Serological diagnosis of newly emerged *Leishmania martiniquensis* infection in BALB/c mice using different routes of parasite inoculation

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Abstract Content

Leishmania martiniquensis is a newly emerged vector-borne protozoan causing autochthonous leishmaniasis in both immunocompetent and immunocompromised human patients in Thailand. Since the true animal reservoir of this pathogen has not yet been identified, fundamental serologic research of *L. martiniquensis* infection using murine experimental model is crucial to determine the possible cross-reactivity of antibodies with available diagnostic methods. The aim of this study was to detect the kinetics of humoral immune responses against *L. martiniquensis* infection in BALB/c mice using 3 serological diagnostic methods. BALB/c mice were experimentally inoculated with 5x10⁶ *L. martiniquensis* promastigotes via subcutaneous (s.c.), intraperitoneal (i.p.) and intravenous route (i.v.). On 7, 14, 28 and 112 days post-infection, dpi, blood was collected to isolate plasma for serological detection. Direct agglutination test (DAT, cut-off at a 1:100 dilution) was compared with a commercial enzyme-linked immunosorbent assay (ELISA) in-clinic test (the SNAP® *Leishmania* Test), and immunofluorescence antibody test (IFAT, cut-off at a 1:100 dilution) for the detection of *L. martiniquensis* antibodies in these mice. On 112 dpi, all *L. martiniquensis*-infected mice (4/4) were seropositive by DAT only via s.c. whereas infected mice were seropositive by IFAT via all routes of inoculation. On 7 and 14 dpi, infection via both i.p. and i.v. routes elicited antibody responses detected by IFAT. In contrast, all mice were seronegative by in-clinic ELISA test. In BALB/c mice, this study is the first to reveal the cross-reactivity of antibodies against *L. martiniquensis* to antigens of *L. donovani* and *L. infantum* presented in DAT and IFAT, respectively.

Keywords: *Leishmania martiniquensis*, BALB/c mouse, diagnosis, serology, DAT, in-clinic ELISA test, IFAT

Abstract No: 2800

8 Sept 2017, 1500 – 1515

Screening diaphragm samples from meat shops for *Toxoplasma gondii* in metropolitan city of Lahore, Pakistan through optimized nested PCR technique

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Abstract Content

Introduction: Toxoplasmosis is a food-borne zoonotic disease that affects one-third of our global population. It is caused by parasite; *Toxoplasma gondii*. Human can be infected by ingestion of contaminated meat of animals. The Biological Hazard Panel of the European Food Safety Authority has recommended that Toxoplasma monitoring programmes should be initiated on slaughtered meats of the animals. **Method:** Diaphragm samples were collected from slaughtered animals from butchers' shops. Twenty grams of meat sample was cut into small pieces with cutter. High concentration of proteinase K and lysis buffer were added in 500 µg of crushed meat sample and it was kept overnight at 56°C for its digestion. 100 µl of the sample was processed for DNA extraction by tissue DNA extraction kit. Primers were designed for nested PCR targeting B1 gene of *T. gondii* DNA. Control Positive DNA from European laboratory and sequencing was used to confirm the *T. gondii*. **Results:** External primers amplified about 390 bp amplicon while internal primers amplified about 100 bp product in the test samples and control positive genomic DNA of *T. gondii* by nested PCR approach. **Conclusion:** Quality of meat was checked by detecting *T. gondii* tissue cysts in slaughtered animals through our method as compared to bioassay in rodents

Keywords: Diaphragm; bradyzoite; Nested PCR; Toxoplasma gondii

Abstract No: 4509

8 Sept 2017, 1515 – 1530

Combining qualitative and quantitative science to develop better knowledge exchange strategies

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Abstract Content

In response to growing concerns of anthelmintic resistance prevalence in roundworms of sheep, it is essential to understand the various factors influencing stakeholder's engagement and adoption of 'best practice' approaches to roundworm management. A three year study using qualitative and quantitative methods centred around focus groups, telephone and paper questionnaires and mathematical modelling was undertaken to develop a better understanding on the factors that influence decision making by farmers, and how various actors determine the importance and implementability of particular strategies. The key model findings were that farmer's base line understanding about roundworm control and confirmation about lack of anthelmintic efficacy in their flock were important drivers to uptake of good practice and this was backed up by the focus groups. It is essential to improve acceptance and cost-effectiveness of diagnostic testing in order to influence adoption of best practice behaviour. Farmers and veterinarians agreed that developing tailor-made control strategies and effective administration of anthelmintics were important worm control measures but differed in their opinion on what was actually implementable on farm. Positive interactions between farmers and advisors are key to resolving the issues raised, to enable the necessary explanation, justification and execution of recommended practices to suit farmer's needs and farming conditions. Finally, by involving primary stakeholders in the recommendation development process it is more likely to engender a collaborative and concerted effort which is critical to development within the agricultural industry. The use of quantitative and qualitative approaches provided a holistic overview on the topic of worm-control.

Keywords: Anthelmintic resistance; Focus groups; Questionnaires; Sheep; Stakeholder engagement;

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Abstract No: 4306

Diurnal activity patterns of the temporary fish ectoparasite, *Gnathia africana* Barnard, 1914, from the southern coast of South Africa

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Abstract Content

Gnathiid isopods are perhaps the most common fish ectoparasite. Gnathiid infestation can cause reduced haematocrit, increased corticosteroid stress hormones, the transmission of blood-borne parasites, and mortality for hosts. On coral reefs, gnathiids are abundant, most active at dusk and dawn, and contribute significantly to trophic dynamics. Gnathiids also inhabit temperate intertidal waters, but their activity patterns and contribution to intertidal trophic dynamics remain unstudied. To provide the first ecological data on temperate intertidal gnathiid activity patterns, 172 gnathiid-free *Clinus superciliosus* were set in an intertidal system in Tsitsikamma National Park, on the southern coast of South Africa, during early-morning, morning, afternoon, early-evening, and evening, high and low tide, and within the inter- and infra-tidal zone to examine gnathiid infestation levels. After exposure, gnathiids from each fish were identified to the species level, counted, and their developmental stage was recorded. All gnathiids were identified as *Gnathia africana*. On average, 1 ± 5 s.d. gnathiids were collected from each fish, and the majority of gnathiids collected were stage 1. Significantly more gnathiids were collected during morning and afternoon compared to all other time periods. The number of gnathiids collected was not influenced by the fish's exposure to high or low tide, or placement within the tide zone. Despite that *G. africana* is released from cleaner fish predation because cleaner fish do not reside in temperate intertidal habitat, *G. africana* abundance is surprisingly small. Future studies should examine if predation on *G. africana* or limited host availability and/or habitat regulate *G. africana* population size.

Keywords: Activity patterns; Clinus superciliosus; diel; intertidal; isopod

Molecular diversity of fish pathogens from the Diplostomidae (Digenea) in South Africa

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South Africa

Abstract Content

One of the most important parasitic diseases in wild or cultured freshwater fish is diplostomiasis which is caused by the digenean larvae from the family Diplostomidae. These parasites have become in recent years a focus of intensive studies on most continents, especially in Europe and North America. Africa remains the continent where this group has not been studied using modern molecular tools in combination with quality morphological analysis, an approach that proved to be extremely successful in similar studies elsewhere. The aim of this study is the morphological and molecular characterisation of diversity of these pathogens in freshwater fishes in South Africa. A total of 84 specimens of 19 species of fish was sampled in the Phongolo River, Ndumo Game Reserve and the Riet River, Mokala National Park during three expeditions in 2016. Specimens of diplostomids were recovered from eyes, cranial cavity, muscles and body surface of seven fish species (*Clarias gariepinus*, *Cyprinus carpio*, *Labeobarbus aeneus*, *Nothobranchius orthonotus*, *Oreochromis mossambicus*, *Synodontis zambezensis*, *Tilapia sparrmanii*). Molecular analysis of representative isolates based on multiple markers including mitochondrial (*cox1*) and nuclear (28S; ITS1-5.8S-ITS2) loci, provided identification for eight species belonging to five genera *Bolbophorus*, *Diplostomum*, *Dolichorchis*, *Posthodiplostomum* and *Tylodelphus*. In this study, representative specimens of each diplostomid species were characterised morphologically and molecularly. The obtained results represent an important step to increase the knowledge on species diversity of these important pathogens of freshwater fishes in Africa. This study was supported by the Claude Leon Foundation Postdoctoral Fellowship (2017-2018) and North-West University, South Africa.

Keywords: Fish pathogens; Diplostomidae; morphology; genetics; South Africa

Molecular characterization of a Profilin-like gene from *Cryptocaryon irritans*

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Abstract Content

Profilin known as a multifunctional actin-binding protein, is integral to actin-based cell motility, cytokinesis, neuronal differentiation and synaptic plasticity. In this study, a putative *profilin*-like gene designated as *CiProfilin* (GenBank ID: JX987286) was screened from a cDNA library of *Cryptocaryon irritans* trophonts. The full-length cDNA of *CiProfilin* was 582 bp, involving an open reading frame of 474 bp, which encodes a polypeptide consisting of 157 amino acids with a predicted molecular weight of 17.3 kDa. The quantification of *CiProfilin* mRNA expression by real-time PCR suggested that *CiProfilin* was expressed in whole life cycle of *C. irritans* with a saliently higher level in trophonts. So as to express the gene in prokaryotic system, site-directed mutagenesis was applied to modify the non-universal codons into universal ones, following by subcloning into *Escherichia coli*. In the presence of isopropylthio- β -D-galactoside, the *E. coli* was induced to express fusion protein as glutathione S transferase recombinant CiProfilin (G-rCiProfilin), which was purified by glutathione Sepharose 4B and removed of the GST tags by thrombin. Subsequently, sodium dodecyl sulfate-polyacrylamide gel electrophoresis confirmed the predicted molecular mass of rCiProfilin and western blot analysis proved the antigenicity of rCiProfilin. CiProfilin was found abundant over the peripheral area beneath cell membrane and cytostome of theronts, implying its pivotal roles in food uptake and parasitic invasion. Moreover, co-precipitation assay revealed the bioactivity of rCiProfilin in actin binding. Our research will help to further elucidate specific roles of CiProfilin concerning the growth of *C. irritans* and the mechanism of its invasion to hosts.

Keywords: Cryptocaryon irritans, Profilin, actin

Effects of altered eutrophication and pollution gradient of lakes on helminth parasites of fish from North West Himalayan region: Major trends from seven years of study

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Abstract Content

The quality of aquatic environment has been considered a major factor that influences the population density of aquatic biota, including parasites. In last three decades parasitologists have shown keen interest to understand the major patterns of parasitism in fish exposed to myriad types of stressors. The water bodies, especially lentic water bodies of North West Himalayan region are showing an alteration in water quality due to accelerated anthropogenic pressure, and therefore these water bodies are ideal for assessing the impact of various stressors on fish parasites. Various field studies were carried out in last seven years to assess the environmental relevance of helminth parasites of fish across different lakes showing pollution gradient. In this paper we discuss major patterns observed in different helminth groups of fish across stress gradient. We further discuss how we have used integrated approach to elucidate the fish and aquatic health. The results depict both positive as well as negative effects of water quality on the helminthes. The effect of enhanced pollution was also observed on the seasonality of some parasite groups. The antagonistic effect of multiple stresses was observed on monogenean and cestode parasites. Health indicators of fish generally decreased across the pollution gradient. The seven year of investigation highlights the importance of helminth parasites as an ideal effect indicators and further envisages the importance of fish-parasite sentinel system for elucidation environmental quality of lake water bodies.

Keywords: Aquatic environment; parasitism; anthropogenic pressure; pollution gradient; antagonistic effect

Abstract No: 4483

**Investigation of a serious *Calicophoron daubneyi* outbreak on an Irish farm:
Genotyping of the rumen fluke metacercariae population found in pasture**

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Abstract Content

While traditionally, rumen fluke species were considered to be of little clinical significance in temperate areas, recent reports of serious outbreaks in both cattle and sheep in regions of Western Europe have brought these parasites into focus. Here, a severe rumen fluke outbreak that occurred on an Irish dairy farm in September 2016 is described. Initially 11 of a herd of 31 seven-month replacement heifers were found dead. The remaining animals presented with inappetence, dullness, diarrhoea and recumbency, frequently with their hind limbs stretched out behind them. Subsequently another 11 animals died or were euthanised. Histological examination confirmed the presence of very large numbers of immature paramphistomes, within the lumen of the duodenum and attached to the mucosa by their acetabula. Subacute and acute parasitic tracts in the liver indicated concurrent liver fluke infections. Occasional nematodes were also present in the small intestinal crypts. Since *Calicophoron daubneyi* is widespread in Ireland, the hypothesis that the severe clinical signs observed were due to the presence of particularly pathogenic genotypes of this parasite was tested. For this purpose, metacercariae were isolated from herbage collected from the farm. Following DNA extraction from 106 individual metacercariae, they were identified to species and genotype level based on sequence analyses of the internal transcribed spacer 2 and cytochrome oxidase subunit I gene regions, respectively. All metacercariae belonged to *C. daubneyi*. The most common genotypes detected were identical to 9 genotypes which had previously been described in Ireland, ruling out an association between virulence and genetic variation.

Keywords: Calicophoron daubneyi, cattle, genotyping

Evaluation of peroxiredoxins of *Babesia microti* (BmPrxs) as novel potential drug target

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Abstract Content

Babesia microti live in host erythrocytes, they must have an effective antioxidant mechanism to cope with such oxidative burdens. Peroxiredoxins (Prxs) as cysteine-based peroxidases, are important antioxidant enzymes that prevent oxidative damage caused by reactive oxygen species and reduce hydrogen peroxide (H₂O₂) to water in both eukaryotes and prokaryotes, the Prxs have great impact on protecting parasites from oxidative stress in the erythrocytic stage. In addition, Prxs regulates H₂O₂ as second messenger to mediate cell proliferation, differentiation, and apoptosis by transmission of redox signals, thus Prxs are essential for the survival and virulence of many parasites. In this study, three Prxs cDNA (Prx1, Prx2 and PrxQ) were cloned from *B. microti* (designated BmPrxs). Recombinant BmPrxs (rBmPrxs) protein were expressed in *Escherichia coli* and purified. Three Prxs native proteins were recognized by the antisera against rBmPrxs, respectively. Indirect immunofluorescence assay results indicated that three BmPrxs were located around the nucleus of *B. microti* merozoites in mouse RBCs, respectively. The mixed-function oxidation assay results suggested that three BmPrxs act as antioxidant enzymes that catalyze H₂O₂ via the parasitic Trx system in *B. microti*. The BmPrxQ has no homology with human and other organisms, the peroxidase activity is irreversibly inhibited by conoidin A, thus we concluded that PrxQ is a potential drug target for antiparasitic therapy in the foreseeable future of clinical use. Further studies to analyze the structural characteristics, antioxidant mechanisms, and other possible roles of three rBmPrxs in *B. microti* are warranted, thereby facilitate development of preventive measures against babesiosis.

Keywords: Babesia microti ; peroxiredoxins ; antioxidant enzyme; drug target

Phylogenetic relationships of *Babesia* species in dogs from Malaysia with other geographically dispersed strains based on the Internal Transcribed Spacer Gene (ITS-2)

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Abstract Content

Babesia species are one of the most common canine tick-borne Piroplasms of pathogenic importance worldwide. Sequence analysis of 24 randomly selected *Babesia* sp. isolates in dogs from Malaysia and other geographically dispersed strains was analyzed based on the partial region of ITS-2 gene. Phylogenetic relationships of the Malaysian *B. gibsoni* and *B. vogeli* strains showed higher genetic similarities to *B. gibsoni* (EU084673) and *B. vogeli* (EU084675) strains from USA when compared to strains from other parts of Asia (China and Taiwan), Europe (Germany) and Africa (Nigeria). On the other hand, a substantial genetic variation has been observed between the Malaysian isolates and with isolates from other parts of the world. Additionally, the estimates of evolutionary divergence revealed that *B. gibsoni* from Taiwan (FJ769391) appeared to be the same with the Malaysian strains; although the phylogenetic tree inferred for *B. gibsoni* showed that they are distantly related. This study has helped elucidate the phylogenetic relationship among various *Babesia gibsoni* and *B. vogeli* strains in dogs from different parts of Malaysia and other parts of the world.

Keywords: *Keywords: dogs, Babesia species, Phylogenetics, Malaysia, ITS-2 gene*

Utilizing mannosyle glycoprotein in the diagnosis of trichinosis in experimentally infected rats

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Abstract Content

Glycoproteins of parasitic helminths play important roles in biology and host-parasite interaction. A method of affinity chromatography was adopted to isolate mannosyl glycoprotein antigen from ***Trichinella spiralis*** crude larval antigen using concanavalin A (Con A) column. The selection of con A column was based on the existence of mannose sugar in *T.spiralis* larval extract as proved by indirect haemagglutination inhibition assay. The isolated mannosyl fraction was evaluated by ELISA and Western blot techniques for the serological diagnosis of trichinosis in experimentally infected rats. Either early in one week post infection (P.I.) or in the late stage, six weeks P.I., the isolated mannosyl fraction proved higher potency in the diagnosis of rat experimental trichinosis than crude extract by ELISA. The isolated mannosyl fraction resolved into four bands of 65 KDa, 54 KDa, 30 KDa and 11 KDa compared with multiple bands of crude extract as observed by SDS-PAGE. A 30 KDa mannosyle glycoprotein (s) isolated from *T.spiralis* crude larval extract proved potentials in early as well as late diagnosis of experimental trichinosis in rats as proved by western blot.

Keywords: Glycoproteins , Trichinella spiralis, Affinity chromatography, ELISA, Western blot.

Rapid Oral Presentation – 7 Sept 2017: Companion Animals

Abstract No: 4004

Repartition of ticks (*Rhipicephalus sanguineus sensu lato* and *Dermacentor variabilis*) on dogs 48h after weekly experimental infestations

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Abstract Content

The repartition of ticks on the body of dogs is important to understand the mode of action of ectoparasiticides. The objective of this work was to investigate the repartition of two species of adult ticks on dogs 48h after experimental infestations. A GLP study was conducted on 8 dogs housed individually and infested with 50±5 adult unfed ticks (1:1 Male:Female ratio) on days -5, 2, 7, 30, 90 and 150 (*Rhipicephalus sanguineus*) and on days 2, 7, 60 and 120 (*Dermacentor variabilis*). The ticks were deposited on the back of the dogs. Tick counts were performed on dogs 48h after each infestation. The ticks were categorized as male/female, live/dead, free/attached, engorged/unengorged and located in one of the 7 areas: outside the hind legs, tail and anal area, lateral area, abdomen and inside hindlegs, shoulder and forelegs, head and neck, and dorsal. No ticks were found engorged. No *D. variabilis* and only up to 4 male *R. sanguineus* ticks were found free on dogs. An average total of 16.0 female and 17.6 male *D. variabilis* and 10.7 female and 13.3 male *R. sanguineus* were found attached per dog. 62.9% (*R. sanguineus*) and 39.5% (*D. variabilis*) of the attached ticks were located in the “head and neck” area; and 22.6% (both) were in the “dorsal” area. These results showed that ticks migrate from their infestation site (the back) to their attachment site within 48h. This repartition of ticks is particularly favourable to the targeted action of acaricide and repellent ingredients delivered topically.

Keywords: tick;rhipicephalus;dermacentor;repartition;dog

Detection of gastrointestinal protozoa in the cat population in the Klang Valley and risk factors associated with infection

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Abstract Content

The common gastrointestinal protozoa in cats that cause diarrhea are *Giardia spp.*, *Isospora spp.* and *Cryptosporidium spp.*, and recently *Tritrichomonas foetus* has been recognized as an emerging protozoa that causes chronic diarrhea in cats. *Tritrichomonas foetus* infection in cats has not yet been reported in Malaysia. *Entamoeba spp.* is found rarely but present in cats. This study aimed to detect gastrointestinal protozoa in the cat population in the Klang Valley as well as the risk factors associated with these protozoal infections. Rectal swabs were performed on 30 diarrheic cats presented to selected veterinary clinics in the Klang Valley to culture *Tritrichomonas foetus*. Another 30 fecal samples were collected randomly and subjected to staining for the detection of other gastrointestinal protozoa. Two out of 30 culture samples were positive for *Tritrichomonas foetus* with a prevalence of 6.7% and both positive samples were from young kittens. *Cryptosporidium spp.* was the only protozoa detected in 3 out of 30 fecal samples through the staining method with a prevalence of 10%. Feces from 25 randomly selected stray cats were also cultured for *Tritrichomonas foetus* and 6 positive cats were identified. This study detected *Tritrichomonas foetus* for the first time in the Malaysian cat population. This necessitates inclusion of this protozoal infection as a differential diagnosis of chronic diarrhea in cats in Malaysia. The overall prevalence of gastrointestinal protozoa in pet cats in the Klang Valley determined in this study was low.

Keywords: *Gastrointestinal protozoa ; Tritrichomonas foetus ; Cat ; Culture ; Staining*

First report of *Trypanosoma evansi* infection in a German Shepherd dog in Vietnam

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Abstract Content

The first case of *Trypanosoma evansi* infection in a dog imported from Germany was reported from the Gaia Pets Clinic in Hanoi, Vietnam. A 2,5 year old male German Shepherd dog was hospitalized with clinical signs of weakness, tachypnea, pale mucous membrane and edema of the back legs. Additionally, numerous dog ticks were found between the dog's toes, behind ears, under armpits and around the tail and head. Trypanosome parasites were observed on a diff-quick stain of blood smear. *T. evansi* that infected to the patient was identified based on morphological analysis. Morphological measurement of *T. evansi* isolated from the infected dog was characterized with body size ($26,38 \pm 3,27 \times 2,45 \pm 0,55 \mu\text{m}$), nuclear size ($2,45 \pm 0,30 \times 1,66 \pm 0,27 \mu\text{m}$), kinetoplastic index ($KI = 1,32 \pm 0,11$) and nuclear index ($NI = 0,78 \pm 0,13$). In this study, hematological and sera-biochemical results of infected dog blood were also analyzed for the evaluation.

Keywords: Trypanosoma evansi; German Shepherd dog; Vietnam

Prevalence of Canine *Leishmaniasis* in Dogs In Sabah

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Abstract Content

Leishmaniasis is a parasitic infection caused by obligate, intracellular protozoan of the genus *Leishmania* (family *Trypanosomidae*). Its spread is caused through the bites of the infected female phlebotomine sand flies. More than 23 species of *Leishmania* have been described, most of which are zoonotic. This parasitic disease is classified as Neglected Tropical Disease (NTD) and can be found in parts of the tropics, subtropics, and southern Europe. Some studies had been carried out to determine the prevalence of canine leishmaniasis and most of the studies were conducted in Europe and other continents. So far, none of the study is carried out in Malaysia, thus its epizootiology is still poorly understood. In this study, fifty canine blood samples were collected to determine the prevalence of canine leishmaniasis in Sabah, where autochthonous foci of canine leishmaniasis have not been reported. Canine leishmaniasis is detected by using polymerase chain reaction (PCR) method and the PCR products were purified and sent for sequencing. This study revealed that seven out of fifty blood samples were positives for canine leishmaniasis by using PCR method. Thus, the prevalence rate of canine leishmaniasis in dogs in Sabah is 14%.

Keywords: leishmania; phlebotomine; zoonotic; epizootiology; polymerase chain reaction

Epidemiological survey on the occurrence of *Angiostrongylus vasorum* stages in natural slug populations in Germany

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Abstract Content

Infections with the French Heartworm *Angiostrongylus vasorum* represent neglected parasitoses of dogs in Germany. Recent surveys indicate that this parasite is spreading in Europe. Actual data on *A. vasorum* prevalence in dogs and foxes (acting as reservoir hosts) revealed several endemic foci in Germany. The life cycle of *A. vasorum* is obligatory linked to an intermediate host being represented by a wide range of slugs and snails. Given that actual data on *A. vasorum* infections in intermediate hosts are missing for Germany, we here conducted an epidemiological survey on slugs in selected regions of Hesse and Rhineland-Palatinate. The slugs were collected throughout the year (winter 2014 until autumn 2015) in areas that were previously proven as endemic for *A. vasorum* fox infections. Overall, a total of 2701 slugs were collected and examined for lungworm larvae applying artificial digestion and microscopy. The current data revealed a total *A. vasorum* prevalence of 4.6 % in slugs based on microscopic analyses. The number of *A. vasorum* larvae per slug varied considerably (1-546 larvae per specimen). Considering the different sampling areas, some hotspots with a rather high *A. vasorum* prevalence in slugs (up to 19.4 %) were identified. *A. vasorum* prevalence varied with the season since highest prevalence was detected in summer (9.1 %) and the lowest number of infected slugs was found in winter (0.8 %). Overall, the current data demonstrate that dogs are at a permanent risk for *A. vasorum* infections (even in winter) when living in the investigated areas.

Keywords: Gastropod-borne diseases, Metastrongyloidea, Angiostrongylus vasorum, lungworm

Kinetics of ticks (*Rhipicephalus sanguineus sensu lato*) spontaneous detachment from dogs after experimental infestations: a meta-analytical approach

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Abstract Content

Once anchored to their hosts, ticks are usually considered to remain on their host until full engorgement. However, previous experiments demonstrated that adult *Rhipicephalus sanguineus* ticks migrate between dog hosts within 5 and 7 days of attachment. The objective of this work was to investigate the kinetics of spontaneous detachment of ticks from dogs when assessed through a retrospective meta-analysis. Five studies, performed on dogs infested with *Rhipicephalus sanguineus*, were selected. All dogs (n=38; 6-8 per study) belonging to control groups were selected. Weekly infestations with 50 adult ticks per dog were performed for 1 to 6 weeks. Tick counts were performed on individually housed dogs at different time points ranging from 30min to 96h post-infestation. The ticks were categorized as live/dead, free/attached. The average number of free and attached ticks was calculated for each time point and each infestation. The number of detached ticks was estimated by difference between attached ticks at two consecutive time-points. The number of detached ticks generally increases within studies between consecutive time-points. For example, there was in average 7.8 detached ticks between 1 and 4h after infestation. Up to 28 ticks detached from a single dog between 24 and 48h post-infestation. These detached ticks were not found free on the dogs and are expected to be in the environment. These results confirmed that ticks spontaneously detach from dogs within a few hours after infestation. An underestimation of tick movements is expected since the methodology did not allow an evaluation of the on-host movements.

Keywords: Ticks, *Rhipicephalus sanguineus*, detachment

A survey of ticks (Acari: Ixodidae) of companion animals in Australia

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Abstract Content

Ticks are among the most important arthropod vectors of pathogens affecting companion animals. Twenty ixodid species have previously been recorded on dogs, cats, and horses in Australia. A nationwide survey of hard ticks that infest these companion animals was conducted during 2012-2015. Ticks collected from companion animals from seven states and territories in Australia were examined morphologically to determine species, instar, and sex, and the results were summarised with SPSS software. The sample collection locations were mapped using QGIS software. Overall, 4,765 individual tick specimens comprising 11 species were identified from 837 companion animal hosts. The most common species included *Rhipicephalus sanguineus* on dogs (73%), *Ixodes holocyclus* on cats (81%), and *Haemaphysalis longicornis* on horses (60%), and one novel host record was obtained for *Ixodes myrmecobii*. Most of the collection locations were within previously described enzootic ranges, however 32 records of *R. sanguineus* on dogs occurred outside of its previously reported Australian distribution range. This study is the first of its kind to be conducted in Australia and our results contribute to the understanding of the tick species that parasitise dogs, cats, and horses in Australia.

Keywords: Ticks; Companion animals; Dogs; Cats; Horses

Filariasis in domestic cats and dogs living in Brugian filariasis endemic area in East Malaysia

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Abstract Content

Lymphatic filariasis has been targeted to be eliminated by the year 2020 by the World Health Organization (WHO). *Brugia malayi* is responsible for 10% of zoonotic lymphatic filariasis in humans and is also restricted to the tropics. Although the main reservoir is a leaf-eating monkey (*Presbytis* spp.), this filarial nematode has also been found infecting cats in Malaysia. To assess the parasite burden, we performed a study on cats and dogs in an endemic area of Brugian filariasis, Kg. Tangkarasun, Beluran, Sabah in East Malaysia. Using microscopic and pan-filarial ITS2-region DIDR-F1 and DIDR-R1 primer, 85 cats and dogs belong to the community were screened for filariasis. Twenty five out of 85 samples were positive with microfilaria on blood smear, while 45 samples were found positive upon molecular screening. *Dirofilaria immitis* (29.4%) were the most common filarids found infecting cats and dogs in Kg. Tangkarasun, followed by *D. repens* (14.1%) and *B. malayi* (9.4%). At least 3 species of filarids are now known infecting pets in Kg. Tangkarasun, Beluran. All three species that were identified possess a potential risk of zoonosis transmission to the community living at the area. It is highly suggest to apply treatment for these infected pets as the vectors are available in abundance at this area. This is the first study to report on occurrence of *D. repens* infection in animal in Malaysia.

Keywords: filaria;cats;dogs;Malaysia

Rapid Oral Presentation – 7 Sept 2017: Drug Resistance & Therapeutics

Abstract No: 4017

First Report of benzimidazole resistance in *Haemonchus contortus* in goats in South Darfur State, Sudan

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Abstract Content

In South Darfur State, Sudan, pastoralists have complained of lack of efficacy of anthelmintics to control nematodes. Albendazole was used to treat goats experimentally infected with *Haemonchus contortus* larvae cultured from worms collected from goat abomasa at different abattoirs and also to treat goats naturally infected with *H. contortus*. The faecal egg count reduction test was used to demonstrate that resistant *H. contortus* were present in some study areas and resistance was confirmed using the egg hatch test. This is the first time that benzimidazole resistance has been reported in Sudan.

Keywords: Albendazole; Resistance; Haemonchus contortus; Goats; South Darfur State, Sudan

Using molecular markers to evaluate anthelmintic susceptibility in the poultry roundworm *Ascaridia galli*

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Abstract Content

Susceptibility of *Ascaridia galli* to benzimidazole (BZ) substances was examined for the first time in commercial laying hens using genetic markers. Mutations at codons 167, 198 and 200 of *A. galli* β -tubulin gene known to be involved in BZ resistance in other nematodes were investigated in six flocks (F1–F6) with different numbers of exposure to BZ (Panacur AquaSol®, Intervet AB, 1 mg/kg body weight). Flocks F1 and F4 were treated with fenbendazole on 3 occasions i.e., 22, 27 and 36 weeks post placement (wpp). Flocks F2 and F5 were treated on a single occasion at 27 wpp and hens in flocks F3 and F6 were not exposed to FBZ. At the end of the study, 10 adult male and 10 adult female worms were collected from each flock for DNA extraction and PCR amplicons generated with specific primers for the partial β -tubulin isotype 1 gene were sequenced (650 bp). All sequences were aligned and mapped against *A. galli* β -tubulin isotype 1 reference gene available in GenBank. So far, no variation was observed in codon positions 167, 198 or 200 in β -tubulin isotype 1 gene. However, our study will continue on investigation of mutations in other β -tubulin isotypes and their role in BZ resistance in *A. galli*.

Keywords: Ascaridia galli; Benzimidazole; Genetic markers; Resistance

Detection of benzimidazole resistance in Slovak goat herds based on comparison of faecal egg count reduction test and egg hatch test

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Abstract Content

Anthelmintic resistance (AR) is a serious threat to animal health and have major economic impact worldwide due to production and financial losses. The aim of study was determine occurrence of AR in 30 goat farms in Slovakia and comparison between two widely used *in vivo* and *in vitro* methods for detection of AR in field conditions. A three year survey was conducted during pasture seasons 2014 – 2016 (April – November). On each farm goats were split into treated and control group. Goats were treated by recommended (5 mg/kg body weight) and double dose of albendazole 10 mg/kg. Correlations between percentage of reduction after therapy in faecal egg reduction test (FECR) and hatching of eggs in different concentrations of benzimidazole anthelmintic in egg hatch test (EHT) have been monitored in all examined farms. Based on the results of EHT benzimidazole resistant nematodes were found on 27 farms. Low levels of resistance (<20% of hatching) were detected on 17 farms and 10 farms had high levels of resistance (>40% of hatching). Results of FECR showed on 17 farms percentage reduction of EPG was less than 95 %. The data show good correlation for BZ-resistance, among 30 goat farms. The hatching obtained by the EHT provides a good estimate of *in vivo* efficacy.

Keywords: Goats,anthelmintic resistance,hatching,efficacy

Hierarchical modelling of paired FECRT data shows an increase in variability following treatment

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Abstract Content

More complex statistical models are needed to analyse paired compared to unpaired FECRT data. Bayesian modelling techniques can be used to implement such models, and allow the different sources of variability within and between animals to be quantified. A FECRT experiment was performed to evaluate the efficacy of Moxidectin in a total of 133 donkeys co-grazed in 7 groups. Two separate pre-treatment FEC samples and three separate post-treatment FEC samples were obtained from each individual, giving 654 FEC observations (11 samples were excluded due to insufficient faeces). These data were analysed using a Bayesian hierarchical model to separate the observed variability between observations into variability between (1) groups, (2) animals, (3) pre-treatment observations within animal, (4) post-treatment observations within animal, and (5) inconsistent efficacy between animals. The mean efficacy at each site was modelled as being independent of the other sites. The mean efficacy was determined to be less than 95% in 2 of the 7 groups of donkeys. The most important sources of variability given above were post-treatment within-animal (4), followed by variability between animals (2), then variation in efficacy (5), then pre-treatment within-animal (3) and finally variation between sites (1). In particular, there was a marked and statistically identifiable rise in variability within animals following treatment. Moxidectin resistance was identified. The results also show that drug efficacy varies between animals within a group, and that anthelmintic treatment induces an increase in variability within animals. These factors should be considered when analysing paired FECRT data.

Keywords: Moxidectin resistance; statistical models; variability; FECRT.

**Molecular development of oxfendazole resistance starting with a susceptible
Haemonchus contortus isolate**

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Abstract Content

Haemonchus contortus is one of the main small ruminant parasites in tropical areas and its control is traditionally done through the utilization of synthetic anthelmintics such as benzimidazoles (BZ). BZ resistance is associated to single nucleotide polymorphisms (SNP) located in the β -tubulin isotype 1 gene: F200Y, F167Y and E198A. Here we describe the rise of resistant mutations within a completely susceptible isolate (Inbred-susceptible-Edinburgh - ISE) in an experimental infection model in 3 sheep treated with increasing oxfendazole doses. After initial infection and rise in fecal egg counts, the drug regimen started with subdoses as low as 30% of the recommended dosage and were gradually increased to 100% over the course of a year. Experimental animals were infected every 1.5 months with 5,000 L3 larvae cultured from feces collected one week after each treatment. After an initial period when resistance was only detected in the F200Y locus, both resistant polymorphisms (F200Y and F167Y) were detected in increasing frequencies after each treatment. Eventually resistance at F200Y stationed at frequencies around 70% and decreased while resistance frequencies at F167Y kept rising and finally stationed at around 75%. Resistance at E198A was never detected in the studied period. Our study provide insights into the speed as to which each polymorphism may rise as BZ dosage increases converting a susceptible parasite population into a highly resistant one within a short time period.

Keywords: Haemonchus, anthelmintic resistance, oxfendazole, beta tubulin, SNP

Rapid Oral Presentation – 7 Sept 2017: Equine

Abstract No: 4335

Experimental infection of Irish horses with *Fasciola hepatica*: O liver fluke, where art thou?

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Abstract Content

Fasciola hepatica is a common parasite of livestock in Ireland, causing significant economic losses and affecting animal welfare. In 2013-14, we conducted an abattoir study to determine the prevalence of *F. hepatica* infection in Irish horses. Examination of 200 horses lead to a prevalence determination of 9.5%. To further examine the susceptibility of Irish horses to liver fluke, an experimental challenge was conducted. Horses (n=10, 9 geldings and 1 mare) were infected with metacercariae (n=1000, 500, or 0), administered orally. Before challenge, horses were given anthelmintics effective against nematodes and *F. hepatica*, and levels of GGT, GLDH and bile acids were analysed. Blood and faecal samples were taken at 4 week intervals, and the horses went to abattoir at 18 wpi. Livers were collected and the liver fluke burden was determined. No liver flukes were recovered from any of the horse livers, regardless of dose of metacercariae. Faecal antigen ELISA was negative for all horses, although serum anti-Cathepsin L1 antibodies were elevated in two of the animals. Taken together these data indicate that while horses are commonly infected with *F. hepatica*, there are unanswered questions surrounding relative susceptibility, potential differential infectivity of different genotypes, and dose effects.

Keywords: Fasciola hepatica; liver fluke; horse

Relationship between management practices and faecal egg counts in yearling thoroughbred horse farms in Ireland

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Abstract Content

Currently, it is generally accepted that traditional approach to equine parasite control with decades of frequent use of anthelmintic drugs has contributed to the development of resistance. The aim was to obtain information on the current parasite control strategies and how it relates to egg shedding in yearling thoroughbred horse farms in Ireland. A questionnaire included general information, grazing and pasture management and usage of anthelmintic drugs. Faecal samples were also collected from yearling horses for faecal analysis using mini-FLOTAC technique. The response rate was quite low, only 78 (8%) questionnaires were returned. Approximately a third (42%) of the respondents reported receiving visiting equines during the year, only 69% and 48% of studs quarantine visiting equines and administer an anthelmintic, respectively. However, the majority (71%) of studs considered spring and autumn to be the most important time for deworming. Removing of faeces from the pasture was implemented by 37.6%. The largest number (85.7%) reported they were wormed 4-5 times yearly. Overall, the ivermectin and moxidectin group were most frequently used anthelmintic. The faecal egg counts (FEC) ranged from (0-2945 eggs per gram) in yearling horses. Several management factors were significantly ($P < 0.05$) associated with egg shedding in yearlings, including rotation between groups, harrowing of pastures, co-grazing with cattle/sheep, removal of faeces, and frequency of treatment. The finding from this study illustrates that stud owners do not follow best practice with regard to parasite control at best are sub-optimal and more education of stud managers is needed.

Keywords: Horse, management practices, faecal egg count

Rapid Oral Presentation – 7 Sept 2017: Herbal Remedies & Antiparasitics

Abstract No: 4960

Neem (*Azadirachta indica*) oil as a sustainable tool against *Varroa destructor*

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Abstract Content

Honeybees are livestock and crop pollinators of increasing economical and ecological importance. Several pathogens affect honeybees and in particular the mite *Varroa destructor* is a major damaging parasite of the hive. Several chemical acaricides are currently used against varroa with increasing concern for mite resistance and accumulation of potentially harmful residues in hive products for human consumption. This highlights the need to develop alternative varroacides from natural sources. Neem tree (*Azadirachta indica*) is a well-known source of insecticidal and miticidal products that are relatively non-toxic to mammals. Neem-derived products have been tested against varroa mites in laboratory and field studies with variable results when considering miticide efficacy and tolerability by honeybees (Gonzalez-Gomez et al., 2012; Anjum et al., 2015). Recently, application of neem oil extract directly on hive combs has been shown as a potentially safe and effective antivarroal strategy (Gonzalez-Gomez et al., 2016). The present study aimed to examine the possible ways of action against mites and synergy with suitable beekeeping methods of an in-hive neem oil preparation. The product was applied twice on hive combs at a concentration of 200 ppm Azadirachtin before egg deposition by queens. A significantly lower varroa load was recorded in treated vs to untreated combs and the effect was linked to a combination of mite repellency and alteration of developmental cycle. The results of the present study may contribute to the promotion of neem oil application as a new strategy to control varroa infestation with low or no residual impact on the hive.

Keywords: Neem; natural acaricide; honeybees; Varroa destructor

The effect of a mixture of medicinal herbs on lambs experimentally infected with gastrointestinal nematode *Haemonchus contortus*

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Abstract Content

The effect of dietary supplementation with a mixture of herbs (*Althaea officinalis*, *Petasites hybridus*, *Inula helenium*, *Malva sylvestris*, *Chamomilla recutita*, *Plantago lanceolata*, *Rosmarinus officinalis*, *Solidago virgaurea*, *Fumaria officinalis*, *Hyssopus officinalis* and *Melissa officinalis*, *Foeniculum vulgare*, *Artemisia absinthium*) on parasitological parameters of lambs infected experimentally with gastrointestinal nematode *Haemonchus contortus* was determined. Herbmix (100g dry matter (DM)/d) was added to the basal diets of meadow hay (600 g DM/d) and a commercial concentrate (350 g DM/d) per animal. Experimental lambs (n = 24) were divided into 4 groups: infected animals (1), infected animals supplemented with Herbmix (2), uninfected control animals (3) and uninfected animals supplemented with Herbmix (4). Groups 1 and 2 were infected orally with approximately 5000 of *H. contortus* L3 larvae susceptible to anthelmintics (MHco3). Herbmix supplementation for Groups 2 and 4 began on day 0 (D0). Feces were collected and eggs per gram (EPG) were quantified on D0, D20, D32, D50 and D60. The mean EPG counts were significantly lower between D32 and D50 ($P < 0.05$) for Group 2. Mean cumulative gain in live weight was higher in infected lambs supplemented with Herbmix (Group 2) ($P < 0.001$). Herbmix represents a promising herbal mixture for the control of nematode infections in organic farming.

The study was supported by Slovak Research and Development Agency Project No. 0169-14 and Grant Agency VEGA, Grant No. 2/0120/16 of the Scientific Agency of the Slovak Academy of Sciences.

Keywords: sheep, Haemonchus contortus, herbs

In vitro, evaluation of combined extracts of *Melia azedarach* and *Azadirachta indica* against larvae of *Aedes aegypti*

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Abstract Content

For the last many year, *Aedes aegypti*, vector of dengue virus, has been incriminated for causing a number of deaths in Pakistan. It breeds on stagnant water bodies near to residential colonies. In the socio-economic system where the livestock and farmers share the same roof, mosquitoes bite is inevitable. So, killing of the mosquitos at there breeding sites is most suitable way. Therefore, it is a need of the hour to find out a most economical and efficient bioinsectisides that would also be preparable at the point of care. This study was carried out to evaluate the potential of *M. azedarach* and *A. indica* (Combined water extracts) against Third instar larvae of *A. aegypti* mosquitoes at 24 and 48 hrs post-exposure. The extract was serially diluted to concentrations 15, 30, 60, 120, 140, 280 µg/ml in distilled deionized water. 20 (n=20) 3rd instars larvae of *A. aegypti* were incubated for 48 hrs. Larval mortality was observed for 24 and 48 hrs post-exposure and LC₅₀ and LC₉₀ were analyzed through probit analysis. The extract showed highest larval mortality against the larvae of *A. aegypti* with LC₅₀ = 22 µg/ml LC₉₀ = 38 µg/ml and LC₅₀ = 39 µg/ml; LC₉₀ = 47 µg/ml at 24 and 48 hrs post-exposure, respectively. This is a first report on the larvicidal activity of the combined effect of *M. azedarach* and *A. indica* against *A. aegypti*. Isolation of the bioactive ingredients of the plants and evaluation of their effect against adult *A. aegypti* is, however, recommended.

Keywords: Melia azedarach, Azadirachta indica, Aedes aegypti, in vitro

Polyphenol content of different methanol:water leaf extracts is negatively associated with its anthelmintic activity against egg hatching of *Haemonchus contortus*

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Abstract Content

In vitro studies of plant extracts suggest a relationship between the presence of polyphenols and anthelmintic (AH) activity against *Haemonchus contortus*. Higher polyphenol content appears to increase the efficacy against *H. contortus* as measured by the larval exsheathment inhibition assay (LEIA), while decreasing the efficacy measured with the egg hatching inhibition assay (EHA). This study identified the relationship between condensed tannins (CT), total polyphenols (TP) and total tannins (TT) contents of methanol: water extracts (70:30) from 10 plant species and AH activity against *H. contortus* evaluated by LEIA and EHA. Methanol:water extracts of *Acacia collinsii*, *Lysiloma latisiliquum*, *Havardia albicans*, *Senegalia gaumeri*, *Mimosa bahamensis*, *Piscidia piscipula*, *Acacia pennatula*, *Gymnopodium floribundum*, *Leucaena leucocephala* and *Bunchosia swartziana* were produced. Positive correlations were found between the effective concentration 50% (EC₅₀) of the extracts and their CT content (r: 0.6809, P <0.05, n = 10) and TP content (r: 0.9152, P <0.05, n = 10). The latter suggest that such compounds limit the activity of extracts against egg eclosion. In addition, a higher TT content of the extracts reduced the ovicidal effect (r: -0.7091; P <0.05, n: 10). There was no association between the CT, TP or TT content of the extracts evaluated and the EC₅₀ in LEIA. Funded by CONACYT-CB-2013-01/221041.

Keywords: Polyphenol content; egg eclosion; methanol:water extracts; *Haemonchus contortus*

Abstract No: 4158

Prevalence of *Toxoplasma gondii* in meat from sheep, goat and cattle from markets and slaughter house in Selangor, Malaysia

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Abstract Content

Toxoplasmosis has been recognized globally as an endemic disease capable of infecting all warm blooded animals and causing major health concerns to humans. Studies in Malaysia have shown that seroprevalence of *Toxoplasma gondii* (*T. gondii*) among healthy people in Malaysia can be as high as 30% indicating that it is common among Malaysians. *T. gondii* can be transmitted to human horizontally through consumptions of undercooked meats, unpasteurized milk or by accidental ingestion of oocysts from the environment. In Malaysia, studies on *T. gondii* in meat have been reported in poultry, wild boar and exotic animals but none in ruminants. The aim of this study is to determine the prevalence of *T. gondii* in ruminant meats in markets and slaughter houses in Selangor. A total of 100 meat samples from goat, sheep and cattle are being collected from various retail markets within the nine districts in Selangor and another 100 meat samples from the slaughter houses. All samples collected will be kept at -20°C until further analysis. Samples will be subjected to a commercially available ELISA assay using meat juice to determine the seroprevalence and a nested PCR for the detection of *T. gondii* DNA. Information on the samples such as species, location, age, breed, sex and source of the animal will be taken when applicable and statistical analysis will be performed to determine the risk factors involved. Findings from this study will further enhance knowledge on epidemiology of *T. gondii* in Selangor and its potential health implications to the public.

Keywords: *Toxoplasma gondii*, Ruminant meat, PCR, ELISA

Case report: Human lingual sarcocystosis cause by *Sarcocystis* sp. from autopsy cases at Hospital Sungai Buloh, Selangor

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Abstract Content

Sarcocystosis is an emerging disease caused by intracellular protozoan of the genus *Sarcocystis* spp. It requires two hosts; intermediate and definitive to complete its life cycle. The parasite can be seen in two forms i.e., muscular and intestinal. A case of lingual sarcocystosis was detected from a cadaver of 48 years old; an Indian male residing in Malaysia due to Hypertension Heart Disease on October, 2015. Autopsy was performed in Department of Forensic Medicine, Hospital Sungai Buloh. Tissue samples were collected from diaphragm, tongue and pectoral muscle of cadavers, fixed in 10% buffered neutral formalin for histopathological study and 90% ethanol for polymerase chain reaction (PCR) examination. The histopathology study revealed a sarcocyst of *Sarcocystis* sp. in the tongue with size of 76.44 x 52.38µ containing banana – shaped bradyzoites. The wall was radially striated with villous like projections and thickness of 1.71µ. Indirect fluorescent antibody test (IFA) was applied to dried blood sample collected onto filter paper and positive for sarcocystosis at 1:50 dilution. However, result from other samples still pending for further investigation.

Keywords: Sarcocystosis; sarcocyst; histopathology; autopsy; Malaysia

First detection of *Toxoplasma gondii* and *Giardia duodenalis* in commercial Green-lipped Mussels (*Perna canaliculus*) in New Zealand

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Abstract Content

Toxoplasma gondii, *Cryptosporidium* spp, and *Giardia* spp. are water-borne protozoan parasites of significant medical and veterinary importance. Worldwide, the (oo)cysts are released into the environment via animal faeces. Contaminated freshwater sources are well-associated with outbreaks of human disease. Overseas, all three protozoan parasites have also been detected in estuarine and marine shellfish. However, in New Zealand, there is very limited information on *T. gondii*, *Cryptosporidium* and *Giardia* spp. in the marine environment and presence of these parasites has not yet been investigated in NZ shellfish. Thus, the aim of this study was to screen NZ commercial mussels for these protozoan parasites. Haemolymph was extracted from 104 live commercially grown Green-lipped mussels (*Perna canaliculus*). DNA was extracted from mussel haemolymph and tested for the presence of DNA from the three parasites using established nested PCR assays. *T. gondii* DNA was detected in 12.5% of mussel haemolymph samples and confirmed by direct sequencing. Unfortunately, molecular genotyping using restriction fragment length polymorphism (RFLP) was not successful. *Giardia duodenalis* DNA was also detected in one mussel haemolymph sample and confirmed by direct sequencing to be subtype BIV. *Cryptosporidium* spp. DNA was not detected in any mussels sampled in the study. The results of this study suggest that farmed/commercial shellfish could be an important route of infection for humans. Further studies are underway to determine if the shellfish are infected prior to harvesting through environmental contamination and whether the presence of parasite DNA equates to viable parasite able to be transmitted to mammals.

Keywords: Toxoplasma, Giardia, Mussels, New Zealand, marine

Rapid Oral Presentation – 7 Sept 2017: Novel Parasite Control Options & Diagnostic

Abstract No: 3942

Assessment of irradiation attenuated *Trypanosoma evansi* induced protective immune response in murine and rabbit model

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Abstract Content

Importance of surra caused by *Trypanosoma evansi* ranks high due to its devastating effects on the livestock health and productivity leading to severe economic losses to the dairy industry. Since development of a protective vaccine through either conventional or by advanced biotechnological approaches is not yet possible, induction of immunity by irradiation attenuated *T. evansi* is thought to be a practical and impressive option. We standardised suitable dose of gamma irradiation for optimum attenuation of *T. evansi* and that was between 450 Gy to 500 Gy. Subsequently immunization and challenge study was conducted in murine and rabbit model showed that irradiated trypanosomes could induce protection ranging from 60 to 100% in experimental mice and 100% in rabbits. In murine, the level of protection was correlated with increased serum concentrations of IgM, IgG, IgG1, IgG2a and IgG2b. Among the IgG isotypes, IgG2a and IgG2b response was predominant. The serum cytokine profile post immunization, as determined by flow cytometry bead based assay, showed a predominant T helper cell Type 1 (Th1) response with significant increase in levels of IFN- γ and TNF- α . In rabbit, immunization with gamma radiation attenuated *T. evansi* induced 100 percent protection following homologous challenge with detectable serum IgG response. The cytokine profile study based on Th1/Th2 ELISA kit in the immunized rabbits showed significant increase in levels of IFN- γ and TNF- α in comparison to IL-10. The conferred protection in immunized mice and rabbit to the lethal homologous challenge was attributed to both the humoral and cellular immune responses.

Keywords: Trypanosoma evansi; gamma irradiation; Mice; Rabbits

Assessment of comparative immunoprophylactic efficacy of gamma irradiated *Trypanosoma evansi* and recombinant PFR in murine model

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Abstract Content

Surra, caused by *Trypanosoma evansi*, is an economically important disease of a wide range of domestic and wild animals. Since trypanosomes can effectively evade the host immune response by displaying a battery of variable surface glycoproteins, attempts for developing a protective immunogen has not been met with success. Paraflagellar rod (PFR) is one of the constituent proteins of the flagella and considered as a vaccine target owing to its strategic location and invariable nature. The immunoprophylactic potential of the recombinant full length PFR1 as well PFR2 proteins was assessed in mouse model. The pathway of the CD4+ T cell response triggered by the immunogens was assessed by studying the profile of a selected group of cytokines, viz. IL-2, IL-4, IL-5, IL-10, IFN- γ and TNF- α using flow cytometry technique. The immediate response in a trypanosome infected mouse is typically a Th1 type, mediated primarily by IL-2 and IFN- γ . The predominant humoral response in the immunized mice was IgG with the subclasses IgG1 followed by IgG2a and IgG2b. Against the backdrop of failed attempts to develop a protective vaccine using native or recombinant proteins of trypanosome origin, development of a live attenuated vaccine using ionizing radiation, is believed to be an impressive and practical option and therefore, immunization trials were conducted in experimental rat model. Higher serum titres of IgG antibodies along with the Th1 polarization in the cytokine response post immunization might have been pivotal in the observed absolute protection in the rats lethally challenged with *T. evansi*.

Keywords: Irradiated vaccine; Immune response; Murine; Surra; PFR

A universal approach to molecular identification of rumen fluke species from different life cycle stages, host species and continents

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Abstract Content

Rumen fluke, or paramphistomes, seemingly exhibit a world-wide distribution; but of the more than 70 species that exist within the superfamily Paramphistomidae, many are endemic to specific geographic areas or hosts only. In tropical regions low productivity and high mortality as a result of paramphistomosis are routinely documented; whilst within Europe paramphistomes are considered relatively harmless. Pathology and severity of paramphistomosis may depend not only on the species and immune status of the host, but also the paramphistome species present; consequently, accurate species identification may be important in predicting disease outcome. Because species identification of paramphistomes is virtually impossible by egg examination and difficult morphologically even at adult stage, we aimed to utilise molecular methods to identify different paramphistome species from various host species and continents using DNA isolated from a range of substrates and life stages including adult fluke, eggs extracted from faeces and cercariae within the intermediate snail host. A total of 329 paramphistomes from 157 individuals (9 definitive host species and 1 intermediate snail host) across 15 countries were successfully identified as 10 paramphistome species through DNA sequencing of the amplicon; demonstrating a generic approach that could be utilised for identification of paramphistomes regardless of host species or specimen type. For the majority of species examined, the ITS-2 region was sufficiently discriminatory, with variation between species exceeding variation within species. However, other targets such as 28S and Cox1 should be explored to complement the available ITS-2 data, particularly where little genetic sequence information is available for comparison.

Keywords: rumen fluke; paramphistomes ; PCR ; sequencing ; ITS-2

Modulation of the porcine gut microbiota by *Trichuris suis* and the prebiotic inulin

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Abstract Content

Feeding of the prebiotic compound inulin has been linked to an increase in beneficial microbes such as *Bifidobacterium* and *Lactobacilli* in the gastrointestinal tract, thereby inducing favourable changes in the host's microbiota and a subsequent improvement of the health of the host. Dietary inulin may also have an effect against gastrointestinal helminths in pigs which may result in lowered egg counts and expulsion of helminths. The effect may be driven by changes in gut microbiota and produced metabolites. To examine this we performed a 2-factorial study in 34 pigs to investigate the interactions between the fermentable fructo-oligosaccharid inulin, *Trichuris suis* and the changes over time in the gut microbiota of selected intestinal locations by utilizing a newly developed high-throughput 16S rRNA sequencing technique. Data were analyzed by metaBION and QIIME. The sequencing method is capable of sequencing 288 samples simultaneously without losing depth, resulting in an average of 23,000 reads per sample. Pigs were infected with 5000 eggs of *T. suis* and necropsied after 4 weeks. Preliminary results show a clear effect on the general composition of the cecal microbiota with an increase in Actinobacteria and Lachnospiraceae for pigs fed the inulin-rich diet. The infected pigs showed a difference in the amount of Entereobacteriales, which disappeared for inulin fed pigs, indicating a shift towards a healthier gut microbiota through dietary inulin, even when infected with helminths. These results indicate an association between an inulin-rich diet and a healthier gut, along with minimizing the adverse effects of a *T. suis* infection.

Keywords: Gut microbiota; Inulin; Helminth infection; 16S amplicon sequencing

In vitro evaluation of the impact of pH on *Fasciola hepatica* metacercarial viability

Grace Cuthill¹ ; Gillian Mitchell¹ ; Philip Skuce¹ ; Neil Sargison¹

¹Disease Control/ The Moredun Institute/ United Kingdom

Abstract Content

Grass silage has been highlighted by farmers as a possible disease risk after several unconfirmed reports of fasciolosis in housed livestock. Silage quality is negatively affected above or below the optimum pH range of 3.7-4.7. There exists little information about the effect of pH on *F. hepatica* metacercarial viability. The effects of lactic acid solutions of pH 4, 5 and 6 were assessed using groups of 100 *F. hepatica* metacercariae. After four weeks the viability of the metacercariae was assessed with an *in vitro* excystment assay. 3% of metacercariae incubated at pH 4 were able to excyst after four weeks incubation. This was a significantly lower excystment rate than those incubated in water and pH6 ($p < 0.01$, $p < 0.05$). *F. hepatica* metacercariae are able to survive optimal pH conditions for silage fermentation after 4 weeks. However, pH is only one factor of silage fermentation and it is likely that other factors such as anaerobic and high temperature conditions would also influence viability. Investigating the ability of metacercariae to excyst after exposure to pH conditions associated with both optimal and sub-optimal pH for grass fermentation would give a better understanding of the effect of one factor of the silage making process on liver fluke metacercarial viability. Further work would include the greater challenge of assessing the overall fermentation process on metacercarial viability and the ability to detect viable metacercariae in silage and forage samples. This work would improve our understanding of liver fluke epidemiology and assist with the development of disease avoidance strategies.

Keywords: Fasciola hepatica; Metacercariae; pH; Silage; Epidemiology

Diagnosis of fluke infective stages in the environment

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Abstract Content

Fasciola hepatica is an issue for livestock health and welfare in over 50 countries. In recent years, the epidemiology of liver fluke has changed with the emergence of drug-resistant fluke populations and increasing reports of 'unseasonal' fasciolosis. Currently, forecasting fluke risk is performed at a relatively crude, regional, scale, based on traditional seasonal risk and prevailing weather patterns. Farmers are concerned about fluke risk specific to their farm. The ultimate indicator of infection risk is the metacercarial challenge on pasture. The aim of this project is to develop methods for the detection and identification of viable metacercariae in the environment. In this presentation, I will describe the development of an *in vitro* excystment method to determine metacercarial viability. I will also present the development of a LAMP (loop-mediated isothermal amplification) assay for the specific identification of *F. hepatica* metacercariae targeting the mtDNA COX-1 gene. I have succeeded in the development of an excystment assay, with up to 95% of metacercariae excysting. I have successfully amplified liver fluke metacercariae DNA using PCR and LAMP. Results relating to the optimisation of the LAMP assay to confirm species identification have yet to be achieved. In the months leading up to WAAVP I shall continue to evaluate these methods for the application to environmental samples. New tools to identify viable liver fluke metacercariae in different environments will improve our understanding of fluke epidemiology. And in turn could help identify disease risk, in time, to inform avoidance strategies and facilitate targeted disease control.

Keywords: *Fasciola hepatica*; *Metacercariae*; *Molecular epidemiology*; *LAMP*; *COX1*

An automated cellphone-based feedback and training system for resource-poor farmers for sustainable animal health and production management

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Abstract Content

Training of particularly Resource-Poor, but also of commercial farmers in sustainable application of optimal farm management systems is severely limited globally by personnel constraints. However, while 80% of even Resource-Poor farmers, South of the Sahara, have access to cellphones, the full utilization of which offers a potential solution to this challenge, these are presently very much underutilized for this purpose. To develop a server-supported automated RFID animal activity monitoring system to receive on-farm generated real-time data from transponders on animals for on-server analysis, interpretation, and training of Resource-Poor farmers. Real-time streaming, to Microsoft Cloud, of activity data from transponders, using computer model evaluation, interpretation, and report-back to farmers and (where necessary) veterinary authorities, on animal and pasture health and production, including suspected disease outbreaks. A simplified APP is being developed for downloading to cellphones, with the emphasis on automated farmer training via simplified expounding of principal factors contributing to every server-generated recommendation. A novel automated animal disease detection and farmer training system, comprising server-generated interpretation of streamed data, accompanied by recommendations on management inputs required and alerts to individual and/or herd/flock-related animal production and health conditions and disease outbreaks. This novel integrated farmer training and support system utilizes the expertise of widely divergent disciplines including veterinary, pasture and animal science and mathematical modelling in software development and networking for remote improvement of farmer expertise in animal-side health and production management. It has potential for efficient remote economic advancement of farmers in animal management.

Keywords: Automated farmer training; Automated decision support; Parasite management; Activity monitoring; Nutrition management

Abstract No: 3807

Efficacy of *Coccidia* vaccine and/or *Clostridia* toxoid on concurrent *Coccidia* and *Clostridia* experimental infection in chickens

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Abstract Content

Concurrent infection with *Coccidia* and *Clostridia* is one of the major diseases affecting chickens causing economic losses. This study aimed to assess the immunological status of chicks experimentally infected with local strains of *Coccidia* and *Clostridia* isolated from diseased broiler chickens. A total of 225 -one day age- broiler chicks were divided into 5 equal groups; (G1): normal control, (G2): infected with *Eimeria tenella* and *Clostridium perfringens* type A, (G3): vaccinated with *Coccidia* vaccine, (G4): treated with *Clostridia* toxoid and (G5): vaccinated with *Coccidia* vaccine and treated with *Clostridia* toxoid. Groups (G2-G5) were challenged orally with (5×10^4) *Coccidia* oocysts at the 21st day post vaccination (dpv) and S/C with 0.5ml of 24 h cooked meat broth 1×10^7 CFU *Clostridium perfringens* type A at the 23rd dpv. Blood samples were collected at the 5th, 13th and the 21st dpv and at the 5th, 7th, 15th and the 21st day post challenge (dpc) for detection of antibody and antitoxin against *Clostridium perfringens* type A toxin using ELISA and SNT. Results revealed that (G5) was the lowest one in the morbidity, mortality, intestinal lesions and food conversion rate, while (G2) showed the highest morbidity rate, mortality rate, intestinal score lesions and food conversion rate. (G5) recorded the highest levels of antibody and antitoxin titers reached to the maximum at the 21st dpv and dpc. In conclusion, vaccination with *Coccidia* vaccine beside *Clostridia* toxoid minimized the morbidity and mortality rates and gave the highest protection against concurrent infection with *Coccidia* and *Clostridia* in chickens.

Keywords: Chickens, concurrent Coccidia and Clostridia, Coccidia vaccine, Clostridia toxoid, immunity.

Abstract No: 5550

Microbiome in health and disease with special reference to parasitic diseases

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Abstract Content

Mammals evolved from microbes and simple multicellular organisms over billions of years. Many microbes including viruses, bacteria, archaea, protozoa, fungi and even some multicellular helminths have co-evolved with humans to survive as commensals in the human body. Some bacteria have evolved to live as peripheral and intracellular symbionts on the protozoal organisms. The human body coexists with a large community of microbes (or a microbial zoo) thriving on the skin and mucosa and participating in host nutrition, metabolism and physiology thereby forming a complex ecosystem or “superorganism”. The perfection of high throughput sequencing (next generation sequencing, NGS) has allowed analysis of this collective microbial genome (microbiome) composition, diversity and dynamics and identification of a diverse collection of eukaryotes (including protists, fungi and helminths) that inhabit the human body. Recent research has shown that many of them are often commensals and sometimes maybe beneficial. The term “eukaryome” has been proposed (Luke J., Svensvold CR., 2015) for this collection of organisms. The higher incidence of autoimmune and inflammatory diseases including Crohn’s disease, ulcerative colitis, various allergies, and rheumatic arthritis in industrialised population compared to population with traditional lifestyle is largely attributable to changes in the gut microbiome. Use of helminths as a prophylactic/therapeutic agent (helminth therapy) has been variably successful at preventing or treating autoimmune and inflammatory diseases. Although less numerous than bacteria the much larger eukaryotes may have a disproportionately larger influence on the microbiome and its function. Removal of the eukaryome leads to negative health consequences.

Keywords: Parasites; protozoa; helminths; microbiome; eukaryome

Development of a novel methodology combining morphological with molecular identification of *Walchia* chigger mites, potential vector of scrub typhus disease

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Abstract Content

Zoonotic scrub typhus is vector-borne disease transmitting by the larval stage of Trombiculid mite called "chigger". Chigger mite identification is needed microscopic process. The classical method of species identification necessary to destroy internal tissue and fix with microscopic slide. It seems to be impossible to get molecular information from specimens. However, chigger mites are member of Chelicerate arthropods which auto-fluorescence property of exoskeleton. It allows visualization of surface of morphological characters by using UV light. *Walchia*, a diverse chigger mite genus on rodent hosts, are good demonstration for the study of morphological and molecular correlation. Total of 59 *Walchia* chigger mites were selected from natural rodents collecting in scrub typhus areas. Individual chigger mites can be identified by both fluorescence and conventional light microscopy for morphotyping to species level with 16 characters. At the same time can be used for DNA extraction. The DNA quality of the specimens has been verified by PCR of COI gene amplification and sequencing. For the result, Approximately 640 pb of COI partial fragments was analyzed. 21 COI sequences of 5 species of *Walchia* chigger mites were presented. Phylogenetic analysis of COI sequences among *Walchia* specimens showed that the morphological and molecular identification are correlated reciprocally. It can be concluded that morphological studies by using UV light combine with visible light can actually preserve the quality of DNA materials enough for COI fragment analysis. This is the novel microscopic method for morphological identification of the chigger species that would preserve DNA for molecular studies.

Keywords: Chigger mite / Novel methodology / Morphology / Autofluorescence / Molecular study / Preserve DNA

Exogenous haem is essential for tick reproduction

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Abstract Content

Haem and iron homeostasis in most eukaryotic cells is based on a balanced flux between haem biosynthesis and haem oxygenase-mediated degradation. Unlike most eukaryotes, ticks possess an incomplete haem biosynthetic pathway and, together with other (non-haematophagous) mites, lack a gene encoding haem oxygenase. We demonstrated, by membrane feeding, that ticks do not acquire bioavailable iron from haemoglobin-derived haem. However, ticks require dietary haemoglobin as an exogenous source of haem since, feeding with haemoglobin-depleted serum led to aborted embryogenesis. Supplementation of serum with haemoglobin fully restored egg fertility. Surprisingly, haemoglobin could be completely substituted by serum proteins for the provision of amino-acids in vitellogenesis. Acquired haem is distributed by haemolymph carrier protein(s) and sequestered by vitellins in the developing oocytes. This work extends, substantially, current knowledge of haem auxotrophy in ticks and underscores the importance of haem and iron metabolism as rational targets for anti-tick interventions.

Keywords: ticks; haem; iron; physiology; RNAi;

Abstract No: 4953

A post mortem case investigation of gastrointestinal parasitism in goats from a farm in northern Perak, Malaysia

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Abstract Content

Gastro-Intestinal Parasitism is a normal situation in our country. Without early detection and treatment, it could lead to mortality of animals as observed in this case. Three Boer goats' carcasses (two adults and one kid) were registered to Pathology Section of Veterinary Research Institute (VRI) Ipoh for post mortem examination with the following symptoms of diarrhea and thin body condition. General examination of the three carcasses revealed that these animals were anorexic (BSC=1) and dehydrated. The fur were rough and ruffled. The mucous membrane were pale especially at both eyes and gums. Watery reddish discharge were observed from the nostrils and greenish coloured diarrhoea from the anus. Post mortem examination revealed that all vital organs were generally pale, congested and abnormal in size. Severe enteritis with haemorrhages and congestion were present in the small and large intestines. The intestines were observed to have contained greenish coloured faeces and distended gas. The wall of intestine were markedly congested and pin-point haemorrhage were observed at the serosal surface. When opened, the serosal layer were haemorrhagic and easily peeled off from the wall. The organs and faecal sample were collected and submitted to laboratories for further diagnosis. Results from Bacteriology laboratory examination showed non-significant findings. Meanwhile, all samples were negative for Virology laboratory examination. The result that narrowed the conclusion of this case was the Parasitology laboratory examination in which that the Boer kid was positive for Strongyle eggs and *Coccidia* oocytes in the intestines, while the two adults were positive for *Haemonchus contortus* worms in the abomasum. Hence, Gastro –Intestinal Parasitism was made as the final diagnosis based on the post mortem and parasitology findings.

Keywords: Gasto-Intestinal parasitism, Strongyle egg, Coccidia oocytes, Haemonchus contortus worms, Boer goats

An outbreak of oriental theileriosis in dairy cattle imported to Vietnam from Australia

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Abstract Content

Oriental theileriosis caused by the members of the *Theileria orientalis* complex, is an emerging tick-borne disease of bovines in the Asia-Pacific region. This study reports an outbreak of oriental theileriosis in dairy cattle imported to Vietnam from Australia. Following clinical and pathological diagnoses, a total of 112 cattle blood samples were divided into three groups and tested using multiplexed tandem PCR. Group 1 were from aborted heifers in Vietnam; group 2 were from cattle before shipment from group 1 cattle and group 3 were from the same batch of cattle but transported to Taiwan. *Theileria orientalis* DNA was detected in 72.3% cattle. The prevalences of *T. orientalis* in groups 1, 2 and 3 were 77.6%, 86.9% and 57.5%, respectively, and the difference in prevalence was significant between groups 1 and 3 ($P < 0.0001$). The infection intensities of genotypes *chitose* and *ikedda* of *T. orientalis* were higher in groups 1 (57,721 and 33,709, respectively) and 3 (5,897 and 61,766, respectively) than those in group 2 (2,071 and 6,331, respectively). Phylogenetic analyses of the major piroplasm surface protein sequences revealed that genotypes *chitose* and *ikedda* found determined herein were closely related to those previously reported from Australia. This first report of an outbreak of oriental theileriosis in imported cattle emphasises improved measures for the export and import of cattle infected with *T. orientalis*.

Keywords: *Theileria orientalis*; Transboundary infection; MT-PCR; conventional PCR; Australia-Vietnam

Epidemiology of gastrointestinal nematode burden and species in breeding-age dairy heifers on pasture in Saskatchewan, Canada

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Abstract Content

There is a dearth of information on the epidemiology of gastrointestinal nematodes (GIN) in dairy cattle in Canada. Considering the potential for significant production impacts and the threat of anthelmintic resistance, the aims of this research were to start addressing this lack of information by determining the GIN burden, predominant species and changes in burden and species over time in dairy heifers in Saskatchewan, Canada. Fresh environmental fecal samples were collected from 30 grazing heifers at monthly intervals (June, July and August) on six dairy farms. Eggs per gram of feces (EPG) were determined using the Modified Wisconsin Sugar Flotation Technique. Predominant nematode species were identified at the farm-level using deep amplicon nemabiome sequencing of the ITS-2 rDNA locus of nematode larvae. Population-averaged geometric mean egg counts were 0.98 (95% CI: 0.76-1.25), 2.65 (95% CI: 2.15-3.25) and 4.13 (95% CI: 3.23-5.28) EPG for June, July and August, respectively. The predominant nematode species on all farms were *Cooperia oncophora* and *Ostertagia ostertagi*, accounting for > 75% of the species on each farm. The results in this study are consistent with the literature for young grazing cattle in temperate climates. Going forward, the combination of fecal egg counts and high-throughput deep-sequencing assays will be used to characterize the nematode burden and species in Canadian dairy cattle more extensively and to better investigate anthelmintic treatment efficacy. In combination, these results will provide much needed information for evidence-based, sustainable GIN control strategies in that industry.

Keywords: Gastrointestinal nematode; epidemiology; dairy; heifer; Saskatchewan

Abstract No: 3398

Pampas fox (*Lycalopex gymnocercus*) new intermediate host of *Sarcocystis svanai* (Apicomplexa: Sarcocystidae)

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Abstract Content

Several *Sarcocystis* spp. have carnivores as definitive host and sarcocysts developed in muscles of intermediate host. However, sarcocysts have been found in muscles of wild and domestic carnivores suggesting they are intermediate host for some *Sarcocystis* spp. Here, we report mature sarcocysts in the muscles of Pampas fox (*Lycalopex gymnocercus*). A total of 36 free-living foxes were analyzed. Different skeletal muscles were assessed by microscopic and molecular methods. Cysts and/or DNA of *Sarcocystis* sp. were detected in 61.1% (22/36) foxes. Histopathology revealed the presence of sarcocysts in 52.8% (19/36) foxes. The tongue and masseter were the muscles more frequently infected. Of all the samples processed by homogenization of pooled muscles of each animal, 45.4% (10/22) evidenced muscle cysts and 68.2 % (15/22) resulted positives by PCR. Five amplicons from individual cysts from different samples were selected for sequencing together with four PCR products obtained from the pooled muscles. All nine sequences shared a high identity among them (99.8-100%) and showed the highest identity by BLAST (99%) with a *S. svanai* sequence (KM362428) from a North American dog. By transmission electron microscopy, the sarcocyst wall was thin (< 1 µm), had minute undulations, with tiny evaginations and without evident villar protrusions (the ultrastructure is referred as "type 1"). *Sarcocystis svanai* infects *L. gymnocercus* with a high prevalence and the presence of mature sarcocysts suggests the role of the Pampas fox as natural intermediate host. The definitive host of *S. svanai* remains unknown.

Keywords: Sarcocystis; muscle; wild canid; ultrastructure; sequencing

Prevalence and chemotherapy of *Plasmodium fallax* in domestic pigeons

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Abstract Content

One hundred and twenty pigeons were brought from six different locations of Lahore to the animal house of Zoology Department of GC University Lahore, Daata Darbar, Tollinton Bird Market, Abdali Chowk, Shahdara, Istanbul Chowk Sectarat and Shadman Bird Market. Pigeons belong to the order *Columiformes* are universal birds and found easily in every area except poles. The avian *Plasmodium* parasites which are ecologically successful Apicomplexans, causes avian malaria. Random and non-random sampling was done to increase the chances of detecting diseases if present. Blood was collected directly from brachial vein and blood smears were prepared and stained in Giemsa's solution and field stain. Among the six selected locations, the prevalence of *Plasmodium species* was significantly higher in Shahdara amounting 65% and in Shadman bird market and Abdali chowk was recorded 50% that was second highest incidence rate. Lowest was recorded in Tollinton bird market 25%, 40% in Data darbar and 45% in Istanbul chowk secteriat. Haemosporidians includes three species, *Plasmodium*, *Leucocytozoon* and *Haemoproteus*. The presence of schizonts indicated infective birds with *Plasmodium species*. The incidence in males was noted 40.9% and in females 34.8% during the 6 months duration of study. In month wise incidence detection highest incidence rate in males was recorded in June 56.9% and in females in May 48.5%. The *Plasmodium species* causes malaria, cause a lower body fat, lower body mass, impair immunological responses, decrease nest success and reduction in the parental caring behavior typically limiting to unique settings such as captive or closely managed flocks.

Keywords: Plasmodium, malaria, epidemiology, pigeons.

Intestinal coccidiosis in Camel (*Camelus dromedarius*): A case report

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Abstract Content

Camel husbandry has a great importance in agricultural activities due to its adaptation power in arid and semi- arid regions across the world. It plays a very important role in transportation as well as production of milk, meat, wool, hair and hide as major source of rural economy. Coccidiosis is usually an acute invasion and destruction of intestinal mucosa by protozoa of the genera *Eimeria spp.* Coccidiosis is seen universally, most commonly in young animals. A carcass of camel aged about 5 years submitted to the Department of Veterinary Pathology, College of Veterinary and Animal Science, Bikaner for Post- mortem examination. The history indicated that the camel was suffering from diarrhoea, pyrexia, inappetence, weight loss and emaciation. Post mortem examination showed lesions in the small intestine which had congested and haemorrhagic mucosa on which there were numerous whitish-grey foci were observed. The intestinal tissue specimens were preserved in 10% formal saline and processed mechanically for paraffin embedding by acetone and benzene technique for histopathological examination. Histologically, there was congestion and haemorrhages alongwith giant schizonts in various developmental stages were seen in the lamina propria of the intestine. There was severe edema of the villi alongwith degeneration and desquamation of intestinal mucosa with infiltration of eosinophils and macrophages were observed.

Keywords: Camel, Coccidiosis, Histopathology

***Neospora caninum*-induced neutrophil extracellular trap (NET) formation in Bottlenose dolphins (*Tursiops truncatus*)**

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Abstract Content

Neospora caninum infections can cause disease and enhanced mortality in domestic and wild animals. As such, *N. caninum* infections have recently been reported to occur in bottlenose dolphins (*Tursiops truncatus*). Neutrophil extracellular trap (NET) formation is an innate defense mechanism acting against protozoans. Aim of the study was to analyze the capacity of *N. caninum* tachyzoites to trigger NETs in the cetacean system. *N. caninum* tachyzoites were exposed to polymorphonuclear neutrophils (PMN) of *T. truncatus* at different ratios and time spans. Extracellular DNA staining was used to illustrate and quantify NETs. Molecular components of NETs [i. e. histones (H1, H2A/H2B, H3, and H4), neutrophil elastase (NE), myeloperoxidase (MPO) and pentraxin (PTX)] were demonstrated via immunofluorescence analyses. Treatments with DNase I and the NADPH oxidase (NOX) inhibitor DPI were performed to characterize the DNA nature and NOX-dependency of cetacean-triggered NETosis, respectively. Overall *N. caninum* tachyzoites-triggered cetacean NETosis revealed as a dose-dependent process. SEM analyses demonstrated cetacean PMN-derived NETs entrapping *N. caninum* tachyzoites and forming delicate DNA networks out of gross and slim fibers. Co-localization studies of cetacean-derived NETs demonstrated DNA structures being decorated with histones, NE, MPO and PTX, thereby confirming typical molecular characteristics of NETs. Parasite-induced NETosis was resolved by DNase I and inhibited by DPI treatments, proving the DNA nature and NOX-dependency of this mechanism in dolphins. Bottlenose dolphin-derived PMN reacted against *N. caninum* by NET extrusion evidencing the importance of this ancient effector mechanism of the cetacean innate immune system against this emerging terrestrial neozoan apicomplexan parasite.

Keywords: *Neospora caninum*; NETs; Dolphins; *Tursiops truncatus*

Gastrointestinal parasites dynamic in drug environment in captive wildlife

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Abstract Content

In zoological parks, the wild animals are under constant stress of captivity and are prone to various infections causing even morbidity and mortality. The collected fresh faecal samples of 6 bears, 10 felines (lion, tiger and leopard) and 14 canines (hyena, jackle, fox and otter) of Surat Zoo, India in the February, May, August and November of 2014-16, were transported to laboratory for the assessment of gastrointestinal (GI) parasites. The animals were doused per os @ 10 mg/kg body weight (b.wt.) with mebendazole (January 2014, August 2015), albendazole (July 2014, October 2015/ 2016), fenbendazole (October 2014, May 2015, March 2016) and @ 200 µg/kg b.wt. with ivermectin (January 2015, March 2016). The coccidia oocysts were noted in 1/6 faecal samples of bear in February of initial two years of the study. The *Spirometra* spp. eggs were evident in 1/10 felines in July, May and March of 2014, 2015 and 2016, respectively while *Ancylostoma* spp. eggs in 1/14 canines in July/ September- 2014 and December-2015. The *Diphyllobothrium latum* eggs were noted in 1/14 canines in September/ November-2014, December-2016. In spite of regular deworming, the animals harbors the GI parasites, warrants the screening of the animals for the development of anthelmintics/ drugs resistance. The defined environmental condition of the zoo caused GI parasites prevalence all around the year, which defies the concept of high prevalence of parasites during rainy season (July-October). The treatment regimen of the 3rd year was more effective in cleaning the GI parasites than the previous two years.

Keywords: Wildlife; gastrointestinal parasites; India; Captive

Bat flies from cave-dwelling bats and their potential role of *Bartonella* spp. transmission on Lombok Island, Indonesia

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Abstract Content

Bartonella spp are Gram-negative bacteria that infect humans and wild mammals. Evidence of *Bartonella* spp infections in bats and bat flies has motivated a growing interest in characterizing their transmission cycles. On Lombok Island many caves house massive colonies of bats that are known hosts of bat flies, presumably transmitted by blood-sucking bat flies. The purposes of this research were to identify bats, bat flies species and determine their role in *Bartonella* spp transmission from the bat caves on Lombok Island. Cross sectional survey was carried out in three bat caves on Lombok Island (Tanjung Ringgit Bat Cave, Lembah sempage Bat Cave, and Pujut Bat Cave) from January to March 2017. The bats were captured by net trap to indentify species of bats and bat flies. Blood samples were collected by puncturing the axillaries vein of bats for blood smears and they were examined using Giemsa staining to know the presence of intraerythrocytic elements suggestive of *Bartonella* spp infection. Among the 23 bats captured and identified as *Hipposideros bicolor* (n= 4), *Eonycteris spelaea* (n=11), and *Taphozous achates* (n=8). The result of identification of bat flies showed that *Nycteribia triangularis*, *Eucampsipoda penthetoris*, and *Megastrebla gigantea* were identified from captured bats. The presence of intraerythrocytic corpuscles suggesting *Bartonella* spp infection were seen in 8.6% (2/23) of the blood samples. These results suggest that blood-sucking bat flies can transmit *Bartonella* spp among cave-dwelling bats and human on Lombok Island.

Keywords: Bat flies, Bartonella, Cave-dwelling bats, Lombok Island

Endo- and ecto-parasites of Small Indian mongoose *Herpestes auropunctatus* on St. Kitts, West Indies

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Abstract Content

Herpestes auropunctatus, the small Indian mongoose, is an invasive omnivore on St. Kitts and many other Caribbean islands. It has played a role in changing animal fauna since its introduction into the region during the 1880s. Mongooses are also known to carry human pathogens and may be of public health importance. The aim of the current study was to detect endo- and ecto-parasites harbored by the mongoose on St. Kitts with a special interest in *Trichinella* spp. and *Toxoplasma gondii*, two worldwide zoonotic parasites. In total, 87 mongooses trapped during April to July 2015 were examined by various methods. The methods and number of animals examined were: 1) pepsin-HCl digestion of skeletal muscles (79) for *Trichinella* spp.; 2) PCR of heart homogenates for *T. gondii* (60); 3) fecal flotation by double centrifugation for parasites of the gastrointestinal tract (75); 4) open trachea, bronchi and lungs for lungworms and flukes (76); and 5) hair plucks (79), ear swabs (79) and general skin examination (87) for mites, ticks, lice and fleas. No *Trichinella* spp., *T. gondii*, lungworms or flukes were found. On fecal flotation, *Cystispora* spp. were found with a prevalence of 69.3% (52/75). The only ecto-parasite seen was *Ctenocephalides felis* (79.3%; 69/87). The data show that mongooses in the coastal regions pose a minimal risk to humans for *Trichinella* spp. and *T. gondii* on St. Kitts, which needs to be confirmed by a further study with a larger sample size from various geographic locations.

Keywords: *Herpestes auropunctatus*; *Trichinella* spp.; small Indian mongoose; St. Kitts; *Toxoplasma gondii*

Haematology, clinical parameters and tick burdens in Roe deer fawns

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Abstract Content

In the summer of 2016 a total of 52 neonate roe deer fawns captured in two different areas in Sweden, Grimsö (n = 24) in South central Sweden and Bogesund (n = 28) in the inner archipelago close to Stockholm have been examined at least once for presence of attached ticks. Clinical parameters (n = 32) have been recorded and a blood sample (n = 49) has been taken and analysed for haematology. The residual number of ticks (after correcting for fawn age) and fawn mortality have been calculated. Death (often by predation) has been recorded for 57.7% of fawns, 41.7% from Grimsö and 71.4% from Bogesund. Statistical analysis of recorded parameters has been performed using capture site (Grimsö and Bogesund) and status (dead or alive at February 2017) as independent variables. Preliminary results do not indicate different tick infestation levels between survivors or dead animals, while it is confirmed that roe deer fawns from Bogesund had higher tick burdens. Haematology seems to reveal that fawns from Bogesund were slightly more anaemic than those from Grimsö, a fact indirectly confirmed by clinical findings. These findings will be discussed also in the light of results obtained from blood samples from neonates from 2013 and 2014 for tick-borne pathogens (i.e. *Babesia*, *Borrelia*, *Anaplasma*). These findings can provide baseline parameters useful for further investigations in roe deer. The marking and handling of roe deer in this study were approved by the Ethical Committee on Animal Experiments, Uppsala, Sweden (Approval Dnr: C149/2015).

Keywords: Ticks, Roe deer; Sweden; Clinical examination, Haematology

The Role of Some Species of Hard Ticks In Transmission of Microfilariae for Camels

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Abstract Content

In this study 2400 males and females of Camels from Asowan, Upper Egypt,were examined for Hard Ticks infestation ,and the results revealed that , 60.96% were infested with hard ticks. The collected males and females ticks were examined for presence of microfilariae. The results idicated that 30.63% of male ticks were infected in guts, 32.1% of male ticks were infected in sperms and 2.5% of male ticks were inected in haemolymph. The hard ticks females were also examined for microfilariae (mfs.) infection and the results showed that 15% of guts , 25.21% of seminal receptacles and 1.88% of haemolymph samples were infected with microfilariae .

Keywords: Camels , hard ticks , mirofilariae

Abstract No: 4250

TsPKA-r: A potential immunodiagnostic antigen for the detection of porcine cysticercosis

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Abstract Content

Cysticercosis, caused by metacestodes of *Taenia solium*, has a significant socio-economic impact and is of considerable importance in public health. However, there are no specific diagnostic antigens to distinguish between *T. solium* and *Taenia hydatigena*. In the present study, cAMP-dependent protein kinase regulatory subunit (TsPKA-r), an excretory/secretory (ES) antigen of *T. solium*, was used to establish a specific and sensitive diagnostic tool for detection of porcine cysticercosis. The full-length sequence encoding TsPKA-r was amplified by PCR, sequenced and then identified by bioinformatics. The fusion protein with 6xHis-tags was expressed in *E. coli*, purified by Ni Sepharose™ 6 Fast Flow and used to test reactionogenicity by immunoblotting. TsPKA-r based indirect enzyme-linked immunosorbent assays (iELISA) showed good performance in recognition of sera of pigs experimentally infected with *T. solium* metacestodes, with 93.88% sensitivity and 96.40% specificity. There were no cross-reactions against the sera samples from pigs experimentally infected with *T. hydatigena*, *Toxoplasma gondii* or *Trichinella spiralis*. These results indicate that the TsPKA-r is a promising immunodiagnostic antigen for detection of porcine cysticercosis.

Keywords: TsPKA-r, T. solium, iELISA, immunodiagnostic antigens

Studies on fish-borne zoonotic parasites in economically important fishes in Egypt

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Abstract Content

Fish can be infected with a variety of parasites that can cause zoonotic infections in humans when consumed raw or not properly cooked. Traditionally, these parasitic zoonoses are most common in developing countries because of inadequate survey, diagnosis and monitoring for many of them. The aim of the present study is to survey the diversity of fish-borne zoonotic parasites in Egypt, particularly in Fayoum governorate, from June 2015 to May 2016. A seasonal investigation was conducted on the occurrence of fish-borne zoonotic parasites in two hundred and fifty fish representing five economically important species (Three species of freshwater fish and two of marine fish). The collected fish were transported alive and kept in the laboratory until they were examined. The length and weight of fish were recorded and their relation to the rate of host infection was studied. Different species of zoonotic trematode metacercariae (Prohemistomatids, Clinostomatids and Heterophyid) in addition to nematode larvae (Anisakids) were detected and isolated. The seasonal prevalence and intensity of each parasite species were recorded. Metacercariae were isolated and recovered using the standard pepsin digestion procedure. Encysted metacercariae, which could not easily be identified morphologically, were excysted by either physical pressure or by placing them in trypsin digestion fluid for 20 to 30 min at room temperature until they emerged from the cyst. The morphological characteristics of the fish-borne nematode larvae as well as the trematode metacercariae and their experimentally obtained adults were described. The study concluded the increase of the prevalence of such detected species in regard to the previous reports.

Keywords: Zoonotic ;Fish ;human ;Parasites ; Egypt

Histopathological changes during *Toxocara canis*- and *T. cati*-induced neurotoxocarosis

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Abstract Content

Neurotoxocarosis caused by migration and persistence of *Toxocara* spp. larvae in brains of paratenic hosts may result in distinct pathological changes. Previous studies allowed preliminary histopathological comparison of *T. canis*- and *T. cati*-induced neurotoxocarosis in the model organism "mouse". To provide a more extensive characterization, brains of *T. canis*- and *T. cati*-infected as well as uninfected mice were investigated 7, 14, 28, 42, 70 and 98 days *post infectionem* (dpi). Two histological sections of cerebra and cerebella, respectively, were stained with haematoxylin and eosin. Demyelination was visualized by Luxol fast blue staining. Distinct pathology in both infection groups was observed starting 7 dpi characterized by haemorrhages, haemosiderophages and eosinophilic vasculitis followed by eosinophilic meningitis in cerebra 14 dpi. At that time, degenerative processes like demyelination, vacuolization and gitter cells were solely detectable in *T. canis*-infected mice. Degenerative processes intensified during the course of infection and were predominantly observed 70 and 98 dpi. In contrast, gitter cells were not observed in *T. cati*-infected cerebella until day 70 pi and day 98 pi in cerebra. Actual occurrence of larvae was mainly determined in cerebella, *corpus callosum* and cerebral cortex after *T. canis*-infection, contrary to cerebellar cortex and *arbor vitae* after *T. cati*-infection. Nevertheless, changes were more severe in cerebella than in cerebra in both infection groups. Further immunohistochemical analyses for e.g. characterization of axonal damage are currently conducted and will be presented. Overall, stronger affinity to the CNS beside diverse regulatory mechanisms may contribute to more pronounced pathology in *T. canis*-infected brains.

Keywords: Toxocara canis; Toxocara cati; neurotoxocarosis; neurodegeneration; histopathology

Role of the tick *Haemaphysalis longicornis* in epidemiology of toxoplasmosis

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Abstract Content

Toxoplasma gondii infection is mainly caused by ingestion of water or food that is contaminated with oocysts excreted by cats or by eating raw meat containing *T. gondii* tissue cysts. However, oral transmission does not explain the common occurrence of toxoplasmosis in a variety of hosts, such as herbivorous animals, birds and wild rodents. Maintenance of *T. gondii* in nature and routes of transmission to domestic and wild animals' hosts are poorly known. This study evaluated the role of ticks in the epidemiology of toxoplasmosis. In this study, real-time was used to detect the presence of *T. gondii* DNA in the field collected ticks. The dynamic amount changes of *T. gondii* in the tick body and its infectivity were studied by microinjection of green fluorescence parasites. Under laboratory conditions, infection and transmission of *T. gondii* in the tick *H. longicornis* were evaluated by the use of traditional parasitological methods coupled with molecular detection. Results showed that *T. gondii* DNA in the tick *Haemaphysalis longicornis* were detected at frequencies of 11.25% and 5.95% in adults and nymphs respectively. The *T. gondii* can survive and remain infective in the tick body for at least 15 days. Blood feeding of infected ticks did not transmit *T. gondii* to hosts; however, ingestion of infected ticks may be a route of *T. gondii* transmission between ticks and other common hosts. We conclude that *T. gondii* infection in ticks could serve as a reservoir for toxoplasmosis transmission.

Keywords: toxoplasmosis; tick; epidemiology

Level of *Toxocara* infection in domestic animals and *Toxocara malaysiensis* in cats in Vietnam

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Abstract Content

Toxocara canis of canids is a parasitic nematode that infects humans and other hosts, causing different forms of toxocarasis. Recently, *T. malaysiensis* is reported in Asia, no information is available about this parasite for Vietnam. This study was conducted to assess *Toxocara* infection from household cats and dogs, level of egg contamination from the local environment and *Toxocara* identification. In two Hanoi city districts, 284 dogs, 253 cats were fecal sampled, and 16 dog hair samples, 48 vegetable samples, 106 soil samples were also collected and examined using the McMaster technique. *Toxocara* adults were collected from the small intestines of 15 cats and 14 dogs, and identified using molecular tools. Prevalence of the infection was 47.8% in cats, and 37.7% in dogs. The eggs were recovered from 35.8% of soil samples, 25 % of garden vegetables, and 9 of the 16 dog hair samples. Risk of the infection was higher for dogs and cats in households with egg-contaminated soil compared to those without evidence of soil contamination. The high prevalence of dog and cat *Toxocara* infection and their indiscriminate defecation behavior contributes significant risk of transmission to humans. On other hand, *T. malaysiensis* was identified as a common ascaridoid of domestic cats (in the absence of *T. cati*), and *T. canis* was commonly found in dogs. These results emphasize the need to explore the zoonotic potential of *T. malaysiensis* in Vietnam and anthelmintic treatment of dogs and cats plus educating household members must be implemented in prevention program in Vietnam.

Keywords: Dog; cat; Toxocara infection, Toxocara malaysiensis; zoonotic potential

***Toxoplasma* GRA15II-polarized macrophages facilitates adverse pregnancy outcomes of mice**

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Abstract Content

Toxoplasma gondii (*T. gondii*, Tg) is an obligate intracellular parasitic which infects most warm-blooded mammals worldwide. Congenital toxoplasmosis is one of the major causes of adverse pregnancy outcomes. It has been known that the virulence of Tg strains is closely correlated to polymorphic effectors in different genotypes of the parasite. Previous studies indicated that GRA15II, one of the effectors secreted by dense granules of tachyzoites, may drive macrophages to M1 polarization via phosphorylating of NF- κ B p65, and induce high innate immunity against Tg infection. Here we explored the putative effect of ToxoGRA15II on abnormal pregnancy by subverting the physiological immune tolerance on maternal-fetus interface. Macrophages presented M1 features following transfection with *gra15II*, showing high production of nitric oxide and inflammatory factors (IL-6, IL-23, IL-1 β , IFN- γ , IL-17). Transfusion of the polarized M1 to pregnant mice lead to the increased absorptivity of fetus, stillbirth, and hemorrhage of placenta, with a high level of IL-17 and decreased number of CD4⁺CD25⁺FoxP3 (Tregs) in splenocytes. We conclude that Th1 predominant response to *Toxoplasma* infection may contribute to adverse pregnant outcomes through subverting the immune tolerance on maternal-fetus interface. This may account for the pregnancies with abnormal outcomes and positive antibodies but failure of parasite isolation.

Key words: Toxoplasma gondii, macrophage, GRA15, adverse pregnancy.

One Health application for prevention of zoonotic intestinal parasites infection in Tha Song Yang district, Thailand

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Abstract Content

Potentially zoonotic intestinal parasites are prevalent in tropical regions, especially among immigrant communities at country borders. This study aimed to determine prevalence and contributing risk factors for intestinal parasites in Tha Song Yang district at Thai-Myanmar border, Tak Province. We collected fecal samples from 513 villagers and 30 domestic animals from six sub-districts to determine prevalence and possible zoonotic transmission. Knowledge and attitude towards risk factors and prevention of these parasites were evaluated by questionnaires. In collaboration with Ministry of Interior (MOI) and Ministry of Public Health (MOPH), we facilitated multi-sectorial brainstorming among villagers, health volunteers, health care providers, and public health authorities, to explore perceptions on disease-associated risks and solutions. Microscopic examination of human fecal samples revealed *Endolimax nana* (17.5%) as the highest prevalence, followed by *Entamoeba coli* (11.75%), and *Blastocystis hominis* (11%). Helminthes species accounted for 10.25%. Interestingly, 90% domestic animal fecal samples showed infective forms of intestinal parasites similar to those of human. However, zoonotic correlation of these parasites was still inconclusive. Questionnaire analysis indicated 70% respondents had inadequate knowledge about disease-associated risk factors and prevention. Multi-sectorial brainstorming revealed water-related problems and improper animal waste management as most prevailing risks. Target-specific solution focused on necessity of assistance from local authorities to improve water quality and waste management. These suggestions, combined with technical findings, were constituted into Policy Brief and notified to MOI/MOPH for strategic planning to reduce disease burden. Further studies include species-specific PCR confirmation and subtyping of samples to assess zoonotic correlation of these intestinal parasites.

Keywords: intestinal parasites; One Health; zoonosis

Survey of brucellosis in cattle in Sub Districts (Akabiluru and Situjuah Limo Nagari) of Lima Puluh Kota District, West Sumatera Province, Indonesia, 2016

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Abstract Content

Brucellosis is a zoonotic disease that can cause economic losses and the impact on human health, especially in developing countries. West Sumatera Province since 2009 has been declared free of Brucellosis, surveillance which is part of the program of prevention and control of Brucellosis has been conducted regularly to maintain free status and protect livestock from Brucellosis. The purpose of this study was the identification of Brucellosis on cattle and description of the farm management in sub-districts (Akabiluru and Situjuah Limo Nagari). Total 100 head of cattle were selected using simple random sampling proportionally. Serum samples were tested for Brucellosis by Rose Bengal Test (RBT) and Complement Fixation Test (CFT). There was no cattle with positive Brucellosis. The results of the bivariate analysis between the characteristics of breeders with farm management at high risk of Brucellosis were females, age of breeders, education and typology. The characteristics of breeders were significantly associated with farm management at high risk of Brucellosis.

Keywords: Brucellosis, Zoonotic Disease, Surveillance,

Prevalence of *Trichuris trichiura* in African Green monkeys (AGM; *Chlorocebus sabaesus*) on St. Kitts, West Indies

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Abstract Content

On St. Kitts, West Indies, soil-transmitted parasites are a concern in the human population with studies suggesting a prevalence of >5%. One source of infection might be African green monkeys (AGM; *Chlorocebus sabaesus*). The population of AGM on St. Kitts is estimated to be similar to that of the human population (40,000) on the island. Due to hurricanes, which destroyed food sources in the mountain region, and agricultural practice changes, AGM have moved to semi-urban and urban areas of the island. Daily human/AGM interactions are common with AGM invading gardens and eating areas. Recent molecular work indicates that the *Trichuris trichiura* of the AGM is the same as that in people suggesting that AGM could be a source of infection for people. In this study, feces of 64 wild caught AGM (January to February 2015) were examined for *Trichuris trichiura* eggs using double centrifugation and Sheather's sugar flotation solution. *T. trichiura* eggs were recovered from the feces of 62 (96.9%) of the AGM. Egg counts ranged from 4 to >2000 with five AGM having counts <100 and 20 having counts ≥1000. Based on these results, AGM could be a major source of exposure for people on St. Kitts. All procedures in this study were performed under Ross University School of Veterinary Medicine and/or Behavioral Science Foundation IACUC approved protocols.

Keywords: Chlorocebus sabaesus; Trichuris trichiura; zoonosis

Should monkeys wash their hands and feet?

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Abstract Content

African green monkeys (AGM; *Chlorocebus sabaesus*), an introduced species to the island of St. Kitts, West Indies, are considered a pest; their population is estimated to be similar to that of the human population (40,000). AGM troops invade outdoor eating areas and destroy farmer's crops. However, they also are a tourist attraction. Tourists feed wild AGM in picnic areas and hold young tame AGM for photographs. AGM are known to have zoonotic parasites, but the risk of exposure is unknown. In this pilot study, the hands and feet of eight AGM (4 wild and 4 tame) as well as picnic benches frequented by AGM troops were examined for parasites. Crude egg/parasite recovery methods were used: hands and feet were gently washed with water (under an approved RUSVM IACUC protocol) and picnic benches were wiped with a damp (0.05% tween) sponge and then rinsed. Collected wash water was analyzed using double centrifugation and Sheather's sugar flotation solution. *Trichuris trichiura* eggs and *Strongyloides* sp. larvated eggs were recovered from the hands or feet of all four wild AGM. Parasites were recovered from two of the four tame AGM, one with *T. trichiura* and the other with hookworm eggs. *Trichuris* sp. larvated and non-larvated eggs and hookworm eggs and larvae were recovered from the picnic benches; however, PCR and sequencing have not been completed to confirm the species. Preliminary study results suggest that these interactions with AGM could be a source of human exposure to zoonotic parasites.

Keywords: Chlorocebus sabaesus; zoonosis

Molecular characterization of *Cryptosporidium* and *Giardia* in farmers and their ruminant livestock from the Coastal Savannah zone of Ghana

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Abstract Content

Cryptosporidium and *Giardia* causes diarrhoea in developing countries including Ghana, however, nothing is known about the species and subtypes of *Cryptosporidium* and *Giardia* in farmers and ruminant livestock in this country. A total of 925 faecal samples from humans (n=95), cattle (n=328), sheep (n=217) and goats (n=285), were screened for *Cryptosporidium* and *Giardia* by quantitative PCR (qPCR) at the 18S rRNA and glutamate dehydrogenase (*gdh*) loci respectively. *Cryptosporidium* positives were typed at the 18S and 60 kDa glycoprotein (*gp60*) loci. *Giardia* positives were typed at the triose phosphate isomerase (*tpi*), beta-giardin (*bg*) and *gdh* loci. The prevalence of *Cryptosporidium* and *Giardia* by qPCR was 8.4% and 10.5% in humans, 26.5% and 8.5% in cattle, 34.1% and 12.9% in sheep, and 33.3% and 12.3% in goat faecal samples, respectively. *Giardia* assemblages A and B were detected in humans and assemblage E was detected in livestock. *Cryptosporidium parvum* was the only species identified in humans; *C. andersoni*, *C. bovis*, *C. ryanae* and *C. ubiquitum* were identified in cattle; *C. xiaoi*, *C. ubiquitum* and *C. bovis* in sheep; and *C. xiaoi*, *C. baileyi* and *C. parvum* in goats. This is the first molecular study of *Cryptosporidium* and *Giardia* in livestock in Ghana. The identification of zoonotic species and *C. parvum* subtype IIcA5G3q in livestock, previously thought to be anthroponotically transmitted, suggests potential zoonotic transmission in Ghana. Further studies on larger numbers of human and younger animal samples are required to better understand the epidemiology and transmission of *Cryptosporidium* and *Giardia* in Ghana.

Keywords: *Cryptosporidium*; *Giardia*; Ghana; livestock; farmers.

Knowledge, attitudes and practices (KAP) of owners, veterinarians and policy makers in relation to animal schistosomiasis and potential zoonotic transmission in Senegal.

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Abstract Content

Background; Livestock schistosomiasis infections due to *Schistosoma bovis*, *S. curassoni*, *S. matthei* and/or hybrids therein, within cattle, sheep and goats are prevalent in sub-Saharan Africa. The pathology, diagnostics and impact of the disease in animals in real world settings has, however, had little attention. Aim; To ascertain the awareness and behaviours of farmers to animal schistosomiasis. Methods; Focus groups (n = 30) were carried out with Farmers in four areas of Senegal (Barkadji, Richard Toll, Dakar and Koungeul). In-depth interviews (n = 32) were conducted with veterinarians, human health workers and policy makers to ascertain knowledge, attitudes and practices in relation to zoonotic diseases. Interviews were recorded, transcribed; transcripts were anonymised and translated. NVivo qualitative data analysis software, QSR International Pty. Version 11, 2016, was used for thematic analysis according to the NIH framework. Provisional Results; The disease schistosomiasis was known by many farmers, and the livestock mortality rates described by farmers and veterinarians were high. Farmers and veterinarians familiar with the disease had good knowledge of the prognosis, pathogenesis and zoonotic component. Clinical signs included ocular musculature changes which have not previously been described in detail. Owners and veterinarians consider this diagnostic for clinical schistosomiasis and an indicator of high infection intensity and poor prognosis without treatment. The impact of the disease is variable between regions, due to access to water. Impact; Farmers in certain areas considered schistosomiasis to be in the top three severe livestock diseases suggesting schistosomiasis has a higher impact in animals than thought previously.

Keywords: KAP, schistosomiasis, livestock, zoonotic, hybrids

Detection of pathogenic *Leptospira* species in urine samples of bovines by Real-time Polymerase Chain Reaction (PCR)

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Abstract Content

Leptospirosis is a globally important emerging zoonotic disease caused by pathogenic spirochetes of the genus *Leptospira*. The major route of infection is by indirect environmental contact with leptospire in urine of reservoir hosts. Therefore, presence of reservoir animals is an important risk factor for human leptospirosis cases. Unfortunately, people living in risk areas have a poor level of knowledge on potential reservoir animals. Objective of the study was to detect pathogenic *Leptospira* species in urine samples of bovines by real-time Polymerase Chain Reaction (PCR). Mid-stream urine samples were randomly collected from 50 bovines in a high risk area for leptospirosis in the District of Gampaha, Sri Lanka. Urine samples were tested by real-time PCR after the DNA extraction. 50 samples, five samples (10%) were positive for pathogenic *Leptospira* by real-time PCR. The prevalence of leptospiral infection was 40%(2/5) and 7%(3/45) in female and male bovines respectively. Based on the bovine study, the real-time PCR data represent 10% positivity. Animals display intermittent shedding of Leptospire. Collection of several consecutive urine samples may present true carriage rate and it will exceed the average positivity (10%) found in the study. None of the bovines in this study had been vaccinated and it is important to vaccinate bovine with a multi-serovar vaccine as it would help to limit the disease burden among livestock and decrease environmental contamination. Results of PCR suggested that these (semi) domestic animals form an infection reservoir for *Leptospira*.

Keywords: *Leptospirosis, reservoir animals, real-time PCR*

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Abstract No: 5524

Caligid copepods infection in cultured fishes and trials for control using some medicinal plants.

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Abstract Content

Fishes are subjected to several types of parasites. Outbreaks among fishes in aquacultures are mainly due to ectoparasites infection particularly of crustacean type that lead to marked economic losses through reduced productivity as well as high mortality rates .On the other hand, marketing problems are also recorded due to the visible large sized species and/or the resulted damages and gross lesions. Recently, usage of herbal treatments were progressed for replacing the chemicals (such as formalin and other drugs) used for controlling parasitic infection in order to avoid their toxic side effects . For this aspect, the present investigation aimed to evaluate the in–vitro and in-vivo efficacy of some plant extracts including ginger (*Zingiber officinale*) and garlic (*Allium sativum*) as natural therapeutics for Caligid species infection among cultured fishes. The obtained results of histopathology and scanning electron microscopy (SEM) supported the detected anti-parasitic effects of the tested extracts on the target species as a promising natural herbal therapy . Studies are underway to conduct in vivo and in vitro experiments for evaluating the efficacy of the same herbal plants for controlling monogenean parasites and also metacercariae species infecting fishes as well as their experimentally obtained adult stages.

Keywords: Caligid, Infections, Fishes, Control, Medicinal plants

Development of Polymerase Chain Reaction for the detection of *Ichthyophthirius multifiliis* in Freshwater Fish

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Abstract Content

Ichthyophthiriasis in freshwater fish is caused by the ciliated protozoa parasite *Ichthyophthirius multifiliis* (*I. multifiliis*). Infected fish results in extensive skin and gill damage, leading to reduced production and significant mortality in commercial freshwater fish. Clinical diagnosis for detection of this parasite requires the presence of trophonts on fish skin epithelium by visual observations. Occasionally, the parasite only infects the gills and there are no obvious gross lesions and in such cases conventional methods are not accurate enough for identification. We present here a sensitive molecular-based diagnostic assay using polymerase chain reaction. The specific primer (ICM) was designed based on the sequence of the 18S ribosomal RNA gene of *I. multifiliis*. A touch-down polymerase chain reaction was developed to amplify a 233 bp PCR product. The result shown that the sensitivity of touch-down PCR assay was determined to be 10 pg of *I. multifiliis* DNA and able to detect 1 trophont of *I. multifiliis* mixed with 1 gram of fish mucous. Moreover, the assay is specific with *I. multifiliis* DNA and no amplification with other external protozoan pathogens commonly found in freshwater fish including, *Trichodina* sp. and *Chilodonella* sp. This study demonstrated that a touch-down PCR assay is sensitive and specific to *I. multifiliis* with has potential applications for clinical *I. multifiliis* diagnosis.

Keywords: *Ichthyophthirius multifiliis*; Polymerase chain reaction; Detection; 18S ribosomal RNA; Freshwater fish

Postulating the viability of parasitic *Sauricofyle sprostoni*, Unnithan, 1972 from *Saurida tumbil* as a potential bio-tagging probe

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Abstract Content

This study proposed the feasibility of monogenean parasite (*Sauricofyle sprostoni*) as a potential bio-tagging probe for *Saurida tumbil* population. *Saurida tumbil* were collected from seven stations at South China Sea, offshore Terengganu waters during the Scientific Expeditions Surveys of Demersal Fish Resources, EEZ, between May to June 2016. The prevalence and mean intensity of the infection rates of *S. sprostoni* were recorded and compared between sampling locations. Although 67 *S. sprostoni* individuals were found in only six stations, there was a significant difference in the infection rate (prevalence and mean intensity) amongst the stations. Additionally, *S. sprostoni* exhibited the following biological tagging criteria; single-host life cycle, easily detected and identified; and showed no pathological damages to the fish host. *S. sprostoni* is distinguished from other monogenean species based on four pairs of clamps with a long peduncle for each clamps. Henceforth, *S. sprostoni* is substantially an important indicator to separate *S. tumbil* population. In light of promising results, future research should explore aspects of other sampling locations and examining more *S. tumbil* specimens.

Keywords: Saurida tumbil; Sauricofyle sprostoni; bio-tagging; monogenean parasite; South China Sea

Molecular characterization of *Thelohanellus wallagoi* (Sarkar, 1985) (Myxosporea: Myxobolidae), parasitizing freshwater fish *Wallago attu* from Meerut District in India

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Abstract Content

The present survey of freshwater fish *Wallago attu* revealed severe infection of known myxosporean species, *Thelohanellus wallagoi* Sarkar, 1985 belonging to genus *Thelohellinus* Kudo, 1933 parasitizing gills, kidney and intestine from Meerut District. This species was described on the basis of morphology of plasmodia, histology findings on locations of plasmodia and DNA sequence data. Plasmodia of *T. wallagoi* are characterized in having spores measuring 9.2-4.8 µm, pyriform in valvular view and lenticular in sutural view having anterior end pointed and posterior end rounded with shell valves. Mature plasmodia are histozoic with single polar capsule measuring 5.4-2.7 µm, having polar filaments of 18 µm. Histology reveal no pathological changes but severe infections. SEM revealed flat surface. Supplemented 18S rRNA gene sequence of *T. wallagoi* did not show a close relationship with any other *Thelohanellus spp.*, represented in gene bank.

Keywords: Myxosporea; fishes; parasites; infection.

Poster Presentation – Asian Parasitic Issues

Abstract No: 4103 (Poster# S1 - 2)

Stray animals (dogs and cats) as sources of soil-transmitted parasite eggs/cysts in temple grounds of Bangkok metropolitan, Thailand.

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Abstract Content

Soil contaminated with helminth eggs, larvae and protozoan cysts is a potential source of infection and poses a threat to the public, especially to young children frequenting playgrounds. The present study determines the infection levels of soil-transmitted parasites eggs/cysts in soil samples from temple grounds in 50 districts in Bangkok metropolitan, Thailand. Nine hundred and sixty soil samples from 96 temple grounds (10 samples per temple) in 50 districts in Bangkok metropolitan, Thailand were screened and the egg/cyst counts per gram (EPG) for each parasite was measured using the double centrifugal flotation technique. Forty-two out of 50 districts (84.99%) were found to be contaminated with eggs from eight nematode genera, two nematode larvae and coccidian cysts. The highest count for parasite eggs found in this study was the one for *Toxocara* eggs (76.19%) followed by strongyle eggs (42.86%) and *Spirocerca* eggs (35.71%) respectively. The incidence of *Toxocara*, *Ancylostoma* and *Trichuris* eggs in soil samples highlights the risk of transmission to the human population, especially monks, nuns and children who live nearby the temple.

Keywords: soil-transmitted parasite; dogs; cats; zoonoses; temple ground; Bangkok; Thailand

Morphological and molecular analyses indicate the conspecific possibility of *Ancylostoma ceylanicum* and *Ancylostoma kushimaense*

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Abstract Content

Ancylostoma nematodes mainly parasitize the small intestines of human and carnivore. *Ancylostoma ceylanicum* parasitizes human as well as Carnidae and Felidae animals, and many human hookworm infections due to the species have been reported in Asian countries. Besides *A.kushimaense* is a hookworm of raccoon dog (*Nyctereutes procyonoides*) which is distributed in the Far East. The two *Ancylostoma* species resemble each other in morphological features, especially teeth in the buccal cavity, a key for species identification. In this study, we compared molecular property as well as morphology between the two species to clarify their classification. Adult males and females of the two species were used for morphological observation and morphometric measurements as well as molecular analyses on nuclear ribosomal ITS1 and ITS2, and mitochondrial *cox1*. No clear difference between the two species was observed in the bursal rays, spicule length and buccal teeth, although body width and a few other measurements were larger ($p < 0.01$) in *A. ceylanicum* than in *A.kushimaense*. The two species had completely identical ITS1 (363bp) and ITS2 (222bp) sequences and those sequences were also identical to ITS1 and ITS2 of *A. ceylanicum* (LC036567), respectively. The *cox1* sequences (367bp) were divided into 8 haplotypes with 97.0~100% homology in *A.kushimaense* and 5 haplotypes with 97.5~100% homology in *A. ceylanicum*. The *cox1* homology was 95.9~98.1% between *A. ceylanicum* and *A.kushimaense*, which was higher than that between the other *Ancylostoma* species. These results could not give any evidence on that *A. ceylanicum* and *A.kushimaense* are distinct species.

Keywords: *Ancylostoma ceylanicum*; *Ancylostoma kushimaense*; ITS1; ITS2; *cox1*

Characterization of *Echinostoma revolutum* and *Echinostoma robustum* obtained from domestic ducks in Bangladesh, on the basis of morphology, nuclear ribosomal ITS2 and mitochondrial *nad1* sequences

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Abstract Content

In the genus *Echinostoma*, a group of species containing 37 collar spines is called *Echinostoma* 'revolutum' group which has received much attention by the researchers. However, precise discrimination of species within the 'revolutum' group is quite difficult because of their morphological similarity. The objective of this study was to characterize the echinostomes of ducks from Bangladesh both by morphology and molecular property. We collected 31 adult echinostomes from intestine of 11 free ranging domestic ducks. After staining with hematoxylin and carmine solution, the flukes were studied under optical microscope for detail morphology and morphometric measurements, and were identified as *E. revolutum* (16) and *E. robustum* (15). The *E. revolutum* and *E. robustum* specimens had no intraspecific variation in the ITS2 sequences. In the *nad1*, *E. revolutum* and *E. robustum* had 9 and 11 haplotypes, respectively. In the ITS2 and *nad1* phylogenetic trees, the *E. revolutum* specimens consisted of a monophyletic group along with *E. revolutum* references, however, the *E. robustum* specimens consisted of a cluster with *E. miyagawai*, *E. friedi*, as well as *E. robustum*. The genetic divergence values suggested that *E. robustum*, *E. miyagawai* and *E. friedi* are conspecific, and therefore, these three *Echinostoma* species should be considered as synonymous. From the phylogenetic trees, Eurasian and American isolates of *E. revolutum* and *E. robustum* were divided into two distinct clades, suggesting that the two species have geographically separated lineages, Eurasian and American. This study may contribute to better understand the species diversity of the 'revolutum' group, especially in Asia.

Keywords: *Echinostoma revolutum*; *Echinostoma robustum*; ITS2; *nad1*; morphology

Intensity of soil-transmitted helminthiasis among the different Negrito sub-tribes in Malaysia

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Abstract Content

In Malaysia, STH infections are generally caused by *Trichuris trichiura*, *Ascaris lumbricoides* and hookworm. This cross sectional study aimed to determine the status of STH infections among Negritos, the smallest tribe encompassing only 3 % of the Orang Asli population in Malaysia. Briefly, stool samples and finger prick blood for Hb assessment were taken. We performed the Kato-Katz technique to determine the prevalence and intensity of STH. Blood samples were analyzed for anemia using a hemoglobinometer. A total of 383 Negrito participants were included with the overall prevalence of 86.2 % (95 % CI: 82.7 %, 89.6 %). Trichuriasis (71.5 %) was the most prevalent followed by ascariasis (45.4 %) and hookworm (20.9 %). Heavy intensity infections were found in 10.2 % of trichuriasis, 17.1 % of ascariasis and 1.2 % of hookworm infection. Most of the infected participants had moderate infections with 49.8 %, 50.9 % and 26.8 % in trichuriasis, ascariasis and hookworm infection, respectively. By sub-tribes, the highest worm burdens (heavy infection) were found in Jahai population with 29.8 % for trichuriasis, 26.3 % (ascariasis) and 14.3 % (hookworm). Heavy intensity infection was not found among the Lanoh sub-tribe. Anemia was detected in 69.2 % of respondents but only 2.3 % of them had severe anemia. This study suggests that the periodic treatment of an antihelminthic drug should be targeted among Negrito especially the Jahai community. Inclusion of appropriate health education in their native language will be beneficial to help reduce the potential of re-infection and control the transmission of helminthiasis.

Keywords: STH; Prevalence; Intensity; Negrito; Malaysia

Poster Presentation – Biotechnology & Genetics

Abstract No: 5473 (Poster# S1 – 4)

Cys-loop receptors domains are immunogenic and confer partial protection against *Rhipicephalus (Boophilus) microplus* tick infestations

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Abstract Content

Rhipicephalus (Boophilus) microplus ticks are obligatory hematophagous ectoparasites of cattle and act as vectors for disease-causing microorganisms. Conventional tick control is based on the use of chemical acaricides; however, their uncontrolled use has increased resistant tick populations, as well as food and environmental contamination. Alternative immunological tick control has shown to be partially effective. The only anti-tick vaccine commercially available in the world is based on intestinal Bm86 protein, but it shows a variable effectiveness depending on tick strains or geographic isolates. Therefore, there is a need to characterize new antigens in order to improve immunological protection. The aim of this work was to evaluate newly identified cys-loop receptors as vaccine candidates. N-terminal domain of a glycine-like receptor and of a glutamate receptor were recombinantly produced in *Escherichia coli*. Groups of three BALB/c mice were independently immunized with three doses of each recombinant protein (20 µg) emulsified with Freund's adjuvant. Both vaccine candidates were immunogenic in mice as demonstrated by western blot analysis. Next, recombinant proteins were independently formulated with the adjuvant Montanide ISA 50 V2 and evaluated in cattle infested with *R. microplus* tick larvae. Groups of three European crossbred calves were immunized with three doses of each adjuvanted protein (100 µg). ELISA test was used to evaluate the IgG immune response elicited against the recombinant proteins. Results showed that vaccine candidates generated a moderate humoral response on vaccinated cattle. Vaccination slightly affected the number of engorged adult female ticks; however it had no effect on oviposited egg masses.

Keywords: Rhipicephalus microplus; Tick; Cys-loop receptor; Vaccine

**Molecular characterization of a novel cys-loop receptor gene from the cattle tick
*Rhipicephalus (Boophilus) microplus***

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Abstract Content

The cattle tick *Rhipicephalus (Boophilus) microplus* is the most economically important ectoparasite affecting the cattle industry in tropical and subtropical areas around the world. The principal method of tick control has relied mainly on the use of chemical acaricides, including ivermectin; however, cattle tick populations resistant to ivermectin have recently been reported in Brazil, Mexico and Uruguay. Currently the molecular basis for ivermectin susceptibility and resistance are not well understood in *R. microplus*. This prompted us to search for potential molecular targets for ivermectin. Here, we report the molecular characterization of a novel cys-loop receptor gene. The characterized mRNA has an ORF 1401 bp that encodes for a 467 amino acid polypeptide, which contains features common to ligand-gated ion channels (LGICs), such as a large amino-terminal extracellular domain, four transmembrane domains, a large intracellular loop and a short carboxy-terminal extracellular domain, with a predicted molecular mass of 52.86 kDa and a pI of 8.32. The deduced amino acid sequence showed around 30% identity to other LGICs previously characterized in *R. microplus*. The polypeptide contains a signal peptide, a PAR motif, which is important for forming anion channels, as well as a conserved glycine residue at the third transmembrane domain, which is essential for high ivermectin sensitivity. PCR analyses showed that characterized receptor mRNA is expressed at egg, larval and adult (ovary and midgut) developmental stages. Our findings suggest that the deduced receptor is an additional molecular target to ivermectin and it might be involved in ivermectin resistance in *R. microplus*.

Keywords: Rhipicephalus microplus; Tick; Cys-loop receptor; Ivermectin

PacBio Circular Consensus Sequencing uncovers the haemoparasite microbiome in South African domestic and wild felids

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Abstract Content

Using universal primers that amplify near full-length 16S or 18S rRNA genes, samples were sequenced by circular consensus proofreading approach on a PacBio instrument, opening a window to the microbiome in domestic cats and wild felids (lion, cheetah, African wild cat, caracal, tiger). A library of barcoded 16S and 18S rDNA PCR amplicons was constructed, bound to P6 polymerase, loaded onto 3 SMRT cells, sequenced using C4 chemistry and observed with 6 h movies on a PacBio RSII. Barcode binning was performed using SMRT Portal V 2.3.0; separated reads were converted into fasta files for analysis using a 16S rDNA database and RDP classifier. A custom database was established to analyse the 18S rRNA sequence data. 21 data sets were obtained: 9 from samples amplified with 16S rDNA universal primer set; 12 from samples amplified with 18S rDNA primer set. Results revealed sequences with similarity to *Rickettsia*-like, *Anaplasma phagocytophilum*, *Babesia microti*, *Babesia odocoilei*-like, *Babesia rodhaini*-like, *Hepatozoon felis* and *Hepatozoon*-like. *Anaplasma phagocytophilum*, *B. microti* and *H. felis* were previously reported from felids. This is the first report on the blood microbiome, including both bacteria and protozoa, in felids in South Africa. These preliminary findings provide an idea as to which blood parasites can occur in felids in South Africa, and also that there are at least 4 (*Rickettsia*-like, *B. odocoilei*-like, *B. rodhaini*-like, and *Hepatozoon*-like) novel haemoparasites circulating in South African felids.

Keywords: Microbiome; South Africa; domestic cats; wild felids

Characterization and function analysis of a novel gene, Hc-maoc-1, in the parasitic nematode *Haemonchus contortus*

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Abstract Content

Enoyl-CoA hydratase (MAOC) is required for the biosynthesis of the fatty acid-derive side chains of the ascaroside via peroxisome β -oxidation in the free-living nematode *Caenorhabditis elegans*. The derivative of dideoxy-sugar, ascarylose is used as dauer pheromones or daumones to induce development of the stress-resistant dauer larvae stage. The full-length cDNA of *maoc-1* gene in *H. contortus* (*Hc-maoc-1*) was 900 bp, which contained eight exons separated by seven introns and possessed the Hotdog domain and the MaoC-like domain, together with several other residues and a hydratase 2 motif. It was transcribed throughout the lifecycle and peaked in the fourth-stage larvae (L4) of *H. contortus*. However, its transcription level decreased in diapausing L4. The protein expression and location of Hc-MAOC-1 were mainly in the intestine of L3 larvae. *C. elegans* was used as a model organism to ascertain the function of *Hc-maoc-1* in *H. contortus*. Overexpression of *Ce-maoc-1* and *Hc-maoc-1* in *C. elegans* showed extended lifespan and increased body size. The protein Ce-MAOC-1 and Hc-MAOC-1 were localized in the intestine with a punctate pattern. In *C. elegans*, knockdown of *Ce-maoc-1* conferred shortened lifespan and body lengths, decreased brood size and increased lipid storage. Our results showed the similar characteristics and functions with *Ce-maoc-1* and provided evidences of the potential functions of Hc-maoc-1 in biosynthesis of daumones in *H. contortus*.

Keywords: Haemonchus contortus; Hc-maoc-1; Caenorhabditis elegans; Ce-maoc-1; Diapause

Serotyping of *Toxoplasma gondii* in mice and determination of association between peptide sequences of different strains

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Abstract Content

The zoonotic intracellular parasite, *Toxoplasma gondii* can cause chronic infection in many species including humans. Approximately 90% of reported *Toxoplasma* isolates in the U.S. are one of four types (I, II, III, or XII) the other types are categorized as non-canonical or exotic. Currently there is not a non-invasive test that can distinguish each strain nor link disease states to a strain type. In this study, our goal was to develop a serological test (enzyme-linked immunosorbent assay) for typing of *Toxoplasma* strains. We used lineage-specific and polymorphic peptides derived from *Toxoplasma* dense granule (GRA3, GRA5, GRA6, GRA7 and GRA15), rhoptry (ROP8 and ROP20) and hypothetical proteins as antigens. Only the polymorphic peptides derived from dense granule (GRA3, GRA6 GRA7 and GRA15) antigens distinguished type II from non-type II infections. However, peptides derived from GRA7 (GRA7-III-224 and GRA7-III-226) had the ability to distinguish the type III from I and II. -Our results clearly indicate that the immunogenicity of a peptide is determined by peptide length. The comparison n of peptide sequences of multiple strains will aid the selection of peptides that may be helpful to discriminate type I, II, III and exotic strain. In the future, linking strain type to disease state would facilitate the development of a rapid, highly sensitive, and non-invasive method for diagnosis of *Toxoplasma* in humans and animals.

Keywords: *Toxoplasma*; Mice; Serotyping; Dense granule antigens; Peptide alignments

Availability of microsatellite markers for discriminating Japanese *Fasciola* clones

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Abstract Content

Elucidation of genetic structure within *Fasciola* populations is important for molecular epidemiology. *Fasciola* flukes occurring in Japan are usually aspermic and considered to produce clonally by parthenogenesis, and they are categorized into five clones based on the genetic types of nuclear ITS1 and mitochondrial *nad1* markers. However, these markers show very low genetic variations and therefore are considered unsuitable to detect additional clones probably existing in Japan and to understand detailed genetic structure of the fluke populations. Recently, microsatellite DNA markers, which show high mutation rates and polymorphic alleles, have been developed for elucidating genetic structure of *F. hepatica* populations. In this study, we compared Japanese *Fasciola* clones detected by the conventional ITS1 and *nad1* markers, and by the microsatellite DNA markers, and evaluated availability for understanding genetic structure of the fluke populations. Eight flukes from different locations were used for analyzing 12 microsatellite DNA markers as well as the conventional ITS1 and *nad1* markers reported previously. The microsatellite DNA markers were amplified by post labeling PCR and the genetic types were determined by fragments size analysis. Three clones were detected from the flukes by ITS1 and *nad1* markers. The polymorphic alleles were detected from 9 out of 12 microsatellite DNA markers and thus, 6 clones were found in the flukes. These results suggested that the microsatellite DNA markers can detect more clones of Japanese *Fasciola* flukes than the conventional markers and are available for elucidating genetic structure of Japanese *Fasciola* population.

Keywords: Japanese *Fasciola*; clones discrimination; microsatellite; ITS1; *nad1*

Implementation of loop-mediated isothermal amplification for rapid identification of *Anisakis simplex* (Nematoda, Anisakidae) from Baltic cod

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Abstract Content

Nematodes of the Anisakidae family are parasites of sea mammals and fish-eating birds and occur commonly in marine waters around the globe. Fish are their intermediate/paratenic hosts. Humans acquire infection after consumption of fish containing viable anisakid larvae. Anisakidosis cases have been noted in many countries all over the world, including Baltic region. The goal of our broad-range study is to develop fast, sensitive and easy to perform method based on LAMP technique, to identify anisakids: *Anisakis simplex* sensu stricto, *Contracaecum osculatum* s.s., *Pseudoterranova decipiens* s.s and *P. krabbei*. Herein we present the first results concerning *A. simplex*. The BeST LAMP Kit (A&A Biotechnology, Poland) was used as a basic component of the developed detection system of the parasite DNA. Different primer pairs, complementary to partial sequences of *A. simplex* mitochondrial *cox1* gene were designed for specific detection of this nematode DNA using isothermal temperature-time profile 65°C - 60 min. Obtained products were analyzed on 1.5% agarose gel. Designed primers were tested with different Baltic anisakids and gave specific product only with *A. simplex* DNA. No cross-reactivity with non-target nematodes was observed. Microscopic and molecular methods of anisakids identification available so far require high qualification of the staff, and/or expensive equipment. Presented technique, after developing systems for remaining Baltic anisakids, offers new perspectives and could be implemented to control safety of fishery products by fish processors and inspection service. This research was supported by The National Centre for Research and Development under the Strategic Program Biostrateg (grant no. 296211/4/NCBR/2016).

Development of high efficacy *in vitro* drug selection method for sequential genetic manipulation of *Plasmodium berghei*

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Abstract Content

The malaria parasite *Plasmodium berghei* is one of the main rodent malaria models. A shortcoming of this model parasite is its low flexibility in genetic manipulation. As this parasite cannot be continuously propagated in cell cultures, *in vivo* drug selection procedures are necessary to isolate genetic mutants. Drugs harmful to rodents therefore cannot be used for drug selection, which restricts the range of genetic manipulation. In this study, we addressed this problem by establishing a novel *in vitro* culture drug selection method, which we used in combination with other established methods to successfully isolate genetically manipulated parasites. The target mutants were enriched to the desired level within two weeks. We show that our system can also be used for sequential genetic manipulation of parasites carrying the traditionally used selection markers, demonstrate the procedure's versatility, and show its use in isolating specific genetically manipulated parasites. This novel *in vitro* selection method increases the number of available selection markers, allowing more extensive genetic manipulation in malaria parasite research.

Keywords: Plasmodium; genetic manipulation; drug selection

Metacaspases of *Toxoplasma gondii* is involved in IMC1 processing and daughter budding

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Abstract Content

Toxoplasma gondii is a major zoonoses infected almost all kinds of warm-blooded animal and human beings. *T. gondii* tachyzoites replicate by endodyogeny, and inner membrane complex (IMC) was indispensable for daughter cell development. The IMC of *T. gondii* is composed of many detergent-insoluble protein. Metacaspases, a kind of cysteine peptidase, have functional properties of apoptosis, cell-cycle regulation and protein degradation. A comprehensive search in *T. gondii* database (ToxoDB) revealed three genes encoding Metacaspase-like protein, which named TgMCA1, TgMCA2 and TgMCA3 respectively in our study. The transcription level of TgMCA3 was low in tachyzoites among the three, so we investigated the function of MCA1 and MCA2 by gene knockout strategy. MCA1 localized in the cytoplasm of the intracellular parasites and translocated into the nucleus in the extracellular parasites. We also found TgMCA1 co-localized with IMC1 during endodyogeny. Lack of MCA1 or MCA2 didn't affect the proliferation of the parasites. But the proliferation was reduced seriously when lack of MCA1 and MCA2 simultaneously. The defect endodyogeny was appear as the abnormal localization of IMC1 in double knockout strain. We also found IMC1 of double knockout strain was sensitive to detergent compared to parent strain through extracting total protein of the parasites with deoxycholate. We conclude that MCA1 and MCA2 play critical role in IMC1 processing and contribute to *T. gondii* endodyogeny.

This study was supported by National Natural Science Foundation of China (No.31672544, No.31372424)

Keywords: Toxoplasma gondii; Metacaspases; endodyogeny

Rhoptry protein 16 associated with pathogenicity of *Neospora caninum*

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Abstract Content

Neospora caninum is a common cause of abortions in cattle and nervous system dysfunctions in dogs. Bioinformatics analysis shows rhoptry protein 16 in *N. caninum* (NcROP16) and rhoptry protein 16 in *T. gondii* (TgROP16) have similar structures and may have similar functions. To our surprise, we found that similar to *T. gondii* RH strain, the *N. caninum* Nc-1 strain could phosphorylate host cell STAT3^{Y705}, but could not phosphorylate STAT6^{Y641}. We constructed NcROP16 gene deleted strains (Δ NcROP16), complement(Δ NcROP16) and overexpression strain. Plaque assays and intracellular proliferation tests indicated that the Δ NcROP16 phenotypes had changed, resulting in smaller plaques and slower intracellular growth. A virulence analysis showed that the cerebral loads of the parasite in mice infected with the Δ NcROP16 strain were significantly reduced compared to the loads in mice infected with the Nc-1 strain. In contrast, the overexpression led to the largest number of parasites observed in the mouse brains. Similarly, the overexpression of ROP16 caused the most powerful virulence in mice. In addition, NcROP16 has protein kinase activity and is involved in the host cell STAT3 signaling pathway. NcROP16 can induce the transcription of Fas and FasL, resulting in host cell apoptosis. The results show that NcROP16 is a key virulence factor in *N. caninum*, promotes the host cell apoptosis and enhances the pathogenicity of the parasites in the host.

This study was supported by the National Key Basic Research Program (973 program) of China (No. 2015CB150300), Beijing Municipal Natural Science Foundation (Grant No. 6131001, 6172023)

Keywords: *Neospora caninum*, *Rhoptry protein 16*, *Virulence factor*, *STAT3*

Molecular diagnostic for levamisole resistance in *Haemonchus contortus*

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Abstract Content

In tropical areas, *Haemonchus contortus* is the leading cause of production losses in small ruminant herds and its control is traditionally done through the utilization of synthetic anthelmintics. Levamisole, an imidazothiazole derivative, is widely used in Brazil and the occurrence of resistance is not uncommon. The genetic base for levamisole resistance in *H. contortus* is still under investigation, but it has been recently associated with a 63 bp deletion in the Hco-acr-8 gene, which codes for one of the subunits of a levamisole-sensitive acetylcholine receptor. Here we describe a real time PCR test for the detection and quantification of the presence and absence of this deletion. Reactions contained 12.5µl 2x Fast Start Universal SYBR Green Master Mix (Roche, West Sussex, UK), 0.3 pmol/µl of each primer (forward and reverse), 50 ng of DNA and water for a total volume of 25µl. Amplification conditions were: 95 °C for 10 min and 35 cycles at 95 °C for 15 s and at 56 °C for 30 s. We tested the assay in two known *H. contortus* isolates, one resistant (Kokstad isolate - KOK) and another susceptible (Inbred-susceptible-Edinburgh - ISE). We also used the test to characterize a local *H. contortus* population previously exposed to levamisole. Preliminary results are in agreement with previously reported data as only the resistant allele was detected in the KOK isolate and both alleles were detected in the ISE isolate suggesting that this test may be useful in the fast detection of levamisole resistance in *H. contortus*.

Keywords: Haemonchus, levamisole, resistance, diagnostic, molecular

Immunodiagnosis and molecular confirmation of *Toxoplasma gondii* infection among patients with end-stage renal disease undergoing hemodialysis

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Abstract Content

Infection is a significant cause of morbidity and mortality in patients with chronic kidney disease (CKD), especially who were under dialysis due to their depressed immunity. *T. gondii* is a ubiquitous parasite that causes severe manifestations in immunocompromised patients. This case-control study was conducted to the immunodiagnosis and molecular validation of *Toxoplasma gondii* infection among patients with end-stage renal disease undergoing hemodialysis. The study population consisted of 260 hemodialysis patients and 259 healthy controls referred to the main dialysis centers of Tehran, Iran during 2016. All participants were studied for anti-*T. gondii* IgG and IgM antibodies by ELISA. As well, the presence of *T. gondii* genomic DNA in whole blood samples of IgM-positive patients and healthy controls was evaluated using GRA6-PCR and SAG1-LAMP assays. The anti-*T. gondii* IgG and IgM antibodies were detected in 67.3% and 7% of hemodialysis patients and 47% and 1.5% of healthy controls respectively. Two of the 18 blood samples from IgM-positive patients and none of the IgM-positive control subjects were positive by GRA6-PCR. Whereas, nine and two blood samples of IgM-positive patients and healthy controls were positive for *Toxoplasma* DNA by SAG1-LAMP technique respectively. The seropositivity of *Toxoplasma* IgM antibody was significantly different between hemodialysis patients and healthy control group which was confirmed by PCR and LAMP assays. The higher prevalence of *T. gondii* infection in hemodialysis patients compared with the healthy controls, propose that these patients can be a group at risk for toxoplasmosis. As screening for toxoplasmosis before dialysis the patients is necessary.

Keywords: Immunodiagnosis; Molecular verification; *Toxoplasma gondii* infection; Hemodialysis patients; SAG1-LAMP

Molecular differentiation of *Fasciola* species and characterization of genetic diversity of *F. gigantica* Using ITS-2 Gene in Southeastern Iran

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Abstract Content

Fascioliasis is caused by two species of parasitic flat worms or trematodes that mainly affect the liver. It belongs to the group of foodborne trematode infections and is a zoonosis, meaning an animal infection that may be transmitted to humans. By using the morphometric method, differential diagnosis between *Fasciola* spp is problematic. The aim of the present study was to provide molecular evidence for the existence of *F. gigantica* in the Southeastern Iran using sequencing analysis PCR-RFLP analysis of ITS-2 regions. Nucleotide sequences of internal transcribed spacer 2 (ITS-2) of nuclear DNA were obtained from 50 adult worms *Fasciola hepatica* and *F. gigantica* of naturally infected cattle and sheep from two slaughterhouses at Zahedan. Molecular method, PCR-RFLP analysis of ITS2 using *pagI* restriction enzyme was used as a Approach for Differential Diagnosis for *F. gigantica*. All of the ITS-2 gene PCR amplicons of 456 bp from *F. gigantica* flukes were cut by the *pagI* endonuclease to fragments of 165 and 291bp. Based on RFLP analysis, 47 of the samples confirmed to be *F. gigantica* and 3 samples were confirmed *F. hepatica* to PCR amplification of ITS-2 gene (not cut by the *pagI* endonuclease). In the current study, sequencing results of the ITS-2 PCR products were used to characterize the genotypic diversity of the *F. gigantica* flukes obtained from endemic areas Southeastern Iran. The results of our study revealed impressive presence of *Fasciola gigantica*, relative to the *F. hepatica* in southeastern Iran.

Keywords: cattle, *Fasciola*, RFLP-PCR, sheep, Southeastern Iran, ITS-2

Immunodiagnosis of *Toxoplasma gondii*-recombinant dense granular (GRA) 5 protein for the detection of toxoplasmosis in hemodialysis patients

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Abstract Content

Serological assays for the diagnosis of toxoplasmosis mostly rely on the tachyzoite specific antigens of *Toxoplasma gondii*, which are difficult to produce by conventional methods. The aim of this study was to clone and express of GRA5 protein of *T. gondii* and evaluate its potential for immunodiagnosis of toxoplasmosis in hemodialysis patients. The GRA5 gene was successfully cloned, expressed and purified by affinity chromatography and the production was evaluated by SDS PAGE, and western blot analyses. The rGRA5 was used for developing an ELISA based on the rGRA5 using sera from patients with toxoplasmosis and healthy controls. Furthermore, 96 serum samples from hemodialysis patients infected with toxoplasmosis and 100 seronegative controls were included to evaluate the sensitivity and specificity of rGRA5 based ELISA. The consistency of the results of two tests was determined by using the Kappa coefficient of agreement. The rGRA5 showed higher and optimum immunoreactivity with 1:200 dilution of serum from *Toxoplasma* infected patients. The sensitivity and specificity of test were calculated as 90 and 92%, respectively. Findings of the present study showed that rGRA5 can be used as a potential immunogenic antigen for developing immunodiagnostic tools for immunodiagnosis of toxoplasmosis in patients including hemodialysis patients.

Keywords: *Toxoplasma gondii*, recombinant dense granular (GRA) 5 protein, hemodialysis patients

Molecular characterization of Echinococcus isolates of equine origin from Egypt

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Abstract Content

Cystic echinococcosis (CE) is a cyclozoonotic infection of a worldwide distribution affecting various species of intermediate hosts and humans. The disease still presents economic and public health problems with low to high degree of endemicity. Equine cystic echinococcosis can be caused by various *Echinococcus* taxa. The present work aimed to update the epidemiological and molecular knowledge about CE infection in donkeys for better understanding of their role in maintaining the *Echinococcus* life cycle. In the present study, 40 donkeys of different ages and sexes were subjected to postmortem examination for the presence of hydatid cysts after slaughtering at Giza zoo, Egypt. 10% of examined donkeys were harbored hydatid cysts in their livers only with fertility rate 100%. Female donkeys showed higher prevalence rate than males and the infection occurred in older donkeys. Using molecular tools, the DNA extracted from the isolated cysts was subjected to PCR amplification and sequencing using a pair of oligonucleotide primers which constructed to target 299 bp within NADH (Nicotinamide Adenine Dinucleotide) dehydrogenase subunit 2 (ND2) gene which considered being specific for *Echinococcus equinus* strain. The sequencing of 299 bp PCR products of ND2 gene showed homology to *E. equinus* (G4 genotype). Findings of this may have important implications for the design and development of vaccines, diagnostic reagents and drugs impacting on the epidemiology and control of echinococcosis.

Keywords: Hydatid cyst; donkeys; PCR; Echinococcus equinus; Egypt

Poster Presentation – Companion Animals

Abstract No: 3417 (Poster# S1 - 11)

Multilocus genotyping of *Giardia duodenalis* isolates from breeding cattery cats in Japan

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Abstract Content

The protozoan parasite *Giardia duodenalis* is a frequently detected intestinal pathogen in a wide range of mammals, including humans and companion animals, and can cause diarrhea. On the basis of molecular biological characteristics, *G. duodenalis* has been recognized as a species complex comprising 8 different genotypes, named assemblages A-H, which have different host ranges. Assemblage F is the predominant genotype in cats. In addition, assemblages A and B, which are well known as zoonotic genotypes, have been detected in cats. Recently, multilocus genotyping has been recommended to determine the assemblages of *G. duodenalis* in isolates. Breeding catteries are the major source of kittens for pet shops, and a high prevalence of *G. duodenalis* has been reported in breeding cattery cats. However, genetic characteristics of *G. duodenalis* isolates from these cats have not been well defined. The present study was performed multilocus genotyping of *G. duodenalis* isolates from breeding cattery cats in Japan. A total of 51 fecal samples from breeding cattery cats, which were positive for *Giardia*-specific antigen, were genotyped by multilocus sequence analysis of 4 loci (SSU rRNA, β -giardin, *gdh*, and *tpi* genes) PCRs. Forty-one samples were successfully sequenced for at least 1 loci. Single genotype isolates were demonstrated in assemblages F (n=28), A (n=5), and C (n=1). Mixed genotype isolates included assemblages F+A (n=4), F+C (n=2), and C+D (n=1). The results of the present study suggest that the risk of zoonotic transmission of *G. duodenalis* from breeding cattery cats to humans should not be ignored.

Occurrence and control of *Trichuris vulpis* and *Uncinaria stenocephala*: A case from a Danish breeding kennel

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Abstract Content

Gastrointestinal parasites may be common in Danish dog kennels with large numbers of animals with access to outdoor areas of soil/grass. However, little is known about how parasite transmission dynamics is affected over time in relation to anthelmintic treatment. In this case study, faecal egg counts (mainly *Trichuris vulpis* and *Uncinaria stenocephala*) of 39 dogs in a breeding kennel were monitored for 19 months (February 2015-August 2016) during which time dogs were treated twice (March and December 2015) with fenbendazole (3-5 consecutive days) and praziquantel (1 day). Soil from all kennel areas was examined for parasite eggs twice (February and June 2015). Treatments were fully effective against *T. vulpis*, but reinfection occurred, and December 2015 (pre-treatment) and August 2016 prevalence and infection levels were identical to those recorded at study start. This is likely to reflect that all outdoor areas were contaminated with fully embryonated *T. vulpis* eggs. In contrast, overall *U. stenocephala* occurrence seemed to be reduced with time though the majority of animals were still positive by August 2016. If the outdoor areas are continuously used, it is unlikely that *T. vulpis* can be eliminated in the kennel by anthelmintic treatment as eggs may survive for years in soil. Treatments may primarily serve to keep infections at a moderate level. However, a concerted effort of hygiene and strategic use and maintenance of outdoor areas combined with strategic treatment may have a greater impact on *U. stenocephala* as transmission relies on more short-lived free-living larvae and animal reservoirs.

Keywords: Trichuris vulpis; Uncinaria stenocephala; kennel; anthelmintic treatment

Distribution of vector-borne infections of dogs in the Moscow region.

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Abstract Content

The blood smears of dogs were examined in Moscow region under light microscope in 2011-2014. If *Babesia* merozoites were detected the samples were examined with IFA-tests SNAP 4Dx against *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Borrelia burgdorferi* s.l. and *Dirofilaria immitis*. The microfilaria were visualized in samples after concentration. Differential staining of acid phosphatases was used to distinguish *Dirofilaria* larva. Anamnesis of infected dogs was analyzed. The ticks were removed from dogs inside veterinary clinic or collected from vegetation in different ecosystems of Moscow region. In total 283 cases of babesiosis of dogs were reported, with 8,0% of cases reported for dogs never leaving city limits. Remaining 92 % of dogs were transported from time to time to countryside. In the majority of dogs the babesiosis is developing without severe consequences, and only in 2,5% of cases the acute renal failure was diagnosed. In 1,7% of dogs an autoimmune hemolytic anemia was observed. About 0,7% of studied dogs were seropositive for *Dirofilaria immitis* (all other pathogens were absent in these animals). In 1,7% of cases the microfilaria of *D. repens* were detected and in 0,7% of cases the microfilaria of *D. immitis* were found. During the survey 1045 ticks were collected (*Dermacentor reticulatus*, *Ixodes ricinus*, *I. persulcatus*). *Rhipicephalus sanguineus* was once found on the dog imported from Thailand. In the natural habitats of Moscow region 480 ticks were collected (*I. ricinus*, *I. persulcatus*, *D. reticulatus*). It is presumed that only *D. reticulatus* is a natural agent of babesiosis transmission in Moscow region.

Keywords: Vector-borne infections, dog, *Babesia*, *Dirofilaria*.

Multilocus genotyping of *Giardia duodenalis* isolates from house cats and pet shop kittens in Japan

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Abstract Content

Giardia duodenalis is a pathogenic protozoan that causes diarrhea in a wide range of mammalian hosts including humans and companion animals. This protozoan species has been divided into 8 genetic assemblages at least, and the host specificity differs depending on the assemblages. However, genetic characteristics of *G. duodenalis*-infected cats have not been completely defined in Japan. The purpose of the present study is to perform multilocus genotyping of *G. duodenalis* isolates from house cats and pet shop kittens in Japan and evaluate the zoonotic potential. Fifty-seven fecal samples from cats (house cats: 13, pet shop kittens: 44), which were positive for *Giardia*-specific antigen as determined by ELISA kit, were the subjects of our analysis. Nested or semi-nested PCRs targeting four loci (18S rRNA, GDH, β -giardin and TPI) were performed on all samples. All DNA amplicons were sequenced for genotyping. Forty-four isolates (house cats: 11, pet shop kittens: 33) were positive in at least one of the PCRs. Assemblages F and A were identified from 33 and 5 isolates, respectively. One of the isolates from house cats was identified as assemblage B. Five isolates had different results, depending on the targeting genes. These were either assemblage F or A. Assemblage F is considered common in cats but rare in humans, while assemblages A and B are well recognized as zoonotic groups. The present study suggested the potential of *G. duodenalis* zoonotic transmission from domestic cats to humans in Japan.

Keywords: Giardia duodenalis, cats, multilocus genotyping

Lungworms in Germany 2002 – 2016: Is there an increase in occurrence and geographical spread?

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Abstract Content

Angiostrongylus vasorum and *Crenosoma vulpis* are metastrongylid nematodes which are considered to be widespread meanwhile in Europe. To study the occurrence of lungworm infections in the dog population in Germany the results of coproscopical examination of fecal samples submitted for routine diagnostics to the Veterinary Laboratory Freiburg were analysed. In total 65,967 canine fecal samples from 2002 – 2016 were examined by flotation and Baermann funnel technique. Lungworm larvae were found in 883 (1.34 %) samples, whereof 606 (0.92 %) and 277 (0.42 %) were positive for *A. vasorum* and *C. vulpis*, respectively. The share of *A. vasorum* positive dogs increased from 0.09 % in the period 2002-2006 to 0.83 % in 2007-2011 and 1.29 % in 2012-2016. The share of *C. vulpis* positive dogs in the period 2002-2006 was 0.15 % in 2002-2006, 0.50 % in 2007-2011 and 0.48 % in 2012-2016. The rates of infection of *A. vasorum* were significantly higher in 2014 ($p < 0.05$) compared to 2002-2007, and in 2015 and 2016 ($p < 0.05$) compared to 2002-2010. For *C. vulpis*, significantly higher infection rates were found only in 2008. There were no significant differences in relation to age or sex. Seasonality of infections was observed for both species, as the infections in summer were significantly less prevalent (*A. vasorum*, $p = 0.0179$; *C. vulpis*, $p = 0.0095$) compared to winter. The data support the hypothesis that the prevalence of *A. vasorum* in the dog population in Germany has increased in the last years. By contrast this could not be confirmed for *C. vulpis*.

Keywords: *Angiostrongylus vasorum*, *Crenosoma vulpis*, dog, faecal examination, epidemiology

Evaluation of the efficacy and safety of an imidacloprid 10 %/moxidectin 1 % spot-on formulation (Advocate[®], Advantage[®] Multi) in cats naturally infected by *Capillaria aerophila*

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Abstract Content

Capillaria aerophila is a parasitic nematode affecting the respiratory tract of wildlife, dogs, cats and, occasionally, humans. Infected cats may be asymptomatic, or show various respiratory signs resembling to a chronic bronchitis. No products are licensed in Europe for the treatment of cat capillariosis to date. The aim of the present study was to provide further evidence on the efficacy and safety of a spot-on formulation containing moxidectin 1% (w/v) and imidacloprid 10% (w/v) (Advocate[®]/Advantage[®] Multi, Bayer) in the treatment of feline *C. aerophila* infection when administered once at the approved dose. Efficacy was tested on days 7 ± 1 and 11 ± 1 following treatment on day 0 and compared to pre-treatment faecal egg counts on days -6 ± 2 and -2 ± 2. Forty-one cats were enrolled in two groups: G1, treated with Advocate[®] (n = 20 cats) and G2, left untreated (n = 21 cats). Cats in G1 were negative for *C. aerophila* faecal egg output at the post-treatment evaluation (efficacy: 100%). Cats in G2 were persistently infected with a mean value of 195.2 EPG. Differences in mean EPG values were statistically significant (p<0.001). Nine out of eleven G1 cats that showed respiratory signs at enrolment fully recovered after the treatment. No adverse events occurred in treated cats. The present study confirmed that Advocate[®] is efficacious and safe in treating lung capillariosis in naturally infected cats.

Keywords: *Capillaria aerophila*; cat; moxidectin

Spreading of *Thelazia callipaeda* in south western France

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Abstract Content

The distribution of *Thelazia callipaeda*, commonly known as “oriental eyeworm”, has been considered for a long time to be confined to the former soviet Republics and Asia where the nematode causes infections in domestic and wild carnivores, rabbits and sometimes humans. However, in the past decade, thelaziosis has been diagnosed in dogs and sometimes in cats from a growing number of European countries including Italy, Switzerland, France, Spain, Portugal, and Greece. In 2006, a survey demonstrated that many autochthonous cases of canine thelaziosis were present in the department of Dordogne (south western France) in three hyperendemic counties where strawberry production was predominant. The objective of the present study was to obtain an updated evaluation of the endemic occurrence of *T. callipaeda* in south western France. In April 2016, an electronic questionnaire (via SurveyMonkey®) was sent to 1670 veterinary clinics from 24 French departments of Western France. Among 279 responses, 97 veterinary clinics reported cases of canine thelaziosis during the last twelve months. Most of them (72/97) reported a limited number of cases (1- 5). Two veterinary clinics in previously-identified hyperendemic counties of Dordogne reported the higher incidence (50 and 68 new cases). Noteworthy, Two clinics located in another department (Landes) also reported many autochthonous cases (40 cases) demonstrating the existence of new endemic foci. The present investigation confirmed that Dordogne is still an endemic area of ocular thelaziosis and that the disease is spreading in new areas of south western France since a decade

Keywords: Thelazia callipaeda, dog, France, spreading

Field efficacy and safety of NexGard[®] (afoxolaner) and NexGard[®] Spectra (afoxolaner + milbemycin oxime) chewable tablets against sarcoptic mange in dogs (canine scabies) in Europe

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Abstract Content

The safety and efficacy of NexGard[®] and NexGard[®] Spectra (Merial) were evaluated in sarcoptic mange infested dogs in a blinded GCP compliant field study. Skin scrapes (five per dog) from dogs presenting signs suspicious of sarcoptic mange were examined to confirm infestation and to establish *Sarcoptes* mite counts. Total 106 dogs were screened in eight clinical centers in Portugal and Germany, and 80 dogs, which fulfilled the inclusion criteria (*i.e.*, *Sarcoptes* mite count ≥ 5 , absence of *Demodex* mites in scrapes), were scored for the presence/extent of specific clinical signs (pruritus, papules and crusts, alopecia), and allocated at random to be treated twice four weeks apart with either NexGard[®] or NexGard[®] Spectra in a 1 to 1 ratio. Four and eight weeks after treatment initiation, *Sarcoptes* mites were counted in five skin scrapes per dog and clinical signs were scored. Thirty-eight NexGard[®]-treated dogs and 27 NexGard[®] Spectra-treated dogs completed the study. Compared to baseline, reduction of mean live *Sarcoptes* mite counts four and eight weeks after treatment initiation was 98.3% and 99.8% for NexGard[®] and 99.4% and 100% for NexGard[®] Spectra ($p < 0.001$), and treatment with NexGard[®] or NexGard[®] Spectra improved clinical signs in all dogs ($p < 0.001$). Overall 98.5% of the owners were very satisfied or satisfied with the performance of the treatments. No adverse reactions related to treatment were reported during the study. In conclusion, results of this field study confirm that two administrations one month apart of NexGard[®] or NexGard[®] Spectra provide an effective and safe treatment against sarcoptic mange in dogs.

Keywords: Afoxolaner; field study; dog; Sarcoptes scabiei

Global clinic-based serologic survey of *Giardia* infections in dogs and cats, 2011-2016

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Abstract Content

Giardia duodenalis is globally distributed and is a common cause of infectious diarrhea in cats and dogs. Various fecal flotation diagnostic methods are widely used but can be prone to false negatives. Therefore, infections are often diagnosed with commercially-available rapid tests that detect *Giardia* antigen in feces. The goal of this study was to determine the rate of positive *Giardia* antigen test results among pet cats and dogs in different regions of the world over a six-year period (2011-2016) by means of a rapid enzyme immunoassay (EIA). The data were obtained from an international database of SNAP[®] *Giardia* results from more than 45,000 cats and 300,000 dogs presumed to have diarrhea at the time of testing. Results were obtained from 37 countries grouped into six regions where results were available for both species. *Giardia* antigen positive rates among canine patients by region were: Asia-Pacific (n=4,112), 17.5%; Latin America (n=1,762), 16.2%; Middle East-North Africa (n=642), 24.3%; North America (n=261,513), 13.8%; Northern Europe (n=18,123), 19.3%; and Southern Europe (n=26,117), 30.2%. *Giardia* antigen positive rates among feline patients by region were: Asia-Pacific (n=1,336), 11.3%; Latin America (n=164), 12.2%; Middle East-Africa (n=239), 10.9%; North America (n=32,843), 9.4%; Northern Europe (n=4,689), 14.7%; and Southern Europe (n=5,868), 16.9%. When comparing results between species using the Chi-squared test, positive rates for felines were significantly lower than for canines in all regions ($P < 0.001$) except Latin America ($P=0.1800$). The results of this study confirm that *Giardia* infections are common in presumably diarrheal cats and dogs worldwide.

Keywords: *Giardia*; Canine; Feline; Parasite; Fecal

Evaluation of the efficacy and pharmacokinetic profile of Advantage Multi® (10% imidacloprid + 2.5% moxidectin) for dogs for the prevention of heartworm disease and infection all month long

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Abstract Content

Advantage Multi® for dogs is approved for use as a monthly topical solution for the prevention of heartworm disease caused by *D. immitis*. A laboratory study conducted in compliance with VICH GL 9, examined the efficacy of Advantage Multi® for the prevention of heartworm disease and infection all month long and included two groups of eight dogs each. All dogs entering the study completed a physical examination including examination for heartworm antigen and *D. immitis* microfilariae. Dogs in Group 1 were treated with Advantage Multi® on study day (SD) -30 as per the label recommendation while those in Group 2 were non-treated controls. On SD 0, dogs in Groups 1 and 2 were subcutaneously infected with approximately 50 infective third-stage *D. immitis* larvae. Results showed no heartworms in the treated dogs compared to six of eight non-treated dogs with heartworm (range of 2-33 worms/dog). The Advantage Multi® treated dogs had significantly fewer heartworms (p

Keywords: Efficacy; Advantage Multi® for dogs ; Prevention of Heartworm Disease and Infection

Thermoregulation controls the developmental host transition in filarial parasites

Dirofilaria immitis

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Abstract Content

The parasitic nematode employing obligate diapause arrests development at the particular stage in each generation. Recovery to the progressive stage from diapause is induced upon host infection by both intrinsic and extrinsic (environmental) elements of host. The dog heartworm, *Dirofilaria immitis* (*D. immitis*) a filarial nematode, develops through a series of four molts during transmission cycle between mammalian hosts and mosquito vectors. L1 and L2 larvae grow up in mosquito body before reaching infective larval stage (L3), and further developmental transition (re-initiation) occurs just following transmission to the host. We found that combination of proper temperature (37°C) and nutrition supply is indispensable to *D. immitis* L3 to resume development with acute expression of stress marker *hsp70*, which was rapidly decreased in *D. immitis*, whereas the sustained expression observed in *C. elegans*. Unordinary duplication of a part of kinase domain ensured the poor activation of stress kinase JNK in response to 37°C. *cuticlin-1* and *cathepsin-L*, identified as thermo-regulated genes, were required for adequate L3 molting. Taken together, our findings suggest that thermoregulation for adapting to environmental shift also exerts control over the developmental transition in filarial parasites *D. immitis*.

Keywords: D. immitis

Retrospective analysis of canine vector-borne diseases (CVBD) in Germany with emphasis on the endemicity and risk factors of leishmaniosis

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Abstract Content

A cross-sectional study was designed to provide data on the detection of canine vector-borne diseases (CVBDs) with special emphasis on leishmaniosis in Germany. Samples were examined for *Leishmania* spp., *Babesia* spp., *Ehrlichia canis*, *Dirofilaria immitis* and *Anaplasma phagocytophilum* during the years 2004-2006 and 2014-2016 (*Leishmania* only). Erythrocyte stages of large *Babesia* spp. or *Babesia* DNA were found in 1.7% of 9,966 blood smears and 3.3% of 15,555 samples examined by PCR, respectively. Antibodies titres (≥ 80) against *Babesia canis* were detected by IFAT in 11.5% of 2,653 serum samples. Out of 570 samples 3.2 % were positive for *Ehrlichia canis* using PCR. Antibodies against *E. canis* and *Anaplasma phagocytophilum* (both at titres ≥ 50) were detected by indirect IFAT in 15.1 % and 41.9% of 18,652 and 794 serum samples, respectively. Using Knott's test 4.5% of 440 blood samples were positive for microfilariae, and *Dirofilaria immitis* antigen was found by ELISA in 1.4 % of 9,381 serum samples. *Leishmania* spp. DNA was detected in 11 % of 301 whole blood or tissue samples examined by PCR. Antibodies against *Leishmania* were found in 23.5% (23,665 samples) and 22.7% (54,103 samples) of blood samples by IFAT (titres ≥ 50) and ELISA (≥ 7 test units), respectively (2004-2006 versus 2014-2016). Antibodies against *Leishmania* (IFAT) were detected in 80.6 % (399/495) of dogs imported from endemic areas, in 57.6 % (34/59) of German dogs travelling outside Germany and in 4 (n=8) German dogs without any history of travelling.

Keywords: Canine Vector-borne Diseases; Germany; Occurrence; Imported infection; Autochthonous infection

Evaluation of the clinical efficacy and safety of an imidacloprid 10 %/moxidectin 2.5 % spot-on formulation (Advocate®, Advantage® Multi) in comparison to an untreated control group in dogs naturally infected by *Capillaria boehmi*

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Abstract Content

The parasitic nematode *Capillaria boehmi* inhabits the nasal passages and sinuses of wild and domestic canids, causing either subclinical or clinically signs of varying severity i.e. sneezing, nasal discharge and impairment of scenting ability. The present study investigated the efficacy of imidacloprid 10% / moxidectin 2.5% spot-on (Advocate®/Advantage® Multi, Bayer) in dogs naturally infected by *C. boehmi*. Twenty dogs were randomly allocated in two groups: T1 (n. 10 dogs) treated with Advocate® at the recommended dose on day 0, and T2 (n. 10 dogs) left untreated. The reduction of the faecal egg counts (EPG) from baseline (days -6 ± 2 and -2 ± 2) to study completion was set as primary efficacy criterion. Clinical assessments and rhinoscopy to visualize the parasites *in situ* were used as secondary efficacy criteria. Out of the ten T1 dogs, eight did not shed eggs on day 28 ± 2 (reduction of EPG 99.66%) while two were still positive and received a second treatment on day 30 ± 3. A further efficacy evaluation was performed on day 42 ± 2 (study completion), when the two dogs scored negative. The mean EPG at study completion was 0 in T1 and 368.49 in T2, and the difference between the groups was statistically significant (p<0.01). The efficacy at study completion was 100%. T1 dogs showing clinical signs on day 0 fully recovered after treatment and no adverse events occurred. The results show that Advocate® is safe and effective in the treatment of nasal capillariosis of domestic dogs

Keywords: *Capillaria boehmi*; moxidectin; treatment; dog

***Angiostrongylus vasorum*, “the French heartworm”: A serological survey in dogs from France**

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Abstract Content

The nematode *Angiostrongylus vasorum* is living in the heart and pulmonary arteries of dogs and other canids. It was first described in dogs from south-western France in the nineteenth century, leading to the byname “the French heartworm”. The occurrence of *A. vasorum* has been increasingly reported from various European countries, but little is known about its current distribution in France. In this first large scale survey, 2289 sera from French dogs were collected for various reasons and tested using two distinct ELISAs for the detection of *A. vasorum* circulating antigen and of specific antibodies, respectively. In total 1.14% of the animals (n=26, 95% confidence intervals, CI: 0.74-1.66%) were positive by both ELISAs, while 0.61% of the tested dogs (n=14, CI: 0.33-1.02%) were antigen-positive and 2.01% (n=46, CI: 1.47-2.67%) were positive for specific antibodies. Regions where antigen- and antibody-positive animals came from were overlapping and distributed over the northern and southern parts of the country, while in central France relatively low numbers of positive dogs were detected. These results confirm the occurrence of *A. vasorum* in dogs originating from different parts of France. Early diagnosis of *A. vasorum* with appropriate tools and resulting knowledge of its presence is essential to ensure adequate anthelmintic treatment or prevention strategies, before the onset of fatal canine angiostrongylosis.

Keywords: Angiostrongylus vasorum, dog, Seroprevalence, France, ELISA

Seroepidemiologic data on *Leishmania infantum* infection of cats in southern Italy

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Abstract Content

Feline leishmaniosis (FeL) has been reported in several countries of the Mediterranean basin, with prevalence rate generally below that recorded in dogs. Though FeL is rarely diagnosed clinically, cats are exposed to sand fly bites in endemic areas since they often access outdoor also at night during the vectors season. Recently, a serological and molecular study conducted in an endemic area of southern Italy (Aeolian Islands, Sicily) showed an overall *L. infantum* prevalence of 25.8% and 41.8% in endemic populations of cats and dogs, respectively. To assess the prevalence of *L. infantum* in cats in Apulia region (southern Italy), 167 owned cats referred to veterinary clinics, were tested for antibodies against *L. infantum* by the indirect fluorescent antibody technique (IFAT) by using a cut-off $\geq 1:40$. The seroprevalence of *L. infantum* infection in cats was 16.7%, with 57.1% of positive animals presenting an antibody titre of 1:40, and the remaining from 1:80 to 1:320. The majority of cats positive for *L. infantum* were more than two years old (n=22/28, 78.6%), of which 12 (54.5%) showed IFAT titres of 1:40, followed by 10 cats (45.5%) with titres ranging from 1:80 to 1:160. The majority of cats with an antibody titre $\geq 1:80$ were older than 2 years of age, suggesting a multiple exposure to sand fly bites. Additional studies are needed to elucidate the epidemiological role of domestic cats as potential secondary reservoirs of *L. infantum*, to better design prevention and treatment strategies for controlling leishmaniosis.

Keywords: L. infantum; Feline leishmaniosis; seroprevalence

**Efficacy of afoxolaner plus milbemycin oxime chewable tablets against adult
Ancylostoma ceylanicum hookworm in dogs**

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Abstract Content

A fixed-combination chewable tablet incorporating afoxolaner plus milbemycin oxime (NexGard Spectra[®], Merial) was tested in induced infected purpose-bred Beagle dogs for efficacy against adult *Ancylostoma ceylanicum* hookworms. Sixteen dogs were inoculated each by oral administration of approximately 500 infective larvae of *A. ceylanicum*. Seventeen days after inoculation, the dogs were weighed and allocated randomly to be treated with afoxolaner plus milbemycin oxime chewable tablets or to remain untreated. Commercial chewable tablets of different strength were combined to deliver doses as close as possible to the minimum effective dose of 2.5 mg afoxolaner plus 0.5 mg milbemycin oxime per kg body weight. Parasites were recovered and counted for determination of efficacy seven days after treatment. All eight dogs that had been left untreated were harboring adult *A. ceylanicum* (geometric mean, 317.8; range, 210 to 428) while only one and nine *A. ceylanicum* were recovered from two of the eight dogs treated with afoxolaner plus milbemycin oxime chewable tablets (geometric mean, 0.5; p<0.0001). Thus, 99.9% efficacy against induced infection of *A. ceylanicum* was obtained by the use of oral NexGard Spectra[®] at the minimum effective dose. Treatment with NexGard Spectra[®] chewable tablets was well accepted and safe.

Keywords: Ancylostoma ceylanicum, milbemycin oxime, afoxolaner, dog

***Cercopithifilaria* spp. in canine populations from Greece**

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Abstract Content

Filarioids of the genus *Cercopithifilaria*, which are transmitted by hard ticks (Ixodidae), parasitize dogs. The adult nematodes usually dwell beneath the cutaneous tissues, and their microfilariae are found exclusively in the dermis. Up to now in Europe, three species of the genus *Cercopithifilaria* have been reported in dogs, i.e. *C. grassii*, *C. bainaе* and *Cercopithifilaria* sp. II. The aim of the present study was to investigate the presence of *Cercopithifilaria* spp. in canine populations from Greece. Skin snips were collected from 80 randomly selected owned or sheltered dogs of both sexes aged from 1 month to 12 years. The vast majority of them has not received recently antiparasitic drugs and had a history of tick infestation. Each skin snip was soaked in saline solution for 2h in an incubator at 37°C and thereafter the sediment were examined for the presence of microfilariae using a light microscope. Dermal microfilariae were detected in 12/80 (15%) skin snip samples. Based on the morphometrical features of 89 microfilariae measured, 82 were identified as *C. bainaе*, and the remaining as *Cercopithifilaria* sp. II. In details, the microfilariae of *C. bainaе* were detected in 12 dermal samples, while a mixed infection by *C. bainaе* and *Cercopithifilaria* sp. II was recorded in one dog only. The results of this study confirm the presence of *Cercopithifilaria* spp. in canine populations from Greece. Therefore, further studies are needed to evaluate the distribution and better understand the pathogenic role of these filarioids in dogs.

Keywords: Cercopithifilaria spp.; Cercopithifilaria bainaе; Cercopithifilaria sp. II; dogs; Greece

Laboratory evaluations of the immediate and sustained effectiveness of lotilaner (Credelio™) against three common species of ticks affecting dogs in Europe

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Abstract Content

Lotilaner is an isoxazoline with rapid onset of action, providing sustained efficacy against ticks; its efficacy against the three most common tick species in Europe was confirmed in two laboratory studies. In each study, 16 dogs were ranked and blocked by pre-treatment tick counts, and randomized to receive either lotilaner chewable tablets (Credelio™) or to be sham-treated. Study 1 assessed efficacy against concurrent infestations with *Rhipicephalus sanguineus* and *Ixodes ricinus*, study 2 against *Dermacentor reticulatus*. Dogs were treated on Day 0 and infested on Day -2, with counts on Day 2, and on Days 7, 14, 21, 28 and 35, with counts 48 hours post-infestations. Efficacy was determined by percent reduction in mean live tick counts. Infestations were adequate at all assessment times. On Day 2, no live ticks were found on any lotilaner-treated dog. Subsequently, in Study 1 lotilaner was 100% effective in eliminating live *I. ricinus* and *R. sanguineus* on all but two occasions, in which efficacy was greater than 98.0%. In Study 2, except for a single unattached live tick on Day 16, efficacy against *D. reticulatus* was 100% at all time-points. Results are consistent with other studies, where efficacy of lotilaner was demonstrated through at least one month for *D. variabilis*, *I. scapularis*, *R. sanguineus*, and *A. americanum*, demonstrating its potential as valuable tool in the treatment of tick infestations of dogs. Lotilaner flavored chewable tablets were well tolerated and effectiveness was sustained through at least 35 days.

Keywords: Lotilaner; Ticks; Efficacy, Dogs; Isoxazoline;

Identification of pathogenic bacteria *Wolbachia* from filarial worms in naturally infected cats and dogs in Thailand

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Abstract Content

Wolbachia is obligatory intracellular bacteria sheltered in many species of filarial nematodes and arthropods. These bacteria are responsible for manipulation of filarial development and fecundity. *Wolbachia* also play important roles in the pathogenesis of heartworm disease and lymphatic filariasis. The aim of this study was to identify the presence of *Wolbachia* DNA in microfilaria from cats and dogs which are naturally infected with filarial parasites in Thailand. Blood samples were collected from microfilaremic cats and dogs that positive for *Dirofilaria immitis*, *D. repens*, *Brugia pahangi*, or *B. malayi*., confirmed by Giemsa and Acid Phosphatase staining technique. To detect *Wolbachia* DNA, PCR using primers specific for *Wolbachia ftsZ* gene was performed. Amplification products were purified and DNA sequencing was performed to confirm that the amplified DNA represented the DNA from *Wolbachia* bacteria. Sequence analysis showed 99-100% sequence identity with *ftsZ* gene sequence of *Wolbachia pipentis* from *Dirofilaria* spp. and *Brugia* spp. available in Genbank. These results confirmed that *Wolbachia* infection is widespread among filarial nematodes. Phylogenetic analysis on *ftsZ* gene revealed that filarial wolbachiae in this study can be divided into two lineages based on the species of their filarial hosts. In addition, almost all species of filariae harboring *Wolbachia* spp. were sensitive to antibiotic treatments. Further study will target on the anti-wolbachiae antibiotic treatments to offer a novel alternative protocol against both adult worms and microfilariae in filariasis.

Keywords: Wolbachia, filaria, phylogeny

Detection of a new canine leishmaniasis focus in Northern Italy

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Abstract Content

Recently, canine leishmaniasis (CanL) caused by *Leishmania infantum* in the Mediterranean region is spreading outside the traditional endemic areas, possibly due to i) changes in the environmental conditions more favourable to the phlebotomine vectors (*Phlebotomus perniciosus*, *P. neglectus* and *P. perfiliewi*) and ii) increasing travelling of infected dogs from endemic to non-endemic areas (Maroli et al, 2008). In a previously considered Leishmania-free area surrounding the municipality of Costa Volpino, Bergamo Province, Northern Italy, owned dogs were investigated during the years 2009-2010 when referred to the local veterinary clinic for routine examination or clinical evaluation of symptoms compatible with CanL. Anamnesis regarding previous travels to known endemic areas was recorded for each dog. Serum samples were tested to detect antibodies against *L. infantum*. In total, five confirmed autochthonous clinical cases in resident dogs were identified, and five asymptomatic resident dogs were found serologically positive. An entomological survey was performed in July 2009 placing sticky traps in localities close to sites where resident dogs have been found infected. A total of 162 sand flies were collected, among which 15 (9.3%) were *P. perniciosus* and 12 (7.4%) were *P. neglectus*, both potential vectors of *L. infantum*. The remaining specimens were identified as *Sergentomyia minuta*. A new collection campaign is planned by summer 2017 to monitor the progress of the site colonization after a 8-years interval. On the basis of serological, clinical and anamnestic results on resident dogs, a new autochthonous CanL focus was thus identified in a previously non-endemic municipality of Northern Italy

Keywords: Leishmania infantum; dogs, autochthonous focus; Northern Italy

Serologic and molecular diagnostic survey of vector-borne disease-causing pathogens in hunting dogs from Southern Italy

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Abstract Content

Canine vector-borne diseases (CVBDs) result from a range of pathogens transmitted to dogs by arthropods. The present study aimed to examine populations of hunting dogs from southern Italy using both serological and molecular diagnostic methods for evidence of infection by known pathogens of CVBDs. Blood samples were collected from hunting dogs (n. 1,191) in the Avellino and Salerno provinces of Campania region of southern Italy. Serological testing for *Ehrlichia canis*, *Anaplasma* spp., and *Borrelia burgdorferi sensu lato*-specific antibodies and *Dirofilaria immitis*-specific antigen was performed using a commercial in-clinic enzyme-linked immunosorbent assay kit, SNAP[®] 4Dx[®] Plus. Molecular diagnostic testing for the blood-borne presence of *Ehrlichia canis*, *E. chaffeensis*, *Anaplasma platys*, *A. phagocytophilum*, *Babesia canis*, *B. vogeli*, *B. gibsoni*, *Dirofilaria immitis*, *D. repens*, *Hepatozoon canis*, and *Leishmania* spp. was performed by real time polymerase chain reaction (RT-PCR) assays. Seropositive rates for the four individual pathogens were: *E. canis* 7.7%; *Anaplasma* spp. 4.2%; *D. immitis*, 1.4%; and *B. burgdorferi sensu lato* 0.3%. PCR positive rates for eight detected pathogens were: *H. canis* 16.8%; *A. platys* 2.4%; *E. canis* 1.9%; *B. vogeli* 1.6%; *Leishmania* spp. 0.4%; *B. canis* 0.2%; *D. repens* 0.2%; and *D. immitis* 0.1%. Co-infections were detected at a rate of 2.3% by serology and 2.7% by RT-PCR with *Anaplasma/E. canis* and *E. canis/H. canis* co-infections occurring most frequently by serology and PCR, respectively. Results of the present survey indicate that hunting dog populations are at risk of CVBDs in southern Italy.

Keywords: CVBD; hunting dogs; Southern Italy; Ehrlichia; Co-infections

Prevalence of feline lungworm *Aelurostrongylus abstrusus* in England

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Abstract Content

The present study was conducted to address a significant aspect of the epidemiology of pulmonary disease caused by *Aelurostrongylus abstrusus* (*A. abstrusus*), a potential serious health problem in feline medicine. Despite the significant impact of infection with the lung worm *A. abstrusus* on the health and welfare of cats there is a lack of epidemiological studies that assess the prevalence and distribution of this parasite in cats in the UK. The aim of this study was to determine the prevalence of *A. abstrusus* in cats from England. From January 2016 – May 2017, faecal samples ($n = 629$) were collected from cats of various ages, breeds, genders, and geographic regions across England. Overall, 14 (2.2%) cats were positive for *A. abstrusus* based on Baermann's technique and microscopic examination. All infected cats had outdoor access or were stray and not dewormed, indicating that lifestyle and deworming are the two major factors that influence the frequency of infection with lungworm in cats. This finding demonstrates a role for *A. abstrusus* as a parasite of potential significance in cats, in particular those that are not dewormed. To reduce the spread of this parasite, an effective feline lungworm control program needs to be implemented. This program should not only include prevention strategies using anthelmintics, but also should strive to enhance diagnostic capabilities and awareness of veterinary professionals of this emerging parasite.

Keywords: Aelurostrongylus abstrusus, prevalence, cat, UK, lungworm

Genetic identification of ticks in domestic canines of Culiacán, Sinaloa, Mexico

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Abstract Content

The ticks are ectoparasites of the dogs and can act as reservoir or vector of zoonotic parasites and other pathogens potentially causal of diseases how Rocky Mountain Spotted Fever, Lyme disease, Typhus, Rickettsial, Tularemia, Babesia and Anaplasma; Generally these diseases are unique to different ticks which carry causal organisms of these such diseases and can be confined to certain regional áreas. the aim of this study was genetical characterization of ticks present in dogs attended at veterinary clinics in the city of Culiacan Sinaloa, Mexico. for it, were collected 314 live ticks present on 157 canines and processed in the Parasitology laboratory of the FMVZ-UAS, after the Morphological identification of Rhipicephalus, were formed 10 groups and the DNA extraction was performed by QIAamp DNA Mini Kit de QIAGEN® and processed by PCR using the oligonucleotide sequences: 16S+1: 5'-CCG GTC TGA ACT CAG ATC AAG T3' y 16S-1: 5'-GCT CAA TGA TTT TTT AAA TTG CTG T-3', of the 16S ARNr mitochondrial. The PCR amplification of target region of the the 16S ARNr, from DNA tick samples, resulted in 10 amplicons of size 460 bp genetically compatible with Rhipicephalus sanguineus of the GenBank sequences; this confirme genetically the presence of ticks Rhipicephalus sanguineus in domestic dogs attended at veterinary clinics in the city of Culiacan, Sinaloa, Mexico, and is important to assess the zoonotic potential represented by this ticks and its impact on human health and canine

Keywords: Ticks, dogs, Rhipicephalus sanguineus,

***Rickettsia rickettsii* in ticks of domestic dogs of Culiacan, Sinaloa, Mexico.**

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Abstract Content

Rickettsia rickettsii is a risk to public health worldwide by affecting broad sectors of the population, mainly because it is transmitted by dog ticks including *Rhipicephalus sanguineus* perfectly adapted to the domestic environment, existing evidence which is exposed to high temperatures tick attack and feeds on humans faster, implying a higher risk of human parasitism by *R. sanguineus* and in areas with hot summers, as Sinaloa state, Mexico, increasing the risk of transmission *R. rickettsii* with possible fatal outcomes; for this study were selected 314 ticks, obtained of 157 dogs attended at veterinary clinics in the city of Culiacan, Sinaloa, Mexico and processed in the Parasitology laboratory of the FMVZ-UAS, DNA extraction was performed by phenol-chloroform technique, generating 10 groups processed by PCR to identify genetically *Rickettsia*, using the oligonucleotide sequences: CS-78: 5'-GCA AGT ATC GAG GGT GAT GTA AT-3' and CS-323: 5'-GCT TCC TTC TTA AAA AAT AAA GGA TCA T-3' of the *gltA* gene, encoding citrate synthase enzyme, present in all rickettsiae. The PCR amplification of target region of the *gltA* gene, from DNA tick samples, resulted in 3 amplicons of size 401 bp and one sequences generated and analysed, was genetically compatible with *Rickettsia rickettsii* of the GenBank sequences; concluding that the presence of *Rickettsia rickettsii* in ticks *Rhipicephalus sanguineus* of domestic dogs attended at veterinary clinics in the city of Culiacan, Sinaloa, Mexico, is confirmed, is important to assess the zoonotic potential represented by this disease and its impact on human health and canine

Keywords: Rickettsia rickettsii, Rhipicephalus sanguineus, vector, dogs

Seroprevalence of canine vector-borne diseases in Greece

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Abstract Content

Canine vector-borne diseases (CVBDs) represent a diverse group of infections, which may affect the canine health and many of them have a zoonotic potential. Greece is a typical Mediterranean country offering a favorable environment for the development and spread of CVBDs. The primary objective of this study was to update the current knowledge on the distribution of *Dirofilaria immitis*, *Anaplasma platys*, *A. phagocytophilum*, *Ehrlichia canis* and *Borrelia burgdorferi* and expose the risk factors of these infections in dogs from all over Greece. In this study, blood samples were obtained from dogs (n=1000) collected over 12 months (January 2016 to December 2016) from all the municipalities of Greece. Dogs were apparently healthy at physical examination and older than 6 months. Serum samples were tested with an in-clinic kit (SNAP[®] 4Dx[®] Plus, IDEXX[®] Laboratories, USA). A total of 233 (23.3%) dogs were found positive to at least one of the tested pathogens. More precisely, 125 (12.5 %) dogs were found to be positive to *E. canis*, 90 (9%) to *D. immitis*, 62 (6.2%) to *Anaplasma spp.*, while only one dog was positive to *B. burgdorferi*. The findings of our study confirmed that canine population in Greece is potentially at risk of acquiring any of the CVBDs. Thus, the early diagnosis and the use of effective prevention or treatment methods are important to achieve successful control of the CVBDs.

Keywords: Canine; vector; borne; diseases; Greece

Significant Differences in Diagnostic Sensitivity between Two Rapid In-Clinic Tests for Antibodies to Tick-Borne Pathogens

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Abstract Content

Diagnosis of tick-borne diseases in dogs is generally supported by hematologic and serologic findings. Commonly used serological tests include immunofluorescence assay (IFA), and/or ELISA. A rapid in-clinic ELISA, SNAP[®] 4Dx[®] Plus (IDEXX), has been reported in published studies to have high sensitivity and specificity for antibodies to several tick-borne pathogens. The aim of this study was to evaluate the performance of a new rapid in-clinic test, Anigen CaniV-4 (BioNote), relative to SNAP 4Dx Plus. Both tests detect antibodies to *Ehrlichia canis*, *Anaplasma phagocytophilum*, *A. platys* and *Borrelia burgdorferi*. A total of 536 canine serum or plasma samples were collected from IDEXX Reference Laboratories (IRL) and individual clinics. The following diagnostic standards were used to characterize all samples – IFA for *E. canis* and *A. phagocytophilum*, species-specific ELISA for *A. platys* and Western blot for *B. burgdorferi*. Because CaniV-4 has a product claim for *E. canis* only (other *Ehrlichia* species excluded), *E. canis* samples were further confirmed by a species-specific ELISA. Compared to the respective diagnostic standards, the sensitivities of CaniV-4 and SNAP 4Dx Plus were 59.3% and 96.3% respectively for *E. canis*, 78.9% and 89.5% for *A. phagocytophilum*, 74.5% and 83.6% for *A. platys*, 33.3% and 90.0% for *B. burgdorferi*. Specificity for all analytes were similar for both test kits. This study revealed significant differences in sensitivity between the two in-clinic tests. The low sensitivity of CaniV-4 for *E. canis* and *B. burgdorferi* antibodies could have important implications for veterinary medicine and for public health.

Keywords: *E. canis*, *A. phagocytophilum*, *A. platys*, *B. burgdorferi*, SNAP 4Dx Plus, CaniV-4

Poster Presentation – Drug Residues in Food

Abstract No: 4970 (Poster# S1 - 23)

Impact of extra-label use of albendazole and fipronil in poultry

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Abstract Content

The increase of argentine poultry production in the last years is closely linked by the use of antiparasitic drugs in order to optimize this production, being the compounds available scarce. Albendazole (ABZ) and fipronil (FIP) are used extra-label for the control of nematode and external parasites, respectively. The goals of the study were: a) to evaluate the ABZ egg residues and its effect on the fertility and hatchability (Study-A); b) to investigate the FIP egg residues profiles after its extra-label administration to laying-hens (Study-B). Study-A: Forty eight (48) breeder hens were randomly divided into four groups and treated with ABZ at either 10, 40 or 80 mg/kg/day in feed over seven days, an untreated group was used as Control. Eggs were incubated under controlled conditions and fertility and hatchability were assessed. Study-B: Hens from a local farm were extralabel treated with FIP in feed. Eggs were collected for a 36 days post-treatment period. In Study-A, while fertility was not affected by ABZ, the hatchability values decreased inversely with the administered ABZ dose level. A statistically significant ($P < 0.05$) reduction on egg hatchability was observed with ABZ treatment at the highest doses (40 and 80 mg/kg/day). In Study-B, residue concentrations of fipronil-sulfone (active metabolite of FIP) were found in yolk egg at higher levels than the Maximum Residue Limits (MRL) allowed. Altogether, these data strongly suggest that extra-label use of ABZ and FIP would generate a high level risk on consumers as well as on poultry production.

Keywords: Albendazole; Fipronil; extra-label; egg residues

Determination of the milk withdrawal period following treatment with the isometamidium-based product Vivedium (Ceva Saúde Animal Ltda.)

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Abstract Content

Isometamidium is an aromatic amidine of phenanthridine with antitrypanosomal activity, being used for a long time in veterinary medicine for the control and treatment of infections caused by *Trypanosoma congolense*, *T. vivax*, *T. brucei* and *T. evansi*. It is known that in a One Health context, it is not only important to ensure that animals are disease-free, but it is also important to ensure that their productions are compliant with the residue limits. To address this concern, this study was conducted to determine the milk withdrawal period following intramuscular application of Vivedium, an isometamidium-based product. Twenty healthy cows received a single intramuscular injection at the highest dose rate of 1 mg/. All animals were milked with automatic machines and milk samples collected at different time-points post-treatment. The residues levels were determined by liquid chromatography with mass spectrometry detector. For the establishment of withdrawal period, the Maximum Residue Limits set by *Codex Alimentarius* were used (100 µg/L for milk). The first choice analytical methodology was linear regression, using the Time to Safe Concentration/TTSC method. All the concentrations were below the limit of quantification (50 µg/kg) from the first sampling time to the end of sampling, therefore it was not possible to calculate withdrawal period using the TTSC method. As the first sampling time (6 hours) was shorter than the standard milking frequency (every 12 hours), it was established that the milk withdrawal period following single administration of VIVEDIUM to dairy cow is zero.

Keywords: Isometamidium; withdrawal period; milk; trypanosoma; one health

Evaluation of Multi-class, Multi-residues screening and confirmation Method (MMM) for 77 veterinary drug residues in pork and chicken muscle using LC-MS/MS

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Abstract Content

Multi-class Multi-residue Method (MMM) is currently developed and used for efficiently regulatory control purpose rather than targeted group of residue analytical method. The sample extraction steps using solvent of acetonitrile:water (4:1) and dispersive SPE C18 for clean up and concentration of different polarities of residues are based on USDA FSIS CLG MRM 1.04. Extracted residues are identified and quantified using LC-MS/MS triple quadrupole under ESI condition. Evaluation of MMM for screening & confirmatory purposes for 77 veterinary drug residues from 11 classes i.e. 58 MRL compounds and 19 prohibited or no MRL compounds was performed regarding 2002/657/EC. Threshold (T) and Cut-off factor (Fm) parameters at screening target concentration levels were determined for screening purpose. Identification and confirmatory parameter i.e. Rt, Ion ratio of 2 product ions, IP and recovery were evaluated for confirmatory purpose. Twenty pork and twenty chicken muscle samples were used for method evaluation. The result of screening evaluation has shown that the method cut-off factors (Fm) of 53 compounds are successfully at MRL or below, but 6 compounds are at 2MRL and 19 of prohibited or no MRL compounds are at different spiking levels. The results of confirmation of 77 compounds have shown at screen target concentration with recoveries in the range 51 – 142 %, linearity (R²) value greater than 0.99. This MMM can be routinely and effectively implemented in residue monitoring plan for screening and confirmation residues in food animal muscle.

Keywords: Multi-class, Multi-residue Method; Veterinary Drug Residue; LC-MS/MS; Screening; Confirmation.

Comparative testing of multiple anthelmintic resistance for gastrointestinal nematodes using different methods in small ruminants of Punjab, India

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Abstract Content

The coincidence in prevalence of multiple anthelmintic resistance for gastrointestinal nematodes (GIN) in small ruminants by three average-based and two individually-based faecal egg count reduction (FECR) tests at five small ruminants farms was evaluated. Non-dewormed animals (≥ 8 week) were randomly assigned to three treatments and one control group ($n=10$). Group I received fenbendazole @ 5mg/kg. & 10 mg/kg., group II received ivermectin @ 0.2mg/kg. & @0.4mg/kg., group III received levamisole @ 8mg/kg. & 12 mg/kg.BW orally for sheep and goat, respectively. Group IV served as un-treated control. Presence of GIN resistant was determined ten days post treatment with three different average-based FECR (FECR₁, FECR₂ and FECR₃) and two individually-based FECR (iFECR₁ and iFECR₂) methods. For prevalence of fenbendazole resistance spearman correlation coefficient for FECR₁ was non-significant with other formulae but for FECR₂ with FECR₃, FECR₃ with iFECR₁ and iFECR₁with iFECR₂ coincidence was significant at 1% while for FECR₂ with iFECR₂ and FECR₃ with iFECR₂ was significant at 5%. For ivermectin resistance spearman correlation coefficients were significant at 1% level. In case of levamisole spearman correlation coefficients showed significant coincidence at 1% for FECR₁with FECR₂ & iFECR₁, FECR₂ with FECR₃ & iFECR₁ and iFECR₁ with iFECR₂ while for FECR₁ with FECR₃ & iFECR₂ coincidence was at 5%. The coincidence of prevalence of anthelmintic resistant (95% CI) among the five farms was non-significant as indicated by Kappa values. Coincidence between the standard average-based FECR and individually based methods suggest that either methods could be applied to small ruminants farmers.

Keywords: Anthelmintic resistance; FECR; Nematode; small ruminants; Punjab;

Poster Presentation – Drug Resistance & Therapeutics

Abstract No: 4512 (Poster# S1 - 26)

Susceptibility patterns of selected *Plasmodium falciparum* clonal parasites from Burkina Faso, Africa to aminoquinoline drugs

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Abstract Content

Amodiaquine is an antimalarial compound chemically and functionally related to chloroquine. Currently, it is used in combination with artesunate as the first choice to treat uncomplicated *P.falciparum* malaria in some countries in Africa and South America. Chloroquine resistance parasites and cross resistance between CQ and AQ or its active metabolite, desethyl-amodiaquine has been observed in Burkina Faso. Pfcrt and Pfmdr1 are genes, genetically link to the resistance mechanism and the underlying mechanisms for cross-resistance are still being discussed. The aim of this study is to assess the susceptibility patterns of clonal parasites *Plasmodium falciparum* compared to reference laboratory strains (DD2, GB4 and HB3). Blood samples of 402 patients from the village Bourasso, Nouna, Burkina Faso were analysed. Genomic DNA was extracted from filter papers using the Chelex-100 method and different *Plasmodium* species were analysed by microscopy and species-specific nested-PCR. The mutation in Pfcrt which was associated to CQ and AQ resistance, were analysed using pyrosequencing and in vitro susceptibility of clonal parasites to CQ, AQ and DeAQ was determined using IC50. Three different phenotypes of *Plasmodium falciparum* (S9, S47, S173) based on the IC50 values were cultured and clonal parasites were obtained. All clones from S9 and S47 harboured Pfcrt CVIET haplotypes while all clones from S173 were CVMNK haplotype. The clones from S9 showed higher IC50 values on average to CQ & AQ compared to clonal parasites of S47. Clonal parasites from S173 has low IC50 values towards CQ. Responses to CQ, AQ and DeAQ varied between the clones.

Keywords: Chloroquine, Amodiaquine, Clonal Parasites, Drug Resistance, Susceptibility

Further evidence of P-glycoprotein involvement in resistance to ivermectin in adult stages of *Haemonchus contortus*

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Abstract Content

The efflux transporter P-glycoprotein (P-gp) has been implicated in drug resistance of nematodes affecting livestock. Increased expression of P-gps in nematodes after *in vitro* as well as *in vivo* exposure to anthelmintics suggests a role of P-gps in the efflux of these drugs. The present work evaluated the expression of different P-gps genes in a laboratory selected ivermectin (IVM)-resistant isolate of *H. contortus* *in vivo* exposed to IVM. Six lambs were experimentally inoculated with a a laboratory selected IVM-resistant isolate (L₃) and adult parasites were collected at 0 (control), 6, 12 and 24 h post oral treatment with IVM (9 X therapeutic dose). The expression profiles of several P-gps was studied by qPCR using ACTB as a reference gene. P-gp1, P-gp3, P-gp9 and P-gp11 gene expression decreased along the different experimental times. P-gp2 increased its expression in a time-dependent manner (181, 200 and 248 % at 6, 12 and 24 h post-administration, respectively). P-gp12 was not detected under our experimental conditions. Overexpression of membrane drug transporters including P-gp have been associated with IVM resistance in different nematodes. However, the high variability observed in the expression profiles of the analyzed genes and the large genetic diversity within the resistant isolates hamper the comprehension of the resistance mechanisms against anthelmintic drugs. Currently, no consistent pattern has been observed between different nematode species in terms of up-regulation of ABC transporters such as P-gp after treatment with IVM. Further studies are needed to improve the understanding of resistance mechanisms in adult-stage of *H. contortus*.

Reduced albendazole efficacy against *Ascaris lumbricoides* in school-aged children from Huye district, Rwanda

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Abstract Content

Preventive chemotherapy of schoolchildren against soil-transmitted helminths (STHs) relies on two benzimidazoles (BZs), albendazole and mebendazole. Anthelmintic resistance (AR) is common in ruminant and equine nematodes but information on drug efficacy against human STH is limited and efficacy monitoring is only rarely implemented to assess mass drug administration programs. In 12 schools in the Huye district of Rwanda, efficacy of a single albendazole dose (400 mg) was evaluated. Faecal samples were analysed using wet mount microscopy and Mini-FLOTAC to determine cure rate (CR) and faecal egg count reduction (FECR). Furthermore, co-infections with *Plasmodium* sp. and *Giardia duodenalis* were determined. *Ascaris* was the predominant STH and DNA from *Ascaris* positive samples collected pre- and post-treatment was analysed for single nucleotide polymorphisms (SNPs) putatively associated with BZ-resistance in β -tubulin genes as known from other helminths. Overall CRs of 69.9% by Mini-FLOTAC and 88.6% by wet mount microscopy were observed. The FECR was 75.4% and 95% confidence intervals were 50.4-87.8% calculated using sample variance, 55.4-88.8% determined by bootstrapping and 75.0-75.7% applying a Markov Chain Monte Carlo Bayesian method. FECR varied widely between schools (range 0-96.8%). No potential BZ-resistance associated SNPs were found in any of the four *Ascaris* β -tubulin genes assessed. Since FECRs below 95% indicate reduced efficacy, these findings suggest presence of BZ-resistance in the local *Ascaris* populations. Impaired efficacy of albendazole is alarming and requires further studies to demonstrate e.g. heritable AR. Our findings call for routine monitoring in preventive chemotherapy programs, particularly if high coverage is achieved.

Keywords: soil-transmitted helminth; benzimidazole; anthelmintic resistance; *Ascaris*; tubulin

Efficacy of ivermectin against gastrointestinal nematodes in donkeys from Central Mexican plateau

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Abstract Content

In this study, 53 stool samples from oral ivermectin treated donkeys were analysed. Deworming treatment with ivermectin is one of the most popular services given by The Donkey Sanctuary-Universidad Nacional Autónoma de México (DS-UNAM) in the states of Querétaro and Hidalgo, located within the Mexican plateau. Fecal Egg Count Reduction Test (FECRT) was performed, as well as larval identification. Surveys were also carried out to the owners in order to reveal the anti-parasitic control applied to their animals. 80% of the owners revealed that they do not use any other anti-helmintic strategy apart from the one applied by the DS-UNAM. The rest of the owners are not aware of the molecule applied, its expiration date, or the correct dose that should be used. Results showed that in this specific population, oral ivermectin has an efficacy higher than 99% on day 14 post-treatment; therefore, we can consider these parasites susceptible to this molecule. The most frequent parasites found during the study were the ones englobed into the sub-family Cyathostomidae, also known as small strongyles. We concluded that the implementation of strategies such as Targeted Selective Treatment (TST) to the role of activities done by DS-UNAM will have great impact on these animals. Even though there is no resistance to oral ivermectin, the present study indicates that anti-helmintic resistance will be developed due to the continuous and uncontrolled use of the mentioned molecule.

Keywords: Donkey, ivermectin, resistance, nematodes, FECRT

Inadequate management of anthelmintic resistance: A real-world case in a cattle commercial farm

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Abstract Content

Routine Faecal Egg Count Reduction Tests (FECRT) in a cattle commercial farm shows the effect of an inadequate management in the introduction of gastrointestinal nematodes resistant to different macrocyclic lactones (ivermectin-IVM-, moxidectin-MXD-, eprinomectin-EPR-, abamectin-ABA- and doramectin-DRM-). The “case-study” farm had been a cow-calf system for many years in the past, with low (1-2) anthelmintic treatments each year. However, it changed its productive activity to grazing cattle fattening in 2015, based on the introduction of steers from different farms, which are treated at arrival with IVM to prevent sarcoptic mange. In 2015, the efficacy (FECRT) against *Haemonchus* spp. was 42% (IVM) and 97% (MXD), while the efficacy against *Cooperia* spp. was 78% (IVM) and 98% (MXD). The presence of resistance to IVM was evident, while MXD maintain high levels of efficacy. The next year, the efficacy against *Haemonchus* spp. dropped to 0% (IVMsc), 72% (MXD), 28% (EPR), 7% (ABA), 0% (DRM) and 5% (IVMoral), while the efficacy against *Cooperia* spp. was varied: 0% (IVM), 97% (MXD), 100% (EPR), 0% (ABA), 0% (DRM) and 0% (IVMoral). The efficacy against *Ostertagia* spp. was 100% for all treatments. The drastic decline in the efficacy of IVM and the very low efficacies obtained for all macrocyclic lactones treatments in 2016 (even for MXD and IVMoral) could be explained by some inadequate management measures: the selection of resistant population by the IVM treatment used as preventive of sarcoptic mange at the arrival of animals (although it was only one treatment), and the lack of quarantine procedures to avoid the introduction of parasite strains highly-resistant to macrocyclic lactones.

Keywords: Anthelmintic resistance; Macrocyclic lactones; Cattle; Management

A survey of *Rhipicephalus microplus* populations for mutations associated with fipronil resistance: Preliminary data

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Abstract Content

Fipronil exert action by blocking GABA-gated chloride channels (GABA-Cl) present in the nervous system of arthropods and mutations in its gene have been associated with fipronil resistance in different pest species (*Rdl* mutation). Mutations associated with fipronil were found in populations of *Rhipicephalus microplus* (Canestrini) from Uruguay and Brazil. *Rdl* mutation was reported recently in populations resistant to fipronil. As source of genomic material it was used ticks (adult and larvae) from a fipronil-susceptible populations and from fipronil resistant strains from Uruguay and Brazil (n=12). Resistance to fipronil in these strains was confirmed with toxicological bioassays. DNA fragments of the GABA-Cl gene spanning the *Rdl* mutation were obtained by PCR and directly sequenced. Five aminoacid substitutions were present in the resistant populations. Two mutations may be linked to resistance. In ticks from Uruguay the A286L mutation was not detected. However, in populations from Brazil, A286S and A286L mutations were found. The association of these mutations with resistance to fipronil was confirmed by genotyping a larger number of individuals over tick samples with different levels of resistance to this acaricide. Other resistance mechanisms have are present.

Keywords: Rhipicephalus microplus resistance fipronil rdl

A novel multiple nucleic acid hybridization technique for visualization of drug target expression in nematode tissues

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Abstract Content

In order to elucidate the mechanisms of drug action and resistance, it is imperative to understand the location of receptor expression in parasites. There is a need for highly sensitive and specific techniques to visualize the localization of putative receptors while minimizing background signal. We present the optimization and utility of a novel multiple nucleic acid *in situ* hybridization technique that allows chromogenic detection of individual mRNA transcripts in formalin-fixed nematode tissues. Following probe binding to target sequences, a subsequent signal amplification step results in the rendering of single mRNA molecules into punctate signal dots that can be observed using a bright field microscope. The high sensitivity of the assay allows for the detection of single RNA molecules, and special probe design confers a high level of specificity. We demonstrate the usefulness of the technique in enhancing our understanding of a mechanism of ML resistance.

Keywords: Localization; drug receptors

Case study: Broad spectrum anthelmintic resistance on a sheep farm in northern NSW, Australia.

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Abstract Content

In June 2016, a Faecal egg count reduction test (FECRT) was conducted on a sheep farm in northern NSW, Australia. Sheep were displaying clinical signs of gastrointestinal infection and not responding to treatment. As a level of anthelmintic resistance was suspected a number of classes of anthelmintics were assessed including; organophosphate, macrocyclic lactone (ML) and in combination an ML, benzimidazole, levamisole and salicylanilide. In addition, the more recently registered classes of anthelmintics, monepantel (amino-acetonitrile derivative) and derquantel/abamectin combination (spiroindole + ML) were included. Ninety merino sheep naturally infected with a field strain of *Haemonchus* spp. were randomly allocated based on Day -7 faecal egg counts to 6 treatment groups (15 animals/group). Sheep were subsequently treated based on label recommendations and individual bodyweight. Faecal samples were collected 7, 14 and 21 days' post-treatment to conduct faecal egg counts and group bulk larval cultures. The FECRT confirmed broad spectrum anthelmintic resistance at this test site with treatment efficacies ranging from 21.3% (monepantel) to 93.8% (derquantel/abamectin combination) against the *H. contortus* strain. Furthermore, resistance to the multi-combination anthelmintic containing 4 active ingredients was evident (52.5%). This broad spectrum of resistance emphasises the importance of integrating alternative sustainable methods in parasite control in order to slow development of resistance and increase the life time effectiveness of anthelmintics.

Keywords: Haemonchus contortus.; Broad spectrum anthelmintic resistance; Faecal egg count reduction test; Australia

Development of a sensitive indirect competitive enzyme-linked immunosorbent assay (icELISA) to determine spinosad

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Abstract Content

Spinosad, a mixture of spinosyn A and D, is a naturally fermentation-derived insecticide, and acts through interaction with the nicotinic acetylcholine receptor (nAChR), which possesses different mode of action of most commercial insecticides. Spinosad is not stable compared with other macrocyclic lactones. To overcome this disadvantage, we need a new formulation that can protect spinosad from fast degradation. A fast, easy and sensitive spinosad assay method will accelerate a new formulation study, and can also be used to monitor spinosad overuse or abuse.. In this study, an indirect competitive enzyme-linked immunosorbent assay (icELISA) was developed with a specific monoclonal antibody against spinosyn A. Briefly, succinoyl-C-17 of spinosyn A was synthesized and coupled with KLH as immunogen and OVA as coating antigen. After immunized female BALB/c mice with the conjugates, hybridoma were selected to produce antibody (mAb 3E6). The half maximal inhibitory concentration is 4.11 ng/ml, and detection range is 3.7-27 ng/ml with R^2 of 0.998. The mAb showed no cross reactivity with other insecticides. Analysis of spinosyn A-fortified water by the icELISA showed average recoveries from 80% to 105%, and gave the correlation coefficient of 0.990-0.991 with a slope of 1.20-1.42 confirmed by high-performance liquid chromatography. The icELISA turned out to be a sensitive and convenient tool for monitoring spinosad residues in water. In addition, it could be used in pharmacokinetics studies to quickly obtain results compared with HPLC when developing different formulations of spinosad.

Keywords: spinosad; icELISA; monoclonal antibody

Therapeutic and persistent efficacy of single dose spinosad o/w microemulsion injection against natural infestations of lice in goats

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Abstract Content

Spinosad is a safe pesticide, it has significant effect on human lice. FDA approved Natroba (0.9% spinosad topical suspension) for the treatment of *Pediculosis capitis*. However, spinosad has low aqueous solubility, which seriously restricts the development and its use in animal parasites control. The present work aimed to use microemulsification technique to prepare a spinosad o/w microemulsion injection to improve its solubility, extend the duration and increase the bioavailability. And efficacy test was carried out to verify the effect against goat biting lice. In this study, the effects of different doses of spinosad o/w microemulsion injection (10 mg/kg, 5 mg/kg, 2.5 mg/kg, 1.25 mg/kg body weight) versus traditional ivermectin injection (0.2 mg/kg body weight) in the treatment of goat lice were compared. The experiment was conducted in two farms. Forty-eight naturally lice infested goats were selected in the experiment in each farm based on pre-treatment lice counts. At day 30 post-treatment, cure rates of the spinosad o/w microemulsion injection treatment against goat lice at 10 mg/kg, 5 mg/kg, 2.5 mg/kg, 1.25mg/kg body weight reached 8/8 (100%), 7/8(87.5%), 8/8(100%), 7/8(87.5%). The spinosad treatment also reduced wool damage in infested goats and helped goats gain weight. For the safety observation, spinosad at 20 mg/kg body weigh (subcutaneous injection) did not show any adverse effects. Results of this study demonstrated that the off-label, experimentaly injectable formulation of spinosad is convenient to use, highly effective and safe for the treatment of the goat biting lice.

Keywords: Spinosad; Microemulsion; injection; lice; goat

Functional investigation of nematode parasite specific acetylcholine receptors

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Abstract Content

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

Keywords: AChR, parasite, *acr-26*, *acr-27*, morantel

Poster Presentation – Equine

Abstract No: 4533 (Poster# S2 - 98)

Prevalence and risk factors for gastrointestinal nematode in stabled horses in Selangor and Putrajaya

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Abstract Content

The objectives of this study were to determine the prevalence and risk factors for gastrointestinal nematode (GIN) infection in stabled horses in Selangor and Putrajaya. Fecal samples from 100 horses in ten different locations were collected. Modified McMaster was performed to detect GIN eggs. Questionnaires focusing on the general information about the management practices on stables were completed. Positive samples were subjected to Baermann technique for faecal culture to identify the genus of nematodes. The overall prevalence of GIN infection in the ten locations as estimated using the Modified McMaster was 38%. The prevalence varied between locations and ranged from 9% to 100%. 100% prevalence was recorded in three locations. Strongyle group was the most common intestinal parasite found in this study. Risk factors for GIN infection were age, deworming interval and anthelmintic used. Adult horse group aged 16-20 years had higher infection rates compared with other groups. Horses which anthelmintic drugs were administered irregularly were more likely to become infected with GIN. Horses in treated with Oxfendazole were likely to become infected than horses that treated with other anthelmintics. GIN genus identified include *Trichonema* (53%), *Ascaris* (5%), *Trichostrongylus* (21%), *Strongyloides* (12%), *Strongylus* (2) and *Poteriostomum* (2%). This information will initially be of great significance to add the existing knowledge of the epidemiology of GIN under local management and climatic condition.

Keywords: Anthelmintic; faecal culture; gastrointestinal nematode; helminth genus; McMaster

Equine cyathostomins – modelling biology and drug resistance

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Abstract Content

A model has been constructed which describes the biology and development of anthelmintic resistance in the equine cyathostomins. Because of the complexity of the cyathostomin species mix and the general paucity of knowledge on their biology the model deals with the complex as a single unit rather than individual species. Biological assumptions were based on a literature search on parasite dynamics of both external and internal stages of cyathostomin parasites. By using temperature and rainfall data to estimate the development of eggs to infective larvae and their subsequent survival and migration on pasture the model can be tailored to any site for which weather data is available. The model was calibrated using historic data and fine-tuned using weather station data from different climatic regions from which parasitology data were also available. This is important because anthelmintic treatment regimes which are suitable for one environment may be completely inappropriate for another. The parasitic phase of the cyathostomin life cycle was modelled using data from several recent and historic necropsy studies with generation of full worm counts of both intestinal and mucosal stages. When adult worms are removed by anthelmintic treatment they are rapidly replaced by the maturation of L3 / L4 stages in the lumen or mucosa, depending on the differential efficacy of different anthelmintics against each of these stages. This presentation will outline the structure of the model, discussing the benefits of using drugs in combination and the advantages of leaving a proportion of horses untreated based on model output.

Keywords: Equine cyathostomins; Population dynamics; Model; Anthelmintic resistance

Equine *Parascaris* spp. – modelling biology and drug resistance

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Abstract Content

A model has been developed for the biology of *Parascaris* spp. in the young horse. The model incorporates four main variables; the rate at which larvae migrate through host tissues to return to the small intestine, the proportion of migrating larvae which succeed in returning to the small intestine, the rate of growth in size of maturing and adult worms and the survival rate of maturing and adult worms. The most influential variable in determining model output is the survival rate of worms in the small intestine, which in the model, declines in response to the increasing age of the horse and the increasing cumulative length of worms in the intestine as a proxy for crowding. Given the importance of this variable to model behaviour and the paucity of experimental data this is identified as a priority for future study. The model was calibrated using necropsy data generated from several published studies. Incorporating genetics for anthelmintic resistance allows for a comparison of the long-term effect of different treatment strategies on the development of resistance. Simulated treatments increase the development of resistance when given to foals under 6 months of age. A comparison of proposed treatments at 2 and 5 months of age to commonly used monthly treatments indicated reduced selection for resistance, especially when using a non-larvicidal anthelmintic for the first treatment. The unselected migrating larvae will mature and become fecund before the second treatment is given, thus leaving some parasites in refugia while maintaining sufficient control of parasitic stages.

Keywords: Horse; Parascaris; Model; Anthelmintic resistance

Seroprevalence of *Toxoplasma gondii* and *Trichinella spiralis* in horses in Xinjiang, Northwestern China

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Abstract Content

Toxoplasma gondii and *Trichinella spiralis* are widely distributed in humans and animals throughout the world, but the data on prevalence of horses in China are limited. In this study, 409 equine serum samples from Xinjiang Uygur Autonomous Region (XUAR), northwestern China, were tested by enzyme linked immunosorbent assay (ELISA) to investigate the antibodies against *T. gondii* and *T. spiralis* in horses of this region. Results showed that 22 samples (5.38%) were positive for *T. gondii* and 14 (3.42%) for *T. spiralis*. Two samples (0.49%) were both positive with *T. gondii* and *T. spiralis*. It was found that the prevalence of *T. gondii* in older horses (>15 years) was significantly higher than that in younger animals. There was no significant difference between the prevalence and the genders of the animals. To verification the results of seroprevalence for *T. gondii*, 10 samples of uterus secrets from abortion mares with positive *T. gondii* antibody, were tested by PCR assay. The internal transcribed spacer – 1 (ITS-1) gene specific for *T. gondii* was amplified with a ratio of 100% (10/10), which was consistent with the result of seroprevalence. The results of present survey indicated that infection with *T. gondii* and *T. spiralis* in horses should not be ignored in Xinjiang, northwestern China. Consumption of horse meat in this region may represent a potential source for human infection with *T. gondii* and *T. spiralis*.

Keywords: Horse; Toxoplasma gondii; Trichinella spiralis; seroprevalence; China

Cryptic species in *Cylicostephanus calicatus*: Application of molecular and proteomic methods for study of cyathostomins

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Abstract Content

Cylicostephanus calicatus is one of the 50 species of cyathostomins which parasitize in the caecum and colon of equines. The morphological description of this parasite is based on only three individuals and might be questionable. MALDI-TOF MS of adult female and male worms from German and Ukrainian origin was carried out in a broad range of 2000-20000 m/z, followed by PCR of the internal-transcribed-spacer-2 (ITS-2) and cytochrome oxidase 1 (COI) of the same individual worms. Different nucleotide sequences were observed for different individual worms all morphologically identified as *C. calicatus*. Ignoring minor variations, both COI and ITS-2 sequences of different supposedly *C. calicatus* specimens grouped into two clearly distinct clusters, and clusters were identical for the mitochondrial and nuclear loci. In particular, nucleotide identity in the COI gene between the two genotypes was only 91%. The ITS-2 regions predominantly differed by a deletion of 90 bp in one of the genotypes while the remaining sequence regions were highly similar. Moreover, MALDI-TOF MS spectra for female and male individuals from the different clusters also showed clearly distinctive patterns. The proteomic method was therefore able to identify the genotype and the sex of the individual worms correctly. The results indicate that *C. calicatus* is in fact a complex including at least two cryptic genetically different species. To prove the hypothesis of a cryptic species, a re-evaluation of morphological traits of these species should be performed and, if necessary, *C. calicatus* must be re-described in parallel to the description of its sibling species.

Keywords: *Cyathostomins*; MALDI-TOF MS; *C. calicatus*; cryptic species

***Parascaris equorum/univalens*: Immunogenicity assessment of soluble antigens using Counter Current Immunoelectrophoresis**

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Abstract Content

The equine pathogenic intestinal parasites, *Parascaris equorum* and *Parascaris univalens* live in the small intestine of horse, donkey, mule and zebra. These parasites usually cause acute infections which lead to leanness and death in acute stages of the disease. Due to the disease difficult to treat, and has high economic losses, the prevention strategies including vaccine development plans seems reasonable. This study was aimed to the immunogenicity assessment of *P. equorum/univalens* soluble antigens using Counter Current Immunoelectrophoresis (CCIEP) method. The adult worms were collected from infected foals and after preparation of the antigen solution (AgS) and examination of enzymes and amino acids compositions, the intraperitoneal injection of AgS was performed to three groups of rabbits. Subsequently, the immunogenicity assessment of AgS was done using antigens detection and CCIEP assays on rabbit's sera samples. CPK, LDH, and G6PD enzymes were detected in AgS using UV, RA-100 instrument, and immunofluorescence assay. Alanine and Lysine were the most amino acids which were detected in AgS by HPLC. The highest antibody levels in the sera sample of rabbits were detected in the group A which received two injections with 3 days interval weekly. Following by intraperitoneal injection of AgS to the rabbits, the antibody against the AgS has created and the antibody titer will growth by increasing the AgS injections. Also, it can be concluded that CCIEP is a quite rapid, sensitive and suitable assay for the immunogenicity assessment of *P. equorum/univalens* AgS as well as vaccine development plans in the future.

Keywords: *Parascaris equorum/univalens*, Immunogenicity assessment, Soluble antigens, Counter Current Immunoelectrophoresis, Vaccine development

Poster Presentation – Equine Parasites

Abstract No: 5610 (Poster# S2 - 33)

Prevalence of *Anoplocephala perfoliata* in Swedish horses – an abattoir study

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Abstract Content

The prevalence of the equine tapeworm *Anoplocephala perfoliata* was 63 % in a study conducted at necropsy at the Linköping abattoir in Sweden in 1992. Since then the number of horses has increased considerably. Also new anthelmintics such as praziquantel has been launched and their use has been intensified in Sweden. The present study included 201 horses (mainly trotters) from two different abattoirs in south-central Sweden. At slaughter the number of tapeworms in the intestines was observed macroscopically along with tissue lesions in the lower part of the ileum, the ileocaecal ostium, caecum and the beginning of the ascending colon. Tapeworms observed were collected, counted and measured to the nearest cm. Furthermore, faeces were collected from the rectum and analysed for tapeworm eggs using both McMaster counting and a qualitative flotation method based on 30 g of faeces and a flotation fluid with a density of 1.29 g/cm³. The prevalence of *A. perfoliata* in the intestines at necropsy was 76%, which was significantly higher both than following detection of tapeworm eggs in faeces with the McMaster method (6%) and with qualitative flotation (28%). Statistical analysis of the data is still pending with more results to come. However, it seems like the prevalence of *A. perfoliata* has increased in Swedish trotters since 1992. Furthermore, as has been observed before *A. perfoliata* infected horses are easily missed based only on faecal diagnosis irrespective of the faecal detection method used.

Keywords: Anoplocephala perfoliata; praziquantel ; prevalence; necropsy

Poster Presentation – Food & Waterborne Parasites

Abstract No: 4511 (Poster# S1 - 33)

Surprise egg: *Prosthogonimus cuneatus*, a trematode

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Abstract Content

In December 2016, a backyard hobby chicken raiser with six during the summer free-ranging hens in southwestern Finland, noticed a flat translucent object, a trematode about 1 cm long inside an egg white. Morphologically the flatworm was identified as *Prosthogonimus cuneatus*. The species is known from a wide variety of bird species. It is also reported as a potentially serious pathogen to domestic chickens, but the majority of recent reports in chicken are from Tropical areas. In Europe and North America, chicken production is mostly industrial, and the parasite's rather fantastic life cycle cannot challenge poultry industrial biosecurity and its disease barriers. The adult *P. cuneatus* reside in the oviduct or bursa Fabricii region of the cloaca. They produce eggs, which develop further if being eaten by aquatic snails, which later excrete swimming *P. cuneatus* cercariae in their faeces. These look for dragonfly larvae or nymphs, also called naiads, which they enter via the anus, encyst in the muscle and form metacercariae, which then mature and are infectious. Birds get infected by ingesting an infected naiad or adult dragonfly. While current commercial poultry industry effectively excludes *P. cuneatus* from chicken, it and other *Prosthogonimus* species are common in wild bird species also in Europe. Increasing interest in animal welfare may also increase backyard and outdoor chicken raising, thus permitting the life cycle. *Prosthogonimus* species are not the only parasites accidentally seen in free-ranging chicken eggs. Understanding these parasites' life cycles is important in ecological egg production.

Poster Presentation – Herbal Remedies & Antiparasitics

Abstract No: 4146 (Poster# S1 - 34)

***Nosema ceranae* control under field conditions.**

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Abstract Content

The ability to reduce infection in *Nosema ceranae*-infected colonies under field conditions was evaluated after administration of tannic acid (Sigma-Aldrich®), toltrazuril (Cevazuril®) and resveratrol (MegaResveratrol®) in spring and autumn. These substances were previously evaluated in toxicity tests under laboratory conditions to verify they were harmless to honey bees. Sixty-two (n=62) colonies located at the IRIAF-CIAPA (Spain) were included in the study. Fumagillin was used as positive control and untreated colonies served as negative control. Parasite loads (calculated as percentage of infected honey bees) and colony strength parameters (total number of bees, total amount of brood, consumption, and honey production) were monitored in all colonies during one year (September to August). *N. ceranae* was present in all colonies throughout the study. The highest load reduction was obtained in both treatments with Fumagillin. Even though the reduction with the other products was lower, colony strength was not affected, meaning the administration of tannic acid, toltrazuril or resveratrol (either by syrup or candy) could be enough to reduce the percentage of infected bees and serve as control for nosemosis C. Our results confirm that a single application of any of these substances during autumn would decrease the infection loads during winter and together with the increasing numbers of newborn uninfected bees in spring, this unique treatment will avoid reaching the threshold to cause death to the colony. The application and monitoring of treatments for longer periods in professional apiaries in southern regions will confirm these results.

Keywords: Nosema ceranae, honeybee, control, nosemosis

Effects of curcumin on *Besnoitia besnoiti* tachyzoite replication, viability and motility

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Abstract Content

Besnoitia besnoiti infections are considered as an emerging disease in Europe, which affects fertility and productivity of cattle. Besnoitiosis may induce severe clinical signs (generalized dermatitis, orchitis and vulvitis) resulting in reproduction disorders and economic losses. The polyphenol curcumin, which represents the main component of *Curcuma longa* rhizome, is well-known for its antioxidant, anti-inflammatory and immunomodulatory properties. Since antiprotozoal effects of curcumin were previously reported for *Eimeria* spp., we here studied its effects on *B. besnoiti* tachyzoites viability, motility, infectivity and replication in endothelium-based *in vitro* cultures. Therefore, different doses of curcumin (1, 2, 4 and 8 µM) were tested. Overall, curcumin beared direct parasitocidal effects since 56% of *B. besnoiti* tachyzoites died after treatment (LD₅₀ of 5.93 µM). Furthermore, we observed a significant reduction of tachyzoite motility at 4 and 8 µM curcumin treatments. In addition, curcumin pre-treatments of tachyzoites led to a significant reduction (e.g. 3.08±2.19%, 2 µM) of infection rates in BUVEC when measured at 24 hours p. i. Consequently, curcumin treatments significantly blocked parasite proliferation at 48 h p. i. In all assays, dose-dependent effects were observed. With the current data we demonstrated that curcumin affects *B. besnoiti* viability, motility and *in vitro* replication. Therefore, feeding of cattle with *Curcuma longa* rhizomes may represent a new strategy for besnoitiosis treatment. However, further studies on curcuma *in vivo* toxicity, anti-proliferative effects and tissue distribution have to be undertaken to confirm the beneficial effects of this compound in the *in vivo* situation.

Keywords: *Besnoitia besnoiti*; curcumin; *Curcuma longa*; antiprotozoal; viability

Excretion profile of two injectable formulations of ivermectin in cattle

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Abstract Content

In Latin America, despite the environmental risk of avermectins, more concentrated formulations have been marketed without environmental regulations by government authorities. In this study, eight non-lactating cows were assigned into two groups. Group one (Ivermectin-1%) was treated with Ivomec® (0.2 mg/kg), and Group two (Ivermectin-3.15%) was treated with Ivomec Gold® (0.63 mg/kg). All cows grazed in paddocks of *Cynodon nlemfuensis* and were given 1.5 kg of commercial concentrate daily. Faecal samples were collected at: -1 (control dung), 3, 6, 14, 28, and 35 days post-treatment (dPT). Analysis of residual ivermectin in faecal samples was performed by HPLC, with fluorescence detection. Ivermectin was undetected in all faecal samples in control dung. The mean ivermectin concentrations (ng/g dry weight ± S.E.) of Group one at 3, 6, 14, 28 dPT were 896.8 (138.7), 569.8 (38.8), 214.8 (73.8), 54.4 (29.9), respectively. Meanwhile, ivermectin concentrations of Group two at 3, 6, 14, 28, 35 dPT were, 774.2 (73.1), 1064.9 (58.9), 790.7 (83.2), 463.1 (46.2), 279.8 (50.37) ng/g d.w., respectively. The maximal ivermectin excretion after treatment with Ivermectin-3.15% barely exceeded that of Ivermectin-1%. However, concentrations of ivermectin remained above 400 ng/g (d.w.) over four weeks after dosing with ivermectin-3.15%. This level of excretion has been proven lethal for sepsid flies and immature stages of Aphodius beetles. In Latin America, the widespread use of long-acting formulations of ivermectin causes concern since higher amounts of this drug will be voided in the environment, maintaining its insecticidal activity against non-target organisms that provide important ecological functions.

Keywords: antiparasitic drug, ivermectin, environmental risk, excretion

**Adverse effects of injectable formulations of ivermectin on the dung beetle
Onthophagus landolti (Coleoptera: Scarabaeidae)**

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Abstract Content

Two bioassays were carried out to assess the adverse effects of cattle treatment with either ivermectin-1% (0.2 mg/kg) or ivermectin-3.15% (0.63 mg/kg) on the larval survival and reproduction of *Onthophagus landolti*. Beetles were exposed for a 10-day period to feces of treated cattle starting at -1 (controls), 3, 6, 14, 28 and 35 days post-treatment (dPT). Adult survival of *O. landolti* was not affected by either of the two treatments. Also, we evaluated the effect of ivermectin residues on the attractiveness of cattle dung to *O. landolti* beetles by an olfactometer. Residual ivermectin after treatment with the ivermectin-1% almost completely suppressed fecundity of females at 3, 6 and 14 dPT, and inhibited fecundity of *O. landolti* at 28 dPT in 42.3%. Meanwhile, residual ivermectin after treatment with ivermectin-3.15% almost completely suppressed fecundity of females at 3, 6, 14 and 28 dPT, and reduced fecundity of *O. landolti* at 35 dPT in 84.9%, relative to controls. Larval survival was significantly reduced only at 3 dPT with ivermectin-1%. Meanwhile, ivermectin-3.15% significantly reduced larval survival at 6, 14 and 28 dPT. L-I and L-II larval stages were more sensitive to residual ivermectin than L-III. No preference of *O. landolti* to dung with residual ivermectin or control dung was found. In conclusion, ivermectin in cattle feces after treatment with ivermectin formulations has a significant adverse effect on fecundity and larval survival of *O. landolti* up to four weeks post-treatment. Moreover, ivermectin residues do not affect the attractiveness of cattle dung to *O. landolti*.

Keywords: Ivermectin, Beetle, Onthophagus landolti, environmental risk

The use of carvacrol encapsulated with yeast cell walls to control resistant strains of *Rhipicephalus microplus* (Acari: Ixodidae)

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Abstract Content

The chemical control of *Rhipicephalus microplus* has selected for resistant populations. The phenol compound carvacrol can serve as an alternative method for the control of *R.microplus*; however, environmental actions can increase the volatility of this compound. A microencapsulation technique using yeast cell walls can prolong the acaricide action of carvacrol and acts as a physical barrier against environmental action. The aim of the present study was to investigate the activity of carvacrol and the encapsulation of carvacrol with yeast cell walls on resistant *R.microplus*. Carvacrol was encapsulated with *Saccharomyces cerevisiae* cell walls. The acaricide activity and the volatility were measured using a larval immersion test on *R. microplus*. The efficacy of the encapsulation was confirmed by Fourier transform infrared spectroscopy and scanning electron microscopy. Fourier transform spectroscopy showed similar vibrational peaks between the analyzed samples, supporting the scanning electron microscopy results that there was encapsulation. The size difference between the yeast cell walls(diameter $2.5\pm 0.2\mu\text{m}$) and the encapsulated carvacrol(diameter $4.5\pm 0.5\mu\text{m}$) was statistically significant($P>0.001$). The encapsulated carvacrol showed the highest larvicidal activity against *R.microplus*, exhibiting LC_{50} of 0.80mg/mL, while the carvacrol LC_{50} was 1.83mg/mL. The yeast cell walls promoted low volatility of carvacrol, maintaining a high acaricidal activity for up to 60h, and the reduced efficiency of carvacrol (18%) in the 10h post test was significantly different($P>0.001$). The high acaricidal activity and its prolongation when the encapsulated carvacrol was used showed that this technique can be viable for the development of a new delivery system for an active compound to control *R.microplus*.

Keywords: monoterpene; formulation; acaricide; tick

**Association of synthetic anthelmintics and natural monoterpenes against
*Haemonchus contortus***

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Abstract Content

The resistance of *Haemonchus contortus* to synthetic anthelmintics is an increasing concern and different strategies are being evaluated. The present trial studied the in vitro effect of the association of synthetic compound and natural monoterpene on eggs and larvae of *H. contortus*. The monoterpenes carvacrol, thymol, r-carvone, s-carvone, citral and p-cymene, and the synthetic anthelmintic ivermectin, and albendazole were used. Egg Hatch Test (EHT) and Larval Migration Inhibition Test (LMIT) were performed. The lowest efficient concentration of monoterpenes in EHT ($\leq 11\%$ of efficacy) and LMIT ($\leq 18\%$ of efficacy) was used in association with different concentration of synthetic compound. The IC50 and Synergism Rate (SR) were calculated. The highest efficiency of monoterpenes in EHT was obtained with r-carvone (IC50 = 0.25 mg/mL) and s-carvone (IC50 = 0.79 mg/mL) and in the LMIT with r-carvone (IC50 = 0.60 mg/mL). The best association was observed in the EHT with albendazole (thymol SR: 2.9 and r-carvone SR: 1.6) and ivermectin (citral SR: 1.9 and carvacrol SR:1.7). No synergistic effect was obtained using the LMIT. The combination of synthetic compound and natural monoterpenes could be positive to gastrointestinal nematodes control: However this strategy should be carefully analysed due to the possibility of antagonistic effects among the different compounds.

Keywords: carvacrol; thymol; citral; nematode

Antagonistic effect of the combination of acai (*Euterpe oleracea*) and ivermectin against nematode larvae of sheep

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Abstract Content

Ethnoveterinary medicine is the study on how natural remedies are used by local communities to control animal diseases. The Amazonian fruit acai (*Euterpe oleracea*) is very popular to the Brazilian culture and is widely used in indigenous practices, including medicine. The objective of this work was to determine the effect of acai against nematode-larvae using an *in vitro* model. Furthermore, this study analyzed the efficacy of its combination with ivermectin (IVM), a large-spectrum anthelmintic drug. The larvae used for the test were *Haemonchus contortus* (75%), *Trichostrongylus axei* (22%) and *Bunostomum* (3%). The study used acai ranging from 0.8 to 50mg.ml⁻¹ – G1 and G2 with acai in the same quantities adding 200ug/ml of IVM in each treatment. The larval migration on agar test (LMAT) was used for this experiment. Acai alone had an efficacy ranging from 13.6 to 83.2% showing a strong concentration-dependence effect. The experiment with IVM gave unexpected results. The efficacy went from 68.7 (0.8) to 10.4% (50), numbers that opposed G1. The results indicated a concentration-dependent antagonism effect among the products, as the treatment with less acai had more effect – when IVM sensed less plant-interference – whilst the treatments with more acai blocked almost all expected action of IVM. The results demonstrate that acai had a favorable anthelmintic property and could be developed and used against gastrointestinal nematodes in farm animals.

Keywords: macrocyclic lactone; drug-drug interaction; Haemonchus contortus; sheep parasites

**Antiparasitic activity of essential oil from *Curcuma longa* Linn. on
Ichthyophthirius multifiliis in goldfish**

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Abstract Content

Ichthyophthirius multifiliis (Ich) is the most common ectoparasitic pathogen in cultured fish. The effective and non-toxic chemotherapeutant is not available for treatment of ichthyophthiriasis. The present study aims to evaluate the parasitocidal effect of essential oils from *Curcuma longa* Linn. on the infective stage of Ich in goldfish. The essential oils were isolated from the turmeric rhizomes using simultaneous steam-distillation and analyzed using gas chromatography coupled to a mass spectrometer (GC-MS). The tested dispersions were prepared by using 10, 30 and 60 ppm essential oils in distilled water together with propylene glycol. Cytotoxic effect of the dispersions was measured on fish mononuclear cells (MN) by MTT reduction assay. *In vivo* toxicity bioassay was also determined in goldfish. A time kill analysis was performed to find out the killing time of 60 ppm turmeric oil against Ich theronts by light microscopic examination. The result demonstrated that the major phytochemical constituent of the turmeric oil was ar-turmerone (36.37%). The highest concentration of turmeric oil exhibited weak induction of MN cytotoxicity and no visible toxic effect was also observed in goldfish. The dispersions induced theront death in a time dependent manner. Killing ability of 60 ppm turmeric oil was significantly higher than that of the 10 and 30 ppm turmeric oils and 50 ppm formalin ($P < 0.05$). This study suggests that the dispersions prepared from non-toxic turmeric oil have potential for the control of ich infection in goldfish. However, the study in pharmaceutical development and the clinical application need to be further investigated.

Haematological parameters in *Eimeria tenella* infected broiler chickens treated with *Khaya senegalensis* (DESR. A. JUSS.) n- butanol leaf extract

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Abstract Content

The haematological parameters in *Eimeria tenella* infected broiler chickens post-treatment with n-butanol leaf extract of *Khaya senegalensis* were evaluated. One hundred and twenty experimental day-old broilers sourced from the university research center were divided into six groups namely A to F. The birds in groups A to E were infected at 4 weeks of age with 1.0×10^5 sporulated oocysts / ml / bird and leaf extracts of *Khaya senegalensis* at three doses of 11 mg/kg, 33 mg/kg and 99 mg/kg were used to orally treat the groups (A,B and C) respectively. Group D were treated with Amprolium. E served as the positive and F negative controls. Blood samples were collected weekly from each group using the wing veins and analysed for packed cell volume, haemoglobin concentration, differential leukocyte count and total protein. The findings in this study showed that anaemia was ameliorated as the values only decreased slightly below the values of the control group and this could be attributed to the beneficial effects of *Khaya senegalensis* in improving the PCV. The lowest PCV was 24.66% at the third week post-infection in the 11 mg/kg treated group. A decreased haemoglobin count (9.86 ± 2.19 vs 10.02 ± 1.95) in the 11mg/kg group when compared with the negative control was observed. The differential leukocyte count values showed statistically no correlation when compared with the different treatment levels. It is recommended that more research be conducted into the biochemical effects of the plant *Khaya senegalensis* on the blood parameters and their corresponding health benefits.

Keywords: Haematology; broiler chickens; Eimeria tenella; Khaya senegalensis; extracts

In vitro determination of plant extract efficacy against *Cryptosporidium*

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Abstract Content

Cryptosporidiosis is a significant disease in humans and animals, causing diarrhea, mortality and substantial economic losses. However, treatment options are inadequate while cryptosporidia are naturally resistant to most anti-protozoal drugs. To date there has been limited investigation into the potential of plant secondary metabolites e.g. condensed tannins (CT) to treat *Cryptosporidium* infections. *In vitro* growth of *C. parvum* and *C. hominis* in human ileocaecal adenocarcinoma (HCT-8) cell culture has been performed routinely for several years to ascertain possible pharmaceutical candidates. We utilized this assay to assess anti-cryptosporidial efficacy of characterized fractions of extracts from CT containing plants including e.g. white clover, cinnamon, blackcurrant leaf, birch leaf, walnut leaf, goat willow leaves, goat willow sprigs and weeping willow catkins which were previously tested *in vitro* against gastrointestinal nematodes of small ruminants. In our initial investigations cell cultures were seeded with 2500-10000 oocysts of various *C. parvum* and *C. hominis* strains and parasite development was monitored by immunofluorescence microscopy. Replicate samples (n = 5) of each extract in 3 different concentrations were tested using *Cryptosporidium* infected, untreated and nitazoxanide treated cells as negative/positive controls, respectively. Minimum inhibitory concentrations and detrimental effects on the cells were determined. By employing the established developmental times of the various life-cycle stages of the parasite in the assay it is possible to selectively apply plant extracts to these specific stages to assess any varying effect. The assay also benefits from a relatively fast incubation time, enabling rapid accumulation of data and immediate reassessment of promising candidates.

Keywords: Cryptosporidium; bioactive plant extracts; cell culture; in vitro

Chemical composition and anthelmintic activity in vitro of the essential oil of *Mentha piperita*: preliminary data.

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Abstract Content

Research towards plant compounds with activity against worm parasites has been carried out for a number of decades; however it is especially important now when drench resistance has compromised the effectiveness of present control methods. In this study we characterized the composition of *Mentha piperita* essential oil and evaluated its anthelmintic effect against developmental stages of trichostrongylids from naturally infected goats (95% *Haemonchus contortus* and 5% *Trichostrongylus* spp.) through the egg hatch and larval development assays. The major constituent of the tested substance quantified by gas chromatography was menthol (58.35%). The lowest concentration of essential oil with 100% efficacy in all *in vitro* tests against caprine trichostrongylids was 0.35%. However, the equivalent dose of menthol alone (0.4%) was not fully effective against nematode egg eclosion. The anthelmintic activity of the essential oil of *M. piperita* showed good effectiveness in previous studies but also showed the presence of toxic compounds such as pulegone which could be toxic to goats according to allometric extrapolation. Our results show that the major component in the essential oil is not the only one responsible for the antiparasitic effect of *M. piperita*. Also, our data support the idea there is a wide variation in the composition of essential oils obtained from different sources. Thus, it is important that in assessing anthelmintic activity of plant candidates, the chemical composition should be thoroughly investigated before proceeding to the *in vivo* step.

Keywords: herbal; compounds; nematode; eggs

In vitro anthelmintic properties of chicory plant against nematode parasites of cattle

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Abstract Content

Producing a cheap and safe anthelmintic drug is the aspiration of many involved in this field because of the negative impact of nematodes on livestock production globally. Ethanol extract of chicory plant was tested *in vitro* against nematode parasites. Faecal cultures were treated at three concentrations (5, 10 and 20%). Results showed chicory plant had varied ($P < 0.001$) effects on nematode larvae from cattle at the three concentrations. It's clear that increasing of the concentration caused stronger larvicidal effects (75.7–86.9%). The findings suggest that chicory plant possesses anthelmintic properties against nematode parasites of cattle *in vitro*. The active component in chicory plant is tannin. Studies to measure the amount of tannins to avoid its negative effect on feed intake and digestibility are need it. Also, further *in vivo* studies need to be done to investigate the effect on faecal egg count, weight gain and health parameters.

Keywords: Plant extract; Nematodes; Anthelmintic; Chicory; Cattle

Poster Presentation – Human Food & Water Borne Pathogens

Abstract No: 3915 (Poster# S1 - 41)

Detection and prevalence of protozoan parasites in ready-to-eat packaged salads on sale in Italy.

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Abstract Content

Protozoan parasites commonly infect humans and animals, and numerous foodborne outbreaks associated with the consumption of fresh produce contaminated with *Giardia duodenalis*, *Cryptosporidium* spp., *Cyclospora cayetanensis* and *Toxoplasma gondii* have been reported worldwide. To investigate the prevalence of protozoans in 'ready to eat' (RTE) salads on sale in Italy, 648 packages were purchased from Industrial and Local brands. Nine individual packages from each brand were collected per month, pooled and subjected to microscopy and molecular analyses. 864 slides were microscopically examined to detect *Cryptosporidium* spp. and also *Blastocystis hominis* and *Dientamoeba fragilis*. Molecular tools identified *G. duodenalis* assemblage A, *Cryptosporidium parvum* and *Cryptosporidium ubiquitum*, *T. gondii* Type I and *C. cayetanensis*. *B. hominis* and *D. fragilis* were also molecularly confirmed. The overall prevalence of each protozoan species was 0.6% for *G. duodenalis*, 0.8 for *T. gondii*, 0.9% for *Cryptosporidium* spp., and 1.3% for *C. cayetanensis*, whereas the prevalence of *B. hominis* was 0.5% and that of *D. fragilis* 0.2%. Using microscopy and/or molecular tools, we found that 4.2% of the samples were contaminated by at least one protozoan species, and 0.6% of samples presented coinfections of two protozoan species, with a number of oocysts ranging from 62 to 554 per g of vegetable for *T. gondii*, and 46 to 1.580 for *C. cayetanensis*. This is the first large-scale study on the presence of protozoans in packaged salads in Europe. RTE sanitation processes from harvesting to packaging does not guarantee a product free from protozoans of fecal origin.

Keywords: Protozoans; Prevalence; ready-to-eat salads; Italy

Evaluation of the efficiency of detection methods of *Anisakidea* larvae in cod's fillets in aspect of threat to consumers' health

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Abstract Content

Anisakidosis is a foodborne disease caused by viable Anisakidae larvae ingested when eating marine fish infected with these parasites. *Anisakis simplex* and *Pseudoterranova* spp. larvae are found in the Baltic cod fillets, however, their prevalence differs in particular fishing grounds. According to European Commission regulations, fish intended for human consumption originated from areas when Anisakidae occurs should be visually inspected for the presence of anisakid larvae before being released to consumption. A total number of 552 cod individuals caught in 2016 were examined. Fish were degutted and fillets were examined according to EC recommendations. Next, each fish was digested in HCl-pepsin solution to determine whether some nematodes could remain undetected by the recommended method, and thus, whether eating of these fish can poses a threat to the consumers health. Cod caught in Gulf of Gdańsk were free of parasites. Single infected fish were found in Gdansk Deep (1 of 42), Slupsk Furrow (2 of 147) and Kolobrzeg-Darlovo (2 of 98) fishing ground. Infected cod were found in fishing grounds in the Bornholm vicinity. The prevalence of infection of fish from these fishing areas inspected visually was approximately 7% while after digestion it increased almost twice. Moreover, in some fillets parasites were detected only after digestion. Obtained results show that cod infected with anisakid larvae, even after visual inspection, still poses a potential threat to consumers. This research was supported by The National Centre for Research and Development under the Strategic Program Biostrateg (grant no. 296211/4/NCBR/2016).

Keywords: anisakidosis; cod fillets; larvae detection

Seroprevalence of *Toxoplasma gondii* in meat from goat and cattle from wet markets in Klang Valley, Malaysia

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Abstract Content

Toxoplasmosis has been recognized globally as an endemic disease capable of infecting all warm blooded animals and causing major health concerns to humans. Studies in Malaysia have shown that seroprevalence of *Toxoplasma gondii* (*T. gondii*) among healthy people in Malaysia can be as high as 30% indicating that it is common among Malaysians. *T. gondii* can be transmitted to human horizontally through consumptions of undercooked meats, unpasteurized milk or by accidental ingestion of oocysts from the environment. In Malaysia, studies on *T. gondii* in meat have been reported in poultry, wild boar and exotic animals but none in ruminants. The aim of this study is to determine the seroprevalence of *T. gondii* in ruminant meat in wet markets in the Klang Valley area. A total of 170 meat samples from goat and cattle were collected from various wet markets within the area. All samples collected were kept at -20°C until further analysis. Samples were subjected to a commercially available ELISA assay using meat juice to determine the seroprevalence *T. gondii*. With the increase of meat intake, findings from this study will further enhance knowledge on the epidemiology of *T. gondii* in ruminants meats used for human consumption in Klang Valley and its potential health implications to the public.

Keywords: *Toxoplasma gondii*, ruminant meats, ELISA

Poster Presentation – Livestock Parasites

Abstract No: 5612 (Poster# S1 - 43)

Availability of *Lymnaea rubiginosa* and other snails around cattle grazing areas

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Abstract Content

Freshwater snail (*Lymnaea rubiginosa*) is an important intermediate host for fascioliasis. A study was conducted to detect the availability of *L. rubiginosa* at a cattle farm in Kuala Terengganu which were found to be positive for fasciola infection. Water bodies located around the grazing areas were inspected. A total of 11 sites were chosen and characterized as paddy field, mud flat, irrigation canals, ditch and artificial drainage. At each site, snails were collected and water parameters such as pH, temperature and calcium content were recorded. Snails collected from these sites were identified as *Bellamya* sp., *Batillaria* sp., *Pomacea* sp., *Melanoides* sp., *Indoplanorbis* sp., *Melanopsis* sp., *Lymnaea rubiginosa*, *Physa* sp., *Gyraulus* sp., *Telescopium* sp. and *Achantina* sp. *L. rubiginosa* were found at the drainage area abundant with *Ipomoea* spp. The water temperature for this site was 26.7°C, with pH of 6.81 and 33.06 mg/L calcium. Results from this study indicate that *L. rubiginosa* is present within the vicinity of the cattle grazing area. Therefore, inspection of *L. rubiginosa* should be conducted around cattle farms in Terengganu where fasciola infection is a problem.

Keywords: Snail, liver fluke, fasciola, cattle, parasite

**A survey of gastrointestinal parasitic infection on small ruminant farms in
Seberang Perai Selatan District, Penang, Malaysia**

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Abstract Content

In Malaysia, Helminthiasis due to strongyles such as *Haemonchus contortus* and coccidiosis caused by *Eimeria* sp. have been reported to cause severe economic losses in the small ruminants livestock industry. This paper reports the occurrence of gastrointestinal parasite infections on small ruminants situated in Seberang Perai Selatan district, Penang. Faecal samples were obtained from a total of 193 animals was random selected from 14 ruminant farms. The results of this survey indicate that helminthiasis and coccidiosis is rampant in sheep and goat farms. The most common infections diagnosed were helminthiasis infection (77.72%) and coccidiosis (60.10%) followed by *Moniezia* sp. (5.18%). From this study, it shows that parasitic diseases can be managed by good animal husbandry in farms since high parasitic infections were observed in farms that were poorly managed based on nutrition, hygiene and basic animal husbandry practices. The smallholders depended on health and extension services from the State Veterinary Department. A continuous monitoring of small ruminant farms by the Department of Veterinary Services will provide important information for assisting farmers with managing the spread of parasitic infections and maintaining the productivity of animals.

Keywords: Gastrointestinal parasite, Coccidiosis, management

Characteristic histopathology of hepatic distomiasis in goat suggestive of pre-cancerous lesion

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Abstract Content

Hepatic distomiasis is trematode parasitic infestation caused by *Fasciola hepatica* and *Dicrocoelium dendriticum* in human and animals. The involvement of liver fluke, *Dicrocoelium dendriticum*, *Fasciola hepatica* in progression of tumor has been poorly investigated so far. The present paper discussed pre-carcinogenic histologic characteristics in liver of goat, severely affected with *Dicrocoelium dendriticum* and *Fasciola hepatica*. The study of two liver sample of goat collected at slaughter house showed moderate to severe jaundice along with some nodular swelling in one case. Atypical bile duct hyperplasia, peri-cholangitis, and severe chronic cholangiohepatitis were common histological feature produced by the parasite, showing its presence in bile duct even with an aberrant localization in blood vessels observed for *D. dendriticum*. There were development of adenomatous hyperplasia and periductal fibrosis besides inflammatory cellular exudation of lymphocytes. Cholangitis and pericholangitis were more severe in the case of *Fasciola* along with prominent multinodular lobulation. Abundant fibrous connective tissue proliferation with formation of lymphoid follicle showed severe chronicity of the lesion. Adenomatous hyperplasia and cholangiofibrosis in bile duct may arise of dysplastic changes from lining cell which may get neoplastic transformation. It is concluded that long standing irritation of flukes in bile duct produce marked alteration in bile duct and may leads dysplastic transformation to neoplasia.

Keywords: Dicrocoelium; Fasciola; Goat; Precancerous lesion

Poster Presentation – Novel Parasite Control Options & Diagnostic

Abstract No: 4285 (Poster# S1 - 44)

Potential role for specific IgA in protective mechanisms of a recombinant *Teladorsagia circumcincta* vaccine prototype

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Abstract Content

Nowadays, the search for antigens to elaborate effective vaccines against gastrointestinal nematodes (GINs) that can generate protective immune response, reducing the use of drugs and the development of resistance to them, is a priority. However, finding immunogenic molecules that allow to obtain its recombinant homologue capable of stimulating the response is an arduous task. Nisbet *et al.*, 2013, developed and tested a protective recombinant prototype. However, they observed individual variability in its response. The objective of this work was to reproduce the experimental design of Nisbet *et al* 2013 in two Canarian sheep, Canaria and Canaria Hair Breed, with differences in susceptibility to GINs. The comparative study of the immune response developed in these sheep breeds, within and between breeds, could provide relevant information that would allow to optimize this vaccine prototype. The immunization produced a reduction in the length and the intrauterine eggs of the worms in the vaccinated group of the Canaria sheep, whereas in the Canaria Hair Breed only a non-significant reduction of worm burden was observed. Significant and negative correlations between antigen-specific IgA and parasitological variables were observed in both vaccinated groups. These data could suggest an effective orientation of the immune response in parasitological control in vaccinated animals and a role of IgA in this protection. Acknowledgements: European Union's Horizon 2020 research and innovation programme under the grant agreement No. 635408 (PARAGONE)

Keywords: IgA, L4 antigen, sheep, Teladorsagia circumcincta, recombinant vaccine

Assessment of modified Baermann methods for diagnosis of first-stage helminth larvae

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Abstract Content

Lungworms and French heart worms are often diagnosed as first-stage larvae (L1) using different modifications of the Baermann method. The current study compared four different versions (sedimentation glass (SG), wide plastic bag for food (FB), narrow pastry piping bag (PB) and a commercial plastic funnel (PF)) for recovery of *Angiostrongylus vasorum*, *Dictyocaulus arnfieldi* and *Dictyocaulus viviparus* in combination with different incubation (8 and 24h) and storage (5°C for 10, 17 and 24 days) times. Using the PF many samples were false negative and recovery was very low. The overall majority of *D. arnfieldi* and *A. vasorum* L1 were recovered within 8 hours using the SG, FB and PB, whereas *D. viviparus* yields were more substantially improved by 24h incubation. The SG yielded more *A. vasorum* than the FB and PB, which were equally effective. Though the GB also tended to give a higher recovery of *D. arnfieldi* there was no overall substantial difference compared to the plastic bags. The GB and FB gave comparable results for *D. viviparus* after 24h while the outcome for the PB tended to be lower. In general, *A. vasorum* experienced the most severe mortality during storage followed by *D. viviparus* whereas *D. arnfieldi* had the best long-term survival. Overall, it is recommended to combine the SG with less than 10 days storage and 24h incubation. The PB was probably too narrow to allow consistent efficient L1 recovery, but the FB can be a low cost disposable option whereas the commercial PF is not recommended.

Keywords: Baermann; first stage larvae; incubation time, storage time

A nano-pesticide formulation for enhanced longevity and efficacy of blowfly treatments

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Abstract Content

Recent developments in nanotechnology have advanced modern agriculture through creation of new agrochemical agents and new delivery mechanisms. Nanoparticles have novel properties that can increase a pesticide's efficiency by protecting active ingredients against degradation. The aim of this study was to determine the benefits of using silica nanoparticles to encapsulate ivermectin, a photosensitive pesticide used in the control of sheep myiasis. Three different types of ivermectin-loaded silica nanoparticles with tunable surface roughness and chemistry (smooth, rough and hydrophobic rough) were evaluated against first instar *Lucilia cuprina* larvae using a sheep serum assay, before and after UV irradiation. Un-encapsulated ivermectin and empty silica nanoparticles were included as controls. UV irradiation caused a 78% reduction in the efficacy of free ivermectin, whereas the ivermectin encapsulated in the silica nanoparticles retained its activity. The distribution and cuticular penetration of the different silica nanoparticle types on *L. cuprina* were determined using fluorescein-5-isothiocyanate (FITC)-labeled particles and a fluorescence microscope. Knowledge of nanoparticle uptake and distribution pathways in parasites will inform formulation and application methods for the development of next-generation nano-pesticide formulations with improved efficacy.

Keywords: Nanopesticides; Insect pest control; Nanoparticles

Use of fluorescent lectin binding to distinguish eggs of gastrointestinal nematode parasites of sheep

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Abstract Content

Fluorescently labelled lectins may form the basis of a rapid test to identify, to genus, parasite eggs from faecal samples. The binding of a panel of 19 lectins to carbohydrates on the eggs of economically important nematode parasites of sheep has been assessed as the basis of a rapid test to distinguish parasite eggs, at least at the genus level. A total of six lectins can be used to identify eggs of six nematode parasites: peanut agglutinin (PNA) for *Haemonchus contortus*; *Lens culinaris* agglutinin (LCA) for *Teladorsagia* sp; *Aleuria aurantia* agglutinin (AAL) for *Trichostrongylus* sp; *Psophocarpus tetragonolobus*-II (PTLII) for *Nematodirus* sp; *Lotus tetragonolobus* lectin (LTL) for *Cooperia* sp and wheat germ agglutinin (WGA) for *Chabertia ovina*. For LCA, weak binding was also observed to *Trichostrongylus* sp and *C. ovina* eggs, and for LTL, weak binding was observed to *C. ovina* and *Nematodirus* sp eggs. Nematode eggs from two field collected faecal samples were identified using lectin binding, PCR and visual techniques. The results were identical except for the *Nematodirus* sp. eggs which did not lyse and hence could not be identified by PCR. This result indicates the utility of this method for future development into a test for rapid determination of parasite eggs to genus from field samples.

Monthly administration of milbemycin oxime plus afoxolaner chewable tablets (NexGard Spectra®) to prevent *Angiostrongylus vasorum* infection in dogs

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Abstract Content

A randomized, negative control, blinded study was conducted to evaluate the preventive efficacy of monthly administered NexGard Spectra® (milbemycin oxime and afoxolaner) chewable tablets against canine *A. vasorum* infection in a multiple challenge (trickle infection) model. Twenty beagle dogs were challenged orally with approximately 40 third-stage larvae of *A. vasorum* once every other week on seven occasions. Half of the dogs were administered NexGard Spectra® as close as possible to the minimum recommended dose (0.5 mg/kg and 2.5 mg/kg milbemycin oxime and afoxolaner, respectively) four times from Day 0 onwards at monthly intervals while the remaining dogs served as untreated controls. Starting six weeks after first challenge, feces were collected bi-weekly and examined for *A. vasorum* larvae. For parasite recovery and count, dogs were necropsied eight days following the last treatment. No clinical signs were observed in any of the dogs throughout the study. Geometric mean adult *A. vasorum* counts were 66.4 and 3.4 for the untreated and treated dogs, respectively ($P < 0.0001$). Thus, preventive efficacy of monthly NexGard Spectra® treatment was 94.9%. In addition, NexGard Spectra® treatment reduced *A. vasorum* larval excretion by 99.9% ($P < 0.0001$). At necropsy, considerable lung lesions were observed in the untreated dogs while NexGard Spectra®-treated dogs did not show marked lung lesions. These results demonstrate that NexGard Spectra®, when administered at monthly intervals, can effectively prevent canine *A. vasorum* infection.

Keywords: Dog, Angiostrongylus vasorum, milbemycin oxime, prevention

Acaricidal activity against *Rhipicephalus microplus*, *Rhipicephalus sanguineus* y *Amblyomma* spp. of extracts from *Diospyros anisandra* (Ebenaceae) collected in different seasons

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Abstract Content

New strategies for ticks control which are less hazardous and more environmentally friendly are searched for; such as the use of plant extracts. Several studies have evaluated the acaricidal activity of plant extracts on larvae *Rhipicephalus microplus* ticks. However, there are other tick species of importance for livestock and the health of pets. This study evaluated the acaricidal activity of methanolic extracts from bark of *Diospyros anisandra* against larvae of *Rhipicephalus microplus*, *Rhipicephalus sanguineus* and *Amblyomma* spp collected in dry and rainy seasons. Methanolic extracts of bark from *D. anisandra* were obtained in dry and rainy seasons in Yucatan, Mexico. From bovines and dogs, adult ticks of the tick species mentioned were collected. To evaluate the acaricidal activity, the larval immersion test was used at different concentrations (2.5, 5.0, 10 and 20%). In addition, lethal concentrations at 50% (LC50) were obtained with Polo plus software. Mortalities of 64.5-100%, 31.5-100% and 34.0-100% for *R. microplus*, *R. sanguineus* and *A. spp.*, respectively, were observed in the dry season collection. The extracts collected in rainy season showed efficacies of 31.7-99.2%, 23.9-100% and 35.2-87.0%, respectively. The LC50 obtained from the dry and rainy collections of *D. anisandra* were 2.2 and 3.1; 3.5 and 4.3; 3.0 and 4.1 for *R. microplus*, *R. sanguineus* and *A. spp.*, respectively. The bark of *Diospyros anisandra* seems to be a good alternative for controlling these ticks, due to its high acaricidal activity and it does not present differences in its effectiveness by collection season.

Keywords: Diospyros anisandra, Rhipicephalus microplus, Rhipicephalus sanguineus, Amblyomma spp., methanolic extract.

Anthelmintic effect of *Diospyros anisandra* on larval development and hatching of cyathostomins eggs (Nematoda: Cyathostominae)

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Abstract Content

Cyathostomins are the nematodes with the highest prevalence in horses. Various anthelmintics have been used for its control; however, resistance to benzimidazoles, pyrimidines and macrocyclic lactones has been reported. Control alternatives include the use of plant extracts with anthelmintic properties. The objective of the present study was to evaluate the *in vitro* anthelmintic effect of *Diospyros anisandra* methanolic extract on larval development and hatching of Cyathostomins eggs. Extracts collected during the dry and rainy season were evaluated by hatching inhibition test at different concentrations (600µg, 300µg, 150µg, 75µg, 37.5µg, 18.7µg and 9.3µg per ml). In addition, lethal concentrations at 50% (LC50) were obtained with Polo plus software. Extracts from *D. anisandra* bark showed 95% of hatching inhibition (HI) in both seasons from the concentration of 37.5µg/ml, while leaf extract showed a HI > 90 % in both seasons from the concentration of 75 µg/ ml. The LC50 of bark and leaf from *D. anisandra* were lower for the collections of rainy season (10.2 and 18.4 µg / ml, respectively) compared to the dry season (32.8 and 28.1 µg/ml, respectively). Additionally, it was demonstrated that extracts of *D. anisandra* have high ovicidal effect (≥96% at 37.5µg / ml). The results of the present study showed that the extracts of bark from *D. anisandra* collected in rainy season have high anthelmintic effect *in vitro* on the larval development and hatching of Cyathostomins eggs, representing a possible alternative for the control of these nematodes.

Keywords: Anthelmintic effect, Cyathostomins, Diospyros anisandra, equines, extracts.

Dany's Bienenwohl® (containing 3.5% oxalic acid) in the treatment of varroosis in honey bees under field conditions in Germany

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Abstract Content

In total 45 bee colonies were enrolled at 2 study sites, one in Bavaria (Site 1), and one in Lower Saxony (Site 2). At site 1 the bees were of the breed *Carnica*, kept in Deutsch Normal 1.5 hives, at site 2 the colonies were of the breed *Buckfast*, kept in Dadant hives. On day 0 all colonies were free of brood; each 15 were allocated per random to either T1 (Dany's Bienenwohl of Dany Bienenwohl GmbH, Germany; 6 ml per comb gate), T2 (Ecoxal® of Ceva Salud Animal S.A., Spain, 50 ml per colony), or T3 (physiological saline, 6 ml per comb gate); all were treated by trickling. Perizin®, Bayer, was applied by trickling as follow up treatment to all colonies 12/14 days after the initial treatment. Dead mites were counted daily from day 1 onwards, up to day 12 at site 1 and day 14 at site 2, when the follow up treatment was administered to all colonies as a reference treatment to determine the number of residual mites for 7 days. The mean % mite reduction was 97.98% in T1, and 91.39% in T2 as compared to T3. The %mite reduction in T1 was superior compared to T2 (difference T2-T1 = -6.58%, p=0.0007). Overall safety was similar for both treatment groups regarding queen survival, colony strength and development of brood in spring. Dany's Bienenwohl® was highly efficacious and safe in the treatment of Varroosis in honey bees caused by *Varroa destructor* under field conditions in Germany.

Keywords: Oxalic acid, varroosis, Varroa destructor, efficacy

New insights into helminth secretome for potential immunotherapeutic and diagnostic tool

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Abstract Content

Parasitic helminth infections in livestock are responsible for significant economic losses particularly in developing and tropical regions of the world. Soil-transmitted helminths affect more than 1.5 billion people causing great socio-economic impact. Among these, hookworms, roundworms and whipworms are of particular importance due to their high endemicity worldwide. In the last few years there have been significant advances in the field of extracellular vesicles research as potential targets for new specific treatments and diagnostic tools. In the present study we aimed to characterise the factors involved in *T. muris*-host relationships. The study of the genome and transcriptome of *T. muris* has provided meaningful insights of whipworm infections. We provide the first high-throughput proteomic analysis of the soluble proteins present in the ES products and have thoroughly characterised the proteomic and genomic content of the EVs secreted by the whipworm to gain a more comprehensive picture of the genomic composition of the *T. muris* EVs. In the present study, we have provided important information regarding the molecules secreted by *T. muris*. This result agrees to the functional annotation performed on the *T. muris* proteins predicted from the genome. The identification of the secretome content will prove useful not only for the design of novel approaches at controlling whipworm infections, but also to understand the way the parasite promotes an optimal environment for its survival. This work provided new information on potential drug targets and important traits that drive chronicity in Trichuriasis.

Recombinant Glutathione S-Transferase adsorbed to aluminum hydroxide: A vaccine candidate against *Fasciola hepatica* in mice

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Abstract Content

Fasciolosis is a parasitic zoonosis caused by infection with *Fasciola hepatica*. Disease control using Triclabendazole (TCBZ) results in the development of anthelmintic resistance against the drug. Vaccination would be an attractive option to pursue in fasciolosis control to reduce the need for anthelmintics. We evaluated the immunogenicity and protection conferred by a recombinant Glutathione-S Transferase- Mu (rFhGSTMu) protein against *F. hepatica* in mice. The recombinant enzyme was produced in *Escherichia coli*. IgG and IgG subisotypes were measured using an ELISA. Liver damage was estimated by the determination of serum Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (AP) activity. Balb/c mice were distributed across four groups (n=10/group) immunized subcutaneously at weeks 0, 2 and 4 as follows: Group 1: rFhGSTMu + Freund Incomplete Adjuvant (FIA); Group 2: rFhGSTMu + Aluminum Hydroxide (AH), Group 3: rFhGSTMu + Quil A and Group 4 (control group) was injected with saline. All groups were challenged two weeks after the last immunization with six metacercariae of *F. hepatica*. All vaccine formulations induced IgG specific antibodies with a mixed IgG1/IgG2a response. rFhGSTMu + AH induced significant reduction in worm counts (90%). Other formulations, however did not induce a significant reduction in worm counts (0 to 10% similar to the unvaccinated control group). Liver enzyme activities in the group immunized with rFhGSTMu + AH were significantly lower than values recorded in the other groups. Our results indicated that rFhGSTMu formulated in AH is a potential vaccine candidate against *F. hepatica* in the mouse model.

Keywords: *Fasciola hepatica*, GST, Vaccine, Mice

Efficacy of afoxolaner (NexGard® and NexGard Spectra®) for the treatment and control of generalised demodicosis due to *Demodex canis* in dogs under field conditions

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Abstract Content

A multi-centric study was conducted in France, Italy and Poland to evaluate the efficacy of monthly administered afoxolaner in its chewable tablet formulations (NexGard Spectra® and NexGard®) for the treatment of canine generalized demodicosis under field conditions. In total, 50 dogs confirmed positive for *Demodex* mites by pre-treatment deep skin scrapings, and presenting clinical signs of generalized demodicosis were included. Dogs were allocated to be treated with either NexGard Spectra® (≥2.5mg/kg afoxolaner + ≥0.5 mg/kg milbemycin oxime) or NexGard® (≥2.7mg afoxolaner/kg) in accordance with label instructions. Of the 50 dogs enrolled, 48 completed the study; 19 dogs were treated with NexGard Spectra® and 29 dogs received NexGard®. Deep skin scrapings and clinical examinations were performed monthly in order to evaluate the reduction in mite counts and the resolution of clinical signs (pruritus, alopecia, erythema, pustules, papules or scales/crusts). On Day 84, the percentage reduction of mite counts in dogs treated with both NexGard Spectra® and NexGard® was >90%. A significant clinical improvement was also observed. In conclusion, this field study demonstrates that monthly administration of oral afoxolaner in NexGard® or NexGard Spectra® may have interesting potential in of the control of canine generalized demodicosis.

Keywords: Dog, Demodex canis, afoxolaner, treatment

Transfection of buffalo flies, *Haematobia (irritans) exigua*, with wAlbB *Wolbachia*
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Abstract Content

Buffalo flies (BF), (*Haematobia (irritans) exigua*) and horn flies (HF) (*Haematobia (irritans) irritans*) are hematophagous parasites of pastured cattle that are responsible for significant production losses and animal welfare impacts in cattle across the world. Numerous strategies have been devised to manage BF and HF, but control continues to rely heavily on the use of chemicals, with ongoing issues of resistance. The endosymbiotic bacterium *Wolbachia* has wide ranging effects on host biology including the induction of cytoplasmic incompatibility, modification of host fitness and impacts on vector competence, which suggests the potential for use in *Haematobia* control programs. We have established *Haematobia* (BF and HF) cell lines and successfully transfected them with the mosquito cell-adapted wAlbB strain of *Wolbachia*. Micro-injection methodologies have been designed, and injection of BF embryos with wAlbB has commenced. We have also determined small cage mating methodologies to test maternal inheritance, cytoplasmic incompatibility, and fitness effects of wAlbB infection in BF. Future work will be focussed on microinjection of BF embryos with *Haematobia* cell line-adapted *Wolbachia* to develop transfected BF lines, and biological studies to determine the fitness effects of *Wolbachia* infection, towards the design of *Wolbachia*-based control programs for BF.

Keywords: Haematobia; Wolbachia; Diptera; Rickettsia

Response to Barbervax® vaccination in ewe hoggets

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Abstract Content

The Barbervax® vaccine for control of *Haemonchus contortus*, is registered for use in hoggets following vaccination as lambs with immunity induced and maintained by 4 vaccinations at 6 weekly intervals. In hoggets and older sheep booster vaccinations elicit a marked antibody response long after cessation of protective immunity and it has been postulated that that natural challenge assists in maintaining immune responsiveness. To investigate this possibility we designed an experiment to test two hypotheses. Firstly, that immunity will persist for longer than 6 weeks after a full vaccination course in year 2 and secondly that a constant natural challenge will enhance the immune response to a pre-lambing booster vaccination. Blood and faecal samples were collected at 4-weekly-intervals after the 4th vaccination for worm egg count (WEC) and specific antibody ELISA from 3 groups of 20 fine-wool Merino ewe hoggets (vaccinated, unvaccinated and vaccinated + LA-Moxidectin). LA-Moxidectin was administered to provide a total of 18 'worm free' weeks after the 4th vaccination. Hypothesis 1 was not supported with little evidence of vaccination-induced reduction in WEC or persistence of it, and no significant correlation between WEC and antibody titre ($P > 0.05$) although vaccinates had significantly higher antibody titres ($P < 0.05$) at weeks 4, 12 and 16. Hypothesis 2 was also not supported as antibody levels 5 weeks after a pre-lambing booster vaccination, 21 weeks post the last (4th) vaccination, were significantly increased in the vaccinated groups from weeks 2-5 post booster, with no difference between groups that received LA-Moxidectin or not.

Keywords: Barbervax®; Haemonchus contortus; nematode infection; vaccination

Improving the design of the Composite FECRT to detect triclabendazole resistance in fluke Populations

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Abstract Content

The liver fluke (*Fasciola hepatica*) is a parasitic trematode of major importance to the livestock industry in the UK and worldwide. For sheep, triclabendazole (TCBZ) is the drug of choice as it kills both adult and juvenile stages of the parasites. However, TCBZ is becoming increasingly ineffective due to heavy reliance on anthelmintic resulted in emergence of drug resistance fluke populations in the country. Thus, reliable assays to detect anthelmintic resistance in populations of parasites are required for assessment of effective control of parasitic infections. The aim of this study was to improve the design of the Composite FECRT (cFECRT) to detect TCBZ resistance in fluke populations. For composite faecal samples, 5g of faeces from each of the 10 sheep per group, 2 groups per farm, were used to make the 50g composite samples and analysed for fluke eggs by using a sedimentation method. If the total composite pre-treatment count is 100 eggs per gram of faeces or greater, a second set of samples from the same animals is collected 21 days after treatment with TCBZ. The hypothesis is that if it was necessary to sample the same 20 sheep before and post-treatment, or if two random groups of 20 sheep could be sampled for the two counts and the optimum time to collect the post-treatment sample. Bootstrap analysis showed that the same 20 sheep had to be sampled pre-treatment and at 21 dpt. The results also showed that sample testing at 21 dpt can help avoid false positive results.

Keywords: h;a;k;i;m

***Haemonchus contortus* control and performance of dairy goats vaccinated with Barbervax®: preliminary data**

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Abstract Content

The search for vaccines against worm parasites has been carried out for many decades but it's especially important now that drench resistance compromised present control methods. Here we present preliminary results of a trial designed to evaluate Barbervax®, a vaccine derived from gut proteins of *H. contortus* in the control of this nematode in dairy goats. Female Saanen (n=20) and Anglo Nubian (n=20) goats aged six months were assigned by breed, body weight and fecal egg counts into four experimental groups (n=10) vaccinated or not, starting with three priming doses 21 days apart followed by boosts every 6 weeks. All animals grazed on the same bushland area and were subjected to natural plus artificial worm infection. Over the pregnancy and lactation we monitored FAMACHA®, egg counts, blood values and overall performance. Vaccinates of either breed had significant reductions in egg counts compared to controls, with 65.3% ± 10.7 for the Anglo Nubians and 67.6% ± 8.9 for Saanen but blood parameters and FAMACHA scores were positively affected by the vaccine only in the Saanens. Protection was maintained during *per partum* and lactation. Milk yield and other performance parameters were not affected by the vaccination regimen. Unlike anthelmintics, Barbervax® does not have a withdrawal period and so it could be useful in the management of *Haemonchus* infection during milk production.

Keywords: goats; nematode; H11; H-Gal-gp

Application of MALDI-TOF mass spectrometry for the detection of filariae in *Aedes aegypti* mosquitoes

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Abstract Content

Filariosis is a disease group affecting humans and animals, caused by nematode parasites of the order Filariidae. These parasites can be transmitted, essentially, by mosquitoes during blood meals of infected female specimens. Screening vectors for these filariae currently relies on time- and resource-consuming methods such as dissection and polymerase chain reaction-based method. Here, we applied matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) to assess whether this tool can generate changes in the protein profiles of *Aedes aegypti* experimentally infected with filarial nematodes (*Dirofilaria immitis*, *Brugia malayi* and *B. pahangi*) compared to those uninfected testing different parts of mosquitoes. First we created a reference mass spectra database from *Ae. aegypti* infected or not by filariae using MS of 47 specimens' compartment. In a second step, we tested the remaining mass spectra (350) in a blind validation test. Regardless the filariae species, the better correct classification rate were obtained from the thorax-head part with 94.1 and 86.6, 71.4 and 68.7% for non-infected and *D. immitis*, *B. malayi* and *B. pahangi* infected mosquitoes respectively. The results obtained demonstrate that MALDI-TOF-MS can be used as a first-order sorting tool to screen field mosquitoes for the presence of filariae.

Keywords: MALDI-TOF mass spectrometry, Aedes aegypti, Dirofilaria immitis, Brugia malayi, Brugia pahangi, Real-time PCR, Entomological surveillance

The influence of mechanochemical and drug delivery technology on the efficacy of benzimidazole anthelmintics

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Abstract Content

The studies were made on 70 sheep infected by gastrointestinal nematodes suborder Strongylata. The supramolecular complexes of albendazole and fenbendazole with polymer polyvinylpyrrolidone produced by mechanochemical modification of substance in activator of percussive-grating type were used in tests. Sheep of different groups of 10 animals each received per os supramolecular complexes of albendazole and fenbendazole at the doses of 2,0 mg/kg of active substance (AS) in comparison with the basic substances of albendazole and fenbendazole which were administered orally at doses of 2,0 and 10 mg/kg. Control animals did not receive the drug. The efficacy of drugs was determined on the basis of the results of coproscopic study of feces by flotation methods before and 17 days after treatment. The supramolecular complex of albendazole at the dose of 2,0 mg/kg showed 98,3% efficacy against *Nematodirus* and 100 % against other intestinal nematodes. Substance of albendazole at doses of 2,0 and 10 mg/kg showed 23,4 and 98,0 % efficacy respectively. The supramolecular complex of fenbendazole showed 99,1 % efficacy against *Nematodirus* spp. at a dose of 2,0 mg/kg of AS and 100 % efficacy against other species of intestinal nematodes. Basic drug – substance of fenbendazole showed 97,7 % efficacy at the dose of 10 mg/kg and 17,8 % at the dose of 2,0 mg/kg. The efficacy of supramolecular complexes of albendazole and fenbendazole was 5 times higher with the efficacy of the basic drug. The study was supported by RSF project No 14-16-00026.

Keywords: Mechanochemical technology; supramolecular complexes; efficacy; anthelmintics

Specific antibody detection in dogs with filarial infections

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Abstract Content

Intra vitam diagnosis of canine *Dirofilaria* spp. is based on the presence and identification of microfilariae or the detection of blood circulating mature female *D. immitis* antigens. However, reliable diagnosis during the long prepatent period of >180 days is currently unfeasible.

Murine monoclonal antibodies (mAbs) were generated against adult male *D. immitis* E/S products. A sandwich-ELISA was developed using mAbs for on-plate purification of *D. immitis* somatic antigen to catch specific antibodies in dog sera. Sensitivity was determined with sera of 32 *D. immitis* and 15 *D. repens* patent infected dogs and specificity with 69 randomly selected canine sera from a Swiss filarial-free region. Sera of *D. repens* experimentally infected dogs were used to determine time course of antibody development. Cross-reactions were tested with canine sera infected with various helminth species.

Sensitivity of the ELISA (mAb Di36/1) for *D. immitis* infection was calculated to be 93.8% (95% CI: 79.2–99.2%), sensitivity on *D. repens*-infections reached 100% (95% CI: 81.9–100%). Specificity was determined to be 98.6% (95% CI: 92.2–100%). Further (cross-)reactions occurred with canine sera of other filarial infections, and occasionally with naturally but not experimentally helminth-infected dogs. Antibody development for *D. repens* infections occurred between 24-80 days post inoculation.

Previous ELISAs for canine antibody detection of *Dirofilaria* infections resulted in unsatisfactory sensitivities and specificities. The presented ELISA with its high diagnostic values may be applicable as a supplementary or alternative diagnostic tool for the documentation of contact rate and infection pressure in hitherto *Dirofilaria* non- or low-endemic regions.

Keywords: *Dirofilaria*, diagnostics, antibody detection, ELISA,

Case Report of *Cochliomyia hominivorax* (Diptera, Calliphoridae) larvae infestation in two dogs successfully treated with oral Afoxolaner (NexGard®).

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Abstract Content

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 their 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 dosed as close as possible to 2.5 mg/kg to treat screwworm on two dogs naturally infested with *Cochliomyia hominivorax* larvae in the municipality of Campinas, São Paulo state, Brazil. First case was a 5-year-old male German Shepherd mix weighing 35 Kg with a high infestation of larvae on an extensive ear lesion. This dog received approximately 2.75 mg/kg of afoxolaner once orally (one 3g soft chew containing 68mg + one 1.25g soft chew containing 28.3mg). The second case was a 2-year-old female Rottweiler mix weighing 36 Kg with a moderate infestation of larvae on a small round lesion on the lateral thoracic region. This female dog received 2.99 mg/kg of afoxolaner once orally (one 3g soft chew containing 68mg + one 1.25g soft chew containing 28.3mg + one half-gram soft chew containing 11.3mg). No adverse events were observed after treatment. Three hours after treatment some larvae started to die and in both cases all larvae were killed 24 hours post-treatment (i.e. 100% efficacy). 327 dead larvae were recovered from the lesion of the first case (51 identified as L2, 144 were L3, 132 not evaluated) and 44 larvae were recovered from the second case (11 were L2 and 33 identified as L3). Afoxolaner (NexGard®) at doses close to the minimum active dose of 2.5 mg/kg was rapidly efficacious for the treatment of two dogs with moderate to severe infestations with *Cochliomyia hominivorax* larvae, by eliminating all larvae within 24 hours after a single oral treatment.

Keywords: myiasis; new world screwworm; ectoparasite control

Efficacy of eprinomectin (EPRINEX MULTI) against sarcoptic mange and multi-host ixodid ticks in milking sheep

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Abstract Content

Eprinex-Multi, as the only endectocide approved for use in dairy ewes, provides an attractive opportunity to control ectoparasites which commonly occur during lactation. This study aimed to assess on a farm level the therapeutic/prophylactic efficacy of eprinomectin on *Sarcoptes scabiei* and multi-host ixodid ticks infestations in naturally infected sheep administered as pour-on at the dose of 1.0mg/kg BW. Mange study was conducted in 2 flocks enrolling a critical study group of 25 sheep per flock. Animals were treated on D0 and D14 and efficacy was assessed at D7, D14, D28 and D56, by evaluating the presence of mites and lesions severity (score 1-4). Tick study was conducted in 4 flocks enrolling 24 treated and 6 untreated animals per flock. Animals were treated on D0 and efficacy was assessed on D2, D4, D7 and D14. Tracer animals were used to monitor tick challenge and ticks species present. A reduction of 76.4% for the first flock and 92.3% for the second flock in mite counts at D7 and the absence of mites after D14 for both flocks was documented. Clinical observations confirmed full recovery of the lesions in both flocks by the end of the study. Ticks identified were adults and nymphs of *Haemaphysalis sulcata*, *Hae. punctata*, *Hyalomma excavatum* and *Rhipicephalus spp.* The efficacy of Eprinex-Multi varied considerably reaching up to 73.85% at SD4 for therapeutic efficacy and 85.97% at SD14 for persistent efficacy. This variability could be related to the level of on-going environmental challenge and management practices applied in each flock.

Keywords: Sarcoptes scabiei, ixodid ticks, dairy sheep, eprinomectin

***Trypanosoma evansi* was found as vesicle like form in the cytoplasm of nucleated cells of horses, Sukhothai, Thailand**

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Abstract Content

An outbreak of Surra in a horse farm, Thailand was reported. Clinical signs such as weakness, weight loss, high fever, lachrymation, imbalance movements and collapse of horse were reported. Eight out of twelve horses were found heavily positive for *Trypanosoma evansi* by Woo's method. *T. evansi* infection was also confirmed by thin blood smear (TBS), buffy coat smear (BCS), polymerase chain reaction (PCR) and mouse inoculation methods (MI). Of 7/11 horses were positive by TBS and 11/12 horses were positive by PCR. A strong phagocytic activity of the white blood cells was observed. *T. evansi* detected in the blood stream were found as round forms containing a nucleus and a kinetoplast, within vesicle like structure in WBC's cytoplasm. *Trypanosoma evansi* was also found extracellularly as coiled shapes with existence of a nucleus, kinetoplast with a flagellum lined around the cell, the aspect of amastigote form. Intracellular form that we have found was unusual and rare form. Therefore, this is the first report of *T. evansi* infection as vesicle like form in the cytoplasm of mononuclear cells in horses. Despite high level of parasitaemia observed and low dose treatment further on administered (1.75 mg/Kg Diminazene aceturate), most of the horses survived, which suggest that the phagocytosis activity was efficient enough to support control of the parasite in most of these horses. Whether, such "amastigotoid" forms are deleterious forms that will always be lysed, or, whether they might be dormant forms that could reinvade the blood later on could not be established.

Keywords: *Trypanosoma evansi*, Intracellular, Surra, Horse, phagocytosis, Vesicle like form

Real time PCR for quantitative and qualitative detection of *Leucocytozoon sabrazesi* in chicken

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Abstract Content

Avian leucocytozoonosis in Northern Thailand is mainly caused by *Leucocytozoon sabrazesi* infection that has impacts on productivity losses and economic consequences. However, effective chemotherapeutic drug for *L. sabrazesi* is still uncertain. Accurate quantitative diagnostic method is necessary for therapeutic development. Due to various limitations of conventional microscopic examination, a real time PCR technique (qPCR) was developed for quantitative and qualitative detection of *L. sabrazesi*. Specific oligonucleotide primers to mitochondrial *cytochrome b* gene of *L. sabrazesi* have been designed. To evaluate the analytical sensitivity and specificity of qPCR to detect *L. sabrazesi*, PCR were benchmarked. Comparison to PCR targeting the same gene, qPCR revealed 10 times lower of detection. The novel qPCR could amplify specifically to *L. sabrazesi*; whereas PCR cross-amplified *L. caulleryi* and *Plasmodium gallinaceum*. To estimate the agreement of qPCR and microscopic examination for qualitative detection of *L. sabrazesi*, the field trials of 404 blood samples was examined. Results showed the substantial agreement of qPCR versus microscopic examination (k=0.625, p<0.005; Epi Info™ v7.0). To estimate the agreement of qPCR and microscopic examination for quantitative detection of *L. sabrazesi*, the 50 positive blood samples was inspected. Linear regression analysis revealed that qPCR and microscopic examination were significantly positive related (R=0.84; p<0.001; GraphPad Prism®). In conclusion, this study provides a powerful qPCR technique for the quantitative measurement that may be applicable in the research of therapeutic development for *L. sabrazesi* infection. In addition, combining qPCR with microscopy can provide an accurate diagnostic tool for disease surveillance and control strategies.

Keywords: *Leucocytozoon sabrazesi*; real time PCR; PCR; cytochrome b; microscopic examination; quantitative; diagnosis

Poster Presentation – Parasite Diagnostics

Abstract No: 5640 (Poster# S2 - 102)

Development of a multiplex real time TaqMan assay for simultaneous detection of two major diarrhoeal parasites *Cryptosporidium* and *Giardia*

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Abstract Content

Both *Cryptosporidium* spp and *Giardia* spp are known to cause diarrhoea in both human and animals, which are also important for environmental quality monitoring. An accurate diagnosis of aetiologies as in case of concurrent infections is very crucial in taking treatment management decisions in both hospital as well as community settings. Presently there is a lack of molecular diagnostics and so also multiplex assays for routine diagnosis of these neglected diseases particularly in resource poor areas of the developing world. This study aimed to develop a sensitive and specific multiplex qPCR assay to detect both parasites simultaneously, so that an accurate and rapid diagnosis of either mono or dual infection could be available. Specific primers and TaqMan probes were designed for *Cryptosporidium* 18S rRNA and *Giardia* spp GDH genes. For each gene, the qPCR cycle threshold values of dilution points were plotted as a function of the logarithm of the input DNA quantity (10^1 to 10^{-5} ng/ μ l). Detection limit was calculated to be 0.0004 ng/ μ l for both assays in contrast to 0.4 ng/ μ l as in case of conventional PCR. Diagnostic sensitivity and specificity of the real time PCR assays were calculated to be 100%. This multiplex TaqMan assay was successful in a single step detection of the said two pathogens which is advantageous over traditional detection techniques being in practice till date. Future development should be screening application of this multiplex assay in routine as well as in epidemiological detection possibly with additional pathogen targets for their simultaneous detection.

Keywords: Diarrhoea, TaqMan, qPCR, Cryptosporidium, Giardia

Poster Presentation – Parasitic Disease & Animal Welfare

Abstract No: 3988 (Poster# S1 - 57)

Coccidia infections in Danish farmed mink

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Abstract Content

Although Danish farmed mink are frequently infected with Coccidia, knowledge of factors affecting the infection is scarce. Thus, we studied age, geographical and season-related factors affecting coccidia prevalence. Unsporulated oocysts excretion was quantified microscopically (n=4142) every 7-14th day (April-October 2016) from bitches and cups on 30 farms (n=335 mink) from South- or North Jutland, or Zealand. Minimum once, 60.9% (n=204) mink excreted *Eimeria*, 56.7% (n=190) *Isospora* and 20.9% (n=70) excreted both coccidia. Positive mink were identified on all farms. *Eimeria* prevalence was higher on the Zealand farms (25.4±2.2%, P<0.0001) compared to South- and North Jutland farms (5.4±2.9%; 7.5±4.1%). *Isospora* prevalence was similar regardless of farm locality (12.2±2.9%, 11.8±3.5%, 9.2±7.1%). *Eimeria* prevalence peaked in June-July (12.6%-24.9%), while *Isospora* prevalence peaked in July-August (12.1%-27.6%). More cups (19.5%) than bitches (4.6%) were *Isospora* positive, while *Eimeria* prevalence was similar for cups (15.7%) and bitches (10.5%). For cups, *Eimeria* prevalence peaked when cups were 7-11 weeks old and again when 18-24 weeks old. *Isospora* prevalence peaked in cups 13-15 weeks old. Three *Eimeria* types were characterized by size and wall thickness (unverified by PCR); A, B and C. Types B and C (40.9%, 39.8%) were more prevalent than A (19.3%). Bitches were primarily infected with type B (50.4%), while type C (48.0%) predominated in cups. Type B infections dominated in mink from Zealand (56.5±13.7%), while mink from Jutland were primarily infected with type C (55.6±28.6%; 81.9±19.4%). Farmed mink showed high coccidia prevalence with seasonal- and age-related *Isospora* prevalence, and seasonal- and geographical-related *Eimeria* prevalence.

Keywords: Farmed mink; Coccidia; prevalence; Eimeria; Isospora;

Prevalence and risk factors of camel hydatidosis in North Darfur State, Sudan

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Abstract Content

A cross-sectional epidemiological study was conducted to estimate the prevalence and investigate the risk factors of hydatidosis in camels slaughtered at Elfashir Abattoir in western Sudan. Total of 387 camel carcasses investigated for hydatidosis during November 2010 to February 2011 and 213 camels found to be infected with hydatid cysts (55%). One hundred and twenty three (57.7%) camels were infected in lungs, 29 (13.6%) in livers, 5 (2.3%) in spleens, 38 (17.8%) in both lungs and the livers, 14 (6.6%) in lungs and spleens, 2 (1%) in livers and spleens and 2 (1%) in the three organs. Out of 966 hydatid cysts observed, 408 cysts (42.2%) were viable, 197 (20.4%) were not viable, 157 (16.3%) were sterile and 204 (21.1%) were calcified. The classification of 966 hydatid cysts according to their fertility status showed that from 183 (19%) cysts in the liver, 100 (54.6%) were viable, 28 (15.3%) non-viable, 25 (13.7%) sterile and 30 (16.4%) were calcified. While from 751 (77.7%) cysts in lungs, 288 (38.3%) were viable, 162 (21.5%) were non-viable, 129 (17.2%) were sterile and 172 (23%) were calcified. From 32 cysts (3.3%) in spleens, 20 (62.5%) were viable, 7 (21.9%) were non-viable, 3 (9.4%) were sterile and 2 (6.2%) were calcified. Age and presence of other diseases as risk factors were found significantly associated with hydatidosis with a p-value of 0.002 and 0.001, respectively. The potential risk factors as sex, body condition, origin and colour were found non significant.

Keywords: Hydatidosis, Prevalence, Dromedary Camels, North Darfur, Sudan.

Abstract No: 5518 (Poster# S1 - 59)

Early diagnosis of *Toxocara vitulorum* among calves in Turkey dairy farms by detection of specific antibodies by using Western Blot technique

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Abstract Content

Toxocara vitulorum causes serious problems in Turkey. This study was aimed to prepare two antigens, Larva extract (Ex) and Peri-enteric (Pe) for Bovine Toxocariosis serodiagnosis. Feces collected from a total of 2393 calves 0-6 months in 22 provinces. Fulleborn Flotation Technique using saturated sodium chloride solution used for fecal examinations. Twenty-eight calves found positive with *T. vitulorum* (1,17). McMaster technique performed for parasite egg count per gram of feces and maximum (epg) found 10250. Feces from 2393 calves collected and tested. Both mature parasites and feces collected from positive calves drenching anthelmintics and eggs incubated at room temperature for parasitic larval recovery within 17 -40 days. Peri-enteric fluid recovered from mature parasites. Ex antigen from larva and Pe antigen from peri-enteric fluids prepared. Protein concentration measured by spectrophotometer. Different protein bands with molecular weights of 14-150 kDa for Ex antigen and 12-141 kDa for Pe antigen by SDS-PAGE electrophoresis separation. Negative sera did not show bands with Ex antigen in immuno-blotting analysis, while positive sera showed protein bands of 14, 35, 56, 63, 68 and 94 kDa molecular weights. Also negative sera did not react with Pe antigen, while positive sera showed protein bands to 96 and 110 kDa. Purification of *Toxocara vitulorum* larval extracts is one of the promising areas of parasitic research in future. Possibilities of antigens extraction from the mature *Toxocara vitulorum* for a perfect and effective antigenic determination for an accurate and effective diagnosis and control for Bovine Toxocariosis.

The magic and mystery of Galectin-11 gene variants in parasite control

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Abstract Content

To understand galectin's role in innate immunity, we take advantage of our model system that we have investigating for several years. Galectin-11 (LGALS-11) is specifically expressed by epithelial cells in ruminants upon parasitic infection as a part innate immune response. Many parasitic diseases such as gastrointestinal strongylid nematode (GIN) worms have a destructive long-term impact on animal health and is estimated to cause billions dollars of losses per annum due to poor productivity and death of ruminants. X-ray crystallographic and DNA sequencing studies of LGALS-11 identified amino acid variations in residues, they play important role in function of LGALS-11. Quaternary solution structure analysis confirmed that the genetic substitution in LGALS-11 affects the dimer and tetramer formation. We further observed that this natural genetic variation in the dimer/tetramer formation property resulted in differential effects on the parasitic larvae, of *Haemonchus contortus* *in vitro*. Genetic variant-1 (tetramer) limits motility by paralysis and larval development compared to variant-2 (monomer/dimer). Further studies have focused on which parasitic glycoproteins are interaction partner of LGALS-11. In addition, we have now started to investigate the role LGALS-11 in self-recognition of glycans in the mucosal layer of the stomach. It is clear that LGALS-11 play important role in host-parasite interactions. Due to the increased use of the limited number of anti-parasitic drugs to control these infections have lead to drug resistance in most of these species of GIN worms. Results of this study could lead to the identification of vaccine antigens or breeding animals with parasitic resistance.

Keywords: galectin-11; anti-parasitic activity; oligomerization; carbohydrate specificity; X-ray crystallography; gastrointestinal parasite

**Prevalence of gastrointestinal parasites and confirmation of the existence of
Spirocerca lupi in dogs in Vietnam**

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Abstract Content

The aim of this study is to confirm the prevalence of gastrointestinal parasite in dogs in Ha Noi and Bac Ninh, Vietnam. Gastrointestinal have been incriminated as the major impediment to dog health worldwide (Smith, 1991), including *Spirocerca lupi* esophageal strictures and cause sudden death in dogs. 145 dog intestine samples were collected and examined to check the presence of parasites. The prevalence of gastrointestinal parasites were very high including *Ancylostoma* spp. (74,48%), follows by *Toxocara* spp. (13,79%), and 4,83% of dogs was infected with *Spirocerca lupi*, the worm cause haemothorax and acute death. It was observed oesophagous nodular lesions of dogs infected with *Spirocerca lupi*. In each nodular, about 13-30 worms was appeared. Histopathology showed parasites section surrounded by inflammatory cells (including neutrophils, eosinophils and lymphoplasmacytic cells), infiltration of mononuclear cells and replacement of the elastic tissue with collagen, fibrocytes and necrotic cells. The infection rate of gastrointestinal parasites was very high in dog. Early detection of *Spirocerca lupi* infection will be helpful for treatment in such endemic areas.

Keywords: Gastrointestinal, histopathological, Spirocerca lupi, dog

Importance of egg per gram threshold determination in goats from Mexican plateau: To deworm or not to deworm?

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Abstract Content

Previous studies show that 86% of Mexican use products against internal parasites and 71% against external parasites. In contrast, only 2% mention that parasites are a problem. In the Mexican Plateau, deworming is a practice by imitation or custom. The threshold of nematode eggs per gram of feces (HPG) to apply an antiparasitic has been considered from 500 to 1000 HPG, without studies supporting this. The present work was carried out in the CEIEPAA FMVZ UNAM herd with 300 goats in the Mexican plateau (Querétaro, Mexico) at an altitude of 1880 m in a semi-dry, temperate (BSh) climate and within 18 °C temperature average. Stool samples were obtained and the amount (HPG) of each individual was determined; coprocultures were also performed. Considering the phenomenon of overdispersion of the excretion of HPG in goats, it was determined that in the period of greater humidity and heat (August-September) the threshold of HPG in goats from the plateau is 2000 HPG, since 26% of the animals are eliminating more than this threshold. On the other hand, in the period of lower humidity and cold (December-February) the threshold is 500 HPG with 21% of the animals eliminating more than this amount of HPG. The species of nematodes present are *Haemonchus*, *Trichostrongylus*, *Teladorsagia*, *Cooperia*, *Oesophagostomum* and *Chabertia*. Deworming depends on the therapeutic threshold of HPG, the percentage of overdispersion, the parasites present, the time of year.

Keywords: goats, nematodes, egg per gram threshold, deworming

**Distribution of gastrointestinal nematodes egg counts in sheep from Mexico
Centersouth and its implications of parasitic integrated control**

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Abstract Content

The study was carried out during fourteen months (September 2015 to November 2016) with more than 300 Dorset, Suffolk and crossbred sheep, from the herd CEIEPO FMVZ UNAM (Morelos, Mexico) at an altitude of 3250m, in a temperate (Cwb) climate and within 12°C temperature average. Stool samples were collected monthly and the amount of gastrointestinal nematode (GIN) eggs per gram of feces (EPG) was determined. The year was divided into 3 seasons: season 1 characterized by warm-rainy climate (S1); season 2 with low humidity and cold (S2); and season 3 with dry temperate climate (S3). In S1, of the total lambs (n = 343), 81% had 0 to 500 HPG; In S2 (n = 329), 83% had 0 to 500 HPG; In S3 (n = 261), 86% also had 0 to 500 HPG. The above agrees with the phenomenon of overdispersion of NGI infection in sheep, since few actually have high loads. A selective treatment directed against NGI can be performed since in the three seasons the percentage of animals with > 500 HPG was 19%, 17% and 14% respectively. This phenomenon of overdispersion over the fourteen months of this study also allowed us to suggest that the HPG threshold to be used may be > 500, which is typical of the region with its particular climatological and breed conditions. Considering climate and own thresholds are fundamental tools for parasitic integrated control.

Keywords: sheep, nematode, overdispersion phenomenon, treshold epg, parasitic integrated control

Tick-borne haemoparasite occurrence in Eastern Rock Sengi (*Elephantulus myurus*) of South Africa

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Abstract Content

Sengis, or elephant shrews, of the order Macroscelidea, are small insectivorous mammals endemic to Africa. Several studies have shown that sengis are parasitised by large numbers of ixodid ticks. Previous studies also provided strong evidence that the eastern rock sengi (*Elephantulus myurus*) may be a natural reservoir hosts of *Anaplasma bovis*, a rickettsial pathogen of cattle. Despite the importance of sengis as hosts of immature ticks and the association of these tick species to known pathogens, limited information is available on the role of *E. myurus* as a reservoir of tick-borne pathogens. The aim of the study was, therefore, to determine the tick-borne haemoparasite diversity in eastern rock sengi of South Africa by screening 66 blood samples for the presence of *Theileria*, *Babesia*, *Ehrlichia* and *Anaplasma* spp. using the Reverse Line Blot (RLB) hybridization assay. PCR products hybridized with the *Theileria/Babesia* genus-specific probe in 1.5% (n=1) of the samples and 33.3% (n=22) hybridized with the *Ehrlichia/Anaplasma* genus-specific probe. The PCR products failed to hybridize with any species-specific probes. This could suggest the presence of a novel species or variant of a species. A total of 31 (47%) of the blood samples tested negative or below the level of detection of the assay. The parasite 16S rDNA of selected samples was subsequently amplified, cloned and sequenced; results confirmed the presence of *A. bovis* DNA. An RLB probe for the specific detection of the *A. bovis*-like strain has since been developed.

Keywords: *Anaplasma bovis*; Reverse Line Blot hybridization assay; *Elephantulus myurus*; Tick-borne diseases; Haemoparasites

Experimental *Ancylostoma ceylanicum* infection in dogs and cats: Infection rate, worm length and fecundity

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Abstract Content

Ancylostoma ceylanicum (AC) is a common hookworm parasitizing dogs and cats in southeastern Asia which readily establishes in humans. Deliberate infections in the course of anthelmintic studies allowed to study some biologic parameters of the infection in dogs and cats as there is only limited comparative data. Eight (5M, 3F) dogs and eight (4M, 4F) cats, ~7-10 months old and nematode-naïve, were inoculated with ~500 or ~300 larvae, respectively, of a canine-source AC from Thailand which has been passaged in cats for three years before inoculation. Five weeks after inoculation, fecal egg counts (EPG) were established, and hookworms were recovered from the animals, counted by sex and measured (total length, width, spicule length). Infection rate was significantly ($p < 0.05$) higher in dogs than in cats (64.95% vs. 32.38%) and male and female AC recovered from dogs had a larger size (length, width, spicule length) than worms recovered from cats ($p < 0.05$). Male-to-female ratio, fecundity (EPG divided by female worm burden) and number of eggs produced per female per day did not differ significantly between dogs and cats. Male hosts tended to have higher infection rate and female AC burden than female hosts (61.61% vs. 32.02%, $p = 0.071$; 135.78 vs. 74.43, $p = 0.080$). Significant host sex × species interactions were recorded for infection rate, total AC count, and AC size. In conclusion, results indicated that both dogs and cats are highly susceptible to AC and that AC infection is potentially subject to regulation by host species (host size?), host sex and density-dependent processes.

Keywords: Ancylostoma ceylanicum, dog, cat, biology

Selection and evaluation of endogenous control genes for real-time polymerase chain reaction gene expression studies in *Theileria parva*

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Abstract Content

The real-time PCR (qPCR) technique is considered to be the most accurate and most reliable for measuring gene expression based on its sensitivity, real time detection of reaction progress, speed of analysis and precise measurement of the target material in the sample. In fact, qPCR is the preferred and ideal method when dealing with a gene of extremely low expression levels. However, the reliability of any relative qPCR experiment depends on an invariant endogenous control for normalization and validation of results. Several genes have previously been used as endogenous control genes in expression studies of the tick-borne hemoparasite, *Theileria parva*, the causative agent of the fatal cattle theileriosis in Africa. However, there is no record of the evaluation of these genes in literature. Thus, candidate housekeeping genes for this parasite were evaluated in this study. *Theileria parva* housekeeping genes that were evaluated using qPCR included β -actin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 28S rRNA, cytochrome b and fructose-2.6-bisphosphatase (F6P). RefFinder, a web-based tool that integrates the currently available major computational programs, geNorm, Normfinder, BestKeeper, and the comparative $\Delta\Delta C_t$ method, was employed to analyze the expression stability of the candidate reference genes and rank them accordingly. This comprehensive analysis revealed varied expression of cytochrome b, F6P and GAPDH genes between two *T. parva* isolates investigated, while, 28S rRNA and β -actin were the most stable. Therefore, 28S rRNA and β -actin genes can be considered suitable candidates to be used for normalization of qPCR results for gene expression studies in *T. parva*.

Keywords: Theileria parva; gene expression; endogenous control genes; housekeeping genes; qPCR

Blood parasitic surveillance of cattle in Sub-district (Situjuah Limo Nagari and Akabiluru) of Lima Puluh Kota District, West Sumatera Province, Indonesia, 2015

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Abstract Content

Blood parasitic Diseases is endemic diseases, especially in tropical and subtropical regions around the world. Blood parasitic Diseases are important in Indonesia among others anaplasmosis, babesiosis and Theileriosis. Blood parasitic diseases can economic impact on the livestock sector businesses because it can harm of cattle. The survey aimed to determine the prevalence of blood parasites, as well as factors that affect the incidence of blood parasites in sub-districts (Akabiluru and Situjuah Limo Nagari). Total 100 head of cattle were selected using simple random sampling proportionally. Blood samples were collected for detection of Parasitic Diseases by looking in a microscope. Data analysis included: univariate and bivariate analysis with chi-square (χ^2) and odds ratio (OR). The results of the prevalence was 97%, the incidence of blood parasites due to infection by Babesia sp. (11%), Theileria sp.(89%), and Anaplasma sp. (50%). Factors associated increase the incidence of blood parasites are typology breeder, breeding experience, feed, water availability, frequency of bathing cows, the frequency response vector, septic tank, the type of flooring, feeding systems, knowledge of blood parasites, and how the use of insecticides. The results of this survey can be concluded that the incidence of blood parasites in beef cattle is caused by multifactorial.

Keywords: Blood parasite, the prevalence, risk factors

Risk factors associated with *Schistosoma spindale* infection in Malaysian cattle

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Abstract Content

To date, there is limited information on *Schistosoma spindale* infection among cattle in Malaysia. A total of 266 rectal fecal samples were collected from six farms. Overall infection rate of *S. spindale* was 6% (16 of 266). This trematode was more likely to co-occur with other gastro-intestinal parasites (i.e., *Dicrocoelium* spp., *Paramphistomum* spp., strongyle, *Eimeria* spp. and *Entamoeba* spp.). Multivariate analysis confirmed that among the cattle in Malaysia, the age (cattle with two year old and higher: OR=21; 95% CI=2.48-179.44; p<0.05) and weight (weighing 200kg and lower: OR=17; 95% CI=3.38-87.19; p<0.05) were risk factors for *S. spindale* infection among Malaysian cattle. This work is important to fill the gaps of the prevalence and associated risk factors of *S. spindale* in cattle.

Keywords: Schistosoma spindale; Malaysian cattle

Occurrence of human infective *Cryptosporidium* and *Giardia* in urban rodents in Kuala Lumpur, Malaysia

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Abstract Content

The role of rodents in the zoonotic transmission of *Cryptosporidium* and *Giardia* in Malaysia remains unknown. A total of 137 faecal samples of urban rodents were collected from two areas in Kuala Lumpur, the capital city of Malaysia. Genotyping of 18S rRNA of *Cryptosporidium* spp. on 134 samples revealed 29.8% of infection rate comprising *Cryptosporidium* spp. (15 isolates), followed by *Cryptosporidium* rat genotype II (13 isolates), *C. muris* (7 isolates), rat genotype III (4 isolates), and *C. meleagridis* (1 isolate). In addition, 17.1% of 134 studied samples were positive to *C. parvum* subgenotype IIa through amplification of the gp60 gene. With regards to *Giardia duodenalis* detection on tpi gene, only one sample was positive, inferring the presence of *G. duodenalis* genotype B. The finding of this study provides important implications for infectious disease control in the country.

Keywords: Cryptosporidium; Giardia; rodents

Isolation and characterisation of *Eimeria tenella* isolated from a broiler farm in Malaysia

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Abstract Content

Eimeria tenella is considered one of the most important species of *Eimeria* parasites that cause avian coccidiosis due to its widespread and highly pathogenic traits. Currently, the main control method is through preventative chemotherapy using anticoccidial drugs which is subjected to development of resistance. Vaccination is a viable option but successful development of cost-effective anticoccidial vaccines requires comprehensive knowledge on the *Eimeria* species present in the local population particularly the pathogenic *E. tenella*. In this study, an *E. tenella* population, EtSik was isolated from a local broiler farm and characterised. Parasites collected from the faeces samples were purified and propagated in specific pathogen-free chickens housed in a purposed-built coccidia-free environment chicken facility. Morphological characterisation showed that the mean dimension of the EtSik population oocysts is 23.65 (± 1.99) μm x 19.86 (± 1.81) μm . Polymerase chain reaction with *E. tenella* species specific primers of the EtSik genomic DNA produced a band size which correlates with the expected product size. Analyses of the genetic diversity of the EtSik strain was performed using the internal transcribed spacer 1 (ITS-1) gene which was amplified, cloned and fully sequenced. Comparison of the EtSik ITS-1 sequence with selected ITS-1 sequences available in the GenBank database revealed the presence of a total of 38 single nucleotide polymorphisms and 5 insertion/deletion sites. Sequence identity with available sequences ranged from 95-99%. Further characterisation of this *E. tenella* population will provide useful information required in developing more efficient strategies for the control of avian coccidiosis.

Keywords: *Eimeria*; protozoan parasites; coccidiosis; poultry production; food security

A survey of endoparasite and ectoparasite infections of wild rats caught in areas of Ipoh and Kuala Lumpur, Malaysia

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Abstract Content

A survey of 95 wild rats were captured from various locations was conducted to determine the diversity and distribution of ectoparasites and endoparasites infesting wild rat population around Ipoh and Kuala Lumpur. The rodents captured were *Rattus norvegicus* and post mortem was carried out immediately, with skin and organs examined for parasite infection. Ectoparasites recovered were blood sucking louse (*Polyplax spinulosa*) and mites (*Myocoptes musculus*). Endoparasites recovered were nematodes (*Syphacis muris*, *Trichuris* sp., *Strongyloides* sp. and *Strongly*) and three species of intestinal protozoan parasite (*Blastocystis* sp., *Trichomonas* sp., and *Coccidia*). Low diversity of ecto and endoparasites were observed infecting wild rats population caught in Ipoh as compared to Kuala Lumpur.

Keywords: wild rats, ecto and endo parasites

Geographical distribution of parasitic diseases in Perak from 2012 to 2016

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Abstract Content

Establishing the current status and distribution of parasitic diseases is essential for developing and implementing parasite control. Although Malaysia is known to have a moderate prevalence of parasitic infection, a precise estimate of the total diseases burden has not been fully described. In this study, Geographical Information System (GIS) used to collate and map the distribution of the parasitic diseases such as coccidiosis, fascioliasis, helminthiasis, blood protozoa and trypanosoma specifically in the state of Perak. Data from sample submission form based on the cases and samples received in Veterinary Research Institute (VRI), Ipoh from the year 2012 until 2016 was collected and assigned according to the districts to generate a distribution map of parasitic diseases. The results showed that helminthiasis (56.3%) and coccidiosis (26.7%) occurred in the most districts in Perak and it is observed that the highest number of parasitic infection was in goat and most cases were reported from the district of Kuala Kangsar. Details on parasitic diseases involved the information on infected species, breed and sex are discussed. Further study on data related to the ecological and climatic features to parasitic diseases can support the understanding on the relationships of parasite exposures and is expected to improve the disease control activities in the near future.

Keywords: parasitic diseases, gis, disease control

Results of a pilot study regarding *Dermanyssus gallinae* in the Greek laying hen industry

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Abstract Content

Dermanyssus gallinae, also known as the poultry red mite (PRM), is a blood sucking ectoparasite, widespread in many parts of the world. PRM poses an increasing economic threat, especially for the laying hen industry, due to its haematophagous activity. It is responsible for reduction of the egg production (quality and quantity) and susceptibility of the poultry health status. Moreover, PRM has public health implications. The aim of this pilot study was to investigate the prevalence of *D. gallinae* infestation of the Greek laying hen industry and provide information on the importance of PRM control. In our study, 12 Greek laying hen farms were visited in Central Macedonia (Northern Greece) and 5 cardboard traps (15X40cm) were placed in each farm in different sites, including beneath feed troughs, inside cage fittings and fastening clips, under egg conveyer belts and under manure belts. The traps (60 in total) were examined for the presence (counting and identification) of *D. gallinae*. According to our results, all farms were infected with this ectoparasite (100% prevalence). The average number (\pm SD) of mites per trap was 356 ± 26 . It is, therefore, evident that PRM is a major problem for the Greek laying hen industry and coordinated action must be taken. An increase of *D. gallinae* prevalence rates may have an epidemiological impact on several animal and human diseases, as PRM can be a potential vector for several pathogens (One Health).

Keywords: Dermanyssus gallinae, poultry, Greece

Poster Presentation – Poultry & Swine

Abstract No: 4246 (Poster# S1 - 65)

Seasonal variation of *Leucocytozoon* infection pattern in backyard chickens in Chiang Mai Province, Thailand

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Abstract Content

Leucocytozoon parasites infect many species of avian hosts, including backyard chickens, and are accompanied by heavy economic loss on the poultry industry in Thailand. *In this study*, we investigated monthly dynamic changes of *Leucocytozoon parasites* infections from backyard chickens in Chiang Mai province of Thailand, over one year, by NESTED PCR based method after testing PCR primer pairs based on parasite mitochondrial cytochrome b (*cytb*). Consequently, the 476 blood samples from backyard chickens in 15 districts of Chiang Mai province were collected during October 2015 to September 2016. The prevalence of blood parasites in backyard chickens founded in the farm and in the sample was found to be 95.93% (118/123) and 72.27% (344/476) respectively. The prevalence of parasite founded in dry (October-April) and wet season (May-September) are 78.57% and 68.52%, respectively. The infection rates were investigated each month for a full year and showed that backyard chickens were infected with *Leucozytozoon* year-round, with peak infection in late October. The higher infection rates in dry season were consistent with increasing temperature and surrounding environmental factors. Our data suggest that transmission of the parasites to backyard chickens is likely to occur year-round on free-range farms as long as insect vectors are available. In summarize, the *Leucocytozoon* infection in backyard chickens significantly relate to the seasons ($P < 0.001$). Our data will provide important information for controlling parasite transmission and disease management in backyard chickens of northern Thailand.

Keywords: Chiang Mai ; backyard chickens ; *Leucocytozoon* ; season

The effects of anticoccidial efficacy of PowerFeel™

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Abstract Content

PowerFeel™ (PF) from NEL BIOTECH is a new product that is based in a new patented compound, which possesses unique qualities as an ionized alkali mineral complex, that promote vital biological processes as well as environmental pollution prevention and pollution control. Effects of PowerFeel™ reduce animal waste odor emission, reduce occurrence rate of various diseases, improve feed efficiency rate, improve carcass quality, and activate immune cell. One hundred and ten 1-day-old broiler chickens (ArborAcre) were divided into 9 experimental groups such as, Control, Infected Control, Salinomycin Control, Salinomycin Infected, Salinomycin PF Control, Salinomycin PF Infected, Halofuginon Control, Halofuginon Infected, Halofuginone PF, Halofuginon PF Infected groups. All chickens were challenged with 3×10^5 oocysts /chicken of *Eimeria tenella* at 31-day-old. All experimental chickens were supplied the feeds contained anticoccidial feed additives such as Salinomycin, Halofuginon and PowerFeel™ (PF) from NEL BIOTECH during all experimental periods. The survival rate, body weight gain, OPG (oocysts per gram), feed conversion rate, oocyst index, lesion score and anticoccidial index were investigated and calculated in the all experimental groups. Correlation between body weight gains, lesion scores and OPG of individual chicks was lack in this results, Analyzing the data of the survival rates, lesion scores, body weight gains and anticoccidial index, The PowerFeel™ had a good anticoccidial effect.

Keywords: PowerFeel™ (PF), anticoccidial efficacy, Eimeria tenella, Salinomycin, Halofuginon

The acaricidal speed of kill of orally administered fluralaner against poultry red mites (*Dermanyssus gallinae*) on laying hens and its impact on mite reproduction

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Abstract Content

Dermanyssus gallinae, the poultry red mite, is a haematophagous ectoparasite that has a rapid proliferation rate with a negative impact on birds' health, welfare and productivity resulting in severe economic consequences for poultry farmers. Fluralaner, a novel systemic ectoparasiticide, was evaluated for its potential effect on mite vitality and reproduction after oral administration to laying hens. One group (n=8) was orally treated with fluralaner by gavage (0.5 mg/kg twice 7 days apart), the other group received no treatment. Each group was infested with *D. gallinae* at various days after the initial administration. Engorged mites were collected 2.5 hours after infestation and incubated. Mites were assessed for vitality at 4, 8, 12, and 24 hours after each infestation. Tubes containing eggs and/or living mites were incubated another 8 days for assessment of mite reproductive capacity. Fluralaner demonstrated a fast speed of kill (98.7% - 100%) within 4 hours post infestation for 12 days after treatment initiation. At 15 days after treatment initiation, 100% efficacy was achieved within 24 hours post infestation, and no mite oviposition occurred during this period. Nineteen days after treatment initiation, the mites' ability to generate nymphs was reduced by 90.8%, which decreased at later infestations. Fluralaner administered orally to hens, twice 7 days apart, provides efficacy against experimental poultry red mite infestation for at least 2 weeks. The demonstrated rapid speed of kill results in substantial depletion of the mites' oviposition and suggests that fluralaner can be an effective tool in the control of *D. gallinae*.

Keywords: fluralaner; Dermanyssus; poultry red mite; speed of kill; efficacy

***Eimeria tenella* oocyst excretion and riboflavin intake in infected chickens**

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Abstract Content

The effect of riboflavin supplement on the oocysts output in the chickens experimentally infected with 1,000 sporulated oocysts of *Eimeria tenella* was evaluated. Seven-day-old chickens were divided into 5 groups of 22 chickens per group. One group was inoculated with *E. tenella* oocysts and another was not. These 2 groups were given a basal diet and served as positive and negative control, respectively. The 3 remaining groups were inoculated with *E. tenella* and then administered a basal diet supplemented with riboflavin at the level of 0.8 g·kg⁻¹ feed or with the anti-coccidial drug amprolium at 0.125 g·kg⁻¹ feed or with riboflavin (0.8 g·kg⁻¹ feed) plus amprolium (0.125 g·kg⁻¹ feed). The amprolium dose used was that of the preventive dose. Throughout the experimental period from day 1 to day 7.5, parameters such as mortality, bloody diarrhea and oocysts output were recorded. Bloody diarrhea was observed at 5 days after infection in all challenge groups except for the amprolium group and the riboflavin plus amprolium group. Mortality in positive control group was 4.54% but 0% in the remaining group. The number of oocysts per bird in the riboflavin group were significantly higher than the positive control group (p<0.05). However, there was no significant difference in oocyst output between the amprolium group and riboflavin plus amprolium group (p>0.05). In conclusion, addition of riboflavin at the level of 0.8 g·kg⁻¹ to basal diet can increase oocysts output in *E. tenella* infected chicken but has no effect on the efficacy of the anti-coccidial drug.

Keywords: Eimeria tenella; riboflavin; chicken; amprolium; efficacy

Intestinal helminthiasis in chicken (*Gallus domesticus*): A case report

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Abstract Content

Intestinal parasites (helminths) are a very common problem in poultry. The presence of a few parasites usually do not cause any problems to the chicken. However, large numbers of parasites have been found to cause a devastating effect on the growth, egg production and overall health of the chicken. A 4-month-old chicken carcass had been presented to Pathology Section of Veterinary Research Institute (VRI) Ipoh for post mortem examination with the complaint of inappetance, bloody diarrhea, inactive and saliva drooling from its beak. Post mortem findings are such, very pale in general when the carcass was opened. Abnormalities were seen in all vital organs that includes the heart, lung, liver, kidney and spleen. The intestines were observed to be pale, inflamed and enlarged. Slight haemorrhages and necrosis were at the outer serosal wall. Further findings the intestines revealed that it had greenish coloured watery diarrhea. No haemorrhages were observed from the inside serosal wall of intestines. A live helminth approximately about 4cm in length was observed in the intestines. The organs and helminth samples were collected and submitted to laboratories for further diagnosis. Parasitology laboratory examination revealed that the chicken was positive for *Ascaridia galli* (roundworm) and *Raillietina* spp. (tapeworm) infection. Intestinal helminthiasis was made as the final diagnosis based on the parasitology result as both the *Ascaridia galli* (roundworm) and *Raillietina* spp. (tapeworm) were found and identified in the intestines.

**Immunogenicity and protective efficacy of coccidial common antigen
Glyceraldehyde 3-phosphate Dehydrogenase (GAPDH) against challenge with
three *Eimeria* species**

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Abstract Content

GAPDH is an immunogenic common antigen among *Eimeria tenella*, *E. acervulina* and *E. maxima* identified in our previous study. In this study, genes of GAPDH were cloned from *E. acervulina* and *E. maxima* and named as *EaGAPDH* and *EmGAPDH* respectively. The immunogenicity of recombinant proteins of *EaGAPDH* and *EmGAPDH* were analyzed by Western blot using anti-*E. acervulina* and anti-*E. maxima* chicken sera respectively. The transcription and expression of pVAX-*EaGAPDH* and pVAX-*EmGAPDH* in the injected muscles were detected by reverse transcription PCR and Western blot respectively. Changes of T lymphocytes subpopulation, cytokines production and GAPDH-specific antibody induced by pVAX-*EaGAPDH* and pVAX-*EmGAPDH* were determined using flow cytometry, quantitative real-time PCR and ELISA respectively. Finally, protective efficacies of pVAX-*EaGAPDH* and pVAX-*EmGAPDH* were evaluated. The results revealed that the recombinant proteins of *EaGAPDH* and *EmGAPDH* reacted with anti-*E. acervulina* and anti-*E. maxima* chicken sera respectively. *EaGAPDH* genes were successfully transcribed and expressed in the injected muscles. Vaccination with pVAX-*EaGAPDH* and pVAX-*EmGAPDH* significantly increased the proportion of CD4⁺ and CD8⁺ T lymphocytes and the productions of IFN- γ , IL-2, IL-4 TNFSF15, IL-17 and TGF- β 4, and induced high level of IgG antibody compared to pVAX1 and PBS controls ($p < 0.05$). The vaccination increased the weight gains, decreased the oocysts outputs, alleviate the enteric lesions compared to pVAX1 and PBS controls ($p < 0.05$), and induced moderate ACIs. In conclusion, coccidial common antigen of GAPDH induced significant humoral and cellular immune response and effective protection against *E. tenella*, *E. acervulina*, *E. maxima* and mixed infection of the 3 *Eimeria* species.

Keywords: Chicken coccidian; common antigen; GAPDH; mixed infection; protection

Prevalence and burden of gastro-intestinal helminths in small scale chicken flocks in the Mekong Delta of Vietnam

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Abstract Content

In the Mekong Delta of Vietnam and elsewhere in Southeast Asia farming of traditional, small-scale chicken flocks is common. In these systems often birds are not fully confined, and standards of hygiene and disinfection are often deficient, leading to severe exposure to parasitic infestations. These infestations may cause considerable economic losses to the producers due to decreased feed conversion and weight loss, as well as increased susceptibility to disease. The burden of helminth infestations in small-scale chicken flocks in the Mekong Delta is currently unknown. AIMS: We aimed to investigate the prevalence and burden of infestation of gastrointestinal helminths in small-scale (<2,000) meat chicken flocks in Dong Thap province (Mekong Delta of Vietnam). Farms recruited for the ViParc project (www.viparc.org) were visited between March and August 2017 at the end of the production period (just before sale for slaughter). These farms represent a random selection of chicken farms in the area. The gastrointestinal tract of chickens at time of sale was systematically dissected using a sieve and a binocular microscope. All visible helminths were dissected and placed into dedicated Falcon tubes (proventriculum/gizzard, small intestine and caeca), counted and identified based on their morphology. The relationship between parasitic burdens, levels of disease/mortality and weight conversion was investigated. Results from the study will be presented at the WAAVP 2017 Conference. This study will highlight the impact of gastro-intestinal helminths on chicken production and provide the basis to develop appropriate intervention and control strategies feasible for smallholder farmers.

Keywords: parasitic infestation; Mekong Delta; gastrointestinal helminths; chicken

Poster Presentation – Ruminant Livestock

Abstract No: 3959 (Poster# S1 - 68)

The long-term effect of pasture re-wetting on endoparasitic burden in cattle and sheep in Northern Germany

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Abstract Content

The wet grasslands at the German North Sea provide an important habitat for endangered fauna and flora. The focus of a nature conservation program beginning in 2005 is re-wetting of pastures. However, the potential risk for increasing endoparasite infections in grazing livestock compared to drained grassland is a major concern. During the years 2015 and 2016, 428 fecal samples of cattle and 307 of sheep were analyzed by flotation, sedimentation and Baermann technique. Sampling was done three times per year (April, July, November) and from pastures in three stages of re-wetting (conventionally drained; not drained and extensive farming; not drained and 10% of pasture covered by water). Most frequent findings were strongyle (cattle: 38.8%; sheep: 63.8%) and *Fasciola hepatica* eggs (11.5% / 18.2%) as well as *Eimeria* oocysts (24.1% / 32.3%). There were no statistically significant differences in endoparasite excretion between the three pasture types, but a distinct trend to a higher liver fluke prevalence on re-wetted areas in cattle (9.3% on drained pastures compared to 18.8% on intensely re-wetted areas), but not in sheep. Compared to the first three years of the conservation program (Kemper et al., 2009), the *F. hepatica* prevalence of originally 0.4% in cattle rose considerably. In addition, the previous study did not detect rumen flukes, whereas nowadays the detection rate was 6.1% in cattle and 5.2% in sheep. The increasing incidence of trematode infections should be closely monitored in the future to avoid impairment of animal health and production rates.

Keywords: Fasciola; organic; prevalence; cattle; sheep

A case of *Toxocara vitolorum* in a suckler calf in the Netherlands

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Abstract Content

In April 2017, one out of five faecal samples from a group of 3-4 month old calves with diarrhea was found positive for *Toxocara vitolorum* (EPG 3000 in the Mc Master) at the laboratory of GD Animal Health. The infected animal was a three months old Blonde d'Aquitaine calf, housed in a large group of calves together with their mothers. At the time of sampling there were twenty calves under 6 months of age (in total 49 animals) present at the farm. About a week before the faecal sample was taken, the farmer noticed a long worm. *Toxocara vitolorum* is uncommon in the Netherlands. In this case, all animals present on the farm were born in the Netherlands. The mother is born on this farm in April 2013. The last import was November 2012, the last imported animal left the farm in May 2013. Young and adult animals have been sold to other Dutch farms. The mother of this positive calf had a heifer calf last year, which still lives on another farm in the Netherlands. The most probable origin of this infection is an imported animal. The infection remained unnoticed or at least undiagnosed until this year. This case emphasizes the risk of importing cattle. The fact that the infection has been detected only recently allows for the possibility of unnoticed spread to other holdings.

Keywords: Toxocara vitolorum; suckler calf; import;

***Neospora caninum* detection in a goat fetus**

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Abstract Content

Neospora caninum is an apicomplexan protozoan responsible for abortion in ruminants, however caprine neosporosis needs further investigation. An *Anglo Nubian* fetus of approximately 2-month gestation was studied by Indirect fluorescence antibody test (IFAT), Immunoblot (IB), histopathology (HP), immunohistochemistry (IHC) and molecular assays (PCR; microsatellite genotyping). Interferon gamma knock-out mice were inoculated with a pool of organs and bled 21 days after for serological screening. The mother had IFAT titers of 1:3200 and 1:400 for *N. caninum* and *T. gondii* respectively, and the fetus was negative to both parasites by IFAT and IB. The fetus had severe autolysis and a generalized subcutaneous hemorrhage, severe multifocal necrotizing myocarditis and hepatitis, moderate interstitial pneumonia, and nephritis. *N. caninum* tachyzoites in clusters within myocardiocytes, always associated to HP lesions, were stained by IHC. *Neospora caninum*-DNA was detected in heart, lungs, liver, kidney and muscle from the fetus, and was negative for *T. gondii* by PCR. Mice were seronegative to *N. caninum* and *T. gondii* by IFAT. Microsatellite genotyping revealed a unique genetic profile that differed from previously reported *N. caninum* genotypes in other animal species. Our results indicate that *N. caninum* was efficiently transmitted to the fetus. The immature immunological system, as shown by the negative serological results, could explain the severity of lesions and the outcome of the disease. We report the first case of direct detection of *N. caninum* in a goat fetus in Argentina and *N. caninum* microsatellite genotyping in naturally infected goat worldwide.

Keywords: Abortion; *N. caninum*; Histopathology; Immunohistochemistry; microsatellite

Cases of *Theileria* infection in Malaysia 2011-2015

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Abstract Content

Theileriosis caused by *Theileria* spp may infect domestic and wild animals causing animal mortality and losses in production. These diseases are transmitted by Ixodid ticks of genera *Amblyomma*, *Haemaphysalis*, *Hyalomma* and *Rhipicephalus*. Prevalence of theileriosis described in this paper is based on diagnostic cases received in Veterinary Research Institute (VRI), Ipoh from 2011 to 2015. During these 5 years period, a total of 13444 blood samples comprising of from livestock, pets, laboratory animals and wildlife such as deer, bear and tiger were diagnosed at the Parasitology Section, VRI. Giemsa-stained blood smears were examined to detect *Theileria* spp. Animals recorded positive were cattle, deer, goat, buffalo and sheep. The prevalence rate was highest in cattle (10.25 %), followed by deer (8.79 %), goat (3.79 %), sheep (3.78 %) and buffalo (2.13 %). Prophylactic treatment is mainly by acaricides while chemotherapeutic drugs such as buparvaquone are used to treat infected animals. Further investigation on species of ticks that circulating theileriosis should be work out for better control of the disease especially among livestock.

Keywords: theileriosis, tick borne, livestock, domestic animals

A questionnaire survey to assess current worm control practices used by Australian alpaca farmers

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Abstract Content

Gastrointestinal nematodes (GINs) can cause significant economic losses in alpacas. Currently, very little is known about the epidemiology and control of GINs of alpacas in Australia. No information is available on worm control practices (e.g., farmer's knowledge of worms, type of anthelmintics, dose rate, their frequency of use etc.) used by alpaca farmers. This study was conducted to assess the current worm control practices used by alpaca farmers in Australia. An online questionnaire survey containing questions on husbandry, farmer's knowledge of worms and the use of anthelmintics in alpacas was administered to 954 members of the Australian Alpaca Association (AAA). Data analysis revealed that more than 50% respondents observed GINs as a problem and 79% regularly dewormed their alpacas using macrocyclic lactones (MLs). Most of respondents dewormed alpacas using either sheep dose and/ or one-and-half of the sheep dose; however, only 19% of respondents used the actual body weight for calculating the dose of a dewormer. Thirty two percent of respondents followed deworming schedule recommended by a veterinarian. Sixty percent of respondents were unaware of anthelmintic resistance and most of them had never performed a faecal egg count reduction test (FECRT). Thirty two percent respondents had shared paddocks with other livestock species and most of them were not aware of pasture spelling. This study provides invaluable information on existing worm control practices used by Australian alpaca farmers which may be used to develop strategies to control GINs of alpacas.

Keywords: Alpaca; Questionnaire; GINs; Control ; Anthelmintics

Descriptive findings from the analysis of fecal egg counts from beef cattle of the prairie provinces of western Canada between 2012 and 2014

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Abstract Content

It was the objective of this study to describe the prevalence and burden of gastrointestinal nematode (GIN) in beef cattle of the western Canadian prairie provinces between 2012 and 2014. Fresh fecal samples were collected from cow-calf operations from cows, calves and replacement heifers. Individual fecal egg counts (FEC) were performed using a modified Wisconsin method. Gastrointestinal nematode eggs were identified based on morphology as Trichostrongylid type and reported as eggs per 3 grams of feces (EP3G). Prevalence and burden were estimated using generalized estimating equations, accounting for clustering by herd. A total of 4,226 fecal samples were collected from 241 herds. Between 4 and 57 samples (median 20, IQR 6) were collected from each herd. The prevalence of Trichostrongylid type egg positive samples overall was 76% (95% CI 75-78). The predicted prevalence was 72% (95% CI 68-77) in cows, 80% (95% CI 75-86) in calves and 75% (95% CI 69-82) in replacement heifers. The mean Trichostrongylid type EP3G overall was 14.3 (95% CI 12.9-15.6). The predicted mean Trichostrongylid type EP3G was 13.2 (95% CI 12.0-14.4) in cows, 19.3 (95% CI 17.1-21.5) in calves and 10.9 (95% CI 9.6-12.4) in replacement heifers. These study results provide current information on GIN prevalence and burden in beef cattle from the western Canadian prairie provinces. Comparable literature is scarce. In light of emerging anthelmintic resistance and known production impacts of GIN in cattle, the results found here highlight the need for further epidemiologic studies of GIN in beef cow-calf operations in western Canada.

Keywords: Gastrointestinal nematodes; beef cattle; western Canada; prevalence

The abomasal microbiota of sheep with natural infections by strongylid nematodes

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Abstract Content

Parasitic gastroenteritis (PGE) caused by gastrointestinal (GI) nematodes severely affects the livestock industry worldwide. Given the widespread anthelmintic resistance against all classes of parasiticides available on the market, new strategies to combat these diseases are needed. The development of such strategies will depend on a thorough understanding of the interactions between the parasite and the vertebrate host, which includes the gut commensal flora. Indeed, increasing evidence in other host:parasite systems points towards a role of parasite-associated changes in gut microbiota in host immunity and metabolism. However, no data is thus far available on the composition of the gut microbiota of sheep with natural infections by strongylid nematodes. In this study, we utilized high-throughput sequencing of the bacterial 16S rRNA gene to characterize the composition of the abomasal microbiota and compare relative abundances of individual microbial species of 50 farm sheep with varying burdens of infections by trichostrongylid nematodes. Sequence data were analysed with conventional bioinformatics and biostatistical methods. The microbial profiles of the abomasal microbiota of infected sheep will be described in this presentation, and linked to the burden and nature of parasite infections as defined by total worm counts and morphological identifications, respectively. Knowledge acquired from this study represents a solid basis on which to build a better understanding of the intimate relationships between GI nematodes and commensal microbiota, and provides valuable data towards studies aimed to exploit these relationships for the discovery and development of novel control strategies against PGE in ruminants.

Keywords: Parasitic gastroenteritis; Sheep; Trichostrongylidae; Gut microbiota; 16S rRNA

Development of an improved faecal collection system for cattle

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Abstract Content

A key task in many parasitology studies is the collection of faecal material for analysis or to passage isolates. Research has been undertaken to design a more efficient collection device for calves that takes account of the comfort of the animal, the reduction of exposure of staff to zoonotic parasites and a significant reduction in sample loss. Collaboration between parasitology research staff, a fashion technologist, textile technologist and a textile manufacturer led to the development of a new device. Anti-ballistic textiles were chosen for their tenacity, durability and heat dispersal. Testing to international standards in dimensional stability, maximum force and abrasion resistance were undertaken to ensure suitability for animal use. The new device moves away from the harness system previously used and reduces the fitting difficulties for staff and discomfort for the animals. The key challenges tackled were ensuring a good secure fit on the calves, taking account of sensitive areas on their body as well as the ease of positioning and removing the collection device for staff. Unique design features have been optimised to ensure comfort for the animal, functionality in use and flexibility and longevity of the product. The device was tested on 4 calves ranging in age from 1-6 months, and significantly decreased sample loss compared to previous device. As a result of the design and development work already completed in the calf research an additional simplified product to manage canine incontinence and sampling has also been created.

Keywords: faecal collection, cattle

**Analysis of gene expression and protein synthesis related to hypobiosis of
*Teladorsagia circumcincta***

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Abstract Content

Teladorsagia circumcincta is an important causative agent of parasitic gastroenteritis in small ruminants in temperate regions. Under unfavourable environmental conditions, *T. circumcincta* is able to arrest larval development within a host (hypobiosis); molecular and biochemical mechanisms of this phenomenon have not yet been fully described. Hypobiosis is in principle similar to the “dauer larva”, a well defined developmental stage of the *free-living* nematode *Caenorhabditis elegans*. The expression of *daf* genes in *T. circumcincta* infective larvae was analyzed using the microarray chip designed for *C. elegans*. *T. circumcincta* larvae induced to hypobiosis (I) were compared to *T. circumcincta* larvae with standard development (S). In the group “I” we identified 33 genes as significantly differentially expressed; in the group “S” 12 genes were downregulated and 21 genes were upregulated. The microarray results were correlated to the dataset of proteins mass spectrometrically identified in excretory-secretory products of nematodes from both groups. Proteins considered to be involved in hypobiosis pathways were detected, but no significant differences in relative expression of these proteins between compared groups were observed. Only four differentially expressed genes detected by the microarray analysis were assigned to at least one protein that was identified by mass spectrometry, but showed opposite relative expression. Only one gene-protein pair exhibited the equal expression trend in both the microarray and the mass spectrometry data. We can conclude that further qPCR gene expression analysis of gene candidates should be the next step in investigation of hypobiosis molecular background.

Keywords: Hypobiosis; *Teladorsagia*; dauer larva; *Caenorhabditis elegans*; LC-MS/MS; RNA microarray

Characterization of a secreted cystatin of *Haemonchus contortus*, and its immune-modulatory effect on goat monocyte

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Abstract Content

Haemonchosis is a disease of small ruminant caused by *Haemonchus contortus* (*H. contortus*), and it is most important and alarming challenges to the small ruminant's production. Modulation and suppression of the immune response of the host by nematode parasites have been reported extensively and the cysteine protease inhibitor (cystatin) is identified as one of the major immunomodulators. In the present study, cystatin from *H. contortus* (HCcyst-3) was cloned and expressed in a histidine-tagged fusion soluble form in *Escherichia coli*. The inhibitory activities against cathepsin L, B, as well as papain, were identified by fluorogenic substrate analysis. The immunomodulatory effects of HCcyst-3 on cytokine secretion, MHC molecule expression, NO production and phagocytosis were observed by co-incubation of rHCcyst-3 with goat monocytes. Results demonstrated that the native HCcyst-3 protein was predominantly localized at the body surface and internal surface of the worm's gut. We demonstrated that rHCcyst-3 could be distinguished by antisera from goat experimentally infected with *H. contortus* and could uptake by goat monocytes. The results showed that the engagement of rHCcyst-3 decreased the production of TNF- α , IL-1 β and IL-12p40, however, it significantly increased the secretion of IL-10 and TGF- β in goat monocytes. After rHCcyst-3 exposure, the expression of MHC-II on goat monocytes was restricted. Moreover, rHCcyst-3 could up-regulate the LPS induced NO production of goat monocytes. Phagocytotic assay by FITC-dextran internalization showed that rHCcyst-3 inhibited the phagocytosis of goat monocytes.

Keywords: Haemonchus contortus; cystatin; monocyte

The influence of natural *Fasciola hepatica* infection on liver pathology in cattle

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Abstract Content

Fasciolosis is an important zoonotic disease prevalent world-wide and causes considerable economic losses to the livestock industry. This study was conducted to determine the macroscopic and microscopic liver damages of cattle with natural chronic *Fasciola hepatica* infection. A total of 77 slaughtered cattle livers with cholangitis were randomly collected. Based on analyses of serum ELISA it was observed that 77% (59/77) of the sampled animals had antibodies and were positive to *F. hepatica*. The livers from positive animals were examined macro- and microscopically, and assigned to graduate I to III for the classification of cholangitis and fibrosis. Furthermore, three samples of each category for histological evaluation of fibrosis and inflammation were taken. The most prevalent macroscopic lesion in livers was light cholangitis (51%), however, 29% and 20% of livers had moderate and severe cholangitis. The animals with higher number of *F. hepatica* had severe cholangitis with calcifications in bile ducts. It was shown, that fibrotic (periportal and periductal) lesions were more intense compared to inflammatory lesions. In addition, microscopically mononuclear cell infiltration, necrosis, biliary duct proliferation and arteriosclerosis were observed. The cholangitis degree corresponded to the *F. hepatica* worm burdens and fibrosis in the liver tissues.

Keywords: Fasciola hepatica, cholangitis, pathology, cattle

Risk mapping and major predictors of liver fluke and rumen fluke infections in Ireland

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Abstract Content

Fasciola hepatica has always represented an important threat to Irish livestock probably because the climate is particularly conducive to its intermediate host, the snail *Galba truncatula*. The emergence of another parasitic trematode, *Calicophorum daubneyi*, has raised the question whether the two parasites, which occasionally share the same niche during their life cycles (in the snail intermediate host and the ruminant final host), interact in some way. In this study, Geographical information Systems (GIS) were used to spatially analyse their distributions using large climatic and environmental datasets. Based on data from passive veterinary surveillance and targeted surveys, liver fluke and rumen fluke infection in cattle and sheep were modelled in order to compare their risk maps. Climatic variables (rainfall and temperature), together with vegetation appear to be the most important risk factors for both flukes, while environmental factors such as soil type, habitat or land use were less significant. However, the two parasites differed with regard to their spatial predicted occurrence; while rumen fluke is predicted to have a widespread distribution, liver fluke exposure appears to be more clustered. Whether this is due to any biological interaction, competitive advantage of one fluke species over the other and/or differences in control measures remains to be determined. It is important to stress that, according to current veterinary advice flukicides effective against *C. daubneyi* should only be used after clinical disease has been diagnosed. In contrast, treatment against *F. hepatica* is routinely used as a prophylactic measure on farms with a history of infection.

Keywords: Liver fluke; Rumen fluke; GIS; Risk mapping; Risk factors

Histological study of both fertile and infertile *Echinococcus granulosus* cattle hydatid cysts reveals pathological differences in laminated and adventitial layers

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Abstract Content

Cystic Echinococcosis, a worldwide-distributed zoonosis caused by the cestode *Echinococcus granulosus*, is an endemic disease in Chile in human and in animals, with cattle being the most affected of the intermediate hosts. Through routine abattoir visits, our laboratory has gathered a large number of hydatid cysts samples, all of which have been paraffin embedded, cut in 5 µm thick sections and stained with hematoxylin-eosin. 6 fertile cysts and 74 infertile cysts from the same *E. granulosus* strain were analyzed by a trained pathologist and the adventitial layer components were characterized. The laminated layer of fertile cysts had an average thickness of 203.87 ± 99.87 µm, while infertile cysts laminated layer had 191.77 ± 111.88 µm of average thickness. Our results show that the adventitial layer cell composition varies between fertile and infertile cysts, and within infertile cysts. Inflammatory infiltrate comprised mostly of lymphocytes and giant cells were correlated with thinner laminated layers whereas thicker laminated layers were associated with fibroblasts and collagen fibers in the adventitial layer. These findings support the idea that infertile hydatid cysts may be the result of the bovine immune system attack on the metacestode.

Keywords: Echinococcus granulosus; hydatid cyst fertility; bovine immune response; histology

Transcriptomic analysis of the systemic response to *Fasciola hepatica* infection in Sheep

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Abstract Content

Fasciola hepatica is an important cause of disease in livestock and in man. Modulation of immunity is a critical strategy used by this parasite to facilitate its long-term survival in the host. Understanding the underlying mechanisms at a system level is important for the development of novel control strategies, such as vaccination, as well as for increasing general understanding of helminth-mediated immunoregulation and its consequences. This study investigated the global gene expression changes of ovine peripheral blood mononuclear cells (PBMC) response to both acute & chronic infection and revealed 6490 and 2364 differential expressed genes (DEGS), respectively. Several transcriptional regulators were predicted to be significantly inhibited (e.g. IL12 and IL18) or activated (e.g. miR155-5p) in PBMC during infection. Ingenuity Pathway Analysis highlighted a series of immune-associated pathways involved in the response, including 'Transforming Growth Factor Beta (TGF β) signaling', 'Production of Nitric Oxide in Macrophages', 'Toll-like Receptor (TLRs) Signaling', 'Death Receptor Signaling' and 'IL17 Signaling'. To extend our understanding, we employed InnateDB to further analyze the DEG dataset and identified 2,458 and 224 molecular interactions in the context of innate immunity from the acute and chronic stages of infection, respectively. To the best of our knowledge, this study is the first system-level analysis of the regulation of host innate immunity during *F. hepatica* infection. We plan now to use a similar approach to characterise the systemic response in cattle, which may identify key attributes of the difference in the response between the two species.

Keywords: Fasciola hepatica ; immune response; transcriptomics; innate immunity; vaccines

Development of a longitudinal study to determine the timing of seropositivity to *Dictyocaulus viviparus* in UK dairy herds (MILC1 study)

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Abstract Content

Disease caused by the bovine lungworm, *Dictyocaulus viviparus*, can be explosive and financially devastating to dairy farms. Predicting if, and when, disease outbreaks are likely to occur is very challenging. In adult herds, farmers tend to either give blanket treatments at randomly chosen moments in time or treat at the onset of clinical signs. There is a need for an integrated, real-time forecasting tool that can drive accurate risk management decisions and help create targeted, effective treatment plans. A longitudinal study of 15 dairy herds in the UK was developed. Lungworm positive dairy farms from the UK were selected. Farm-level data was collected and bulk tank milk samples were tested every fortnight throughout 2 consecutive grazing seasons (2016 and 2017). Antibody levels were measured using the MSP-ELISA (SVANOVA®). The date that the herd first became seropositive (D_p) and the duration of the disease-free period whilst on pasture (T_p) were correlated with a purpose-made mathematical model to predict the timing of seropositivity to *D.viviparus*. *We will present an analysis of work which was begun in 2016 but will be finalised during 2017.* The ability to predict sharp rises in pasture contamination levels from temperature and rainfall conditions will prove invaluable to the accurate control of lungworm. Quantification of the risk from lungworm will allow a measured and timely control strategy to be implemented by farmers and veterinarians alike. The results from this longitudinal study have informed and validated a mathematical model which predicts peak abundance of lungworm on pasture.

Keywords: lungworm; forecasting; mathematical model; risk perception; climate

Evaluation of an insecticide-impregnated ear tag (Patriot™) for controlling horn flies and face flies among cow-calf pairs in the United States

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Abstract Content

Horn flies (*Haematobia irritans irritans*) and Face flies (*Musca autumnalis*) are common insects in grazing animals in most of the world. The current study was designed to evaluate the efficacy of Patriot™ Eartags in reducing horn fly and face fly pressure in cow calf pairs in Missouri, USA. Seventy cow calf pairs were randomly assigned to one of two treatment groups i.e. Patriot Group and Placebo Group. On study day 0, cows in Patriot treatment group were ear administered two insecticide impregnated ear tags (1 in each ear Patriot™ Insecticide Cattle Ear Tags) while the calves were administered one tag (1 tag/calf). Placebo group animals were administered placebo ear tags in a similar manner as Patriot treatment groups (two tags/cow, 1 tag/calf). Fly counts were performed on ten randomly identified cows within each group throughout the study. Beginning on Day -10, fly counts were performed three times (day -7,-3 and 0) to ensure that fly pressure was sufficient prior to Day 0. Post-treatment fly counts were performed weekly on the same 10 cows within each group. The mean horn fly counts per animal in Patriot group were below economic threshold during first 13 week of study period and were slightly above 215 .5 in week 14 and 209.6 in week 15. The highest percent face fly efficacy was observed in study week 5 which was 72.93. The results indicate that Patriot™ Insecticide Cattle Ear Tags were highly effective in mitigating horn fly and face fly pressure for up to 15 weeks.

Topical 0.5% w/v eprinomectin (EPRINEX® Pour-on, Merial): Efficacy against gastrointestinal and pulmonary nematodes and pharmacokinetics in sheep

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Abstract Content

The efficacy of topical eprinomectin (EPN) administered to sheep at 1 mg/kg body weight was evaluated in two laboratory studies and one multicenter field study. In addition, pharmacokinetics of EPN were determined. The laboratory studies demonstrated >99% efficacy ($p < 0.05$) against adult *Dictyocaulus filaria*, *Haemonchus contortus*, *Teladorsagia circumcincta* (pinnata/trifurcata), *Trichostrongylus axei*, *T. colubriformis*, *T. vitrinus*, *Cooperia curticei*, *Nematodirus battus*, *Strongyloides papillosus*, *Chabertia ovina* and *Oesophagostomum venulosum*, and inhibited fourth-stage *Teladorsagia* larvae. A total of 196 naturally infected sheep were included in the field study at two sites each in Germany and in Italy. Blocks of four sheep were formed on body weight and within each block one animal was randomly assigned to serve as control (untreated) and three animals were assigned to be treated with EPN. Examination of feces 14 days after treatment demonstrated overall 98.6% reduction of fecal strongylid egg counts ($p < 0.0001$). Pharmacokinetics of EPN were determined in eight adult female Merino cross sheep based on plasma samples collected up to 21 days following treatment. The main pharmacokinetic parameters were: C_{max} 6.20 ± 1.71 ng/mL, AUC_{last} 48.8 ± 19.2 day*ng/mL, T_{max} 3.13 ± 2.99 days and $T_{1/2}$ 6.40 ± 2.95 days. No treatment-related health problems or adverse events were observed in any study. In conclusion, these studies demonstrated EPN administered topically at 1 mg/kg body weight to be highly efficacious against a broad range of ovine gastrointestinal nematodes and *D. filaria* lungworms and well tolerated by sheep of different ages, breeds, gender and physiological status.

Keywords: Eprinomectin, topical, gastrointestinal nematodes, lungworms, pharmacokinetics, sheep

Buffalo flies, *Haematobia irritans exigua* - a significant cattle parasite set to invade southern cattle production areas in Australia?

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Abstract Content

Invasive species introduced into new regions with broad new resource bases and fewer natural regulating influences can become highly destructive and difficult to control. Buffalo flies (BF) and closely related horn flies (HF), *Haematobia irritans irritans*, both significant pests of pastured cattle, have proven to be extremely invasive species. Horn flies entered the US from Europe in 1886 and have now spread to cattle production areas in most of North, Central and South America. Buffalo flies entered Australia near Darwin in 1838, but spread has occurred more slowly with critical periods of range expansion often associated with favourable weather events. Buffalo flies reached Bundaberg (24.8°S latitude) in eastern Australia by 1946, but no further spread was recorded for the next 30 years. However following a series of mild seasons in the late 1970's spread resumed and BF have encroached 1000 km southward in the last 40 years. The results of CLIMEX modelling suggest that global warming will result in the development of areas with weather conditions suitable for colonisation by BF as far south as South Australia and south western Western Australia by 2030. Provided with a rich resource base of susceptible *Bos taurus* cattle, which currently receive few parasiticide treatments for internal or external parasite control, BF could become a significant cattle pest in southern beef and dairy areas as global temperatures increase.

Keywords: Diptera; cattle; climate change; invasive

Impact of a refugia-based strategy in stocker cattle on species diversity, pasture contamination, and development of resistance

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Abstract Content

Studies in sheep clearly demonstrate that refugia-based strategies reduce the rate with which anthelmintic resistance develops. In this study we tested a selective non-treatment refugia-based strategy in cattle. Mixed stocker calves (184-334kgs) were purchased at sale barns in the southern USA and allowed to contaminate a newly sown wheat/rye grass pasture. Stockers were then allocated randomly into three treatment groups; 100% treated with Dectomax®, 100% treated with LongRange®, and 90% treated with LongRange® (refugia group), and moved onto three separate newly sown wheat/rye grass pastures for 112 days. Worm-free tracer calves (Group 1) grazed the original contaminated pasture for four weeks; half were then treated with LongRange® and half left untreated, followed by necropsy and worm recovery. After 112 days of grazing, stockers were removed and three sets of tracers (Group 2) were placed on each of the three pastures, and the same tracer calf protocol was repeated. Surprisingly, *Ostertagia ostertagi* mucosal L4 numbers were not significantly different between treated and untreated calves in both Group 1 and 2 tracers ($p=0.7, 0.88$). In contrast, the percent reduction for adult *O. ostertagi* in the group 1 tracers was 99.6%; whereas in group two tracers, the percent reductions for the three sets were not significantly different and averaged only 91.2%. Further statistical analysis is in progress to assess the effects of the refugia strategy, however, these efficacy data suggest that resistance to avermectin drugs is emerging in *O. ostertagi* populations in southern US cattle. This is very concerning and requires additional investigation.

Keywords: Refugia; cattle management; anthelmintics; trichostrongyles

The common liver fluke in dairy cattle – its infection dynamics and impact on the milk production

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Abstract Content

The common liver fluke (*Fasciola hepatica*) is the causative agent of a production-limiting parasitic disease (fasciolosis) affecting grazing livestock worldwide. Control of fasciolosis in livestock is often based on annual mass deworming of the animals during the housing period. However, treatment options for dairy cows are limited compared with those for beef cattle and sheep, due to concerns about residues in milk. Economic impacts of liver flukes on dairy production are under debate. The costs of this parasitic disease and the benefits of deworming have never been revealed under Swedish conditions. In this project, we will evaluate the effects of a targeted treatment strategy of heifers and dry cows during the housing period. The aims are to monitor the epidemiology and to quantify the impacts on milk production for two consecutive years. A total of four farms with robotic milking and a recent history of fasciolosis have been recruited to the study. So far these farms have only been sampled on three occasions at the same time as all heifers and dry cows were treated orally with 10 mg albendazole per kg bodyweight. Presence of liver fluke infection has been confirmed by investigation of coproantigens in both heifers (79%, 0%, 21% and 91%) and cows (72%, 48%, 57%, 89%), as well as by detection of antibodies in bulk tank milk (OD values 0.74, 0.71, 0.89, 0.93). It is still too early to make any general conclusions from this study.

Keywords: Fasciola hepatica; dairy cattle; Sweden

A retrospective study of the confirmed positive parasite registered cases in pathology section

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Abstract Content

Parasites are a major cause of disease and production loss in livestock, frequently causing significant economic loss and impacting on animal welfare. The impact of internal and external parasitism on productivity of farm animals are considered for cattle, sheep, pigs and poultry. A retrospective study was conducted at the Registration Unit of Pathology Section, Veterinary Research Institute (VRI) Ipoh. Information were obtained from the database of LIMS (Laboratory Information Management System) and analyzed. The aim of the study was to investigate the number of positive parasite cases that was registered and diagnosed in the year 2015 in VRI. Total of parasite cases that include both negative and positive cases registered were 466 and out of it 105 were confirmed positive for various diseases which comprise of 104 endoparasites and 1 ectoparasites cases. The most common endoparasites (helminthes and protozoa) diagnosed were Coccidia, strongyles, Strongyloides spp, Monienzia spp, Haemonchus spp., liver flukes, Theileria spp. and Trypsnosome spp. The most common ectoparasites diagnosed was *Rhipicephalus microplus*. Samples that frequently registered were blood in EDTA, fecal and organs. In conclusion, parasite infections in farm animals are very common and therefore planned preventative programs are necessary to minimize the risks of parasitic disease outbreaks and losses of animal production.

Eggs characteristic for diagnosis of ruminant endoparasites

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Abstract Content

Conventional microscopic examination was used to diagnose most of the helminth infections in ruminants by identifying the characteristic of helminth's eggs in the faeces. The major types of eggs that present in ruminant faeces include nematodes, trematodes and cestodes. Nematodes have been characterized as a tube within a tube, referring to the alimentary canal which extends from the mouth on the anterior end, to the anus located near the tail. Most of nematode eggs identified were Strongyle-type egg, *Strongyloides* spp., *Ascaridia* spp. and *Toxocara* spp. While commonly diagnosed trematodes in ruminants was *Fasciola* spp. Trematodes also called flukes because of their conspicuous suckers, the organs for attachment. On the other hand, cestodes which usually called tapeworms are long, segmented flat worms that attach themselves to animal intestines. Commonly encountered cestodes in ruminants were *Moniezia expansa* and *Moniezia benedeni*. Other than helminth eggs, coccidia oocysts also commonly found in ruminants faeces. The eggs' characteristic of different endoparasites in ruminants faeces were displayed in this paper. Conventional microscopic examination continues to be first-line diagnostic tools in most parasitological laboratories. Although basic microscopy is able to detect most prominent parasites, new technology could be implemented for genetic study of the parasites.

Keywords: ruminants; endoparasites; eggs characteristic.

Geographic distribution modelling for ruminant liver flukes (*Fasciola hepatica*) in South-eastern Europe

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Abstract Content

Fasciola hepatica is a liver fluke parasite of ruminants and is endemic in many parts of the world. In the present study a cross sectional survey was conducted in Greece and Bulgaria to collect information on the spatial distribution of *F. hepatica* genotypes and haplotypes in order to construct predictive maps for Greece and Bulgaria of different *F. hepatica* genotypes and haplotypes, based on the ecological niche modelling. A total of 204 *F. hepatica* flukes were collected from abattoir surveys in 31 sheep and 9 cattle from various areas of mainland of Greece and Bulgaria. Modelling building was performed using maximum entropy method implemented in Maxent program. The model performance was evaluated using the threshold independent method based on the Area Under the Curve (AUC) of Receiver Operating Characteristics Curve (ROC). The lower (0.832) and the highest (0.947) AUC values were observed in the models for the haplotypes CtCmt1 and CtCmt2.2, respectively. Precipitation and temperature contribute equally to model building of the genotypes based on the 28S rDNA gene. In regard to mtDNA gene region, precipitation is the most important factor in modelling CtCmt1 haplotype range, while temperature appears to be the most important factor in modelling the CtCmt2.1 and CtCmt2.2 haplotype ranges. The highest level of probability for the geographic distribution of *F. hepatica* genotypes and haplotypes covered Southern Bulgaria and Central and Northern Greece regions which contain a high concentration of potential ruminant hosts.

Keywords: Ecological niche modelling; *Fasciola hepatica*; Genotype; Haplotype; Sheep

Molecular survey of theileriosis in Malaysian cattle, sheep and goats

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Abstract Content

Theileria sp. (phylum *Apicomplexa*, order *Piroplasmida*, family *Theileriidae*, subclass *Piroplasmia*) is a tick-borne protozoan which infects a wide range of hosts, including domestic and wild ruminants. As theileriosis may impact livestock industry, improved detection and surveillance of *Theileria* parasites is essential. This study reports the use of molecular approach for detection of *Theileria* sp. in the blood samples of cattle, sheep and goats collected in several animal farms in Malaysia. Animal blood samples were screened for the presence of *Theileria* DNA using a conventional polymerase chain reaction (PCR) assay. A total of 155 (69.2%) of 224 cattle investigated were PCR-positive for *Theileria* DNA. *Theileria* DNA was detected from 90.0% of 40 sheep but none of the 40 goats examined in this study. Sequence analyses of amplified 18S rRNA partial fragments (335-338 bp) confirmed the identification of *Theileria buffeli*, *Theileria sergenti*, and *Theileria sinensis* in the blood samples of cattle and ticks. *Theileria luwenshuni* was identified in the infected sheep. The high detection rates of *Theileria* sp. in farm animals highlight the needs for appropriate control and preventive measures for theileriosis.

Keywords: Theileria; cattle; goat; sheep

Poster Presentation – Teaching and Learning Veterinary Parasitology

Abstract No: 4409 (Poster# S1 - 78)

Veterinary Parasitology teaching - why fight the smart phones?

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Abstract Content

The ubiquitous presence and student usage of smart phones led to consideration of this information delivery channel as a potential support tool for veterinary parasitology learning. An information reference application has been developed to allow veterinary science students to remind themselves of key information about clinically important animal parasites. The development process and early usage patterns and feedback will be summarised in this poster.

Keywords: education learning apps smartphone

Poster Presentation – Wildlife & Exotics

Abstract No: 3416 (Poster# S1 - 79)

Molecular prevalence and characterization of *Cryptosporidium* species among pet birds in Japan

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Abstract Content

The protozoan *Cryptosporidium* species are common in pet birds. Birds are infected with 3 *Cryptosporidium* species (*C. baileyi*, *C. galli*, and *C. mereagridis*), and 11 *Cryptosporidium* genotypes including avian genotypes I-V. These avian *Cryptosporidium* species often induce the mortality, gastrointestinal symptoms, respiratory disease, and renal or cloacal illness. In addition, *C. mereagridis* has a potential for zoonotic transmission. However, only a few studies have reported on molecular analysis of avian *Cryptosporidium* species in Japan. The objective of the present study is to investigate the molecular prevalence and characteristics of *Cryptosporidium* species among pet birds in Japan. A total of 275 fresh fecal samples were collected on one occasion from each birdcage (each cage housed only a single species of bird) in 5 pet shops in Japan. The DNA of *Cryptosporidium* species in feces was extracted using a commercial kit. A nested polymerase chain reaction (PCR) assay targeting the 18S rRNA gene was employed for the detection of *Cryptosporidium* species. To identify *Cryptosporidium* species, positive secondary PCR amplicons were purified and directly sequenced. *Cryptosporidium* species were detected from 8.7% (24/275) of the pet birds analyzed. The sequence analysis identified the following: *C. galli* (4.7%: 13/275), *Cryptosporidium*. avian genotype III (2.9%: 8/275), and *C. baileyi* (1.1%: 3/275). The present study demonstrates a low prevalence of *Cryptosporidium* species infection among pet birds in Japan. Moreover, because zoonotic *C. mereagridis* was not detected, it is likely that the risk of zoonotic transmission of *Cryptosporidium* species from pet birds to humans is low in Japan.

Keywords: Cryptosporidium species, pet bird, molecular prevalence, Japan

Dirofilariasis of carnivores in the central region of European part of Russia.

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Abstract Content

Dirofilariasis of carnivores caused by *Dirofilaria repens* and *D. immitis* is endemic in Moscow region and bordering territories. Previous surveys on the presence of microfilariae in domestic dogs had shown that some cases of infestation were introduced from southern and eastern regions of Russia. No data on distribution of dirofilariasis in dogs and wild canids in central part of Russia were yet reported. The present survey was carried out in 2003-2016 in Moscow, Rjazan and Vladimir regions of Russia. Totally, 427 individuals of a red fox (*Vulpes vulpes*) and 67 individuals of stray and gun dogs (*Canis familiaris*) were dissected and examined on the presence of *Dirofilaria* spp. in heart and cutaneous tissue. *Dirofilaria immitis* were found in one individual of red fox (0.2%) with 10 worms located in its right heart ventricle. Two-three individuals of *Dirofilaria repens* were found in each of 3 infected foxes (0.7%) in cutaneous tissue of underarm and groin areas. In dogs, 7 *D. immitis* were recovered from the right heart ventricle of one animal (1.5%) while *D. repens* were found in 2 dogs (3%) in cutaneous tissue of underarm and back areas (intensity 1-2 worms). The discovery of *Dirofilaria* spp. in wild canids together with the discovery of the nematode DNA in mosquitoes confirms the presence of a local circulation of the causative agents. The higher occurrence of *Dirofilaria* spp. in dogs compared with foxes indicates its higher susceptibility to infection. The study was supported by grant 14-16-00026 from Russian Science Foundation.

Keywords: *Dirofilaria*, red fox, Moscow region.

Incidence of helminthiasis and coccidiosis in captive wildlife in Perak States

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Abstract Content

A study was undertaken to investigate the incidence of intestinal parasites from captive wildlife in Perak States. A total of 164 faecal samples were screened from various animals captivated in wildlife conservation centre, sanctuary and petting zoo. It was discovered that 14.6% of the samples positive for helminthes (7.3 %) and coccidian (7.3 %). While the rest 85.4% were not infected. Helminth taxa isolated were *Strongyles*, *Strongyloides*, *Ascaris* and fluke. Entirely, helminthes infections was detected in mammals while coccidia infection was only in avian. Neither parasitic infection were found in reptiles. *Strongyles* sp. were the most common helminthes observed (50 %) followed by fluke. Although the rate of infection is relatively low, the parasites may cause severe illness in affected animals that could lead to low reproduction and death. Good husbandry practices, regular herd health programmes and sufficient nutrition could reduce medication and animal loss.

Keywords: helminthiasis, coccidiosis, captive wildlife, faecal, intestinal parasites,

Haemosporidian parasites in captive Strigiformes birds in France

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Abstract Content

A limited number of studies about haemosporida infections in birds have been published. However, these infections may cause major damages to avian populations and represent a concern for veterinarians working in zoological parks or wildlife rescue centers. Following the loss of 9 Great grey owls (*Strix nebulosa*) at Mulhouse zoological park in 2013, a prospective epidemiological investigation was performed in captive Strigiformes birds in France in 2016. The purpose was to evaluate the prevalence of haemosporida infection in captive Strigiformes and to estimate the kinetics of infection around the nesting period of birds. Fifteen French zoological parks participated to the study. Blood samples were collected from 122 birds representing 12 species. Direct examination and PCR allowed to identify haemosporida from 44 birds from 10 zoos. Three different species of *Haemoproteus*, as well as one species of *Plasmodium* were detected. The percentage of birds infected by *Haemoproteus* varied accordingly to the period of sampling. Nesting season (May/June) seemed to be at greater risk with an average prevalence of 53.9% (n=69) compared to winter season with an average prevalence of 14.8% (n=122). The prevalence of *Plasmodium* infection in Strigiformes did not exceed 8% throughout the year. This study showed how significant haemosporida infection could be in captive Strigiformes in France. The nesting season was identified as a period of higher risk of infection and consequently the best period to apply prophylactic measures (disinsectisation, antimalarial treatment).

Keywords: Haemosporida, Haemoproteus, Plasmodium, Strigiform birds, Zoological parks, France

Size matters – the bigger ones sucks more blood

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Abstract Content

Ashworthius sidemi is a blood-sucking nematode that occurs primarily in the abomasum of wild ruminants. Even though infections caused by this invasive parasite can pose a serious health threat to ruminants, the biology of *A. sidemi* is still not fully understood. Morphological polymorphism was previously well demonstrated in various gastrointestinal nematodes; however, this phenomenon has yet to be described in *A. sidemi*. Nematodes recovered from the abomasa of the European bison, red deer, roe deer and fallow deer from different localities of the Czech Republic were morphologically identified. Twelve of these nematodes were further subjected to molecular analysis; the mitochondrial ND4 gene, ribosomal ITS-1 and 5.8 gene were investigated. All of the recovered nematodes were identified as *A. sidemi* and they were clearly divided into two groups based on their total body lengths. Significant differences between established groups were detected – males of one group had an average length of 16.7 ± 1.1 mm, whereas their counterparts from the second group had an average length of 25.7 ± 1.1 mm. Clear differences were also observed between both groups of females (18.9 ± 1.5 mm versus 37.4 ± 2.3 mm). Evaluated mitochondrial and ribosomal genes exhibited 99% sequence similarity in all tested nematodes. We assume that the *A. sidemi* population in wild ruminants in the Czech Republic consists of two morphotypes. The newly recognised minor morph is 1.5 to 2 times larger than the major morph. This phenomenon may have pathological as well as epidemiological consequences and requires further investigation.

Keywords: *Ashworthius*; wild ruminants; polymorphism; morphology; morphometry; sequencing

***Taenia taeniformis* and *Spirometra mansonioides* infection in a wild Royal Bengal Tiger (*Panthera tigris tigris*)**

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Abstract Content

The management of wild animals has become more important since they are susceptible to various parasitic diseases both in captive and free range state. In the present study, a wild male Bengal Tiger (*Panthera tigris tigris*) aged about 11 years was rescued from forest, Chickmagalur district, Karnataka and brought to Tiger and Lion safari Unit, Tyavarekoppa, Shimoga, Karnataka. The animal was dull and had poor body condition at the time of arrival, symptomatic treatment was given but the animal was died during the course. On gross examination, the carcass was emaciated, dehydrated, rough hair coat, sunken eyes and no external injury. The post-mortem examination revealed anaemic mucous membrane with slightly enlarged liver. The intestine was opened and found fully packed tapeworms. The intestinal mucosa was inflamed with petichael hemorrhages and thickened sporadically with presence of semi-digested food materials, blood and mucus. The tape worms were collected, washed, processed and stained with haematoxylin and eosin for species identification. Based on the morphological character of mature segments and the scolex, the tape worms were identified as *Taenia taeniaeformis* and *Spirometra mansonioides*. The gut content was collected during post mortem examination for detection of parasitic eggs/ova and examined by sedimentation and floatation techniques as per the standard protocol and found eggs of *Taenia taeniformis* and *Spirometra mansonioides*.

Keywords: Tiger, T. taeniaeformis, S. mansonioides, Shimoga

Characterisation of marsupial piroplasm in kangaroo ticks in Western Australia

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Abstract Content

Piroplasms (*Babesia*, *Theileria* and *Cytauxzoon*), are intra-erythrocytic parasites that can cause piroplasmosis in animals and humans worldwide, and are generally transmitted by ticks. In Australia, these protozoa are commonly studied in host blood samples, however since analysis of these parasites in ticks, particularly from native wildlife, is lacking, knowledge about their potential vectors is limited. The present study aimed to screen and characterise piroplasms in kangaroo ticks, *Ixodes australiensis* (n=107) and *Amblyomma triguttatum* (n=27), collected in Western Australia. Genomic DNA from these ticks was screened by PCR using piroplasm-specific primers targeting the 18S rRNA gene (18S). Approximately 6.7% (9/134) of the kangaroo ticks were positive for piroplasms. All positive samples belonged to the species *I. australiensis* whereas no piroplasm DNA was detected in *A. triguttatum*. Phylogenetic analysis of 784 bp of sequence revealed that the tick-derived sequences exhibited 97.2%, 92.2%, and 92.4% genetic similarity with the marsupial-derived *Theileria fuliginosus*, *T. brachyuri*, and *T. penicillata*, respectively, but clustered in a unique clade. Further and more detailed characterisation of a longer 18S and heat shock protein (HSP70) sequences are ongoing. This study presents the first observation of piroplasm in *I. australiensis* ticks from kangaroos in Western Australia.

Keywords: Ticks; Kangaroo; Piroplasm; *Ixodes australiensis*; Western Australia

***Angiostrongylus vasorum* in captive meerkats (*Suricata suricatta*)**

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Abstract Content

Angiostrongylus vasorum is a cardiopulmonary nematode increasingly diagnosed in many European countries and infecting mostly canids such as dogs (*Canis familiaris*) and foxes (*Vulpes vulpes*). Natural infections have amongst others also been reported in coyotes (*Canis latrans*), wolves (*Canis lupus*) and a red panda (*Ailurus fulgens fulgens*) held in a zoo. We report *A. vasorum* infections in a group of captive meerkats (*Suricata suricatta*) kept at a university facility for behavioural studies. First-stage larvae (L1) of *A. vasorum* were initially detected in a pooled faecal sample. Individual faecal samples, investigated with the Baermann Wetzel technique, revealed that 41% (7/17) of the meerkats were positive, with ranges of 2–125 L1/g faeces. PCR/sequencing of part of the ITS-2 region revealed 99% identity with *A. vasorum*. The infected animals did not show any clinical signs. One meerkat died one day after diagnosis. Histological examination of the lung revealed granulomatous pneumonia caused by *A. vasorum* larvae and eggs. However, the cause of death was a spleen rupture with associated blood loss. All meerkats were treated with 10 mg imidacloprid/2.5 mg moxidectin (spot-on) per animal, after which they became negative in all follow-up examinations up to 1.5 years. Examination of potential intermediate hosts (slugs and snails) is ongoing. Meerkats apparently are suitable definitive hosts with production and excretion of viable L1. Meerkats kept in captivity in areas where *A. vasorum* is endemic and with potential contact to intermediate hosts are at risk for infection. Regular faecal examinations including Baermann Wetzel technique should be considered.

Keywords: *Angiostrongylus vasorum* – lung nematode – meerkat – *Suricata suricatta* – suitable definitive host

Helminth fauna of wild sika deer in Yamanashi prefecture, Japan

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Abstract Content

The helminth fauna of 79 wild sika deer *Cervus nippon centralis* in Yamanashi prefecture, Japan, was investigated between 2014-2016. The results revealed the presence of 1 species of cestode (*Moniezia* sp.), 3 species of trematodes (*Ogmocotyle sika*, *Dicrocoelium chinensis*, *Dicrocoelium dendriticum*) and 7 species of nematodes (*Gongylonema pulchrum*, *Dictyocaulus* sp., *Pygarginema* sp., *Spiculopteragia houdemeri*, *Cooperia* sp., *Trichuris* sp., *Oesophagostomum sika*). *Gongylonema pulchrum*, *Dictyocaulus* sp. and *Pygarginema* sp. were collected from the esophagus mucosa, the lung and the abomasum, respectively. Identification of the helminths were based on their adult morphology and supported by DNA analysis. In our study, although we observed that the flukes of the genus *Dicrocoelium* from the liver showed a comparatively high prevalence (48% ; 38/79), most of the samples were that of *D. chinensis*. We found *D. dendriticum* from only one of deer (1% ; 1/79). *C. nippon* and *D. dendriticum* are known to be distributed all over the world including the Asia. However, our observation of *D. dendriticum* in *C. nippon* might be a new finding for this part of the world. Moreover, the finding of the abomasal spruridid nematode, *Pygarginema* sp. presents a new record of this nematode in Japan.

Keywords: Helminth fauna; sika deer; *Cervus nippon centralis*; *Dicrocoelium dendriticum*; *Pygarginema* sp.

Modern aspects of the epizootology of trichinellosis in the Central Black Earth region of Russia

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Abstract Content

Natural focal trichinellosis is widespread in the Central Black Earth region of Russia. Larvae of *Trichinella nativa* were found in 9 species of mammals - in 7 species of wild predators: fox, raccoon dog, wolf, badger, pine marten, stone marten and 1 species of insectivorous (hedgehog) and 2 species of domestic carnivores (cat and dog). The extensity of infection in wild predators varies from 12,5 to 36,8%, in hedgehog – 5,2%. The fox dominates as the host of *Trichinella* because of relatively high rates of infection (27,6%) and the highest number of foxes among predators. The extent of infection reaches 53,8% in the Voronezh Reserve. The intensity of infection in foxes averages 25,4 larvae per gram of muscle tissue. Thus foxes play a leading role in accumulation and distribution of larvae and in maintaining a stable circulation of natural foci of trichinellosis in the region. Based on these results, an ecological model of the parasitic system of *Trichinella* in the Central Black Earth was developed. The fox as the host-dominant forms the core. The next level is occupied by other 5 species of carnivorous mammals - wolf, raccoon dog, badger, pine marten and stone marten. A hedgehog occupies a detached position. Taking into account the peculiarities of trophic-horological connections, hedgehog accumulates *Trichinella* and transmits them to carnivorous mammals. The main ecological factors and ways of *Trichinella* transmission in populations of these animals are predation, necrophagy and cannibalism. Domestic carnivores can be infected from wild predators. Supported by RSF No14-16-00026.

Keywords: Trichinellosis; epizootology; predators; ecological model

Morphological characterization and prevalence of an *Anatrichosoma* species in Iriomote cats (*Prionailurus bengalensis iriomotensis*)

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Abstract Content

The Iriomote cat (IC), *Prionailurus bengalensis iriomotensis*, is an endangered Japanese wildcat subspecies indigenous in Iriomote-jima, Japan. The current IC population is estimated at approximately only 100–150 individuals. Through conservation activities, we found nematode eggs in the ear discharges of these cats accidentally. We morphologically characterized this nematode and evaluated its prevalence. IC capture was done with the permission of the Ministry of Environment (Japan). Between 2015 and 2017, 13 ICs were captured for a series of ecological surveys or were found dead. Ear swab samples were collected from the captured ICs and microscopically examined. Histopathological sections of ear pinna from a road-killed IC were also evaluated. Many nematode eggs were found in ear wax samples from 9 out of 13 ICs. The four ICs that lacked eggs were juveniles. These eggs had an oval form and were approximately 50 x 75 micrometers in size. Similar to whipworms, they had plug-like structures at both ends of the longer axis. All the eggs contained larvae. Nematode adults were found in the epidermis of the ICs, and numerous crusts and inflammatory reactions were observed around them. Based on its morphological characteristics, this nematode is an *Anatrichosoma* genus member. To the best of our knowledge, biological information about *Anatrichosoma* spp. is scarce. There is only one report concerning *Anatrichosoma* infestation in felids and ours is the first report of it in a wildcat. Further investigations are required to clarify the life cycle, host range and pathogenicity of this parasite.

Keywords: Wildcat, Iriomote cat, Nematode, Anatrichosoma

***Angiostrongylus vasorum* and *Crenosoma vulpis* in foxes (*Vulpes vulpes*) in Norway**

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Abstract Content

Angiostrongylus vasorum has been the focus of several studies due to its apparent emergence and geographical spread throughout Europe. This potentially fatal, snail-borne parasite infects domestic dogs and wild canids causing verminous pneumonia and coagulopathy in conjunction with neurological and gastrointestinal symptoms. Similar, although less severe symptoms, can be caused by the cardiopulmonary nematode *Crenosoma vulpis*. Whereas *C. vulpis* is widespread in Northern Europe *A. vasorum* has not previously been detected in Norway. During January-April 2016 faeces was collected from all regions of mainland Norway from 134 red foxes shot as part of the Norwegian *Echinococcus multilocularis* surveillance program. Following Baermannisation, first-stage larvae (L1) were identified by microscopy based on morphological/morphometric characteristics. Larvae (5 per fox) identical to *A. vasorum* were verified by PCR and partial sequencing of the ITS-2 and the CO1 locus. L1 morphologically identical to *A. vasorum* were detected in 3 (2.2%) foxes. In two foxes from different regions in southern Norway, *A. vasorum* (99% identical to sequences from UK in GenBank) was verified by PCR, whereas the L1 from the third fox were identified as *Varestrongylus capreoli*, a protostrongylid nematode of roe deer. Additionally, L1 of *C. vulpis* were detected by microscopy in 52 (38.8%) foxes. This is the first finding of *A. vasorum* in Norway, and to our knowledge *V. capreoli* has never previously been reported in foxes anywhere. Our findings document the northern expansion of this highly pathogenic parasite and highlight the need for molecular or serological verification of morphological diagnoses.

Keywords: *Angiostrongylus*; red fox; lungworm

Sound features of *Vespa simillima xanthoptera* Cameron

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Abstract Content

Vespid wasps (*Vespa* spp.) are the most noxious pests on apiculture, resulting in significant economic loss. Early monitoring and management is the first step of preventing the damages from Vespid wasps. Sound features generated by wasps or honey bees are different, and their different features could be applied to develop an early monitoring system for the prevention of attacks of wasps to apiary. In this paper, the acoustic signals from wasps and honey bees were measured by a microphone with a preamplifier and an analog-digital converter. The fundamental frequency of the wasp was analyzed to be 100 Hz with the strong harmonic frequencies. On the other hands, the fundamental frequency of the honey bee was 250 Hz with harmonic frequencies. Frequency analysis of the acoustic signals from wasps and honey bees would be suitable for classification of the two species. To identify the acoustic signals of wasps, two fundamental frequencies could be differentiated by setting the threshold of about 150 Hz. The sound features from two bee species would be useful for differentiation and identification of wasps from apiary which may protect the honey bee from the attacks of the wasps, though further systematic signal measurement and analysis are required for the development of the early warning system.

Keywords: Honeybee, vespid wasp, sound,

Prevalence of ectoparasites on laboratory rats at the Laboratory Animal Facility and Management (LAFAM), UiTM, Selangor

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Abstract Content

Ectoparasites are one of the most common health problems among laboratory rodents. A study on prevalence of ectoparasites of laboratory rats was conducted between March and May 2016 at LAFAM. This study was carried out on 187 laboratory rats (75 Wistar and 112 Sprague-Dawley). The rats' fur were brushed using a tooth brush and the scrapings were examined under a light microscope. The results showed that 38.48% of the rats were positive for ectoparasites. The most prevalent ectoparasites in this breeding colony were *Polyplax spinulosa* and *Radfordia affinis* with prevalence of 77.19% and 22.81%. This study indicates that the animals have never been ruled out for any health problems. All rodents should be screen for ectoparasites during the quarantine period.

Keywords: Ectoparasite, fur scraping, rodents, Polyplax spinulosa, Radfordia affinis

Pinworm infection in various species of cockroaches

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Abstract Content

Cockroaches is an important an indicator of sanitation in human society, especially in the kitchen. However, they are also being reared as fodder for exotic pet, especially reptiles. Moreover, the popularity of cockroaches as pet is increasing in some societies. Pinworms of the family Thelastomatidae (order Oxyurida) are known to infect cockroaches. This family is considered to be a large one; having 31 genera. Some species of *Leidynema*, *Thelastoma*, and *Hammerschmidtella* have been reported to be parasitizing in the hindgut of *Periplaneta americana*. Oxyurids of mammals and reptiles are known to show a high degree of host specificity but probably not for thelastomatidae. To examine the prevalence of the pinworms in cockroaches, a total of 79 fecal samples of laboratory-reared cockroaches consisting of 59 species was examined for parasitic eggs. Of the 79 fecal samples, 53 were found to contain oxyurid eggs. This result revealed a high prevalence of thelastomatid parasitic nematodes in laboratory-reared cockroaches. *Leidynema* sp. was found parasitizing in *Neostylopyga rhombifolia*, *Eurycotis decipiens* and *Anallacta methanoides*. *Hammerschmidtella* sp. was found to parasitize in *Periplaneta japanna* and *Eurycotis opaca*. *P. japanna* is a [subtropical](#) field-dwelling [cockroach](#) endemic to southern [Japan](#). Infection of thelastomatid in *P. japanna* may point to the establishment and invasion of alien parasites into the local host. To the best of our knowledge, this is the first report of a thelastomatid nematode infection in *P. japanna*.

Keywords: Cockroach; Pinworm; Japan; thelastomatid nematode

Prevalence of internal parasites in wild boars (*Sus scrofa coreanus*) from South Korea

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Abstract Content

The Korean wild boar (*Sus scrofa coreanus*) is widely distributed in the Korean Peninsula, and its gradual increase in population over the last decade has resulted in a higher frequency of human-wild boar conflict as they wander into parks, public roads and residential areas. Wild boars can also play an important role as the reservoir host for parasitic diseases of domestic pigs and humans such as trichinellosis, ascariasis and toxoplasmosis. In the present study, we investigated the prevalence of internal parasites of Korean wild boars from the Republic of Korea. Gastrointestinal tracts and lungs of 144 Korean wild boars hunted in mountains in the south-western part of South Korea between 2008 and 2017 were examined for their visceral helminths. Results of the survey showed that the lungworms in the genus *Metastrongylus* was the highest in prevalence (72.2%), followed by *Globocephalus samoensis* (50.0%), *Bourgelatia diducta* (49.3%), *Stephanurus dentatus* (37.5%), *Trichuris suis* (25.0%) and *Capillaria* sp. (5.6%), whereas *Ascaris suum* (3.5%) showed the lowest prevalence. Results of this study showed that the risk of exposure to infection of internal parasites in wild boars is considerably higher than domestic pigs in the same region of Korea.

Keywords: Metastrongylus; Globocephalus; Stephanurus; Wild boars; Korea

Poster Presentation – Zoonoses & One Health

Abstract No: 5523 (Poster# S1 - 85)

Molecular epidemiology and diagnosis of leishmaniasis in Egypt

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Abstract Content

Many zoonotic parasites are endemic in Egypt. Intestinal infections of parasitic zoonoses are widespread and the leading cause of diarrhea, particularly among children and residents of rural areas. Some zoonotic parasites are confined to specific geographic areas in Egypt, such as cutaneous leishmaniasis (CL) in the Sinai. Other areas have a past history of a certain zoonotic parasite, such as visceral leishmaniasis (VL) in Alexandria. Now, *leishmania* become wide spread all over Egypt. As a result of the lack of control programs for Sand fly and *stomoxys* species, a marked increase in the prevalence of Leishmaniasis. Animal reservoirs of *leishmania* have been identified in Egypt, especially in rodents and stray dogs, as well as vectors, typically mosquitoes which constitute potential risks for disease transmission. Prevention and control programs against sources and reservoirs of zoonoses should be planned by public health and veterinary officers based on reliable information from systematic surveillance. So, the molecular epidemiological map of the disease and its geographical distribution were studied, select and apply the most sensitive, specific and rapid method for diagnosis.

Keywords: Leishmaniasis; PCR of Leishmania; epidemiological map of Leishmania

Identification of *Echinococcus* spp. in definitive and intermediate hosts in Bhutan

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Abstract Content

Hospital records of cystic echinococcosis in Bhutanese residents indicate the possibility of an indigenous cycle of *Echinococcus granulosus* sensu lato. Therefore, faecal samples were collected from community dogs around slaughter houses and from the capital city of Thimphu (n=138) and in the forest area around a cattle farm in central Bhutan (n= 28). Samples were analyzed morphologically for the presence of taeniid eggs by the floatation and sieving method. Further analysis of samples positive for taeniid eggs by PCR/sequence revealed eleven *Echinococcus* sp. causing cystic echinococcosis infections, seven *Taenia hydatigena* and one *Hydatigera taeniaeformis* infections and additionally in nine samples DNA of *Spirometra* sp. was detected (in 9 cases double infections occurred, in 2 cases no PCR confirmation was achieved). Cysts were collected from locally slaughtered and imported beef and, by direct sequencing, seven (one fertile) and 35 cysts (four fertile), respectively, were confirmed as *E. granulosus* (G1-3). One cyst from local and one from imported cattle (both fertile) were confirmed to be *Echinococcus ortleppi* (G5). Sterile cysts were also collected from local yaks (n=10) and all revealed to be *E. granulosus* (G1-G3). The presence of *Echinococcus* spp. in dogs and ungulates in this pilot study and in ongoing investigations indicates the existence of local transmission for both *E. ortleppi* and *E. granulosus* in Bhutan.

Keywords: Echinococcus; dogs; cystic echinococcosis; taenia; cysts; Echinococcus granulosus

Zoonotic parasite infections in predators in northern Germany

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Abstract Content

Infectious diseases of wildlife are of increasing concern in the One Health concept, especially those with zoonotic potential. Due to increasing utilization of natural habitats and invasion of wildlife into urban regions, the possibility of contacts between humans or domestic animals and wildlife intensifies. The objective of this project was to evaluate parasitic infections in the two most frequent predatory mammals in the Northern German federal state Schleswig-Holstein, the red fox (*Vulpes vulpes*) and the beech marten (*Martes foina*). Between November 2013 and December 2015 a total of 79 red foxes, 19 beech martens and additionally 10 raccoon dogs (*Nyctereutes procyonoides*) were included. Fecal samples were analyzed by combined sedimentation-flotation method, parts of small and large intestine were investigated by washing and scraping technique, and skeletal muscle samples by pepsin digestion. The most frequently detected endoparasites in foxes were *Toxocara canis* (in 46.8% of animals), *Capillaria* spp. (39.2%), *Echinococcus multilocularis* (27.8%), *Alaria alata* and *Uncinaria stenocephala* (both 26.6%). Furthermore, different *Taenia* and *Mesocestoides* spp., *Toxascaris leonina*, *Trichuris vulpis* and coccidian oocysts were found. The examined raccoon dogs revealed the same endoparasite spectrum as the foxes. In contrast, solely *Capillaria* spp. could be detected in a total of 36.8% of beech martens. Pepsin digestion of muscle samples for detection of *Trichinella* spp. did not show any positive results. The study results suggest that in Northern Germany about two thirds of foxes harbor zoonotic parasites, whereas beech martens do not seem to play a decisive role as carrier of zoonotic parasites.

Keywords: fox; *Echinococcus*; *Toxocara*; wildlife; zoonoses

High COI haplotype diversity and molecular phylogeography of *Angiostrongylus malaysiensis* (Nematoda: Angiostrongylidae) in Thailand

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Abstract Content

The rat lungworm *Angiostrongylus malaysiensis* is a metastrongyloid nematode parasite of the Family Angiostrongylidae. It occurs in Thailand, Laos, Myanmar, Malaysia, Indonesia and Japan. In this study, *A. malaysiensis* adult worms recovered from the lungs of wild rats in different geographical regions/provinces in Thailand were used to determine their haplotype by means of the mitochondrial partial cytochrome *c* oxidase subunit I (COI) gene sequence. In addition, COI sequences from GenBank were included for comparison. The results revealed high COI haplotype diversity. Five new haplotypes were identified in addition to the four haplotypes reported in the literature. Four of these five haplotypes – one from Mae Hong Song (northern Thailand), two from Tak (western Thailand), and one from Phang Nga (southern Thailand) – formed a distinct clade with those from Phatthalung (southern Thailand) and Malaysia. The haplotype from Malaysia was identical to that of Phatthalung (haplotype AM1). Phylogenetic analysis revealed that the geographical isolates of *A. malaysiensis* from Thailand and other countries formed a monophyletic clade distinct from the closely related *A. cantonensis*. This study has confirmed the presence of high COI genetic diversity in various geographical isolates of *A. malaysiensis*. The COI gene sequence will be suitable for species differentiation and determination of genetic diversity, population structure and phylogeography.

Keywords: *Angiostrongylus malaysiensis*; haplotype; phylogeography; genetic diversity; cytochrome *c* oxidase subunit I

Molecular and serological investigation of animal reservoirs of visceral leishmaniasis (Kala-azar) in Bangladesh

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Abstract Content

On the Indian subcontinent, visceral leishmaniasis (VL), caused by an intracellular parasite *Leishmania donovani*, is considered to be anthroponosis. The role of domestic animals in its transmission is still unclear. Although cattle are the preferred blood host for *Phlebotomus argentipes*, the sandfly vector of VL in the Indian subcontinent, very little information is available for their role in the disease transmission. In this study, we examined domestic cattle and goats for serological and molecular evidence of *Leishmania* infection in a VL-endemic area in Bangladesh. Blood samples from 400 domestic cattle and goats were collected from houses with active or recently-treated VL and post-kala-azar dermal leishmaniasis patients. The presence of anti-leishmanial antibodies in serum was investigated using using rk39 immunochromatographic strip test. PCR was performed to amplify the ITS-1 gene using the DNA extracted from Buffy coat for the detection of parasite DNA. In this study, 9.4% (n = 18) of the cattle and goats were found to be positive by rK39 dipstick test. Of the 18 seropositive cattle and goats, no parasite DNA was detected in the molecular assay. The study confirmed the presence of antibodies against *Leishmania* parasite in cattle and goats. However, the absence of *Leishmania* DNA in the cattle and goats indicates clearly that the goats and cattle do not play a role as reservoir host. Similar study needs to be undertaken to determine the role of other domestic and wild animals on which sandflies feed.

Keywords: Visceral leishmaniasis, Animal reservoir, Bangladesh

Molecular characterization of *Leishmania* isolates from Bangladeshi patients with visceral leishmaniasis

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Abstract Content

Visceral leishmaniasis (VL), or kala-azar (KA) is an endemic disease of great public health importance in Bangladesh. The clinical manifestation of the disease, response to treatment and lack of an animal reservoir are all similar in eastern India, Bangladesh and Nepal which suggest a common aetiological agent, *Leishmania donovani*. Despite the vigorous need for adequate taxonomic characterization and strain typing of parasite only few attempts had been made to identify the causative species in Bangladesh. The *Leishmania donovani* isolates from different endemic areas in Bangladesh were assessed for their genetic relationship. Twenty-one isolates of *L. donovani* from Bangladesh were subjected to multilocus microsatellite typing (MLMT) using 15 highly polymorphic microsatellite markers. Bayesian model-based and distance-based analysis of the data inferred that the *L. donovani* strains from Bangladesh are identical to parasites in India and Nepal which all form a single, remarkably homogeneous population. In conclusion, our results demonstrate a remarkably homogeneous single clone of *L. donovani*, population in Bangladesh, related to the epidemic spread of VL in the region.

Keywords: Visceral leishmaniasis, Leishmania donovani, Bangladesh, homogeneity, population genetics, epidemiology

***Trichinella spiralis* suppresses collagen-induced arthritis via CD4⁺ T cell hyporesponsiveness**

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Abstract Content

CD4⁺ T cells play a major role in the pathogenesis of rheumatoid arthritis (RA). The aim of this study was to examine the regulatory role of CD4⁺ T cells in collagen-induced arthritis (CIA) in mice infected with *Trichinella spiralis*. Male C57BL/6 mice were infected with *T. spiralis* 2 weeks prior to being immunized with chick type II collagen. Arthritic condition was scored for evaluating the occurrence and severity of arthritis. Histopathological changes in the paws, serum levels of anti-C II antibodies and cytokine production by concanavalin A (ConA)-stimulated spleen cells were examined. Furthermore, proliferation and cytokine production of CD4⁺ T cells from infected CIA mice in response to *in vitro* C II stimulation were assessed. The results showed significantly lower arthritic scores and histology scores in CIA mice infected with *T. spiralis* compared with uninfected mice. The levels of Anti-C II IgG and T helper type 1 (Th1)-derived IgG2c, and Th1 (IFN- γ) and pro-inflammatory (IL-17, TNF- α) cytokines were significantly lower in infected CIA mice compared with uninfected mice. In addition, *in vitro* C II-specific proliferative response and cytokine (IFN- γ and IL-17) production of CD4⁺ T cells were decreased in mice infected with *T. spiralis*. In conclusion, *T. spiralis* infection suppresses collagen-induced arthritis, which might be associated with *T. spiralis*-induced CD4⁺ T cell hyporesponsiveness to C II.

Keywords: Trichinella spiralis; Rheumatoid Arthritis; Type II collagen; CD4+ T cell

Molecular prevalence of *Toxoplasma gondii* DNA in goats' milk and seroprevalence in Northwest Tunisia

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Abstract Content

This study aimed to assess the contamination risk by *Toxoplasma gondii* infection, by consuming raw milk from naturally infected Tunisian goats. The survey was conducted in Northwest of Tunisia (Jendouba Governorate), from February to May 2014, including 77 lactating goats among six herds. From each goat, blood and milk (50 ml) samples were collected. Sera were separated and examined for IgG antibodies against *T. gondii* using an ELISA kit (ID Screen® Toxoplasmosis Indirect, Montpellier, France) according to manufacturer's instructions. After centrifugation of raw milk, DNA was extracted from the resuspended pellet using Wizard Genomics DNA (Promega, Madison, Wisconsin, USA) extraction kit and stored at -20° C until used. For detection of *Toxoplasma gondii* DNA, a nested PCR was performed to amplify fragment of 227 bp belonging to ITS1 gene and coding for the 18S - 5.8S rRNA. Positive ELISA results were found in 31.2 % of goats. Seroprevalence was significantly higher in the delegation of Tabarka than in Hammam Bourguiba ($p=0.001$). Herds containing more than one cat were characterized by the high rate of toxoplasmosis ($p=0.004$). Our study showed that drinking unpasteurized goat's milk could cause a source of toxoplasmosis infection. A total number of 6 samples milk was positive ($7.8\pm 3\%$). Goats with history of abortion showed a high rate infection ($p=0.01$). Our survey showed that raw milk from Tunisian goats can be contaminated, thus it may present a real risk to public health.

Keywords: Toxoplasma gondii; goat; milk; ELISA; PCR; Tunisia

A *Toxoplasma gondii* atypical isolate reveals similar behaviour to the most virulent reference strain.

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Abstract Content

Diverse *T. gondii* genotypes have shown different virulence in mouse model. The aim of this study was to evaluate the *in vitro* invasion and replication of an atypical *T. gondii* isolate obtained from *Macropus rufrogriseus* (*TgMR*) in Argentina. A plaque assay using the reference strains RH (type I), ME49 (type II) and VEG (type III) was conducted. Vero cells were cultured in 24-well plates (10^5 /well) for 24 hours and infected with 10^5 tachyzoites/well. The plaque was incubated for 18 hours at 37°C and fixed with cold-methanol. Immunofluorescence with anti-*T. gondii*-Alexa Fluor and DAPI staining were used. Invasion and replication were determined by counting parasitophorous vacuoles (PV) and the number of tachyzoites/PV, respectively. Significant differences in the invasion capacity ($p < 0.05$, Kruskal-Wallis test) among strains were detected, being lower in both *TgMR* and RH (average 211 PV) compared to ME49 and VEG (1265 and 540 PVs, respectively). The ME49 and VEG strains had low replication capacity (1 or 2 parasites/PV), whereas RH and *TgMR* strains had high replication (≥ 4 parasites/PV). The *TgMR* genotype is III for most markers and I for C 29-2 (#14 or 138 TOXO-DB). Similar genotype was isolated from chickens from South America and a human in Argentina. The *TgMR* isolate has a similar *in vitro* behavior to RH strain: lower invasion capacity than ME49 and VEG strains, but a higher replication rate. These results suggest *TgMR* as a potential high virulent isolate, however further studies in mouse model should be conducted.

Keywords: Toxoplasmosis; atypical isolate; invasion; replication; virulence

Evaluation of biological behaviour of atypical *Toxoplasma gondii* isolates

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Abstract Content

Toxoplasma gondii infection in animals and humans is mainly chronic and asymptomatic, however during pregnancy or immunosuppression it can produce severe lesions. The aim of this study was to evaluate the biological behaviour of two atypical isolates obtained from chickens from Argentina using a mouse model. Four groups of nine Swiss mice were inoculated with three doses (100, 10³ and 10⁴ parasites/mouse) of Beverley strain (reference genotype II), C24 (genotype #123 at TOXODB) and 11/9gall (genotype #19) isolates and PBS as negative control. Animals were daily observed and mortality was recorded (acute infection considered as death within 7-15 dpi). Blood was taken at acute and chronic (30 dpi) infection for IgG detection by IFAT. Histopathological lesions were evaluated on brains stained with H&E. ANOVA, Kruskal-Wallis, and Dunn's Multiple Range tests were used for statistical analysis (GraphPad Prism 5.0). Statistical significance for all analysis was established with P < 0.05. The isolate 11/9gall produced higher mortality (89%) than Beverley and C24 (55%), and no differences among doses were observed. Antibodies were detected in lower concentration in 11/9gall infected mice probably due to the acute mortality. Beverley strain induced significant major inflammatory response on acute infection (congestion, gliosis and perivascular mononuclear cuffings) than 11-9gall (P< 0.0001, Kruskal Wallis). It is important to analyse the interaction between the immune response and atypical *T. gondii* isolates to improve the mouse model for animals and humans toxoplasmosis.

Keywords: Toxoplasmosis; atypical isolates; mouse model: behaviour

The incidence of zoonotic hookworm infection after anthelmintic treatment in Sukabumi District, West Java Province Indonesia

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Abstract Content

Hookworm in dogs is very important not only from the point of animal health but also from the perspective of public health, since all species of hookworms in dogs are zoonotic potential. Infection of hookworm in dogs is endemic in Southeast Asia and the prevalence in West Java Indonesia reached 92.5%. This study was aimed to measure the incidence of hookworm infection after anthelmintics treatment and to identify the factors which influence the incidence. The incidence was measured after 3 months of dog mass deworming in Sukabumi District, West Java. A cross-sectional study was conducted by collection of 100 dog stool samples to identify hookworm eggs using simple flotation methods and by interview of dogs owner to identify the factors that influence the incidence. The data were analyzed descriptively and analytically using Chi Square Test and odds ratio. The results showed that the incidence of hookworm infection after anthelmintic treatment was still 21.0% (95%CI: 14.2– 30.0%). The incidence in puppies (12.0%) was higher than adult (9.0%), highland area (17.0%) was higher than seaside area (4.0%), hunting dogs (14.0%) was higher than house guarding dogs (7.0%), and contact with stray dogs (20.4%) was higher than no contact (2.0%). The significant factors that influenced the hookworm incidence was the topography ($X^2=4.448$, $p=0.035$) whereas highland area had risk 3.381 (95%CI: 1.043–10.960) times than in seaside. The conclusions were anthelmintics treatment still can't eliminate hookworm infection because of several influence factors and the significant factor was the topography of dogs reared area.

Keywords: zoonotic hookworm; incidence; anthelmintic; dogs; influence factors

Treatment of human fascioliasis using triclabendazole in Cajamarca, Peru

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Abstract Content

Human fascioliasis is a significant parasitic disease affecting people and livestock in Cajamarca, Peru. Prevalence rates in humans range between 5 to 24% being particularly high in children of school age. Prevalence in livestock is as high as 90%. The aim of the present study was to determine the clinical efficacy of the human formulation of Triclabendazole (TCBZ) (Egaten®) for treating human cases of fascioliasis. Twenty six human cases of *Fasciola hepatica* infection were identified positive to *F. hepatica* eggs in their faeces using the sedimentation method and confirmed using the Kato Katz technique. Patients were randomly allocated to two treatment schemes of thirteen patients each. All the patients received post prandial treatment with Egaten® for two consecutive days. Group A were treated with 10 mg/kg of body weight of TCBZ and group B were treated with 20 mg/kg. All the patients were submitted to parasitological diagnostic testing at 15, 30 and 45 days post treatment. Twelve of the thirteen patients of group A were egg-negative 45 days after treatment (92.30% cure rate). Nine of the thirteen patients of group B were egg-negative 45 days after treatment (69.23% cure rate). While the higher dose rate appeared more effective, the difference in cure rate was not significant ($p>0.05$). In conclusion, treatment with TCBZ over two days with 10 mg/kg is effective in the treatment of human fascioliasis. Treatment failure observed in some cases may be associated with resistance of *F. hepatica* to TCBZ as previously reported in bovine infection.

Keywords: Fasciola hepatica; Human infection; Triclabendazole; Cajamarca; Peru

Effects of recombinant *Toxoplasma gondii* Citrate synthase 1 on the cellular functions of murine macrophages in vitro

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Abstract Content

In the present study, the Citrate synthase 1 gene of *Toxoplasma gondii* (*T. gondii*) (TgCS1) was cloned and characterized. TgCS1 gene showed an open reading frame of 1665bp nucleotides encoding for a 555 amino acids protein with 61kDa. Using western blotting assay, the recombinant protein was successfully recognized by the sera of rats experimentally infected with *T. gondii*, while the native protein in the *T. gondii* tachyzoites was as well detected by sera from rats immunized with the recombinant protein of TgCS1. The protein binding with murine macrophages was confirmed by immune fluorescence assay. After incubation with rTgCS1, the result showed that rTgCS1 protein had a dual function, low concentration could increase the phagocytosis, but high concentration inhibited the phagocytosis of macrophages. Research on murine macrophages apoptosis illustrated that 5µg/mL, 10µg/mL and 20µg/mL rTgCS1 protein can significantly induce early apoptosis of Ana-1 cells, and 5µg/mL rTgCS1 protein can significantly induce late stage of apoptosis of Ana-1 cells (** $p < 0.001$) and the other concentrations of rTgCS1 protein had no significant effect on cell apoptosis. In the detection of cytokines, the result indicated that the productions of interleukin-10, interleukin-1β, transforming growth factor-β1, tumor necrosis factor-α of macrophages were increased after the cells were incubated with rTgCS1. However, the productions of NO and cell proliferation of the macrophages were significantly reduced. All of the above results suggested that the TgCS1 had significant effects on the cellular functions of murine macrophage in vitro.

Keywords: Toxoplasma gondii; Citrate synthase 1; cellular functions; murine Macrophages

Risk factors of hookworm infection in dogs in Sukabumi Regency, West Java Province

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Abstract Content

Hookworms infection remains as an important parasitic infections affecting animal and human health worldwide. However, information on the prevalence and the risk factors of hookworm infection on dogs in Indonesia is very limited. A cross sectional study to estimate the prevalence and to identify risk factors of hookworms infection on dogs was conducted in rural area with different topography, Sukabumi Regency, Indonesia. A Total of 204 dog stool specimens were examined for hookworm eggs using simple flotation methods. Risk factors of hookworms infection for dog were assessed in relation with type of topography area (high and low land), purpose of having dog, dog demography, dog reared management and deworming. The data of related risk factors were collected through dog owners interview's with completed a questionnaire. The prevalence of hookworm on dogs in Sukabumi was 24.5 % (95% Confidence Interval [CI]=19.1-30.8). This prevalence was associated with topography of the area, the age of dog, and type of dog keeping methods. The prevalence of hookworms was higher in the high land area than low land area (OR=5.935, 95% CI=2.764-12.744). Logistic regression identified puppies as a high risk group to hookworms infection (OR= 2.041, 95%CI=1.035-4.055). The dog which kept in the cage/tied had higher risk than free roaming dog (OR=3.66, 95%CI=1.479-9.091). This study indicates that transmission animal hookworms in the Sukabumi was relatively high and involved some risk factors. Therefore, prevention and control strategies of hookworm infection in animal should formulate in order to prevent infection both in dogs and human.

Keywords: Hookworms; dog; prevalence; risk factors; Sukabumi; Indonesia

Anthelmintic resistance in experimentally infected wild and domestic ruminants detected by in vitro methods

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Abstract Content

Experimental animals three roe deer (*Capreolus capreolus*), three fallow deer (*Dama dama*) and three mouflons (*Ovis musimon*) were infected with the sensitive MHco3 strain of *H. contortus* and similarly 3 roe deer, fallow deer and three mouflons were infected with the MHco4 resistant strain. Simultaneously six lambs were also infected with sensitive and resistant *H. contortus* strains. During the 99 days the course of the intensity of parasitic infection was observed in all species. The level of benzimidazole resistance in mouflons and sheep were analysed with egg hatch test (EHT) and larval development test (LDT). The highest intensity of infection in mouflons was detected on day 48 post infection with resistant strain 26500±1500 eggs per gram (EPG) and susceptible strain 3800±450 EPG. The roe and fallow deer had very low infection intensity until day 58 (50-150 EPG). After 58 day *H. contortus* eggs were not observed probably due to phenomenon of selfcure. In mouflons and sheep the ED₅₀ values in EHT of susceptible *H. contortus* strain varied from 0.043±0.005 to 0.064±0.008 mg/ml of thiabendazole (TBZ) which correlated with a low level of resistance obtained by LDT (LD₅₀ 0.007±0.002 - 0.009±0.001 mg/ml TBZ). In contrast, higher ED₅₀ (0.091±0.001 to 0.111±0.004 mg/ml TBZ) and LD₅₀ values (0.021±0.002 to 0.031±0.001 mg/ml TBZ) were determined both in mouflons and sheep in the *H. contortus* resistant strain. Both *in vitro* tests confirmed the same level of benzimidazole resistance for both species.

The study was supported by VEGA 2/0120/16 and Slovak Research and Development Agency 0169-14.

Keywords: Haemonchus contortus, wild ruminants, anthelmintic resistance

Assessment of soil contamination with parasites of public health significance by adopting flotation techniques

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Abstract Content

Soil is one of the important means of environmental transmission of parasites to man and animals. Prevalence of zoonotic soil transmitted parasites (STP's) in Rayalaseema region of Andhra Pradesh State of India was carried out by adopting three flotation techniques standardized by Kazacos (1983), Lorcaïn (1994) and Santarem (2009). Efficacy of these techniques was compared after processing 144 soil samples seeded with two different concentrations (100/200) of the eggs of *Toxocara*, *Ascaris* and *Ancylostoma* species and oocysts of *Eimeria* species. Analysis of variance was used to compare the results. Irrespective of parasitic species or load, the mean recovery rate of 50 ± 4.32 , 33.57 ± 5.08 and 22.33 ± 2.37 percent was obtained for Lorcaïn, Kazacos and Santarem methods, respectively. The Lorcaïn technique was having higher efficiency and henceforth this technique was employed to screen the naturally contaminated soil samples. The overall prevalence of STP's was found to be 29.19 percent (n=148) out of 507 soil samples screened. Thirteen parasitic species including seven nematodes, two cestodes and four protozoans were isolated. Prevalence of *Toxocara* spp. (9.07%) was recorded as the highest followed by *Ancylostoma* spp. (8.48%), *Trichuris* spp. (3.35%), *Entamoeba* spp. (2.36%), *Strongyloides* spp. (2.20%), *Ascaris* spp. (1.57%), *Balantidium* spp. (1.18%), *Isospora* spp. (1.18%), *Eimeria* spp. (0.98%), *Capillaria* spp. (0.78%), *Taeniidae* (0.78%), *Oxyuroidea* (0.19%) and *Hymenolepis* spp. (0.19%). Comparatively higher prevalence of soil borne parasites was observed in urban localities (31.78%) than in rural areas (22.53%). The present findings represent relatively higher soil contamination with different parasitic forms imposing a significant threat to public health.

Keywords: Soil; Zoonotic; Parasites; Prevalence; STP's.

Circulating pathogenic *Leptospira* in reservoir animals and humans in the District of Gampaha, Sri Lanka

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Abstract Content

Leptospirosis is a worldwide zoonosis caused by infection with pathogenic *Leptospira*. The second highest incidence of leptospirosis is recorded in the District of Gampaha, Sri Lanka. Detection of *Leptospira* in reservoir animals and humans is important to control the transmission of the disease. The objective of this study was to detect *Leptospira* in reservoir animals and humans in a high risk area in the District of Gampaha, Sri Lanka. Blood samples were collected from 38 rodents and confirmed the infection by qualitative Polymerase Chain Reaction (PCR) assay and Microscopic Agglutination Test (MAT). A panel of acute and convalescent blood samples were collected from leptospirosis suspected patients. Acute and convalescent human blood samples were tested by PCR-MAT and MAT respectively. Pathogenic *Leptospira* infection was confirmed in 12%(4/34) of rodents only by PCR assay and 72%(65/90) of suspected patients by PCR and/or MAT. Pathogenic *Leptospira* was present in a significant proportion of the rodents (12%) and humans (72%). It is important for farmers and other populations in the risk areas to be aware of the disease. Personal protective equipment should be worn when farmers and other labourers in high risk areas. A good waste management system is needed to decrease the rodent population. Pathogenic *Leptospira* is circulating in rodents and humans in the study area. Disease control methods need to be strengthened. International Atomic Energy Agency (TC/SRL 5-042), International Centre for Genetic Engineering and Biotechnology (CRP/SRI 8/02) and National Science Foundation, Sri Lanka (RG/2009/BT/01).

Keywords: Leptospira, Rodents, humans, PCR, MAT

There's a macaque in my kitchen: Gastrointestinal parasitism in people and urbanized Long-tailed macaques (*Macaca fascicularis*) in Maha Sarakham, Thailand

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Abstract Content

Increasing contact between human communities and wild primate populations poses disease transmission risk, but the degree and nature of this risk remains poorly understood. We performed a community-based assessment of intestinal parasitism and primate contact in Maha Sarakham, Thailand, an area where long-tailed macaques (*Macaca fascicularis*) of the Kosumpee Forest Park and human residents of adjacent villages occupy an overlapping living space. Human (n=115) and macaque (n=102) fecal samples were collected and analyzed for gastrointestinal helminths by Formalin-Ethyl Acetate sedimentation. Household interviews assessed potential factors associated with parasite transmission. Overall, 12% of human stool samples tested positive for parasites, including *Strongyloides* spp., *Opisthorchis viverrini*, *Taenia* spp., and *Ascaris lumbricoides*. Generalized linear multivariate modelling indicated that odds of infection was higher among those reporting presence of macaques and/or their feces at home (OR=13.9, 95% CI: 1.1-183); however it was lower among those reporting occupational exposure to animals (OR=0.04, 95% CI: 0.003-0.43). Overall, 44% of macaque fecal samples contained at least one gastrointestinal helminth species. *Trichuris* sp., hookworms, and *Schistosoma* spp. were the predominant parasites detected, with higher prevalence in juveniles (<1 year) than adults (≥1 year). Hookworm intensity was significantly higher in juvenile macaques (p=0.02). While human infection was linked to increased exposure to macaques, parasite transmission within shared environment appears to be minimal. Respondents identified positive and negative aspects of living around macaques and identified food shortages and population growth as primary causes for human-macaque conflict. Future work should address the negative aspects of human-macaque interaction to promote healthy co-existence.

Keywords: Thailand; parasites; macaques; One Health

Dynamics of the survival of *Dirofilaria repens* microfilariae in a dog in Ile-de-France

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Abstract Content

Dirofilaria repens is one of the differential diagnosis that must be done when blood microfilariae are detected in a dog. This was the case in a 20 month-old Tosa dog that was presented to the Small Animals Veterinary Hospital of Alfort, France in February 2016, for a subcutaneous nodule on the head. The nodule was punctured with a small needle and polynuclear granulocytes as well as microfilariae were detected after Giemsa staining. After the surgical removal of the skin nodule, one nematode of 8 cm of length was found inside and further identified by PCR (12S and *cox1*) as *D. repens*. Counting of microfilariae in the blood sediment by the Knott technique has been performed every 3 months, for one year. In the absence of any specific treatment, a constant decrease of microfilariaemia (mean of 176, 137, 114 larvae/mL) has been observed for the first 6 months and a sharp decrease (mean of 11 and 0 larvae/mL) for the next 6 months. The dog was imported from South of Romania, at the age of 3 months, and since then, the dog never left Ile-de-France region. Since several cases of subcutaneous dirofilariosis in dogs and cats have been reported so far both in Romania and in Ile-de-France region, it is not possible to accurately identify the place where the initial contamination occurred. However the region of south Romania, where the dog was born is highly endemic for mosquitoes.

Keywords: Dirofilaria repens; Zoonose; Dog; Europe

Temporal pattern of and *Rickettsia* spp. infection in *Amblyomma sculptum* and *Amblyomma dubitatum* in two distinct environments in northeastern Brazil

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Abstract Content

Amblyomma ticks are recognized vectors of several rickettsiae and understanding their temporal patterns is crucial for predicting transmission risk. We assessed the temporal pattern of *Amblyomma sculptum* and *Amblyomma dubitatum* in two different environments. We also tested if these ticks were infected by *Rickettsia*. From January 2015 to December 2016, ticks were collected monthly using dry ice-baited traps in northeastern Brazil. The first collection site (CS1) was a grassy habitat in a rural area and the second (CS2) was an Atlantic Forest remnant. Ticks were morphologically identified and tested by PCR testing for *Rickettsia* spp. (*gltA* gene). In total, 17,196 ticks were collected. In CS1, 74 males, 94 females, and 468 nymphs of *A. sculptum* and 864 larvae of *Amblyomma* spp. were collected. In CS2, 116 males, 69 females, and 1,063 nymphs of *A. dubitatum* and 14,448 larvae of *Amblyomma* spp. were collected. *Amblyomma sculptum* showed a bimodal tendency, with a major peak in the second semester. Yet, *A. dubitatum* presented a well-defined unimodal pattern, with a marked peak in the first semester. Of 78 *A. sculptum* and 46 *A. dubitatum* adults tested, 32.1% and 69.6% were *gltA*-positive, respectively. Regarding nymphs, minimum infection rates of 0% (174 nymphs tested) and 18.1% (188 nymphs tested) were determined for *A. sculptum* and *A. dubitatum*, respectively. This study confirms the year-round presence of *A. sculptum* in northeastern Brazil. Because *A. sculptum* is a major vector of *Rickettsia rickettsii*, further studies are needed to characterize the *Rickettsia* spp. circulating in this tick population.

Keywords: Amblyomma; ecology; temporal pattern; Rickettsia

Molecular characterization of species and genotypes of *Cryptosporidium* in animals inhabiting three main catchments in South-East Queensland (QLD), Australia

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Abstract Content

Cryptosporidiosis represents the major public health concern of water utilities worldwide. As animals in catchments can shed human-infectious *Cryptosporidium* oocysts, determining the potential role of animals in dissemination of zoonotic *Cryptosporidium* to drinking water sources is crucial. As part of an ongoing comprehensive quantitative survey of *Cryptosporidium* species in different catchments and states in Australia, a total of 653 animal faecal samples including cattle, sheep, pig, horse and wildlife samples from three main catchments in South-East Queensland, Australia were screened for the presence of *Cryptosporidium* using a quantitative PCR (qPCR). Subtyping of *C. parvum* and *C. ubiquitum* isolates was conducted at the glycoprotein 60 (*gp60*) locus. *Cryptosporidium* species were detected in 16.7% of samples (95%CI:13.9%-19.8%) screened. Five species were identified; *C. parvum* (n=45)(6.9%,95%CI:5.1%-9.1%), *C. bovis* (n=27)(4.1%,95%CI:2.7%-6%), *C. ryanae* (n=30)(4.6%,95%:3.1%-6.5%), *C. ubiquitum* (n=6)(0.9%,95%:0.3%-2%), and a single case of a *C. molnari*-like genotype in a bird. The presence of zoonotic *Cryptosporidium* in particular *C. parvum*, suggests that animals inhabiting water catchments may contribute to contamination of catchments with human-infectious *Cryptosporidium* oocysts. The public health implications of the identification of zoonotic *Cryptosporidium* species and subtypes in animals in Queensland drinking water catchments will be discussed.

Keywords: *Cryptosporidium*, Water catchments, Zoonotic

Reducing the risk of spreading of dangerous parasitic zoonoses of dogs and cats by breeders responsible use of antiparasitics

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Abstract Content

The aim of this prospective study was checking of use of antiparasitics for dogs and cats in accordance with the approved package leaflet (PL). These antiparasitics are veterinary medicinal products (VMP), which are frequently available without veterinary prescription. Dogs and cats can be hosts of dangerous parasites that pose threat not only to their health, but also human health (*Toxocara* spp., *Taenia crassiceps*, *Echinococcus* spp.). Breeder questionnaires were used to collect essential data. The faeces samples from dogs and cats were examined by flotation, Baermann and PCR methods. Obtained data indicate that most of animals were without health problems, deworming was executed 1-2 times per year, less frequently 3 or 4 times a year or irregularly. The results of coprological examination were negative for most animals. The study reveals using anthelmintics in accordance with the approved PL and epidemiological situation seems to be favourable. On the other hand, breeders do not reflect the urgency of targeted therapy (*Trichuris*, lungworms) and an adaption of drug administration to the age and way of life of animals (*Toxocara* spp.); farm and hunting dogs (*Echinococcus* spp., *T. crassiceps*). The lack in animal health education of breeders and need of closer cooperation between breeder and veterinarian was identified. Communication between Medicines Agency and pharmaceutical industry is necessary to increase awareness of breeders through PL, which should best reflect the situation in clinical practice, alert the breeders for conditions of increasing risk of parasitic infections and make reference to regular cooperation with the attending veterinarian.

Keywords: zoonoses, antiparasitics, dogs, cats

Molecular characterisation of *Cryptosporidium* spp. in lambs and goat kids of unorganized farms of Jammu region, North India

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Abstract Content

Cryptosporidiosis is characterized by acute gastrointestinal disturbances, mucoid or *haemorrhagic* watery diarrhoea, fever, lethargy, anorexia and loss of condition. From India, *Cryptosporidium* spp. in cattle has been reported in many studies but information in small ruminants is scanty. In the present study faecal samples of 178 lambs and goat kids, aged upto 2 months old were collected from privately owned sheep and goat farms of Jammu region. Microscopical screening of faecal samples by diethyl ether sedimentation followed by modified Zeihl-Neelsen stain revealed presence of *Cryptosporidium* oocysts in 28.65% (51/178) animals. The prevalence was higher in lambs than the goat kids. According to age, highest infection was recorded in <1 months animals (35.41%). Significantly ($p < 0.05$) higher infection was observed among diarrhoeic animals (41.66%) than the non diarrhoeic animals (22.03%). Mean oocyst intensity observed was 2, but the animals having mucus and diarrhoea in the faecal samples showed mean oocyst intensity of 3. The samples found positive by acid fast staining were subjected to molecular characterization by a nested PCR of the small subunit rRNA gene (18S). The presence of 830 bp product confirmed for the positivity of *Cryptosporidium* spp.. These positive samples were further subjected to restriction fragment polymorphism analysis with SspI, and VspI restriction enzymes. The PCR-RFLP showed that lambs and kids were infected with *Cryptosporidium parvum*, and *Cryptosporidium ubiquitum*. The results of the present study conclude that identification of *Cryptosporidium* in small ruminants warrants better care of farm animals to avoid contamination and illness in susceptible population.

Keywords: Cryptosporidiosis, Lambs, Kids, Molecular characterisation, India

Assessment methods for recovery of eggs and larvae of zoonotic nematodes in sand beach

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Abstract Content

The aim of this work was to evaluate the recovery of *Toxocara canis* eggs and *Ancylostoma caninum* larvae using experimentally contaminated beach sand as substrate by means of different enrichment techniques. Three degrees of contamination (DC) were established: low, medium and high: 10, 50 and 100 eggs or larvae for each 50g of sand respectively. Firstly, the Willis method was evaluated with the following solutions: NaCl, NaNO₃, ZnSO₄, MgSO₄, sugar+NaCl, with and without the addition of Tween® 20. After that, the sedimentation-flotation method was evaluated by using the three most efficient solutions in the recovery by the Willis method (NaNO₃, MgSO₄, sugar+NaCl, with and without Tween® 20). Also, the Willis method was evaluated by these solutions and the Baermann method for the recovery of larvae. Data was analyzed by a method of multiple linear regression. More eggs were recovered with the Willis method than with the sedimentation-flotation technique. The effect of adding Tween® 20 to the solutions had variable results according to the solution used, showing a negative interaction (MgSO₄, sugar+NaCl), positive interaction (NaCl, NaNO₃) or neutral interaction (ZnSO₄). Larvae were also recovered with the Willis method in all the solutions evaluated, but the percentages were lower than those obtained with the Baermann method. The Willis method was the most sensitive, simplest and economic method for recovering *T. canis* eggs using NaNO₃+Tween® 20. Although this technique allows the recovery of *A. caninum* larvae, its sensitivity is lower than that of the Baermann technique.

Gastrointestinal helminth parasites of pets as a potential risk for human health in Bogor, Indonesia

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Abstract Content

Gastrointestinal helminth parasites in pets can potentially infect humans and cause serious public-health problems. The presence of information of gastrointestinal helminth parasites in pets is important for owner education dealing with parasite prevention. The retrospective study was conducted in The Veterinary Teaching Hospital, Veterinary Medicine Faculty, Bogor Agricultural University. This study covered available data from January 2014 to December 2016 including the result of laboratory examination of helminth infection from cat and dog patients which parasite infection suspected or coming for general checkup. Data on pet species, age, sex and status of helminth infection were retrieved and analyzed by using Chi-Square Test. A total of 27 dog and 78 cat patients was reported at animal hospital for internal parasite examination. Among these dog and cat patients, 48.12 % dogs and 50 % cats were positive infected by helminths. The detected parasite with their frequencies in dog patients was hookworms (25.93 %) and *Toxocara canis* (22.22 %). Cat patients were found more parasitized with *T. cati* (37.18%) than hookworms (8.97%) and *Dipylidium caninum* (5.13%). *T. canis* and *T. cati* were detected more frequently in dogs and cats respectively with less than one years of age ($P < 0.05$). The sex or breed groups didn't significantly affect the prevalence of parasites. The presence of these zoonotic helminth parasites in pet patients examined indicates a potential public health problem in Bogor. Hence, The efforts should be made to educate pet owners to prevent such parasitic diseases.

Keywords: gastrointestinal, helminth, pet, dog, cat, Bogor

Modulation of macrophage phenotype during liver fluke (*Fasciola hepatica*) and bacterial co-Infection

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Abstract Content

Fasciola hepatica releases excretory/secretory (FhES) and tegumental coat (FhTEG) molecules that drive Th2 and regulatory responses with suppression of protective Th1/Th17 immune responses. This is associated with the switching of macrophage phenotypes from classically activated (M1) to alternatively activated (M2). Previous research has reported that hosts co-infected with liver fluke and secondary bystander respiratory infections, such as *Bordetella pertussis* and *Mycobacterium bovis* fail to clear these bacterial infections, which require Th1 immunity protection. Recent *in vivo* findings have shown that chronic helminth infections may have implications in vaccination against *Streptococcus pneumoniae*. We aim to investigate the impact of liver fluke-mediated immunomodulation on the host's immune response to *S. pneumoniae* and *M. bovis*. We have established an *in vitro* co-infection model using a J774.2 macrophage cell line infected with fluke and *M. bovis* antigens. Macrophage activity was assessed from supernatants and cell lysates by measuring nitric oxide (NO) (M1 marker) and arginase (M2 marker) levels. Preliminary results show that NO was significantly down-modulated in both co-infection cultures indicating the ability of fluke molecules to suppress classical activation of macrophage in co-infection consequences. Interestingly, although fluke antigens alone failed to induce macrophage arginase production, ES exposed cells co-infected with *M. bovis* antigens showed significantly increased arginase production while tegumental antigen co-infected cultures produced significantly less arginase than macrophages exposed to *M. bovis* antigens alone. Better understanding of co-infection may lead to improved management and treatment of human and animal disease. Thus, further *in vitro* and *in vivo* models are currently under investigation.

Keywords: *Fasciola hepatica*; bacterial co-infection; immunomodulation; macrophage; immune response

Microsporidia and *Zingiber officinale* (ginger): An emerging parasite and an old medicinal plant in vivo trial

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Abstract Content

Microsporidia, of the genus *Enterocytozoon*, are important cause of life-threatening diarrhea in immunocompromised hosts. There are controversies on the use of albendazole in treatment, whereas, fumagillin was to be more effective but with undesirable side effects. Ginger has been used as an antimicrobial agent since ancient times. However its potential therapeutic effect against *Enterocytozoon bieneusi* has not been tested. This study was done to investigate the effect of ginger as a prospective therapy for microsporidia versus fumagillin in immunocompetent and immunosuppressed mice. Also, to report the synergistic effect of the two compounds together in a drug-combination regimen. *Enterocytozoon bieneusi* was the species identified in the stool samples collected from immunocompromised patients and was used to initiate the in vivo infection in albino mice. Animals were divided into three major groups. Group I: Normal, non-infected non-treated, control group; group II: infected, immunocompetent group; and group III: infected, immunosuppressed group. Each infected group was subdivided into four equal subgroups a, b, c and d which comprise non-treated, fumagillin-treated, ginger-treated, and combined ginger/fumagillin treated mice respectively. Evaluation of the ginger efficacy in infected mice was achieved by assessment of fecal spore shedding, intestinal spore load, and biochemical assay which aimed at estimation of the malondialdehyde level and total antioxidant capacity. Spore count in both stool and intestinal sections and malondialdehyde level decreased significantly with ginger treatment. Best results were obtained when ginger is combined with fumagillin in all measured parameters. Ginger could be a good enhancer for fumagillin efficacy to eradicate infection.

Keywords: *Enterocytozoan bieneusi*; ginger; fumagillin; synergistic action; antioxidant.

Prevalence of antibodies against *Toxoplasma gondii* in the small ruminant population of Oman

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Abstract Content

Toxoplasmosis is a worldwide disease caused by an obligate intracellular protozoan parasite, *Toxoplasma (T.) gondii*. This parasite can infect any warm-blooded vertebrate and is a pathogen of medical and veterinary significance. In this study, a total of 5413 small ruminants (2853 goats from 569 farms and 2560 sheep from 531 farms) were randomly sampled all over Oman and tested through a commercial ELISA (LSIVet™ Ruminant Toxoplasmosis Serum ELISA kit, Life Technologies, France). Prevalence (%) along with 95% confidence interval (CI) was calculated and Chisquare analysis ($p < 0.05$) was conducted to test the significance of association between herd and individual level variables. Furthermore, univariable analysis was also conducted and Odds ratios (OR) along with respective 95% CI was calculated. At herd level, the prevalence was recorded as 35.7% and 36.5% in goats and sheep respectively ($P = 0.782$). The seroprevalence was significantly different in goats (ranged from 3.5 to 56.6%) and in sheep (ranged from 6.3 to 62.5%) from farms of various governorates of Oman ($p < 0.001$). The individual level prevalence was recorded as 16.7% in goats and 17.2% in sheep ($p = 0.624$). Univariable analysis indicated that higher prevalence of antibodies against *T. gondii* was observed in small ruminants above 3 years of age, females and those belonging to farms where cats were frequently observed. The study indicates that *T. gondii* infection is endemic in the small ruminants of Oman and a carefully planned control program is required to safeguard the human population from this important zoonosis.

Keywords: *T. gondii*, seroprevalence, ELISA, small ruminants, Oman

Knowledge, awareness and practices regarding cystic echinococcosis among livestock farmers in Basrah Province, Iraq

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Abstract Content

Cystic echinococcosis is endemic neglected parasitic zoonoses in many of the of Middle East countries. We conducted this targeted survey among livestock farmers in Basrah province, Iraq, to evaluate their knowledge and awareness about the disease, and to investigate risky practices that could contribute to spread and persistence of the disease. Among all the participants ($n=314$), 27.4% owned dog on their farms. Among farmers owning dogs, 76.7% (66/86) never tied up their dogs, 43% (37/86) indicated feeding uncooked animal viscera to dogs, and the majority (96.5%) do not de-worm their dogs. Among all the respondents, 9.8% (31/314) indicated eating raw leafy vegetables without washing. Almost third (32%) of the livestock farmers obtain water for domestic use from a river, while 94.3% (296/314) of them do not boil water before use for domestic purposes. Half of the interviewed livestock farmers in Basrah were not aware about how humans get infected with hydatid disease, and 41.4 (130/314) did not realize that CE is a dangerous disease to human health. Almost third of the farmers who own dogs regarded de-worming of their dogs as a low priority practice. This study highlights gap in knowledge about cystic echinococcosis among the study samples. Risky practices regarding dog keeping management and food and water handling practices were identified. The insight from this research could be used to develop health promotion tools and to improve the delivery of a health education strategy relevant to cystic echinococcosis control in Iraq.

Keywords: Cystic Echinococcosis; Iraq; Knowledge; Practices; KAP

Resistance development in *Aedes aegypti* (Linnaeus) against metofluthrin in mosquito coil

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Abstract Content

This study was conducted to determine the resistance development rate in *Ae. aegypti* to metofluthrin in mosquito coil in the presence of selection pressure. Adult mosquitoes were exposed to coil burnt up to 2.41 minutes in the glass chamber. The burning time of the coil would induce 50% mortality based on earlier test. The surviving mosquitoes were reared to produce next generation. Each generation has undergone similar selection pressure continuously for 10 generations. To measure the resistance development rate, each generation of mosquitoes after selection pressure were exposed to 0.5 gram of coil according to standard test protocol of SIRIM. The knockdown rates were recorded every minute up to 20 minutes. The mosquitoes showed different level of susceptibility to metofluthrin when compared to reference strain. Bioassay results revealed that KT_{50} values of metofluthrin-selected *Ae. aegypti* ranged between 1.75 – 2.35 minutes for 10 consecutive generations. Complete knockdown of reference and selected strains were achieved within 20 minutes of exposure period. However, metofluthrin-selected *Ae. aegypti* exhibited lower mortality (92.45%) compared to reference strain (96.61%) at 24 hours post treatment. This study revealed the potential resistance development in *Ae. aegypti* after 10 generations of selection pressure by metofluthrin in mosquito coil.

Keywords: Aedes aegypti; metofluthrin, mosquito coil, selection pressure, Malaysia

Larval susceptibility status of dengue vector, *Aedes albopictus* (Skuse) against organochlorines and organophosphates in Sarawak, Malaysia

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Abstract Content

This study was conducted to determine the susceptibility status of *Aedes albopictus* larvae against various organochlorines and organophosphates in Sarawak, Malaysia. Ovitrap surveillance was conducted in 13 districts across Sarawak to obtain the *Aedes* specimens. Larval bioassay was performed according to WHO standard protocol. Field collected *Ae. albopictus* larvae were tested against diagnostic dosage of 8 larvicides belonging to organochlorines and organophosphates, namely DDT 0.012 mg/L, dieldrin 0.050 mg/L, bromophos 0.050 mg/L, chlorpyrifos 0.012 mg/L, fenitrothion 0.020 mg/L, fenthion 0.025 mg/L, malathion 0.125 mg/L and temephos 0.012 mg/L. Mortality of larvae were recorded at 24 hours post treatment. The results revealed that *Ae. albopictus* from Sarawak were completely susceptible to bromophos and temephos (mortality = 100%), and highly resistant to DDT, chlorpyrifos and malathion (mortality ranged from 0 – 20%). However, the larvae showed various level of susceptibility to fenitrothion and fenthion. This study indicated that bromophos and temephos were still effective to control *Ae. albopictus* in Sarawak, Malaysia.

Keywords: Aedes albopictus; susceptibility status; organochlorines; organophosphates; Malaysia

Th1 and Th2 immune response in experimental *Leishmania martiniquensis* infection in BALB/c mice

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Abstract Content

Leishmania martiniquensis infection has been reported in human and domestic animals of Martinique Island, Germany, Switzerland, USA, Myanmar and Thailand, with various clinical manifestations including asymptomatic, CL, VL, and atypically disseminated cutaneous and visceral co-existing forms. In the present study, we conducted an experimental infection in BALB/c mice by intravenous injection with *L. martiniquensis*, aimed to improve the understanding of the pathogenesis and immune responses to infection. A quantitative real time PCR (qPCR) specific to *L. martiniquensis* was developed to quantify the parasite burden, and reverse-transcriptase qPCR was employed to determine the expression of mRNA of cytokines and iNOS in the liver and spleen. In the liver, parasite burdens gradually increased and peaked at 4 week-post infection (WPI), but the parasite was finally undetected at 16 WPI. This resolving infection was associated with the development of hepatic granulomas and the high expression levels of IFN- γ , TNF- α , IL-12p40, IL-2, and iNOS, suggesting the development of Th1 immune response. In contrast, parasite persistence was observed in the spleen, associated with splenomegaly. Parasite burdens in spleen was minimal at 1 WPI, gradually increased and peaked at 16 WPI. High expression levels of TNF- α at 2 and 4 WPI, and of IL-10 at 16 WPI was detected and may associated with the progressive breakdown in splenic architecture, lymphoid depletion and parasite persistence. In summary, experimental *L. martiniquensis* infection in BALB/c mice revealed only the visceral infection that may be appropriate to use it as a model for studying self-healing or subclinical infection.

Keywords: *Leishmania martiniquensis*; experimental infection; qPCR; parasite burden; RT-qPCR; cytokine

An investigation on bacterial arthropod-borne diseases in West Malaysia

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Abstract Content

The impact of bacterial arthropod-borne diseases (tick, flea, mite-borne diseases, spotted fevers, relapsing fevers, bartonellosis, anaplasmoses and ehrlichioses) on public health is significant but often neglected. Most of the bacterial arthropod-borne diseases are underdiagnosed due to the lack of appropriate diagnostic assays. In this study, serological methods such as indirect immunofluorescence assays (IFA) and enzyme-linked immunosorbent assays were used to determine the exposure of various Malaysian populations to bacterial arthropod-borne diseases (spotted fevers, anaplasmoses, ehrlichioses and scrub typhus). Entomological surveys were conducted to identify potential vectors. Polymerase chain reaction assays followed by sequencing were used for detection and identification of the causative agents in the arthropod samples. A significant proportion of rural dwellers (including indigenous people and animal farm workers) demonstrated IgG seropositivity to the causative agents of spotted fevers, anaplasmoses, ehrlichioses and scrub typhus, as compared to the blood donors from the urban area. Vector surveillance studies provide evidence on the detection of the relevant bacteria in ticks, fleas and mites. Public awareness and information on diagnosis, treatment and preventive measures are essential to reduce the exposure of the local population to bacterial arthropod-borne diseases.

Keywords: bacteria, zoonotic diseases, tick, flea, mite

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