

Breakout Sessions

21.08.23

Breakout 1: OA01 Equine Parasites

OA01.01

Cyathostomin communities in Horses in Ireland – Opening the Black Box_Byrne, Ms.
Orla_University College Dublin

Co-Authors - Ms. Disha Gangotia, Dr. Annetta Zintl, Mr. Liam Kiser, Ms. Fiona O'Neill, Mr. Daniel McSweeney, Ms. Olivia Boxall, Ms. Stacey Dunne, Ms. Breanna-Rose Lamb, Dr. Nicola Walshe, Prof. Grace Mulcahy.

Cyathostomins are among the most important parasitic infections of equids worldwide, with the potential for both chronic and acute disease. Understanding of the epidemiology, host-parasite relationship and pathogenesis of equine cyathostomin infection is complicated by the large numbers of cyathostomin species and, the occurrence of mixed infections. Studying the communities of cyathostomins, the interaction both within and between communities and with their host, has the potential to contribute to understanding of equine cyathostominosis.

We sequenced the ITS-2 region of individual adult worms found in the caecum, right dorsal colon and left ventral colon of horses harvested from a small abattoir study and then compared community composition within the three intestinal compartments in our study population. We plotted phylogenetic relationships between the helminths according to intestinal section of each horse and also the relationship of the communities between individual horses. Finally, we compared the helminth communities found in this study with communities characterised by morphological analysis in horses in Ireland over 20 years ago.

The prevalence and abundance of cyathostomin species found in Ireland was similar to those found in studies in a number of different geographic locations, as per a recent meta-analysis with the exception that *Coronocyclus coronatus* was more abundant than in other studies. We also observed both positive and negative interactions between individual species in the community composition, notably, a positive association between *Cylicocyclus insigne* and *Cor. coronatus*, and a negative association between *Cylicostephanus longibursatus* and both *Cor. coronatus* and *Cylicocyclus ashworthi*.

In conclusion, studying communities of cyathostomins represents an opportunity to gain insight into the extent to which communication within and between species can influence pathogenicity, age susceptibility and the epidemiology of diseases such as acute larval cyathostominosis.

OA01.02

Age-related excretion of strongyle eggs in young horses_Hertzberg, Dr.
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As part of a retrospective nationwide study (2010 to 2016), results of 2690 faecal egg counts (FEC) of horses up to 7 years of age were analyzed. The mean (arithmetic) strongyle egg excretion measured in eggs per gram of faeces (EPG) in the 8 age groups (< 1, 1, 2, 3, 4, 5, 6, 7 years) were (CI) 198 (122 -306), 481 (386 - 601), 388 (323 - 457), 332 (278 - 395), 293 (240 - 358), 205 (169 - 248), 127 (101 -158) and 88 (68 - 113) EPG. The highest strongyle egg excretion was measured in the yearlings, the reduction until year 7 was 82%. Mean values of the age groups '< 1 - 3 years' and '4 - 7 years' were significantly different with 373 and 171 EpG respectively (p<0.0001). In the range up to 6 years, the foals had the lowest mean FEC. The main reasons are

most likely the relatively low roughage intake from pasture parallel to milk nutrition and the low infection pressure from herbage induced by the mares, most of which only show a minor egg excretion. The substantial increase in strongyle egg excretion after weaning is reflecting the period when the majority of foals are transferred to the rearing stables. The proportion of negative samples in the McMaster analysis (sensitivity 50 EpG) increased from 12 to 71% between age 1 to 7 years. At the same time, the proportion of results with a strongyle EpG \geq 200 fell from 42 to 13%. This indicates that the majority of horses can significantly reduce strongyle egg excretion between the age of 3 and 7, while a small proportion of animals have long-term elevated values, possibly still as adults. The clear differences in strongyle egg excretion between the age categories will serve as a basis for the establishment of more differentiated control measures within the segment of young horses than have been practiced so far.

OA01.03

Cytochrome oxidase C subunit 1 marker region as target for nemabiome-sequencing based cyathostomin population characterization_Von Samson-himmelstjerna, Prof. Georg_Freie Universität Berlin

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In grazing horses cyathostomins (small strongyles) occur ubiquitously, residing in the large intestine of horses from all age groups. They are represented by over 50 accepted species exhibiting a unique multi-species complex. Although in the vast majority of infected horses cyathostomins do not appear to cause any clinical symptoms. In exceptional cases, due to the simultaneous excystation of a multitude of larvae encysted in the large intestinal mucosa into the gut lumen, the so called larval cyathostominosis can occur. Particularly in young horses this may lead to severe diarrhoea, weight loss and often even death. To prevent the clinical manifestation, on most equine enterprises anthelmintic treatments were performed routinely and frequently since decades, which has resulted in the widespread evolution of resistant cyathostomin populations. As neither the strongyle-type eggs shed by cyathostomins nor the larvae developing in the environment from these eggs can be reliably assigned to species level, molecular tools have been developed to allow species identification. The availability of meaningful, standardized and medium to high throughput tools for cyathostomin species identification is of key relevance for investigations on species-specific pathogenicity, ecological aspects but also anthelmintic resistance studies. To this end herein we employed the mitochondrial cytochrome c oxidase I (COI) gene sequences of approx. 20 cyathostomin species. Previously we have shown that the use of this marker provides higher cyathostomin species resolution as compared with e.g., the ribosomal internal-transcribed-spacer 2. We developed a high-throughput sequencing based approach using Illumina MiSeq v3 600. COI amplicons were sequenced and a bioinformatic data processing pipeline was developed which provided a tool to distinguish between the different cyathostomin species. We demonstrate that the metabarcoding-dual-indexing approach we developed allows to assign DNA extracted from pools of cyathostomin larvae obtained from different countries to cyathostomin species level and to characterize the respective species complexes on individual host level.

OA01.04

Does selective anthelmintic treatment according to fecal egg count provide better worm control in horses than fixed “blanket” treatments in the autumn and spring? A case study from Louisiana, USA_Vatta, Dr. Adriano_Louisiana State University

Co-Authors - Ms. Brooke Delcambre, Ms. Victoria Bailey, Ms. Michelle Vetter, Ms. Bryce Gilbert, Dr. Adriano Vatta., Dr. Erin Oberhaus

Modified McMaster fecal egg counts (FECs) were conducted every two weeks from May 2021 to May 2022 for herds of mares maintained at two LSU AgCenter farms (Central, n=26; RBC, n=24) situated in the Baton Rouge metropolitan area, Louisiana. Mares at each farm were assigned to either treatment with ivermectin, at the label dosage, when FEC were ≥ 500 eggs per gram of feces (EPG; Group 1) or ivermectin in only the spring and fall (Group 2). Horses positive for *Anoplocephala* spp. were dewormed with pyrantel at twice the label dosage on an ad hoc basis. The egg reappearance periods (ERPs) following ivermectin treatments for Group 2 horses were 6 and 10 weeks, respectively, in the spring and fall at Central, and 6 weeks in both seasons at RBC. Based on examination of data throughout the year, the ERP for double-dose pyrantel was 6 weeks. At both farms, higher mean FECs (<850 EPG) were occasionally noted in Group 1 mares, but the mean FECs were almost always at or below the 400 EPG level. Mean FECs in Group 2 at both farms were higher ($P<0.05$) than those in Group 1 at several of the sampling points during mid-July to early November 2021. Group 2 mean FECs during this period were generally $\geq 1,000$ EPG (Central) or ≥ 800 EPG (RBC). A decrease in mean FECs was noted for all groups during January 2022, with counts increasing again at the end of February. On the farms studied, selective treatment according to FEC appeared to maintain egg shedding on pasture at consistent and lower levels than “blanket” deworming in the spring and fall did. Fecal egg counts in horses receiving twice yearly treatments reached levels that were, at times, twice as high as the selectively treated horses.

OA01.05

Status of anthelmintic resistance and egg reappearance periods of equine cyathostomins on Australian thoroughbred farms_Abbas, Dr. Ghazanfar_The University of Melbourne

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This study aimed to investigate anthelmintic resistance in cyathostomins of horses using conventional and molecular techniques. Seventy-four faecal egg count reduction tests were conducted for eight anthelmintic drugs at 22 Thoroughbred farms across Australia. The modified McMaster technique was used to perform faecal egg counts pre- and 2-weeks-post-treatment, and the percentage fecal egg count reduction (%FECR) was calculated using a Bayesian hierarchical model. Resistance to a particular anthelmintic drug was confirmed if it failed to meet both criteria of $<95\%$ FECR and $<90\%$ of the lower limit of 95% confidence interval (LCL). Second internal transcribed spacer (ITS-2)-based DNA-metabarcoding was used to determine the species composition of cyathostomins present pre- and post-treatment. The highest efficacy was observed for combinations of ivermectin and pyrantel as well as abamectin and morantel, with $>97\%$ FECR at 2-weeks post-treatment for both combinations in 15 trials. Among macrocyclic lactones, resistance was observed for ivermectin (2/15 trials; FECR range:

82-92%; LCL range: 80-89.5%), abamectin (2/4; 73-92%; 65-88%) and moxidectin (3/14; 88.6-91%; 84-89%). Low efficacy of oxfendazole was found in 6/6 trials (FECR range: 0-56%; LCL range: 0-31%) whereas oxfendazole-pyrantel combination was effective in only 3 out of 18 trials. Shortened egg appearance periods of 4-6 weeks were observed in 31 trials following treatment with ivermectin, moxidectin and combinations of anthelmintics when resistance was not detected at 2-weeks post-treatment. DNA metabarcoding of faecal nematode eggs revealed that *Cylicocylus (Cyc.) nassatus*, *Cylicostephanus (Cyls.) longibursatus* and *Coronocylus coronatus* were the most prevalent species at 2-weeks-post-treatment whereas *Cyc. nassatus*, *Cyls. longibursatus* and *Cyathostomum catinatum* were dominant among first appearing species at 3-weeks post-treatment and beyond. These findings indicate the extent of anthelmintic resistance in cyathostomins prevalent in Australian Thoroughbred horses and pave the way for developing tailored parasite control guidelines to manage anthelmintic resistance.

Keywords: Horse strongyles, Anthelmintic resistance, Macrocyclic lactones, DNA metabarcoding, Australia

OA01.06

Loss of parasite control due to shortened equine strongylid egg reappearance periods _Nielsen, Dr. Martin_University of Kentucky

Co-Authors - Dr. Dave Leathwick, Dr. Christian Saueremann.

Over the past three decades, equine strongylid egg reappearance periods (ERPs) have shortened substantially for macrocyclic lactone anthelmintics. The ERPs of ivermectin and moxidectin were originally reported in the 8-10- and 12-16-week ranges, respectively, but they are frequently found to be around 4-5 weeks in recent studies. This loss of several weeks of suppressed strongylid egg output could have substantial implications for parasite control. This study made use of a computer simulation model to evaluate the impact of shortened ERPs on the anthelmintic performance of ivermectin and moxidectin against equine cyathostomins. The original ERPs were set to 7.1 and 15.4 weeks for ivermectin and moxidectin, respectively, while the reduced ERP was set to 4.6 weeks for both actives. Simulations were set to compare model outputs between original and reduced ERP scenarios and results expressed as percent increase in strongylid egg output, infective third stage larvae on herbage (L3h), and encysted early third stage larvae (EL3). For each drug, simulations were evaluated for two different treatment scenarios (2 and 4 treatments annually), two different age groups (yearlings and adults), and for four different climates (cold humid continental, temperate oceanic, humid subtropical, and hot/cold semi-arid). Across all simulations, there was a substantial increase of the three evaluated parameters. Overall, the loss of anthelmintic performance was at a magnitude of 10 times larger for moxidectin compared to ivermectin with percent increases in the 1000-6000% and 100-300% ranges, respectively. This performance loss was climate dependent, and was also affected by treatment intensity, but not by horse age. This is the first study to evaluate consequences of shortened ERPs in horses and demonstrated a substantial loss in anthelmintic performance resulting from this development.

OA01.07

Occurrence of *Anoplocephala* spp., *Strongylus* spp. and Cyathostominae in German horse farms using coproscopic, serological and molecular approaches_Schmidt, Ms. Jennifer_Institute for Parasitology, Freie Universität Berlin

Co-Authors - PD Dr. rer. nat. Jürgen Krücken, Dr. Eric Bousquet, Dr. Jürgen Bartz, Dr Corrine J. Austin, Kristy L. Lightbody, Prof. Jacqui B. Matthews, Prof. Martin K. Nielsen, Georg von Samson-Himmelstjerna.

Effective and targeted deworming management plays an essential role in horse husbandry, as horses usually suffer from multispecies infections. Recently, an unexpectedly high frequency of infections with *Anoplocephala* spp. and the large strongyles has been observed in several studies from Germany, which pose a particular health risk due to their high pathogenicity. In order to collect current prevalence data on the different equine helminth species, we conducted a large cross-sectional study in Germany. Faecal, blood and saliva samples were collected and analysed from 970 horses from 96 stables from the federal states of Brandenburg, Saxony, Lower Saxony, North Rhine-Westphalia, Schleswig-Holstein and Bavaria. Using Mini-FLOTAC, the egg counts per gram of faeces (EPG) were determined for the respective worm eggs. A prevalence of 47.4% (95% CI 44.3 - 49.8%) was found for the gastrointestinal strongyles with farm level prevalence of 95.8%. Prevalence's of *Anoplocephala* spp., *Parascaris* spp. and *Oxyuris equi* were only 0.8% (95% CI 0.4 - 1.5%), 0.5% (95% CI 0.2 - 1.1%) and 0.2% (95% CI 0.06 - 0.6%), respectively. Worm egg isolation followed by DNA isolation was carried out on farm level. Real-time PCRs were performed to determine the prevalence of *Strongylus vulgaris* (using a species-specific probe), as well as of *Strongylus edentatus*, *Strongylus equinus* and *Strongylus asini* using high-resolution melting curve analysis. PCR results will be compared with serological data obtained using the SvSXP ELISA for *Strongylus vulgaris*. Antibodies against *Anoplocephala* spp. in saliva were determined using the EquiSal Tapeworm Test, and demonstrated a prevalence of positive results (188/970 horses) of 19.4% (95% CI 17.3 – 21.7%) with a farm level prevalence of 38.5%. Risk factors for infections with large strongyles and *Anoplocephala* spp. and high strongyle egg excretion were identified based on analysis of questionnaire data regarding, for example, husbandry, hygiene, pasture management and deworming program.

21.08.23

Breakout 2: OA02 Ticks of ruminants

OA02.01

Low genetic diversity and population structuring of *Amblyomma hebraeum* and *Rickettsia africae* from coastal and inland regions in the Eastern Cape Province of South Africa_Pillay, Dr. Alicia_University of KwaZulu-Natal

Alicia Pillaya*, Nkululeko Nyangiweb,c and Samson Mukaratirwaa.d

Amblyomma hebraeum is the main vector of *Rickettsia africae*, the causative agent of African tick bite fever in southern Africa. Because pathogen dispersal is known to be influenced by tick adaptations to climate or host species, this study aimed to analyse the genetic diversity of *A. hebraeum* and *R. africae* infection of ticks collected from cattle in the Eastern Cape province of South Africa. DNA was extracted, amplified, and sequenced for the COI and ITS2 markers from *A. hebraeum* samples and the 17 kDa and ompA genes for rickettsial detection. Between six and ten haplotypes were identified from 40 COI and 31 ITS2 sequences; however, no population structuring was observed among sites ($\Phi_{ST} = 0.22$, $p < 0.05$). All *A. hebraeum* isolates clustered with southern Africa GenBank isolates. *Rickettsia africae* was detected in 46.92% (95% CI = 41%–53%, $n = 260$) of ticks. All *R. africae* isolates clustered with strain PELE and Chucks, which were reported previously from South Africa. These results confirm that *A. hebraeum* populations are undergoing a recent population expansion driven by cattle movement, facilitating local and long dispersal events across the Eastern Cape province. This is of great public health importance which may affect tourists visiting these regions and requires further long-term surveillance of ATBF patients, and *R. africae*-infected ticks.

OA02.02

The influence of cattle age on hard tick (Ixodidae) infestations in South Africa_Terblans, Ms. Elizna_University Of The Free State

Co-Authors - Dr Ellie MSP van Dalen, Mr Leon Kruger.

Ectoparasites, especially ticks, are responsible for substantial economic losses in the livestock production industry. These losses include a decrease in production output and an increase in production cost and tick-borne diseases. The growing global human population critically necessitates the upkeep of sustainable food production. Current farming practices in South Africa rely heavily on controlling ticks with acaricides. However, many tick species have already developed resistance to most of these acaricides and alternative methods for tick control are urgently needed. To combat the rising problem, the study focused on using the natural resistance of cattle against ticks, known as host resistance, to limit the use of acaricides and reduce the rate of ticks developing resistance against these acaricides. The aim of this study was to determine if the tick load on cattle was influenced by the age of the animals. A group of 30 cross-bred female cattle were selected from the same farm near Bloemfontein in the Free State province. The animals were divided into three groups based on their reproductive status and included reproductive immature, reproductive mature non-pregnant and pregnant animals. All the visible ticks were removed from each animal once a month for a period of six months during spring and summer. The ticks were placed in 70% ethanol and were identified up to species level with a dissection microscope. Tick species observed during this time include *Rhipicephalus appendiculatus*, *R. evertsi evertsi*, *Hyalomma truncatum*, and *H. rufipes*. An age-related increase in the tick load was observed with the lowest infestations found in the reproductive immature group (23,7%),

followed by the reproductive mature non-pregnant (34,3%) and the highest infestation in the pregnant group (42%). The results from the study will contribute to the formulating of an integrated tick control strategy that limit the amount of acaricides used.

OA02.03

Ecological competition and acaricide resistance of two *Rhipicephalus (Boophilus)* species collected on commercial farms in South Africa_ Van Dalen, Dr. Ellie_ University Of The Free State, South Africa

Co-Author - Dr Candice Jansen van Rensburg.

The development of tick resistance to chemical control plays a major role in the increasing global economic impact of ticks on cattle farming. Reports on acaricide resistance of *Rhipicephalus decoloratus*, endemic to Africa, are scant compared to the related and globally distributed *Rhipicephalus microplus*. *Rhipicephalus microplus* is an alien species to Africa and was first introduced in the late 1800's by cattle imported from southern Asia via Madagascar, and around 1980 from cattle imported from Brazil. In South Africa, the invasion of the alien species was mostly reported from traditional communal grazing areas with rapid invasion and sometimes total replacement of the native species. In resistance screening of both *Rhipicephalus (Boophilus)* species to field concentrations of cypermethrin, amitraz, and chlorfenvinphos, using LIT, only 3.7% of the total (383) tick collections submitted from commercial farms in South Africa were *R. microplus* populations. A further 1.6% showed the co-existence of the two species. Comparing the level of resistance between the two species to the acaricides showed a mean phenotypic resistance of 66.2% of *R. decoloratus* populations to cypermethrin and 26.5% to amitraz. This was significantly higher than for *R. microplus* populations with 23.0% of the populations resistant to cypermethrin and 4.1% resistant to amitraz. Closed commercial farming areas seem to have a preventative advantage over communal grazing systems for the invasion of *R. microplus* and the displacement of *R. decoloratus*. This can be attributed to the low introduction of cattle from outside herds that could introduce *R. microplus* onto a commercial farm, and the susceptible status of the introduced *R. microplus* to acaricide control practices on a specific farm. Regular monitoring of the presence and resistance status of these two species can be of high importance to prevent unnecessary financial losses due to insufficient control and disease transmission.

OA02.04

Tick-borne pathogens and body condition of cattle in smallholder rural livestock production systems in East and West Africa_Madder, Prof. Maxime_Clinglobal

Co-Authors - Dr. Josephus Fourie, Mr. Alec Evans, Prof. Dieter Heylen.

Most of the African population lives in rural areas and depend on crop and livestock production for their livelihoods. Given their socio-economic importance, we initiated a standardized multi-country surveillance study to assess the current status of tick-borne haemoparasites of cattle in sub-Saharan Africa. We assessed pathogen prevalences in the blood of 6,447 animals. In addition, we screened for intrinsic (sex, weight, body condition) and extrinsic (husbandry, tick exposure) risk factors as predictors of infections with tick-borne haemoprotozoan (TBHP). There was a large macro-geographic variation observed in *Anaplasma marginale*, *Babesia bigemina*, *B. bovis* and *Ehrlichia ruminantium* prevalences. Most correlated with the co-occurrence of their specific sets of vector-competent ticks. Highest numbers of infected cattle were found in Ghana and Benin, and lowest in Burkina Faso. While *Theileria parva* was seldom found (Uganda only, 3.0 %), *A. marginale*

was found in every country with a prevalence of at least 40 %. *Babesia bovis* infected individuals had lower body condition scores. Age (as estimated via body weight) was higher in *A. marginale* infected cattle, but was negatively correlated with *B. bigemina* and *E. ruminantium* prevalences. *Ehrlichia ruminantium* infection was more often found in males, and *A. marginale* more often in transhumance farming. High levels of co-infection, especially the combination *A. marginale* × *B. bigemina*, were observed in all countries, except for Uganda and Burkina Faso. Tick-borne pathogens of cattle are ubiquitous in African's smallholder cattle production systems. Our standardized study will help a wide range of stakeholders to provide recommendations for TBHP surveillance and prevention in cattle, especially for *B. bovis* which heavily impacts production and continues its spread over the African continent via the invasive *Rhipicephalus microplus* tick.

OA02.05

Tick communities of cattle in smallholder rural livestock production systems in Sub-Saharan Africa_Heylen, Dr. Dieter_University Of Antwerp

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Abstract

Background: The majority of the African population lives in rural areas and depends on agriculture for their livelihoods. To increase the productivity and sustainability of their farms, they need access to affordable yield-enhancing inputs of which parasite control is of paramount importance. We therefore determined the status of current tick species with the highest economic impact on cattle, by sampling representative numbers of animals in each of seven Sub-Saharan countries.

Methods: Data included tick species' half-body counts from approximately 120 cattle at each of two districts per country, collected four times in approximately one year (to include seasonality). Study sites were chosen in each country to include high cattle density and tick burden.

Results: East Africa (Ethiopia, Uganda and Tanzania) showed overall a higher diversity and prevalence in tick infestations compared to West African countries (Benin, Burkina Faso, Ghana and Nigeria). In East Africa, *Amblyomma variegatum* (vector of *Ehrlichia ruminantium*), *Rhipicephalus microplus* (*Babesia bovis*, *Babesia bigemina*, *A. marginale*), *R. evertsi evertsi* (*A. marginale*) and *R. appendiculatus* (*Theileria parva*) were most prevalent tick species of economic importance. While the latter species was absent in West Africa, here both *A. variegatum* and *R. microplus* occurred in high numbers. *Rhipicephalus microplus* had spread to Uganda, infesting half of the cattle sampled. *Rhipicephalus appendiculatus* is known for its invasive behaviour and displacement of other blue tick species, as observed in other East and West African. Individual cattle with higher body weights, as well as males, were more likely to become infested. For six tick species, we found reduced infestations when hosts' were treated with anti-parasiticides.

Conclusions: Outcomes allow the determination of possible changes in presence and prevalence of ticks in each of the countries targeted, which is of importance in the light of human-caused climate and habitat alterations or anthropogenic activities. As many of the ticks in this study are vectors of important pathogens, but also, as cattle may act as end-hosts for

ticks of importance to human health, our study will help a wide range of stakeholders to provide recommendations for tick infestation surveillance and prevention.

Key words: *Amblyomma variegatum*, *Rhipicephalus microplus*, *Babesia bovis*,
Rhipicephalus appendiculatus, vector competence, sub-Saharan Africa

OA02.06

Cattle tick management in South East Queensland, Australia_ Dr. Luc DUREL_Virbac
S.A., France

Co-Authors - Dr. Matthew Ball, Dr. Bruce WATT.

Cattle tick, *Rhipicephalus* (*Boophilus*) *microplus*, is the most significant endemic cattle disease in Australia. A strategic program is essential for cattle health, welfare and productivity. Unfortunately, cattle tick control is challenging in South East Queensland (SE QLD) due to a preference for *Bos taurus* cattle, chemical resistance and reduced plunge dips. Many cattle producers lack a strategic program, treating once or twice when they see peak tick numbers ('hit and miss' approach). Producers are encouraged to reduce overall paddock contamination by strategic spring adulticide treatments. These treatments aim to prevent over 100 days of egg paddock contamination. This level of suppression of egg shedding onto pasture will control cattle tick numbers to prevent production loss but will not eradicate ticks. Suppressive spring treatment options include amitraz spray or dip once every 21 days for four to five treatments, a pour-on macrocyclic lactone (ML) once every 21 days for four to five treatments, a short-acting ML injection once every 28 days for three to four treatments, or a long-acting moxidectin injection twice 56 days apart. Fluazuron can be used 60-80 days after the adulticides to complete a spring-summer program and introduce a different class of chemical. A single adulticide in autumn may be needed before tick numbers naturally decline in winter. Producers are encouraged to submit cattle ticks for resistance testing (Larval Packet Test, or Larval Immersion Test). Resistance has been identified to synthetic pyrethroids, organophosphates, amitraz and fluazuron. No resistance has been identified to MLs but anecdotal reports suggest reduced protection periods on some properties. Programs need to include a ML with at least one other effective chemical. Some properties have reduced overall chemical usage by paddock rotation and spelling. They reserve suppressive programs for young growing stock that 'clean' the paddocks for later grazing by cow and calf units.

21.08.23

Breakout 3: OA03 Parasite Epidemiology

OA03.01

***Angiostrongylus cantonensis* (Nematoda: Angiostrongylidae) an emerging cause of human angiostrongyliasis in India.** Pandian, Mr. Divakaran_Czech University Of Life Sciences

Co-Authors - Prof David Modrý, Dr. Tomáš Najer

Angiostrongyliasis is an emerging zoonotic disease caused by larvae of metastrongyloid nematode *Angiostrongylus cantonensis*. Its obligatory heteroxenous life cycle involves rats as definitive, mollusks as intermediate, amphibians and reptiles as paratenic hosts. In humans, infection manifests as *Angiostrongylus* eosinophilic meningitis (AEM) or as an ocular form. As there is no comprehensive account on the disease in India, our study aims to evaluate distribution of human angiostrongyliasis, its clinical course and possible sources. Systematic literature search identified 17 reports describing 36 human cases from 1966 to 2022. AEM accounted for 29 cases (82 %), 5 cases were reported as ocular, 1 case was combined, 1 case unspecified. The presumed source of infection was reported in only 5 cases. Importantly, 19 AEM patients reported a history of eating raw monitor lizard tissues. As top predators, varanids (*Varanus* spp.) cumulate high numbers of L3, responsible for acute disease in humans. The source was not identified in ocular cases. Absolute majority of cases were diagnosed based on findings of nematodes and clinical pathology (dominated by CSF eosinophilia). Only 2 of the cases were confirmed as *A. cantonensis*, one is by immunoblot and the other by q-PCR. Cases of angiostrongyliasis were reported in Delhi, Karnataka, Kerala, Maharashtra, Madhya Pradesh, Puducherry, Telangana, and West Bengal. With more than 1.4 billion inhabitants, India represents one of the least studied areas regarding *A. cantonensis*. Many cases probably remain undiagnosed/unreported. As most cases have been reported in the state of Kerala, further research can address this region. Besides examination of rodent and mollusc hosts, monitor lizards can be exploited as effective sentinels. DNA-based diagnostic methods, such as q-PCR and LAMP should be introduced into clinical diagnostics of suspect cases, as well as into studies addressing the genetic diversity and species identity of nematodes tentatively identified as *A. cantonensis*.

OA03.02

Prevalence, distribution and clinical impact of canine tick-borne diseases in North-West Australia Barbosa, Dr. Amanda_Murdoch University

Co-Atuthor - Samuel Elliot, Dr Matthew Fox, Lauren Yates, Malvina Tan, Ben Soh, Joyce Lin, Dr Jill Austen, Dr Nicole Laing, Prof. Peter Irwin, Dr. Charlotte Oskam

There is increasing concern about canine tick-borne diseases (CTBD) in Australia, particularly in remote Indigenous communities across the northern and central areas where dog numbers are high, ticks are superabundant and tick prevention is scant. This retrospective cohort study aimed to investigate the prevalence, spatial distribution and clinical impact of CTBD in the North-West of Australia. A total of 452 canine blood samples were collected from the Kimberley, Gascoyne, Goldfields-Esperance, and Mid-West regions between 2018 and 2022, as part of Murdoch University's Canine Neutering Outreach Program. DNA extractions were performed using the DNeasy® Blood & Tissue Kit, followed by species-specific PCRs, and Sanger sequencing. At total of 312 dogs (69.2%; 95%CI: 64.8%-73.5%) harboured infection with at least one pathogen

species. The prevalence of *Anaplasma platys*, *Babesia vogeli* and *Ehrlichia canis* across all years were 21.2% (95%CI: 17.6%- 25.3%), 15.9% (95%CI: 12.7%-19.6%) and 16.45% (95%CI: 12.9%- 20.5%), respectively. Interestingly, this study identified one case (0.3%) of *E. canis* in the Kimberley region, in September 2019, which suggests an earlier incursion of this pathogen in Australia compared to May of 2020, when the first official case of CME was reported. Noteworthy, by 2022, the prevalence of *E. canis* had increased significantly to 31.7% (95%CI: 22.8%- 41.7%), with extended geographical distribution to include the Mid-West region. Mixed infections with *A. platys* and *B. vogeli* were detected in 6% (95%CI: 3.6%-9.6%) of samples collected throughout the study period, whilst 25% (95%CI: 14%-38.9%) of the dogs harboured co-infections with *A. platys* and *E. canis*. Dogs infected with at least one pathogen were almost twice as likely to develop low packed cell volume compared to negative dogs (RR: 1.78, 95%CI: 1.3-2.5). This study provides important baseline data to inform future clinical epidemiological studies, as well as effective strategies for control and management of CTBD in North-West Australia.

OA03.03

Molecular characterization of Wolbachia in hard ticks of small ruminants in Tamil Nadu, India _Arunkumar, Prof. Selvarayar_Tamil Nadu Veterinary And Animal Sciences University,

Co-Author - Dr R Vishnupriya, Prof BR Latha

This investigation was conducted to detect *Wolbachia* in hard ticks of sheep and goats from the districts of Tamil Nadu by molecular method. A total of 2120 hard ticks were collected from body of sheep and goats and identified morphologically. Isolation of salivary glands and mid gut was done from the ticks and DNA was also extracted. For the detection of *Wolbachia*, PCR amplification by targeting WSP gene was carried out with species specific primers. The amplified PCR products of *Wolbachia* from midgut of *Haemaphysalis bispinosa* ticks was 631bp. The obtained PCR product was sequenced and phylogenetic tree was also constructed. A total of 98 pooled mid gut samples of ticks from sheep were negative for *Wolbachia*. Whereas in goats, out of 151 pooled midgut samples of ticks, 2 pooled samples were positive for *Wolbachia*. No amplification was observed from salivary glands DNA of tick species from sheep and goats. This is the first report on molecular detection of *Wolbachia* in *H. bispinosa* ticks of goats in India.

OA03.04

Relationship between the number of counted larvae and the estimated proportion of gastrointestinal nematode species in a pooled fecal sample _Kaba, Prof. Jarosław_Warsaw University Of Life Sciences

Co-Authors - Zofia Nowek, Dr Adrian Potarniche, Prof Michał Czopowicz, Dr Kinga Biernacka, Dr Agata Moroz-Fik, Dr Olga Szalus-Joradnow, Dr Pawel Górski, Dr Tomasz Nalbert, Prof Iwona Markowska-Daniel, Prof. Jarosław Kaba

The gastrointestinal nematode (GIN) genera common in goats vastly differ with respect to pathogenicity. Therefore, identification of GIN population to at least genus level is essential to assess clinical significance of the infection or effectiveness of the treatment. The only practical way it can be done is to microscopically identify the larvae in fecal cultures. However, the number of larvae that should be counted to obtain sufficiently accurate characteristics of GIN population has never been determined. This study was carried out to assess how an increase in the number of counted larvae changes the accuracy of estimation of the prevalence of GIN

species. Pooled faecal samples based on sample from 4-63 goats were collected in 37 goat herds and coprocultures were prepared. After 7-day incubation the larvae were extracted by Baermannization and 500 subsequent GIN larvae were identified to the species/genus level by differentiation of the L3 morphological features in the light microscope (400× magnification). The true proportion of each GIN species in a total population of the herd was determined. Absolute deviation of apparent proportion from the true proportion was calculated for each GIN species in a cumulative manner. The accuracy of estimation of proportion of particular GIN species in a pooled faecal sample increased along with the number of counted larvae. Counting 100 larvae provided estimation of the true *H. contortus* proportion with >90% accuracy in only 24/37 herds (65%, CI 95%: 49%–78%). Counting 300 larvae increased the proportion of accurately examined herds to 95% (CI 95%: 82%–99%; 35/37 herds). Our study showed that >300 subsequent larvae have to be counted to achieve the 90% accuracy in more than 90% of herds. The study was financed from the grant UMO- 2020/37/B/NZ6/00457.

OA03.05

The emergence of thelaziosis caused by *Thelazia callipaeda* in companion animals from the USA_ Dr. Ranju Manoj_Cornell University, USA

Co-Authors - Ms Holly White Rebecca Young, Dr. Morina Pierce, Dr. Sophia Wilcox, Prof. Domenico Otranto, Dr Manigandan Lejeune

Thelazia callipaeda, for a long time known as the oriental eyeworm, was considered an exotic parasite in North America until the first dog case from New York State in 2020. Since then, we recorded *T. callipaeda* infection in 11 dogs (9, 2, and 1 from New York, New Jersey, and Nevada, respectively) and two cats (both from New York). Specifically, we report the first autochthonous feline cases documented from NY State in 2022. The first cat case was a sixteen-year-old neutered male domestic shorthair from Orange County, New York, who presented with clinical signs of epiphora, squinting, and swollen right eye with mucus accumulation, which did not resolve with ophthalmic antibiotic ointments. However, the second case, a 2.5-year-old spayed female domestic shorthair cat from a multi-cat household in Columbia County, had no clinical signs. Both cats had no travel history outside New York State. The retrieved worms were *T. callipaeda* based on transverse cuticular striations and vulvar position. A representative specimen from each cat was PCR confirmed as *T. callipaeda* based on *cox1*, 12S, and 18S rRNA DNA markers. The *cox1* sequences from this study clustered in a monophyletic clade with haplotype1 from Europe, reaffirming the possibility of a point introduction in NY State and its subsequent spread in the USA. The current report of *T. callipaeda* from two cats and ten dogs suggests that this parasite is emerging in Northeast USA, where prior studies confirmed the occurrence of a known vector (*Phortica variegata*). Though travel history is unknown, the record of a dog case from Nevada in the mid-Western USA is alarming. Active surveillance, accurate diagnosis, and prophylactic treatment must be in place to limit the spread of this zoonotic pathogen. Increased awareness and one health approach are needed to contain this multi-host parasite from emerging across the USA.

OA03.06

Sheep parasite species throughout New Zealand._Waghorn, Dr. Tania_AgResearch

Co-Authors - Dr Alex Chambers, Ms Charlotte Bouchet

Anthelmintic resistance continues to increase in prevalence and severity around the world, necessitating changes in parasite control approaches away from an almost total reliance on chemical inputs. Managing parasites with fewer anthelmintic inputs requires a better understanding of parasite epidemiology, especially in situations where some species exhibit high levels of resistance and other species remain highly susceptible to anthelmintics. The seasonality of parasite species abundance has been difficult to establish because visual speciation of infective stage larvae is difficult. Here we apply relatively new DNA methods to a survey of species relative abundance on sheep farms across New Zealand. Nine farms distributed around New Zealand were enrolled in the project and tasked with sending in 10 fresh faecal samples from each of 3 stock classes of sheep (mixed age ewes, lambs and 2nd year (2Tooth) ewes) on their property. This occurred each month for approximately 18 months. Faecal nematode egg counts were performed on each sample and the remaining faeces was cultured by stock class, resulting in 3 cultures per farm per month. The resulting 3rd-stage larvae (L3) were extracted, identified to genus-level morphologically and counted, before up to 2000 L3 were aliquoted for nemabiome sequencing. The mixed-species pools were identified to species-level by deep amplicon sequencing. As expected, initial results indicate differences in the species composition between age classes of sheep and regional variations, probably associated with climatic differences. Full results will be presented.

OA03.07

WAAVP Africa Network Survey: Priorities for control of veterinary parasitic diseases across Africa _Majekodunmi, Dr. Ayodele O._WAAVP Africa Network

Co-Authors - Dr Patrick Vudriko, Dr Abel Biguezoton, Dr Naftaly Githaka, Dr. Daniel Masiga, Prof Hussaina Makun, Dr Jahashi Nzalawahe, Dr Nlingisisi Babayani, Dr Furaha Mramba, Prof Souaibou Farougou, Dr. Lenaig HALOS, Prof Allal Dakkak, Prof. Rosina Krecek, Professor Richard Wall, Dr Johnson O.Ouma

The African continent has a rapidly growing population which will reach ~2.5 billion by 2050, resulting in increased demand for animal protein. Local production must rise to meet this demand and take advantage of the inherent opportunities for economic development, nutrition security and food security. At the same time, Africa's huge burden of veterinary parasites is likely to worsen, in association with climate change, intensification of livestock production and acceleration of anthelmintic and insecticide resistance. Informed, concerted and inclusive management practices are required to combat veterinary parasites to mitigate these impacts. The vision of WAAVP-Africa Network is to generate knowledge, expertise & influence through an engaged community of experts, for sustainable management of veterinary and zoonotic parasites in Africa, hence improving food security, animal welfare and the environmental sustainability of animal husbandry. An online Pan-African stakeholder's survey was launched in December 2022 (English version) and February 2023 (French version) to identify areas of veterinary parasite control that should be prioritized across the continent. More than 100 participants from diverse professions, in over 22 African countries have provided insights on the most economically important veterinary parasites, the biggest gaps in knowledge and expertise, the most impactful interventions, and how these vary across the continent. Preliminary analyses have identified the top priority parasitic diseases including ticks and tick-borne diseases in cattle and companion

animals, gastrointestinal helminths in small ruminants and gastrointestinal protozoans (coccidia) in poultry. The most important interventions identified were to improve knowledge on current parasite epidemiology, to adopt the One Health approach and to improve access to new parasiticides and vaccines. The survey remains open to allow more participants from a broader range of countries and stakeholders to provide their insights.

21.08.23

Breakout 1: OA04 Protozoan Parasites

OA04.01

Giardicidal effectiveness of a pyrantel pamoate + praziquantel + febantel + ivermectin association (Ourofino Saúde Animal Ltda.) in naturally infected dogs_Cruz, Dr. Breno Cayeiro_Ourofino Saúde Animal Ltda.

Co-Authors - Dr. Igor Renan Honorato Gatto, Ms. Milenni Garcia Michels, Ms. Maira Neto Zampier Farias de Souza, Dr. Ferdinando Nielsen de Almeida, Brena Gava Guimarães, Dr. Katherina Coumendouros, Dr. Fábio Barbour Scott

The giardicidal efficacy of an association (praziquantel, pyrantel pamoate, febantel ivermectin – Ourofino Saúde Animal Ltda.), administered orally for three consecutive days in dogs naturally infected with *Giardia intestinalis*, was evaluated. 24 Beagles, aged 4-146 (50.50±50.57) months, weighing 4.3-16.3 (11.23±2.94) kg, were selected, having feces collected and analyzed (centrifugal flotation with concentrated zinc sulfate solution [1.18 g/mL]) for three consecutive days before treatment (only dogs with three positive *G. intestinalis* cysts results underwent stratification in two experimental groups, with all 24 eligible dogs being weighed and randomized). On 0, +1 and +2, twelve dogs from one group received the association through daily administrations based on body weights: 250 mg tablets for each 2.5 kg, 1000 mg for every 10 kg, or 3000 mg for every 30 kg; all of which could be halved, to better match recommended doses of pyrantel pamoate; praziquantel; febantel; ivermectin (when calculated doses couldn't be achieved, they were rounded down). After three consecutive daily treatments, animals had their feces collected on days +3, +4, +5, +6 and +7. Based on elimination of cysts in this period, the products' giardicidal efficacy was established. On day +7, clinical examinations were performed on each dog, medicated or negative control, concluding their experimental participation. Geometric means of *G. intestinalis*. cysts observed (Control and Treated Groups, respectively) were: pre-treatment average = 7.38 and 7.51; +3 = 6.90 and 0.46; +4 = 5.64 and 0.26; +5 = 7.09 and 0.00; +6 = 5.50 and 0.00; +7 = 7.12 and 0.00. Efficacy indexes obtained were, respectively: 93.41%; 95.47%; 100.00%; 100.00%; and 100.00% (average effectiveness: 97.78%). These results support the satisfactory giardicidal efficacy of the investigational product, an excellent alternative against *G. intestinalis* infections, since WAAVP guidelines for evaluating drug efficacy against non-coccidial gastrointestinal protozoa require at least a 90% post-treatment efficacy, considering geometric means.

Keywords: Efficacy; *Giardia intestinalis* cysts; Non-coccidial gastrointestinal protozoa

OA04.02

The sublethal concentration of chlorine improves the cytotoxicity of *Acanthamoeba castellanii*_Bahrami, Dr. Somayeh_Shahid Chamran University of Ahvaz

Co-Authors - Professor Mehdi Zarei, Professor Hossein Hamidinejat, Professor Fiona L. Henriquez

Chlorine is a common disinfection agent commonly added to public water facilities and supplies. In this study, we examined the cytopathic and phagocytic properties of *Acanthamoeba castellanii* trophozoites following exposure to sublethal concentrations of chlorine. Two hours of exposure to five ppm hypochlorite calcium was considered the sublethal concentration for *A. castellanii* trophozoites. Assays on cytotoxicity and adhesion in RAW 264.7 macrophages, osmosensitivity,

and thermotolerance were conducted to determine whether treated trophozoites were more pathogenic than untreated ones. The phagocytic characteristics of treated cells were evaluated by assessing bacterial uptake. Antioxidant activity and oxidative stress biomarkers were compared in treated and untreated trophozoites. We also determined the mRNA expression of the genes for mannose-binding protein (MBP), cysteine protease 3 (CP3), and serine endopeptidase (SEP). Trophozoites treated with chlorine exhibited more extensive cytopathological effects, resulting in the detachment of macrophage monolayers. Treated trophozoites could not grow at high temperatures (43 °C). Besides, they showed osmotolerance to 0.5 M D-mannitol but not to 1 M. In comparison with untreated cells, chlorine-treated cells showed higher bacterial uptake rates. In treated and untreated cells, glutathione and glutathione/glutathione disulfide ratios were significantly different as a result of chlorine treatment, antioxidant enzyme activities, total antioxidant capacity, and malondialdehyde levels increased significantly. In chlorine-treated trophozoites, virulence genes were upregulated. Chlorine can form resistance and virulent amoebae if it is not used at a proper concentration and exposure time.

OA04.03

The Key Molecular Biological Characteristics between *Trypanosoma musculi* and *Trypanosoma lewisi*_ Prof. Zhao-Rong Lu_Sun Yat-Sen University, China

Trypanosoma musculi, the mouse trypanosome and *T. lewisi*, the rat trypanosome have been known for many decades. Interestingly, these two trypanosomes have been recognized as a host specific trypanosome. In fact, *T. musculi* cannot infect rat while *T. lewisi* can't infect mouse either. Current evidence indicated that although they have similar morphology and life cycle, they show significant difference in molecular bases. At least a dozen of cases of human infected with *T. lewisi* were reported, while none of human infected with *T. musculi* was recorded so far as we known. Evidence demonstrated that *T. lewisi* similar to *T. gambiense*, the pathogen of human African sleeping sickness is resistance to the lysis by normal human serum or APOL-1. However, normal human serum can lyse *T. musculi* both in vitro and in vivo. Due to the obvious differences between these two rodent trypanosomes, we will discuss their main biological characteristics including their genomes and kinetoplast DNA (kDNA, similar to the mitochondrial DNA in other eukaryotes) with details. (Work was supported by National Natural Sciences Foundation of China, No: 31720103918)

OA04.04

Study on mutations in the TaSP gene of *Theileria annulata* in India_Edith, Dr. Ramalingam_Tamil Nadu Veterinary and Animal Sciences University

Co-Authors - Dr. T.J. Harikrishnan, Dr. T.M.A. Senthil Kumar, Dr. P. Kumarasamy

Mutation in the protein coding genes of parasites is one of the mechanisms for evading the host immune responses. *Theileria annulata* surface protein (TaSP) is an immunodominant antigen of *T. annulata*. Study on mutations of the TaSP gene will be helpful to assess the suitability of this molecule as a sub unit vaccine candidate or diagnostic marker. In this study the level of mutations in TaSP gene of *T. annulata* isolates from India was investigated. Totally 50 samples from four states of India viz. Tamil Nadu, Andhra Pradesh, Telangana and Uttar Pradesh were amplified using TaSP gene specific primers to get amplicon of the whole TaSP gene (1065 bp). The amplified products were gel purified and cloned using INSTA cloning kit. Plasmid extracted from positive clones containing the TaSP gene insert was sequenced using Sanger's dideoxy method. The sequences were aligned with reference sequence and analysed using DNASTAR lasergene software. Mutations were identified in 55 places in the study sequences. Apart from

mutations, insertions in six places were also observed in the coding sequences. Because of these insertions, 316 amino acids were coded in the study sequence on translation instead of 314 amino acids in the reference sequence. Changes in the number, positions and composition of amino acids due to these insertions and mutations were confirmed by structural analysis of the reference and study sequences using TMHMM 2.0 program. Variations in the 3D structure of the protein were also observed between reference and study sequences by I- TASSER analysis. This is the first report on insertions in the TaSP gene of *T. annulata* isolates from India. However, further extensive study is warranted to estimate the extent of mutations and insertion in the TaSP gene sequences to correlate the effect of these mutations and insertions on the functions of the immunodominant TaSP antigen.

OA04.05

***Tritrichomonas foetus* long read genome sequencing methods.**_Tabor, Prof. Alicja_The University Of Queensland

Co-Authors - Dr Gry Boe-Hansen, Prof Michael McGowan, Dr Kieren McCosker, Mr Tony Cavallaro

Bovine trichomoniasis has been found to be highly prevalent in North Australian extensively grazed beef herds. Three Australian strains of *Tritrichomonas foetus* were sequenced using long read sequencing Oxford Nanopore Technologies (ONT) to determine the conservation among Australian isolates to support vaccine development. A lab strain, and two field isolates from the Northern Territory and Queensland respectively were selected for sequencing. DNA extraction processes were modified to avoid cytosolic nuclease activity to yield high quality long DNA strands. The total lengths varied from 104.7, 110 and 111.7 Mbp with 368, 194, and 226 contigs respectively. These genome assemblies have improved previous *T. foetus* and *Trichomonas vaginalis* genomes with 10 to 100- fold less contigs assembled from our data. Preliminary genome comparisons with *T. foetus* Strain K (reference genome) showed that the Australian genomes did not have or only partially had copies of Iron only hydrogenase large subunit C terminal domain protein. One Australian strain had a longer NADP-reducing hydrogenase subunit HndC and the Australian lab strain lacked a copy of the glycerol- 3-phosphatase gene. The two field isolates had a genomic similarity of 99.2%. We report a significant improvement on *T. foetus* genome assemblies using ONT long read sequencing. This research was supported by Meat & Livestock Australia and the Queensland Government.

OA04.06

A case report of *Theileria orientalis* genotype Ikeda in a dairy cow in New York State, USA.___Frye, Dr. Elisha_Cornell Animal Health Diagnostic Center

Co-Authors - Dr. Kevin Lahmers, Dr. Keith Carlson, Ms. Diane Deleo, Dr. Manigandan Lejeune

A dairy farm housing 1,100 lactating cattle in New York State observed weakness and death of 6 mature Holstein cows during July 1 – July 5, 2022. This farm historically shared hypodermic needles between animals. Milking cows are housed indoors, but heifers are sent to local offsite heifer raisers who purchase cattle from across the US, and pasture graze cattle. An adult cow (Cow #1) examined antemortem was found to be extremely pale, tachycardic and tachypneic. Blood collected prior to death was watery and dark grossly. On necropsy the cow had friable spleen and liver, severe icterus, and an enlarged heart. Field necropsies performed on two other cows (Cow #2, #3) had similar findings. A complete blood count from cow #1 revealed a severe

regenerative anemia with a hematocrit of 9%. *Anaplasma marginale* was detected on blood smear evaluation. The spleen tested positive for *A. marginale* and *Theileria orientalis* by polymerase chain reaction (PCR). Cows #2 and #3 were PCR positive for *A. marginale* but negative for *T. orientalis* on splenic samples. *Theileria orientalis* was confirmed to be genotype Ikeda. Within 2 weeks, a total of 30 cows were affected, 15 of which had died. The source of the *T. orientalis* and *A. marginale* infection may have been the off-site heifer raiser that also purchased animals from other states, but reusing needles might have facilitated pathogen transmission. The farm discontinued use of shared needles to mitigate further transmission of disease. *Theileria orientalis* was first discovered in the US in beef cattle in Virginia in 2017. *Haemaphysalis longicornis*, the known vector for *T. orientalis*, has been present in NY since 2017. This was the first detection of *T. orientalis* in NY, and the first detection of the highly pathogenic Ikeda genotype in the northeastern US.

21.08.23

Breakout 2: OA05 Tick Diseases Transmission

OA05.01

Application of quantitative proteomics to discover biomarkers for tick resistance in cattle_Raza, Dr. Ali_The University of Queensland

Co-Authors - Dr Amanda Nouwens, Dr Muhammad Noman Naseem, Mr Muhammad Kamran, Ms Emily Mantilla Valdivieso, Dr Edward Kerr, Dr Constantin Constantinoiu, Prof Nicholas Jonsson, Dr Peter James, Prof Ala Tabor

Breeding for tick resistance is a sustainable alternative to control cattle ticks due to widespread resistance to acaricidal drugs and the lack of a protective vaccine. The most accurate method used to identify tick resistance phenotype is the standard tick count, but this is labour-intensive and hazardous to the operator. Efficient genetic selection requires reliable phenotyping, such as biomarker(s), for accurately identifying tick-resistant cattle. Although breed-specific genes associated with tick resistance have been identified, the mechanisms behind tick resistance still needs further investigations. This study applied quantitative proteomics to examine the differential abundance of serum and skin proteomes using samples from naïve tick-resistant and -susceptible Brangus cattle at two-time points following tick exposure. The proteins were digested into peptides, followed by identification and quantification using sequential window acquisition of all theoretical fragment ion mass spectrometry. Resistant naïve cattle had a suite of proteins associated with immune response, blood coagulation and wound healing that were significantly (adjusted $P < 10^{-5}$) more abundant compared with susceptible naïve cattle, for example, complement factors (C3, C4, C4a), beta-2-glycoprotein-1, and fibrinogen. These findings were validated by identifying differences in the relative abundance of selected serum proteins with ELISA. The proteins showing a significantly different abundance in resistant cattle following early and prolonged tick exposures (compared to resistant naïve) were associated with immune response, blood coagulation, homeostasis, and wound healing. In contrast, susceptible cattle developed some of these responses after prolonged tick exposure. Resistant cattle were able to translocate immune-response related proteins towards the tick bite sites, which may prevent tick feeding. Physical barrier (skin integrity and wound healing) mechanisms and systemic immune responses are key contributors to resistance. Immune response-related proteins such as C4, C4a, AGP and CGN1 (naïve samples), CD14, GC and AGP (post-infestation) should be further investigated as potential biomarkers for tick resistance.

OA05.02

The role of *Ixodes Scapularis* neutral sphingomyelinase (nSMase) in tick-flavivirus interactions_VR, Dr. Kundave_Tanuvas

Co-Authors - Dr. PK Ramkumar, Dr. MK Vijayasarithi, Dr S Murugavel, Dr R Velusamy

Ixodes scapularis is a well-known vector for several pathogens such as *Borrelia burgdorferi*, *Anaplasma phagocytophilum* and flaviviral infections. Tick-borne virus such as Langat virus (LGTV), a member of Flaviviridae is used as a prototype for the study of Tick-Borne Encephalitis Virus (TBEV). The aim of the research was to elucidate the role of *Ixodes scapularis* neutral sphingomyelinase (nSMase) in LGTV replication and transmission. Studies were carried out in in-vitro tick cell lines derived from *I. scapularis* ticks, laboratory reared disease-free ticks and mice models. RNAi-

mediated silencing of nSMase gene resulted in reduced LGTV loads in both tick cells and ticks. Furthermore, treatment with GW4869 (exosome-release inhibitor) affected viral burden and *Ixodes scapularis* (nSMase) expression levels. The findings suggest tick-mediated replication of Langat virus and change in tick gene expression during acquisition of flavivirus into ticks. Tick-virus interactions help provide insights into anti-viral pathway(s) in tick-mediated disease transmission.

Keywords: *Ixodes scapularis*, Langat Virus, nSMase, RNAi, GW4869 inhibitor.

OA05.03

Understanding and combating ticks and tick-borne pathogens in the Middle East and North Africa Region through a One Health approach _Willingham, Prof. Arve

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Ticks are ectoparasites of domestic animals, wildlife and humans, and tick-borne pathogens are a persistent threat to biodiversity, food security, and human and animal health in the Middle East and North Africa (MENA) Region. Livestock farming is an important contribution to the economy of the countries in this region. Fifty-five tick species have been documented from livestock including camels, cows, sheep, and goats in MENA. Furthermore, fifteen tick-borne pathogens have been recorded that cause diseases in humans and animals putting livestock and those in contact with them at risk. Tick microbiome diversity and interaction between pathogens and endosymbionts play important roles in tick vector competence and pathogen transmission. However, only a few studies have been conducted on tick microbiome in the MENA Region including Palestine, Saudi Arabia, and UAE. Bacterial genera including *Francisella*, *Acinetobacter*, *Escherichia*, *Staphylococcus*, *Pseudomonas*, *Corynebacterium*, and *Bacillus* were all found with high prevalence in these microbiome studies. Determining the circulation of tick-borne pathogens at the livestock-wildlife interface and their possible transmission to human populations is crucial. To better understand the risk of emergence/re-emergence of tick-borne diseases, there is need for continued research on ticks and tick-borne microbes in the region, optimal environmentally-friendly control measures to prevent disease transmission from ticks to animals and humans through a One Health approach, and improved tick management and mitigation strategies. Such efforts require an interdisciplinary research approach that includes investigating ecological determinants, deploying molecular tools using genetics and genomic approaches, and appropriate monitoring and surveillance methodology for ticks and tick-borne microbes. In the initial phase we are collecting ticks from various animal species including camels, cows, sheep, goats, horses and dogs from the different Emirates of United Arab Emirates. With over 500 ticks collected morphological and molecular identification reveal only 3 species occurring in UAE: *Hyalomma dromedarii* (by far the most prevalent), *Hyalomma anatolicum* and *Rhipicephalus sanguineus* which are being screened for microorganisms.

OA05.04

***Amblyomma* infestation in *Kinixys belliana* and *Kinixys homeana* and its possible role in the transmission of haemogregarine parasites in Ibadan, Oyo state, Nigeria_Adetunji, Dr. Veronica_University of Ibadan**

Co-Authors - Dr Ogundijo Oluwaseun, Dr Alarape Selim

Ectoparasites cause harmful effect and also act as vectors of pathogens which can cause high morbidity and mortality. Tortoises are sometimes kept as pets in homes and as side attraction in zoological gardens, but not enough attention has been paid to the study of the diseases which might have the potential to affect their wellbeing. This study was aimed at determining, the presence and prevalence of ectoparasites (ticks, mite and other arthropods) and evaluate their possible roles in the transmission of haemogregarines in *Kinixys belliana* and *Kinixys homeana* obtained from wildlife markets in Ibadan, Nigeria. In total, one hundred and twenty (120) tortoises (70 *K. belliana* and 50 *K homeana*) were purchased at the Wildlife market in Ibadan intermittently throughout a year and spread over rainy and dry seasons. They were screened for parasites infestation. Ticks were carefully detached from infested tortoises using a pair of forceps and placed into labelled bijoux bottles with preservative solution and kept over ice. The mean prevalence for each tortoises group was determined and recorded. Ticks were identified with naked eyes followed by the use of a simple hand lens at X10 magnification. Parasites' abundance and intensities per sex, species and seasons were determined with Mann U Whitney Test. Sex, seasons and species prevalence were evaluated using Pearson's chi-square test while correlation coefficient test was used in the qualification of data. The level of significance was taken as 5%. Hard ticks (*Amblyomma* species) were the only ectoparasites found on 65/120 (54.0%) of the Tortoises examined. Higher prevalence of *Amblyomma* ticks was observed in *Kinixys belliana* (55.7%) than *Kinixys homeana* (52.0%) male tortoise's recorded higher prevalence 34/56 (60.7%) than females 31/64 (48.4.1%). Overall prevalence during rainy season was 33/60 (55.0) and 32/60 (53.3%) in dry season. The sole presence of *Amblyomma* ticks on tortoise supports their possible role as vector of haemogregarine parasites. A comprehensive health programme aimed at controlling ectoparasites such as ticks on tortoises in zoological gardens and in private facilities is hereby recommended.

Keywords: Ectoparasites, Haemogregarines, *Kinixys belliana*, *Kinixys homeana*, *Amblyomma*

OA05.05

Molecular detection of bovine anaplasmosis in dairy cattle of Bangladesh using multiplex PCR_TALUKDER, Dr. MD_Bangladesh Agricultural University

Co-Authors - Mr Mostak Ahmed, Mr MD Zim, Dr Babul Roy

Bovine anaplasmosis caused by *Anaplasma marginale*, is a tick-borne intra-erythrocytic organism of the host resulting in extravascular haemolysis, acute anaemia, morbidity and mortality in tropical areas. Cattle farming emerges as a profitable business in Bangladesh. This study aimed to determine the prevalence of bovine anaplasmosis in different parts of Bangladesh using microscopic inspection, cELISA and DNA amplification to anticipate the number of cattle at risk. To accomplish random sampling, a GIS-based herd selection approach was applied. Thin blood smears stained with Giemsa, tested for *Anaplasma* under oil immersion. All sera were analyzed for specific antibodies using cELISA kit. After DNA extraction from whole blood, PCR was performed using primer sets {F (5'-GCT CTA GCA GGT TAT GCG TC-3') R (5'-CTG CTT GGG AGA ATG CAC CT-3')} to target the main surface protein-1b gene (265bp)

of *Anaplasma*. Among 61 investigated farms, all farms had seropositive cattle for anaplasmosis. The majority of seropositive animals were located in Khagrachari 48.82%, Gazipur 48.02% and Chattogram 44.49%, while the lowest prevalences were recorded in Netrokona 32.00% and Jamalpur 33.93%. PCR results revealed an overall prevalence of 64.45% and incidence of *A. marginale* significantly highest in Khagrachari 77.65% (95% CI = 70.63-83.67%) of southeast zone and lowest in Jamalpur 43.75% (95% CI = 34.39-53.44) of northeast zone. In terms of odd ratio (PCR), multivariate logistic regression models revealed that the risk of anaplasmosis was significantly higher in animals older than one-year (OR=1.54, 95% CI 1.24-1.91), crossbred animals are 3.12 (95% CI 2.43-3.97) times more susceptible to anaplasmosis than indigenous cattle. Increased focus in the management of crossbred cattle, provides less opportunity for pre-exposure to vectors and develops limited immunity, resulting higher prevalence of this vector-borne pathogen as an emerging deadly disease in cattle industry. Genetic diversity and identification of immunoproteins is mandatory to plan control.

OA05.06

Development of an immunochromatographic test for serodetection of *Anaplasma marginale* infection in cattle_Garg, Dr. Rajat_ICAR-Indian Veterinary Research Institute

Co-Authors - Dr. Praveen Singh, Dr. Poonam Kumari, Dr. Hira Ram, Dr. Shobha Yadav

Effective control of bovine anaplasmosis, caused by *Anaplasma marginale*, require highly sensitive and specific molecular and serological tests that can precisely identify the carrier animals and provide reliable information on epidemiological status of *Anaplasma* infection in livestock in endemic regions. In the present study, an immunochromatographic test was standardized and evaluated for serodiagnosis of the infection in naturally infected cattle. The immunochromatographic test (ICT) strips were prepared for the specific detection of antibodies against recombinant major surface protein (rMSP5) of *A. marginale*. The anti-*A. marginale* MSP5 protein IgG antibodies were purified from the serum of a rabbit hyperimmunized with rMSP5 protein. The test was standardized with sera/plasma samples from cattle infected with *A. marginale* (n = 20), uninfected sera from healthy cattle (n = 20) and sera from cattle infected with *Theileria annulata* (n= 5), *Trypanosoma evansi* (n= 2) and microfilariae of filarial nematodes (n= 2). A total of 314 blood samples, collected from cattle suspected for haemoprotozoan infections, were used to evaluate the performance of ICT in comparison to MSP5 based semi-nested PCR and indirect ELISA. Screening of 314 samples revealed that 75.2%, 68.8% and 66.2% of cattle were positive for *A. marginale* infection by semi-nested PCR, indirect ELISA and ICT, respectively. The diagnostic sensitivity, specificity and accuracy of the rMSP5 protein-based ICT was 83.1%, 84.6% and 83.4%, respectively in comparison to semi-nested PCR, while it was 89.8%, 85.7% and 88.5%, respectively when compared with indirect ELISA. There was a substantial agreement (Kappa value= 0.74) between the results of ICT and indirect ELISA. The findings of the present study suggest that the developed immunochromatographic test is rapid, efficient and valuable for serodiagnosis of subclinical bovine anaplasmosis in field conditions.

21.08.23

Breakout 3: OA06 Host responses against helminths

OA06.01

Fatal haemonchosis in Australian beef heifers _Ball, Dr. Matthew_Virbac Animal Health

Severe clinical haemonchosis is less expected in Australian cattle than sheep but does occur in higher rainfall regions. Cattle haemonchosis may be more common due to drench resistance. In 2022 an outbreak of haemonchosis occurred in a group of 10-month-old beef heifers on the North Coast of New South Wales (Australia). At 7 months of age the cattle had been treated with a single active anthelmintic containing abamectin. Two heifers died and surviving heifers were small in size with ventral neck oedema and dark firm faeces. Necropsy findings included pale lungs, clear ascitic fluid and thick mat of *Haemonchus* in abomasum. McMaster Egg Count was 3650 strongyle eggs per gram with 57% *Haemonchus* spp. Haematology identified severe microcytic anaemia with packed cell volume of only 10%. Biochemistry demonstrated severe hypoproteinemia. All heifers were treated with a combination moxidectin/levamisole drench and given a supplementary feeding plan. Six weeks after treatment there had been no further deaths or illness. This case study highlights the severe anaemia haemonchosis can cause in post weaned calves. Recent wet weather contributed to the cause of this outbreak but it is also possible that the abamectin drench given at weaning was ineffective to remove worm burdens. Drench resistance to single active macrocyclic lactones is common in Australia. Drench selection needs to use actives that target likely parasite risk and are expected to have greater than 95% efficacy. The use of ready to use combination or concurrent drench actives is now considered essential for cattle drench programs to ensure high efficacy against key worm species, manage drench resistance and protect productivity. Integrated parasite management (including grazing management, nutrition and worm egg count monitoring) should also be implemented to reduce the over-reliance on chemicals.

OA06.02

Comparative genomics of *Dipylidium caninum*: host association and praziquantel resistance_Jesudoss Chelladurai, Dr. Jeba_Kansas State University College of Veterinary Medicine

Co-Authors - Dr. Aloysius Abraham, Theresa Quintana, William Johnson, Vicki Smith, Deb Ritchie

Dipylidium caninum (Linnaeus, 1758) is a common zoonotic cestode of dogs and cats worldwide. Prior experimental and molecular studies based on partial gene sequences have shown that dog and cat associated genotypes exist in the cestode. There have been no comparative studies at a genome-wide scale. In this study, we sequenced the whole genomes of *D. caninum* isolates obtained from naturally infected dogs and cats in the United States using the Illumina platform. We performed comparative analyses of these genomes against the canine reference draft genome from China. We show that the feline isolates of *D. caninum* were distinct from the canine isolates at Universal Single Copy Ortholog genes. We also show that in variant analyses, the number of SNPs and indels in the feline isolates of *D. caninum* was significantly higher than in the canine isolates. We have previously described cases of praziquantel resistance in canine isolates of *D. caninum*. Praziquantel resistant and praziquantel susceptible *D. caninum* canine isolates were also sequenced using the Illumina platform. We show the distribution of genome level variations

between drug resistant and susceptible isolates and discuss the prospects for future diagnostic tests to distinguish resistant isolates in clinical cases.

OA06.03

Treatment of early immature *Fasciola hepatica* in growing cattle: effect on weight gain and liver weight_Hamel, Dr. Dietmar_Boehringer Ingelheim Vetmedica GmbH

Co-Authors - Manyun Liu, Stephen Yoon, Dr. Steffen Rehbein

Although the negative impact of liver fluke (*Fasciola hepatica*) infection on production and general health in cattle is generally accepted, results of individual research have been variable, with results ranging from important negative impacts on the animal to minimal to no impact. To add information on the impact of *F. hepatica* infection in growing cattle, weight gain and liver weight of young animals experimentally infected with flukes in seven controlled flukicide efficacy studies were analyzed. For the studies, fluke naïve animals were inoculated with approximately 450 to 500 *F. hepatica* encysted metacercariae of recent field isolates. In each study, animals blocked on body weight were randomly assigned into one untreated group (control) and one or more groups which received flukicide treatment when the flukes were four weeks old ('early immature'). The animals were sacrificed eight weeks after flukicide administration, and groups which demonstrated >90% treatment efficacy (range among treated groups, 92.7% to 99.9%; overall reduction 98.1%, $p < 0.0001$) and groups left untreated (total 103 and 47 animals, respectively) were compared. Considering all animals, there was a significant negative association between fluke count and weight gain over the eight weeks post-treatment period ($p < 0.0001$) while fluke count and liver weight were positively associated ($p < 0.0001$). Over the eight-week post-treatment period, flukicide-treated cattle had almost 15% more weight gain than the untreated cattle (50.9 kg vs. 44.4 kg; $p = 0.0003$). Absolute and relative (organ weight divided by body weight) liver weight was lower in flukicide-treated compared to untreated cattle ($p < 0.0001$). Overall, analysis of the post-treatment responses in seven efficacy studies provided evidence of a substantial negative effect of liver fluke infection on the growth of young cattle, likely as a result of pathology of the liver and associated reduction in function as the central organ for protein and bioenergy metabolism.

OA06.04

The impact of breeding for resistance to gastrointestinal nematode parasites: 25 years of WormFEC in the New Zealand sheep industry_McRae, Dr. Kathryn_Agresearch

Co-Authors - Dr Sheryl-Anne Newman, Dr Patricia Johnson, Mr John McEwan

Clinical and sub-clinical diseases present a major constraint to global livestock production. Current control practices of gastrointestinal nematode parasites, based almost entirely on the frequent use of anthelmintics, are becoming unsustainable due to widespread and often severe anthelmintic resistance. Breeding for host resistance is an alternative, sustainable tool that has the advantage of being permanent and cumulative. Genetic progress has been demonstrated in breeding for host resistance using faecal egg count (FEC) in the New Zealand sheep industry, where the WormFEC service has provided breeders with a tool to select for resistance for the past three decades. Alternative phenotypic measures, including anti-parasite antibodies, and selection strategies, including selecting for resilience, have also been explored. Using large industry datasets of FEC-recorded animals, it has been shown that the genetic correlations with other traits of interest, such as dag score or live weight, are either not significant or low, and able to be counteracted using a selection index. While there are alternatives for selecting animals that

are resistant or resilient to parasites, selecting animals that have a reduced FEC has been shown to have benefits at the flock level, due to a reduction in pasture contamination and reduced infection, resulting in improved production. With increasing resistance to anthelmintics, long-term management of parasites requires an integrated control program. Breeding and selecting animals with an increased ability to resist infection is an important part of a sustainable strategy.

OA06.05

Study of the early invasion mechanisms of *Fasciola hepatica* juveniles by quantitative proteomics in an in vivo mouse model_López-García, Ms. Marta_Institute of Natural Resources and Agrobiology of Salamanca (IRNASA-CSIC)

Co-Authors - David Becerro-Recio, Ms. Judit Serrat, María Torres-Valle, Dr. Verónica Molina-Hernández, Dr. Teresa Ruiz-Campillo, Dr. José Pérez-Arévalo, Dr. Álvaro Martínez-Moreno, Dr. Javier González-Miguel, Dr. Mar Siles-Lucas

Fasciola hepatica is the causative agent of fasciolosis, a zoonotic disease responsible for significant economic losses in animal production, as well as a worldwide health problem. Although *F. hepatica* life cycle is well known, molecular interactions governing the migratory process undertaken by the juvenile forms of the parasite from the small intestine to the liver through peritoneum in the vertebrate host remain to be addressed. Therefore, the aim of this work was to establish an in vivo mouse infection model to identify key parasite molecules involved in the migration of *F. hepatica* juveniles through host tissues. C57BL/6 mice were orally infected with 200 metacercariae of *F. hepatica* and parasites were subsequently recovered at 24 hours in the peritoneal cavity (9.38% of recovery rate) and at 8 days post-infection in the liver parenchyma (21.19% of recovery rate). Tegument and somatic-enriched parasite fractions and their respective controls were subjected to quantitative proteomics by sequential acquisition of all theoretical mass spectra or SWATH-MS, an approach that represents a novel technique conferring several advantages over conventional proteomic techniques in terms of accuracy and reproducibility. The proteomic analysis revealed the presence of 1180 proteins in the samples, of which 243 and 532 proteins were differentially expressed in comparison to their respective controls in the parasites extracted from the peritoneum and the liver, respectively. Functional annotation of the highlighted protein ontology terms revealed relevant biological pathways involved in the migration process, such as antioxidant defence, proteolytic, motility, metabolic and transport mechanisms among parasites that invaded host tissues. Overall, our advances will deepen the knowledge of the host-parasite early interactions in fasciolosis to identify new molecular targets for the development of vaccines against this disease.

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OA06.06

***Fasciola hepatica* juveniles interact with the host fibrinolytic system as a potential early-stage invasion mechanism**_Serrat, Ms. Judit_Laboratory of Helminth Parasites of Zoonotic Importance (ATENEA), Institute of Natural Resources and Agrobiology of Sala

Co-Authors - David Becerro-Recio, María Torres-Valle, Fernando Simón, María Adela Valero, María Dolores Bargues, Santiago Mas-Coma, Mar Siles-Lucas, Dr. Javier González-Miguel

The trematode *Fasciola hepatica* is the most widespread causative agent of fasciolosis, a parasitic disease that mainly affects humans and ruminants worldwide. During *F. hepatica* infection, newly

excysted juveniles (FhNEJ) emerge in the host duodenum and migrate towards the intra-hepatic biliary ducts, where adult flukes develop. Provided that crossing of the intestinal wall by FhNEJ is regarded as a “point of no return” in fasciolosis in terms of therapeutic control, understanding the molecular events that drive this process is pivotal for the development of successful control and treatment strategies against this disease. Plasmin, the central enzyme of the mammalian fibrinolytic system, is a broad-spectrum serine protease whose functions are exploited by a number of parasite species for intra-mammalian migration. Therefore, the aim of the present work was to understand whether FhNEJ co-opt the functions of the host fibrinolytic system as a mechanism to facilitate trans-intestinal migration. To this end, a tegument-enriched protein extract of FhNEJ (FhNEJ-Teg) was obtained *in vitro*, and its capability to bind the zymogen plasminogen (PLG) and enhance its conversion to plasmin were analyzed by enzyme-linked immunosorbent, chromogenic and immunofluorescence assays. Additionally, PLG-binding proteins in FhNEJ-Teg were identified by 2D electrophoresis coupled to mass spectrometry analysis, and the interactions were validated using FhNEJ recombinant proteins. Our results show that FhNEJ-Teg contains proteins that bind PLG and stimulate its activation to plasmin, which could facilitate crossing of the intestinal wall by FhNEJ and contribute to the successful establishment of the parasite within its mammalian host. Altogether, our findings contribute to a better understanding of host-parasite relationships during early fasciolosis and may pave the way for the development of more effective treatment and control strategies against this global disease. Funding: RTI2018-093463-J-I00 by MCIU, AEI and FEDER, and CLU-2019-05 and CL-EI-2021-01 by JCYL and European Union ERDF.

OA06.07

Insight to *Platynosomum fastosum* infection in definitive host cats and intermediate host lizard in Malaysia_Abdul Aziz, Dr. Nor Azlina_Universiti Putra Malaysia

Co-Authors - Dr. Malaika Watanabe, Dr. Mazlina Mazlan, Dr. Azlan Che' Amat, Nur Amalina Nasruddin.

Platynosomum fastosum is a hepatic fluke causing platynosomosis or ‘lizard poisoning’ in cats. This disease is generally being underestimated by veterinary practitioners due to lack of awareness and difficulty in diagnosis although the severe cases of platynosomosis could be fatal. This study was designed to detect the presence of cat liver fluke through faecal examination among cats kept in shelters, pet cats and stray cats around Klang Valley, Malaysia. Our study also identify presence of metacercariae from the house gecko trapped inside the building. The detection of liver flukes among stray cats was based on post-mortem examination while fecal samples were subjected to simple floatation and formalin-ether sedimentation techniques for ova detection. House gecko was killed by decapitation and liver and gallbladder were isolated by post mortem. The metacercariae were easily squeezed out from the liver by gentle pressed on the organ using a glass rod, which indicates cats might be infected by biting only a small part of the infected house gecko liver. *P. fastosum* ova were identified in three faecal samples obtained from shelters (2.52%) and three samples collected from veterinary clinics (3.66%) by faecal examination. Twelve cats (23.5%) were positive for *P. fastosum* and ova were found in their bile from post mortem. Macroscopically, affected cats showed mottled liver (33.3%), distended gall bladder with thick tenacious bile (66.7%) that microscopically exhibited hepatic steatosis (25.0%) and hepatitis (33.3%). Fourteen house geckos were found positive for metacercariae of *P. fastosum* with the prevalence of 56% (95% CI 35.3-75.0) following microscopic examination. The histopathological result showed hepatic fibrosis and hyperplasia of biliary epithelium of the lizard liver. This study revealed the detection of *P. fastosum* among cats and demonstrated lizard as important paratenic host in the transmission of *P. fastosum* metacercariae to domestic cats.

21.08.23

Breakout 4: OA07 Diagnosis of GI nematodes of ruminants

OA07.01

Efficacy of fenbendazole against gastrointestinal nematodes in naturally infected goats, in Mozambique _Guinda, Ms. Edna_veterinary Faculty, Eduardo Mondlane University, Freie Universität Berlin

In Mozambique, the excessive use of benzimidazoles to control gastrointestinal nematodes resulted in anthelmintic resistance (AR). In this context, between April 2021-February 2022, through a cross-sectional study, the efficacy of fenbendazole (FBZ, PANACUR®- MSD) 5 mg/kg body weight orally dosage was evaluated in five districts of Maputo province. Where 433 goats from 16 farms (6 commercial and 10 smallholder) between 22 to 30 animals per farm, not dewormed in the past 8-12 weeks, and egg shedding intensity ≥ 150 eggs excreted per gram of faeces, were included. The efficacy was evaluated after 14 days in vivo through faecal egg count reduction test (FECRT) using the Mini-FLOTAC® technique and in vitro by egg hatch test (EHT). For data analysis, the R-package egg counts was used to calculate 95% confidence intervals. The FECRT and EHT detected a high susceptibility on 11/16 (69%) of farms, including nine smallholder farms (on which FECR ranging from 99.12 to 99.99% and EHT 0.029 to 0.076 $\mu\text{g/ml}$ TBZ respectively) and two commercial farms (FECR ranged from 99.54 to 96.74% and EHT 0.039 to 0.079 $\mu\text{g/ml}$ TBZ respectively). AR was detected on four farms (25%), three commercial and one smallholder with FECR ranging from 50.23 to 92.97% and EHT 0.123 to 0.21 $\mu\text{g/ml}$ TBZ. Only in one (6%) commercial farm the FECRT detected resistance (74.2%) and EHT detected sensibility (0.053 TBZ $\mu\text{g/ml}$). The results show statistically significant negative correlation between the FECRT and the EHT data, demonstrated by Pearson's coefficient ($r = -0.0762$, $P < 0.001$). Commercial farms (odds ratio=0.032, relative to smallholder farms) and application of quarantine (odds ratio=0.354) for newly acquired animals were negative risk factors for reduced efficacy. In conclusion, high susceptibility to benzimidazoles was detected in smallholder farms and we hope by molecular testing to identify the most prevalent nematodes and help livestock services to redefine deworming strategies.

Keywords: gastrointestinal nematodes, benzimidazoles, goats, Mozambique

OA07.02

Online tools to supplement the upcoming WAAVP guidelines for calculating prospective sample sizes and classifying efficacy results for faecal egg count reduction tests in ruminants, horses and swine_Denwood, Prof. Matthew_University of Copenhagen

Co-Authors - Prof. Ray Kaplan, Prof. Iain McKendrick, Prof. Stig Thamsborg, Dr. Martin Nielsen, Prof. Bruno Levecke

Anthelmintic resistance continues to be an important issue, and the faecal egg count reduction test (FECRT) is the most important tool for detecting anthelmintic resistance at the farm level. However, there has been substantial debate in the literature regarding the optimal design of FECRT studies and suitable methods for analysing the resulting data. Standardised and validated methods for determining the experimental design of a FECRT and the susceptibility classification of the result are therefore urgently needed. Methods. We present a new approach for determining sample size requirements for the FECRT that is built on a solid statistical framework. These sample size calculations are supported by parameter estimates obtained from 131 FECRT datasets obtained from published data and via the personal contacts of the authors.

Our framework also gives a rigorous anthelmintic drug efficacy classification system for use with FECRT in livestock, which gives a classification of resistant, susceptible or inconclusive based on the observed data. Simulation studies demonstrate that our statistical framework and data analysis method produce appropriate results for synthetic data. The method proposed is simple to understand, and the facility for power calculations gives the opportunity for crucial insight into the design of more efficient FECRT studies. We provide sample size calculations that are tailored to specific host/parasite systems, based on the estimates for expected pre-treatment and post-treatment variability in egg counts as well as within-animal correlation in egg counts. Our methods represent an important step forward for understanding FECRT studies, and are used to underpin the upcoming FECRT WAAVP guidelines for detection of anthelmintic resistance in ruminants, horses, and swine. As part of our wider efforts towards digitalisation of livestock data, the methods discussed have also been made freely available as open-source software via <https://www.fecrt.com>

OA07.03

Phenotypical characterization of eprinomectin-resistant *Haemonchus contortus* isolates assessing motility with WormMicroTracker®. _Petermann, Ms. Julie_National Veterinary School of Toulouse

Co-Authors - Ms. Marie Garcia, Dr Christelle Grisez, Ms Sophie Jouffroy, Pr Philippe Jacquet, Dr Mélanie Albérich, Dr Anne Lespine

Eprinomectin-resistant *Haemonchus contortus* populations are increasingly reported in dairy sheep farms in Southwestern France. In order to adapt drug treatment and recommendations to breeders, it is important to first assess whether the parasite population is susceptible or resistant to the anthelmintics used. This is generally performed using fecal egg count reduction tests (FECRT). However, this method is time consuming and expensive. To improve drug resistance diagnosis, L3 motility was monitored with the WormMicroTracker® device in order to discriminate between resistant or susceptible *H. contortus* isolates using dose response curves. We used three resistant isolates of L3 issued from eggs collected in farms where resistance to eprinomectin was identified by FECRT. The susceptible counterpart used as control was issued from our laboratory. Eighty L3 per well were exsheathed and placed in 96-well plates with increasing doses of either eprinomectin, ivermectin or moxidectin. Larvae motility was monitored for 15 min at 24h and 48h after treatment using the WormMicroTracker® apparatus. Levamisole at 100 µM was used as a positive control. We compared IC50s obtained by generating a dose-response curve and calculated the resistance factor (RF) for each isolate and each molecule. Three replicates of the test were done with the susceptible strain and two of the three resistant isolates and the results were reproducible between experiments. Complete immobility was observed in all isolates in presence of levamisole. The dose-response curves and the calculated IC50 for eprinomectin clearly confirm that the strains derived from resistant farms were highly tolerant to eprinomectin when compared to the susceptible counterpart (RF= 13 to 130). The 3 strains were also resistant to ivermectin (RF= 2.7 to 3.7) and moxidectin (RF= 2.6 to 2.7). We provide here a reliable assay based on larvae motility evaluation which allows to discriminate drug-susceptible worms from eprinomectin-resistant *H. contortus*.

OA07.04

Selective treatment of dairy sheep based on parity and body condition has a limited impact on milk productivity – Lessons learned from five dairy sheep flocks in France_Jouffroy, Ms. Sophie_Ecole Nationale Vétérinaire De Toulouse/ceva/inrae

Co-Authors - Louise Bery, Cecile Rayssac, Kenza Bourrier, Camille Delmas, Marina Abbadie, Melissa Devaux, Christelle Grisez, Dr. Damien Achard, Dr Hamadi Karembe, Dr Anne Lespine, Pr Philippe Jacquiet

The rise of anthelmintic resistance in gastro-intestinal nematodes of sheep is slowly leading to a paradigm change – anthelmintic treatments should be carefully and timely conducted rather than systematic and frequent. While Targeted Selective Treatment (TST) protocols have been widely studied in dairy goats and meat sheep, little has been done for dairy sheep. In the two main French dairy sheep regions, the demand for a simple and reliable protocol is rising. A TST strategy was implemented in 2 farms in the Pyrénées Atlantiques département and 3 farms of the Roquefort area for one and two years respectively. The anthelmintic treatment used was injectable eprinomectin at 0.2 mg.kg⁻¹, its efficacy was measured by FECRT at the beginning of the project. Primiparous ewes were systematically treated, and multiparous ewes were treated according to their body condition, in agreement with the farmers' opinion. Production data were collected and compared using statistical and modelling tools. 12.5% to 69% of ewes were left untreated, depending on the farm. In the Roquefort area, milk yield was negatively impacted when the faecal egg count reached a threshold of 1000 EPG in multiparous ewes and 250 EPG in primiparous ewes. Treating primiparous ewes, multiparous ewes with a body condition ≤ 2.5 and the ewes with the highest milk yield made it possible to target the most heavily infected ewes. Maintaining a refuge population only impacts production between two milk checks by an average of 2%. These results confirm the impact of GIN infection on milk yield, yet in moderate range so that non treatment of selected ewes is compatible with a profitable production.

OA07.05

Anthelmintic action of *Butea frondosa* extract against adult *Haemonchus contortus*: in-vitro studies_Deka, Dr. Neelakshi_College Of Veterinary Science, Assam Agricultural University

Co-Authors - Dr. SC Phukan, Dr. Archana Hazarika

The present study was carried out to evaluate the anthelmintic activity of *Butea frondosa* against adult *Haemonchus contortus* of goats under in-vitro conditions. Adult motility test was performed to carry out the in-vitro studies. Ethanolic extract was prepared and tested for three concentrations viz. 50 mg/ml, 100 mg/ml and 200 mg/ml. The study revealed significant anthelmintic effect of ethanolic extract on live adult *H. contortus* worms in all the concentration, but highest efficacy was observed at 200 mg/ml concentration. Results indicated that *B. frondosa* possess promising anthelmintic activity (in-vitro) and can be used as a dewormer in goats.

21.08.23

Breakout 1: OA08 Molecular tools

OA08.01

Metabolome of canine saliva in dogs with *Babesia canis*: a targeted metabolomics study_Mrljak, Prof. Vladimir_University Of Zagreb, Faculty Of Veterinary Medicine

Co-Authors - Dr Maša Efendić, Dr Blanka Beer Ljubić, Dr Krešimir Martinković, Prof Iva Šmit, Dr Tea Dodig, Dr Gabrijela Jurkić Krsteska, Prof Martina Crnogaj, Prof Jelena Gotić, Dr Josipa Kuleš, Prof Renata Barić Rafaj, Dr Ivana Rubić

Canine babesiosis is an important worldwide tick-borne disease caused by the intra-erythrocyte protozoa of different *Babesia* species. Targeted metabolomics identifies and quantifies the abundance of defined groups of known, chemically characterized and biochemically annotated metabolites. Saliva can be easily used as diagnostic fluid due to its feasible and non-invasive procedure. Because saliva is rich in small molecules, there is a growing interest in using saliva to diagnose diseases or discover the molecular mechanisms of diseases. The goal of the study was to investigate the difference of saliva metabolome between dogs naturally infected with *B. canis* and healthy dogs using a targeted metabolomics approach by UHPLC-MS/MS. The targeted metabolomics was performed in saliva of 14 dogs naturally infected by the parasite *B. canis*, and 14 healthy dogs using the Absolute IDQ-p400 kit (Biocrates Life Sciences AG) developed for high-resolution mass spectrometry. The samples were pipetted on the 96-well plate system for protein removal, internal standard normalization, and derivatization. The metabolite extracts were analysed on a Thermo Orbitrap Q Exactive Plus UHPLC-MS/MS. Metabolites were identified and quantified using the MetIDQ™ software. The targeted metabolomics approach identified Creatinine, Ornithine (Orn), Arginine (Arg), Isoleucine (Ile), Phosphatidylcholine PC (32:0), Hexoses (H1) (including glucose) as significantly increased metabolites in dogs with babesiosis ($p < 0.05$). The most influential metabolites were H1, Arg, and lysophosphatidyl choline (LPC (18:0)). Pathways analysis of significantly identified metabolites suggested that the metabolic changes in dogs with canine babesiosis are linked to arginine biosynthesis and arginine and proline metabolism. In conclusion, the research of saliva samples in dogs infected with *B. canis* demonstrated potential metabolites and pathways significantly changed in dogs with canine babesiosis. The targeted LC-MS metabolomics approach profiled the metabolic change in the saliva of dogs infected with *B. canis*.

OA08.02

Detection of *Tritrichomonas foetus* and *Simplicimonas* from bovine reproductive disorders cases in India: Implications for Diagnostic Strategies_ Bahekar, Dr. Vijay Shriram_National Dairy Development Board, Anand, India

Co-Authors - Dr. Gonuguntla N Hariprasad, Dr. Kota Shri Naga Leela Surendra, T.V.N.S. Saranya, Talla Tusharika, Palamuru Priyanka, Dr. Amitesh Prasad

Bovine Trichomonosis causes reproductive disease in cattle and buffaloes and substantial economic loss to the dairy industry. The use of molecular techniques for diagnosis is gaining popularity due to their high sensitivity & faster detection time relative to the gold standard method of identification of the causative agent by cultural isolation.

This study aimed to investigate the presence of *Tritrichomonas foetus* in cases of repeat breeding and early abortions among bovines. A total of 134 genital samples were collected from

four farms located in different geographical regions of India, namely Telangana, Tamil Nadu, and Maharashtra. The samples were screened using both cultural isolation (InPouch TF test kit) and conventional PCRs targeting the ITS gene. Notably, none of the samples tested positive using the cultural isolation method. Conventional PCRs were performed parallelly using two primer sets recommended by the World Organisation for Animal Health (WOAH): TFR-1 - TFR-2 (for all *Trichomonas* species) and TFR-3 - TFR-4 (specific for *Tritrichomonas foetus*). The use of TFR-1 - TFR-2 primers indicated the presence of *Trichomonas* species in all four farms, with prevalence ranging from 21% to 72%. However, when TFR-3 - TFR-4 primers were used, *Tritrichomonas foetus* was detected only in farm 2 (Tamil Nadu) and farm 4 (Maharashtra), with a positivity rate of 0.93% and 2.8%, respectively. Partial sequencing of the ITS-1 gene using representative PCR products amplified by TFR-1 and TFR-2 confirmed that all the samples positive with TFR-3 and TFR-4 primers belonged to *Tritrichomonas foetus*, while the samples positive only with TFR-1 & TFR-2 primers belonged to *Simplicimonas* genus, a commensal in the gastrointestinal tract of bovines.

The result highlights the specificity of TFR-3 and TFR-4 primers for the detection of *Tritrichomonas foetus*, as recommended by the WOAH and confirms the higher sensitivity of PCR methods over conventional cultural isolation technique. The study also reports the presence of *Simplicimonas* as a commensal in the reproductive tract of Indian cattle and buffaloes.

OA08.03

Molecular phylogeny of *Culicoides* midges in Telangana state, South India._Banothu, Dr. Dasmabai_PVNRTU

Co-Authors - Dr Udaya Kumar manchukonda, Dr. Sreenivasa murthy

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Genus *Culicoides* (Diptera: Ceratopogonidae) are an important vector species, reported mainly from Asia with high potential to transmit arboviral diseases affecting livestock. The most significant economic impact of *Culicoides* lies in their ability to transmit bluetongue disease in small ruminants. In southern India, hyperendemic outbreaks of blue tongue exert high cost to subsistence farmers in the region, impacting on sheep production. Effective *Culicoides* spp. monitoring methods coupled with accurate species identification can accelerate responses for minimising blue tongue outbreaks. To better characterize the outbreak and phylogenetic relations of *Culicoides*, molecular analysis in parallel with a morphology-based taxonomic approach were performed. Phylogenetic analyses of Asian *Culicoides* species were undertaken using the mitochondrial DNA (Mt DNA) Cytochrome C oxidase subunit-I (COI) as a molecular target. Six species of *Culicoides* viz., *C. oxystoma*, *C. imicola*, *C. peregrinus*, *C. similis*, *C. anophelis*, *C. shivasi* were confirmed using ITS 1 region specific primers PanCulF/PanCulR. All the six species got amplified at the range of 400 bp amplicon confirming that all the flies belonged to the genus *Culicoides*. COI gene of *Culicoides* was amplified by universal primers CO1F/CO1R. *C. oxystoma* and *C. peregrinus*, *C. imicola* which yielded specific amplicon size of 658bp. Whereas *C. anophelis*, *C. shivasi* and *C. similis* yielded a specific amplicon at 648 bp. All COI sequences were edited and submitted to GenBank and accession numbers were obtained. Phylogenetic tree was constructed for *C. oxystoma*, *C. peregrinus*, *C. imicola*, *C. similis*, *C. shivasi* and *C. anophelis*. Construction of several trees showed that molecular phylogeny within the genus *Culicoides* correlates not only with morphological-based taxonomy but also with ecological patterns.

Keywords: *Culicoides*, Phylogenetic analyses, Cytochrome C oxidase subunit-I, PanCulF/PanCulR.

OA08.04

Meta-analysis of Cryptosporidiosis in domestic animals in and around Udaipur region_

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Cryptosporidium spp. , causative agent of gastrointestinal diseases in vertebrate animals are of prime importance which causes mortality in livestock, and high fatality in young animals. It is of great importance because of its zoonotic importance. Several *Cryptosporidium* species are known to be zoonotic with animals as major reservoirs. Cryptosporidiosis induces self-limiting diarrhea in immunocompetent individuals, but the infection can be severe and life-threatening in immunocompromised animals. It is one of the most important diseases in young ruminants, especially neonatal calves.

The objective of present study was to do meta-analysis to review the papers on prevalence and molecular data on *Cryptosporidium* infections in selected domestic animals as reservoir source. Data collection was carried out using PubMed, Scopus, Science Direct, Google scholar for a period of 1986 to 2020. A protocol for the literature review was designed. The results showed that overall 11% of investigated species were infected with *Cryptosporidium* spp. In the study considering livestock species as cattle, buffaloes, sheep, goats, pigs, horses, chicken) analysis revealed higher *Cryptosporidium* infection prevalence in cattle (21%) and it was the most commonly infected farm animal.

So it is concluded that domestic animals are considered as potential source of *Cryptosporidium* contamination in the environment and proper control measures should be developed to reduce the occurrence of *Cryptosporidium* infection in these animals.

OA08.05

Transcriptomic variations between mild and severe reactions due to *Theileria parva*

infection_Sibeko-Matjila, Prof. Kgomotso_University of Pretoria

About 40 million cattle are at risk of infection with *Theileria parva*, the most important tick-borne disease of cattle affecting 12 countries in sub-Saharan Africa. The most common disease syndrome of *T. parva* is East Coast fever (ECF), killing one million cattle each year. Cattle infected with the same *T. parva* stock can have varied response, ranging from no clinical signs to severe; however, the host determinants of this varied response have not been fully established. Thus, calves bred from the same sire were experimentally infected with two *T. parva* vaccine stocks, Katete and Chitongo, to study the transcriptome regulation due to infection and mechanisms modulated in mild and severe reaction. RNA extracted from calves that had mild clinical signs (MCS), severe clinical signs (SCS), and non-infected control animals, was sequenced using the Illumina HiSeq platform. Differentially expressed genes (DEGs) were detected between infected and non-infected animals (n=501), and between MCS and SCS (n=186) reaction groups. The Gene ontology and pathway analyses (in KEGG and Reactome) indicated that DEGs in *T. parva* infection are generally involved in 'innate immune response', although it is not considered to play a major role in the host defence against *T. parva* infection. Consistently, 'neutrophil degranulation', an innate immunity pathway, was exclusively enriched in infected calves. The severity of clinical signs was associated with negative regulation of cytokine production and NF- κ B signalling (n=12; 11 downregulated). These results suggests that innate immunity may play a significant role in protection against *T. parva*. Excessive neutrophil

degranulation causes inflammatory tissue injury and is a major contributing factor in pulmonary disorders. This is consistent with the most prominent pathological changes of *T. parva* infection, the severe respiratory distress or failure. In severe disease cases, the immune response regulated by cytokines and the regulation of cell proliferation, facilitated by NF- κ B signalling and thought to contribute to survival, are compromised.

OA08.06

Characterization of non-exposed and hidden antigens from the liver fluke *Fasciola hepatica* Eichenberger, Dr. Ramon M. Zurich University of Applied Sciences

The zoonotic liver fluke *Fasciola hepatica* is responsible for major economic losses to the agriculture industry worldwide. Triclabendazole (TCBZ) has been the drug of choice. However, resistance to TCBZ is becoming prevalent with cases of resistance detected globally. Vaccines as an economically viable strategy have been discussed, where a significant focus is on the development of protein vaccines. Here, we aim to characterize novel protein candidates, which are not presented to the host due to modulated immunogenicity, structural masked epitopes of essential proteins, and hidden antigens. Tegumental- and secreted protein fractions from different life-stages of *F. hepatica* were dissected by size-exclusion chromatography, m-APBA-agarose affinity chromatography, lectin-agarose affinity chromatography, and specific monoclonal antibodies. Protein fractions were further characterized by their humoral- and cellular reaction with the host. Immunological characterization revealed proteins with dominant-, minimal-, and absent humoral immune attraction. Further, *F. hepatica* triggers a modified type 2 immune activation. By stimulating ovine PBMCs, we observed that a fraction dominated by fluke extracellular vesicles induce a completely different immune response compared to other secreted parasite proteins. This distinct response against EVs is hypothesized to be beneficial for vaccine development. The different protein fractions will be further analysed by a quantitative proteomics approach, with a special focus on candidates with minimal- and absent host response as promising vaccine candidates. Detailed analysis of the parasite-host interaction by fractionation of host-interacting antigens (surface- and secreted proteins) with subsequent functional characterization will reveal a detailed catalogue of non-host exposed-, masked-, modulating-, and hidden antigens providing a novel assortment of potential vaccine candidates.

21.08.23

Breakout 2: OA09 Tropical parasites

OA09.01

Animal schistosomes research in Chennai- Camera lucida to

Phylogeny_Narayanaperumal, Prof. Jeyathilakan_Tamil Nadu Veterinary and Animal Sciences University

Animal schistosomes are economically important snail borne trematodes occurring all over India as well as many parts of Asian and African countries. India is uniquely placed with regard to discovery of seven new species of schistosome namely *Schistosoma indicum*, *S. spindale*, *S. bomfordi*, *S. incognitum*, *S. nasale*, *S. nairi* and *Orientobilharzia dattai*. The seed for the research on animal schistosomes in Chennai was sown in the year 1933 by Dr.M.A.N. Rao who discovered the *S. nasale*, the causative agent of nasal schistosomosis in cattle. Afterwards studies were carried about morphology, lifecycle, pathogenesis, diagnosis, treatment and control of *S. nasale* in cattle, buffalo, sheep and goat by various stalwarts of Dept. of Veterinary Parasitology, Madars Veterinary College, Chennai. *Schistosoma nairi* affecting elephant was also discovered from Chennai. Prevalence of other schistosomes namely *S. spindale*, *S. suis* and *S. indicum* was also reported from animals in Chennai. Recently serodiagnostic assays for *S. spindale* and phylogenetic analysis of *S. spindale* and *S. indicum* from cattle were carried out. This paper describes the genesis of research works carried out from the year 1933 to 2023 in Chennai and also the future scope and issues in the area of animal schistosome research.

OA09.02

Progress on sustainable control of goat warble fly infestation in union territory of Jammu and Kashmir, North India_Yadav, Prof. Anish_Sher-e-Kashmir University of Agricultural Sciences and Technology Of Jammu

Co-Authors - Dr. Vikas Yadav, Dr. Shafiya Imtiaz Rafiqui, Dr. Anand Kushwaha, Dr. Rajesh Godara, Dr. Sanku Borkataki, Prof. Shilpa Sood, Dr Rajesh Katoch

Goat warble fly infestation (GWFI) is a subcutaneous myiasis caused by larvae of *Przhevalskiana silenus*, an insect belonging to order Diptera, family Oestridae subfamily Hypoderminae. GWFI is characterised by warbles at lumbar and sacral region of dorsum which harbour the *P. Silenus* larvae or three stages (L1 to L3) for about 8-9 months. The economic loss occurs due to the degradation of hide quality and the deterioration of health and production. The disease is prevalent on north western Himalayan region of Union Territory of Jammu and Kashmir, especially in Bakerwali breed of goats. The disease prevalence ranges from 56.5 to 13% in Jammu and Kashmir resulting in annual economic loss to the tune of about 2.5 million USD by loss in meat, milk and hide. To overcome the losses, sustainable approach towards the disease management at state level has been promoted under the ICAR-National Fellow Project since 2017. In the last six years, a concerted approach has been adopted to provide prophylactic treatment regime, extension education to goat farmers and the Veterinarians of State Animal Husbandry departments and immunosurveillance of the GWFI in different districts of Jammu and Kashmir. In the past 5 years, about 80 clinical camps (covering 237 farmers with goat heads of 47,400) have been organised to sensitize farmers on GWFI with pertinent literature in regional languages and its prophylactic management with low-cost dose regime of Ivermectin at 5 µg/kg bodyweight during July to interrupt the life-cycle of *P. silenus* at L1 stage larvae. The coordinated efforts have resulted in decrease of warble fly prevalence in recent years to about

15.0% in recent survey. The ICAR-NF project has led to the development of novel diagnostics for both field (Dot-ELISA) and mass surveillance (microtitre plate ELISA) based on recombinant antigen Hypodermin C (rHYC) derived from the parasite *P. silenus*. As the hypodermosis eradication campaigns in several European nations indicates the effectiveness of prophylactic ivermectin regimen coupled with mass surveillance, the current developments in Jammu and Kashmir provide prospects of sustainable control of GWFI in the Union Territory.

OA09.03

Molecular identification, associated risk factors and phylogenetic genetic characterization of tick borne haemoparasites amongst naturally infected sheep in North India: A first comprehensive report_Maharana, Dr. Biswa Ranjan_Lala Lajpat Rai University of Veterinary and Animal Sciences

Co-Authors - Dr. Sukhdeep Vohra, Dr. Anita Ganguly, Dr. Harpreet Singh, Dr. Sujoy Khanna, Dr. Aman Nangru

Theileria, *Anaplasma*, and *Babesia* are three important tick-borne haemoparasites exert a substantial negative economic impact on small ruminant production. Yet, no attempt has been made to offer molecular proof of these infections in sheep from northern India. The current study investigated the real picture of tick-borne pathogens and explore various risk factors pertaining to disease occurrence among sheep flocks in Haryana, Northern India. Microscopy of sheep blood samples (n=402) revealed 47.26%, 33.08%, 0.24%, for *Theileria*, *Anaplasma* and *Babesia* spp., respectively while it was 60.94%, 51.24% and 1.24%, respectively, by PCR. Co-infection of *Theileria* spp. and *Anaplasma* spp. were also noted in 16.66% and 25.87% sheep by microscopy and PCR, respectively. PCR-RFLP was used in the present study for species differentiation of *Theileria*. Based on the PCR-RFLP assay, the samples found positive for *Theileria* genus were subjected to PCR using species-specific primers revealed 29.1%, 12.69%, 5.97% and 1.49% were positive for *T. ovis*, *T. lestoquardi*, *T. luwenshuni*, and *T. annulata* respectively. Mixed infection was detected in 11.69% cases. All the *Babesia* genus positive samples were subjected to semi-nested PCR amplified 178 bp DNA fragments specific for *B. ovis*. The samples detected positive for the *Anaplasma* genus were subjected to species specific PCR targeting the MSP4 gene to detect *A. marginale* and *A. ovis*. Results revealed 35.82% sheep positive for *A. ovis* while it was 5.97% for *A. marginale*. Mixed infection with both the haemoparasites was observed in 9.45% of cases. The results are also supported by bidirectional sequencing and Phylogenetic analysis. The presence of haemoparasites in sheep is significantly influenced by a number of risk variables, including age, sex, zone, and others. This is the first comprehensive report on molecular epidemiology and phylogenetic characterization of tick-borne haemoparasites among sheep from North India urges further studies to formulate effective control strategies.

OA09.04

Identification of *Hepatozoon* and *Cercopithifilaria* in ticks infesting dogs of different regions of Iran_Bahraei, Ms. Zahra_Bu-ali Sina University

Co-Authors - Dr. Alireza Sazmand, Dr. Alireza Nourian, Prof. Domenico Otranto

Following previous detection of *Hepatozoon canis* and *Cercopithifilaria baina* in dogs from Iran, this study aimed to identify potential tick vectors collected from dogs from five provinces of Iran. From October 2018 to September 2021, a total of 222 owned and 541 sheltered dogs from Hamedan (n=305), Kermanshah (n= 227), Yazd (n=80), Khouzestan (n=71), and Mazandaran (n=80) were examined for ticks. Collected ticks (n=3339) were identified morphologically, and adults were screened for the detection of *Hepatozoon* and *Cercopithifilaria* either with conventional

PCR (n=119 ticks), tick dissection (n=106), or microscopical examination after fixation in formalin (n=102). A total of 169 dogs (22.1%) were infested with ticks. Tick infestation rate varied in different provinces (*i.e.*, 32.4% in Hamedan, 27.3% in Kermanshah, 10.0% in Yazd), but no ticks were collected in dogs from Mazandaran and Khuzestan. The majority of male ticks were identified as *Rhipicephalus sanguineus sensu lato* (89.3%) and *R. turanicus*, but one dog from Hamedan was infested with *Hyalomma asiaticum* (1 male and 1 female) and *H. excavatum* (1 male). At the molecular examination, *Hepatozoon* was detected in 6.7% ticks, *Cercopithifilaria* in 14.28%, and both parasites in 1.68% *Rhipicephalus* ticks. Parasites were not observed at tick dissection and histological examinations. Nucleotide sequencing and phylogenetic analyses revealed *C. bainaie* and *H. canis* in *R. sanguineus sensu lato* and *R. turanicus* ticks. Data suggests that both tick species above may play a role in the epidemiology of *Cercopithifilaria* and *Hepatozoon* in Iran.

Keywords: *Cercopithifilaria*, *Hepatozoon*, Iran, *Rhipicephalus*, vector-borne pathogens

OA09.05

Diagnosis of canine and feline endo- and ecto-parasites in the tropics: the new Standard Operating Procedures developed by TroCCAP_Mihalca, Prof. Andrei Daniel Usamv Cluj- napoca

Co-Authors - Dr. Filipe Dantas Torres, Prof Gad Baneth, Prof. Domenico Otranto, Prof. Peter Irwin, Prof Rebecca Traub, Prof. Gabriela Perez Tort, Dr. Malaika Watanabe, Prof Bui Khanh Linh, Dr. Tawin Inpankaew, Prof. Barend L Penzhorn, Dr. Pablo Borrás, Prof. Sangaran Arumugam, Dr. Adrian Patalinghug Ybañez, Prof. Jennifer Ketzis

Andrei Daniel Mihalca, Filipe Dantas Torres, Gad Baneth, Domenico Otranto, Peter Irwin, Rebecca Traub, Gabriela Perez Tort, Malaika Watanabe, Bui Khanh Linh, Tawin Inpankaew, Banie Penzhorn, Pablo Borrás, Sangaran Arumugam, Adrian Patalinghug Ybañez, Jennifer Ketzis

The Tropical Council for Companion Animal Parasites (TroCCAP) is an international not-for-profit public company whose mission is to independently inform, guide and make best-practice recommendations to veterinarians and other health professionals in the field of diagnosis, treatment, and control of parasites of dogs and cats in the tropics and sub-tropics. Among the tools used to achieve these goals are free-training and outreach activities (*i.e.*, seminars and workshops) and access to online guidelines. The guidelines present concise but essential and well-structured information on the diagnosis, treatment, and control of dog and cat endo- and ecto-parasites in the tropics and are currently available in eight languages. As most tropical areas constitute middle-to-low-income countries, where expensive and complex diagnostic methods are largely unavailable or unaffordable, TroCCAP advocates for the use of simple, inexpensive and practical methods for the diagnosis of parasitic diseases in companion animals. Considering all these factors and the importance of accurate diagnosis, a new guideline was developed by experts of TroCCAP offering step-by-step instructions that allow veterinarians to perform essential diagnostic procedures using basic resources. The guidelines are designed as Standard Operating Procedures (SOPs) and include a variety of methods for faecal analysis (*i.e.*, simple faecal flotation, centrifugal faecal flotation, Baermann technique, sedimentation technique, acid-fast stain for *Cryptosporidium* oocysts), blood analysis (modified Knott's test, blood smears, buffy coat, direct microhematocrit method), and skin analysis (adhesive tape/acetate strip method, hair pluck method, skin scrape for mites, skin biopsy). For each method, the described procedure includes a brief overview and indications, a list of required reagents and their preparation, the detailed step-by-step procedure, safety precautions and clean up procedures.

Molecular detection of food-borne parasitic contamination in fresh vegetables from fresh markets in Eastern Thailand_ Dr. Chadaporn Nuchjangreed Gordo_Burapha Medical University, Thailand

Co-Authors - Miss Chantima Mora, Miss Natcha Chupongthanet, Dr. Manachai Yingklang, Dr. Chantira Sutthikornchai

Parasitic infection is still a major problem in developing countries, including Thailand. Many outbreaks of parasitic infections in humans have been linked to fresh vegetables. The consumption of raw, undercooked or unclean vegetables is the main way for transmission of food-borne pathogens. The objective of this study was to investigate parasitic contamination in fresh vegetables from three fresh markets in Maung District, Chon Buri Province using both microscopic and molecular approaches. Ten different vegetables including; Cabbage, Chinese cabbage, Morning glory, Sweet basil, Kitchen mint, Asiatic pennywort, Coriander, Culantro, Celery and Rice paddy herb were randomly purchased from the three fresh markets (100 fresh vegetable samples) between August and October 2022. Microscopic methods; Sedimentation with detergent (1% Sodium Dodecyl Sulfate and 0.1% Tween 80 and direct smear) and staining (Rapid DMSO modified acid fast stain for *Cryptosporidium*) and Polymerase Chain Reaction (PCR) technique were used to detect parasitic contamination in raw vegetables. The results found that 4 samples were contaminated with unidentified nematode larvae (4%) from 1 Sweet basil, 1 Kitchen Mint and 2 Culantro by Microscopic method. For the PCR technique, we found 3 samples of Asiatic pennywort were contaminated with the subfamily *Angiostrongylus* sp. (3%), 2 samples of Sweet basil were contaminated with *Cryptosporidium* spp. (2%), whereas 1 Kitchen Mint and 1 sample of Rice Paddy Herb were contaminated with *Giardia duodenalis* assemblage B (2%). The results of this study indicated that these parasites were found in raw vegetables from the three markets in Chon Buri Province, Thailand. This suggests that humans are at risk of getting infections from raw

vegetables. These findings could be applied for prevention and control. Cleaning fresh vegetables or cooking properly before consuming to avoid infection of food-borne pathogens.

Keywords: Fresh vegetables, Food-borne pathogens, Microscopic method, PCR technique, Chon Buri Province, Thailand

21.08.23

Breakout 3: OA10 Swine parasites

OA10.01

African Swine Fever Virus load in hematophagous dipterans collected in outbreaks from Romania: risk factors and implications__Balmoş,, Ms. Oana-Maria_UNIVERSITY OF AGRICULTURAL SCIENCES AND VETERINARY MEDICINE

Co-Authors - Mr. Alexandru Supeanu, Ms. Paula Tamba, Ms. Cintia Horvath, Ms. Luciana Catalina Panait, Mr. Attila David Sandor, Ms. Cristina Daniela Cazan, Mr. Andrei Ungur, Ms. Monica Motiu, Mr. Florin-Alexandru Manita, Ms. Beatris Corina Ancuceanu, Ms. Florica Barbuceanu, Ms. Sofie Dhollander, Prof. Andrei Daniel Mihalca

African Swine Fever Virus (ASFV) has been shown to be mechanically transmitted via hematophagous arthropods to suids. There are few experimental studies, which aimed to determine the role of *Stomoxys calcitrans* (Diptera: Muscidae) in the spread of the virus and to consolidate the hypothesis that such insects have a putative implication in the virus' transmission. However, no study based on the natural infection has documented the presence of viruses in hematophagous arthropods collected from ASFV outbreaks. Insects were collected from thirty farms (16 backyards, 9 type A, and 5 commercial) according to a risk scoring criterion. Standard entomological traps have been used to sample *Culicoides* spp. and *S. calcitrans*. The collected insects were morphologically identified and pooled prior to DNA extraction and PCR in order to evaluate the virus presence and the pig as a source of blood meal for the insects. In total, 3158 insects (*S. calcitrans* n= 198 and *Culicoides* n=2960) were collected in 23 farms of the 30 outbreak farms. Ten species of biting midges were identified. The total number of insect pools showed significant differences according to the month of sampling, with a higher number of pools collected in August and September. Overall, 137 pools out of the 395 examined were positive for the presence of ASFV DNA. There was a higher viral DNA load (Ct value < 24) in farms where pigs were present at the moment of sampling compared to farms where pigs were already culled, in *S. calcitrans* compared to *Culicoides* spp., and in CF and TAF compared to BF. Our data provide new evidence for the possible role of *S. calcitrans* and *Culicoides* spp. as mechanical vectors for ASFV, highlighting the risk associated with higher viral loads that could improve the approach to prevention and control, mainly within commercial farms, through better management of biting insects.

OA10.02

Parasite infections in organic free-ranging pigs in Danish agroforestry systems
_Pedersen, Ms.Amalie_University of Copenhagen

Co-Authors - Ms. Isabella Jørgensen, Mr. Andrew R. Williams, Ms. Mita Sengupta, Ms. Anne Grete Kongsted, Ms. Helena Mejer, Professor Stig M. Thamsborg

Mixed production systems combining pigs with tree production may support self-supply of feed, nutrient recycling, arthropod biodiversity and carbon-sequestration. Trees may also be selected for anti-parasitic effects while pigs eat bark and leaves. This longitudinal study aimed to describe the dynamics of gastrointestinal nematodes in organic farms with pigs ranging on large pastures with partial tree cover. Farm 1 had 650 sows and reared 11,000 piglets annually on pastures with poplar trees (*Populus* spp.)(20% of land cover). Farm 2 had 350 sows and reared 6,000 piglets on pastures with 20-30% cover of poplars. Piglets were moved inside when weaned at ten weeks of age. Farms were visited quarterly for a year and each time, 20 random droppings were picked

from: pregnant and lactating sows, piglets in farrowing paddocks, weaners, and larger finishers (all indoors with outdoor concrete run). Faecal samples were analyzed for nematode eggs by McMaster (threshold: 20 eggs/g faeces (EPG)). Piglets and weaners all had nil/low excretion of *Oesophagostomum* eggs, which is remarkable as lactating and pregnant sows consistently were infected with group mean levels of 2,000-4,000 EPG throughout the year, thus a strong age effect. In contrast, excretion of *Ascaris suum* eggs was more sporadic with moderate levels (1000-2,000 EPG) in certain weaner groups, while most fatteners were infected a low level. Farm 1 routinely dewormed sows 14 days prior to farrowing. Farm 2 routinely dewormed piglets at weaning but began to treat sows during winter. We observed debarking of poplar trees by pigs but leaves could not be accessed. The lack of *Oesophagostomum* infection in young animals probably reflect poor transmission in farrowing paddocks, although heavily contaminated by sows. Infection levels in sows were comparable to other outdoor systems and there was no indication of any immediate effect of tree cover.

OA10.03

Monitoring of ivermectin residues in bovine and pork tissues_Alvarez, Prof.
Luis_CIVETAN

Co-Authors - Dr. Juan Pedro Lirón, Dr. Candela Canton, Ms. Verónica Castilla Gómez de Agüero, Ms. Elora Valderas García, Dr. Marcela Larroza, Ms. Paula Soler, Dr. Carlos Lanusse, Dr. María Martínez Valladares, Dr. Lucila Canton

Argentina is traditionally a beef-producing country. However, in recent years other productions have grown exponentially. Due to improvements in technology or economic issues, consumer preferences have changed for cheaper meats. Animal production systems are closely linked to veterinary drug use for prevention, control, or treatment of parasitic diseases. Consequently, if good agricultural practices are not respected, products obtained from these productions could present residues above the Maximum Residue Limits (MRLs). In this context, ivermectin (IVM) is one of the most widely used to treat parasitic diseases. Consequently, the current study aimed to assess the presence of IVM residues in bovine and pork tissues for local consumption in Buenos Aires province (Argentina). Samples of bovine/pork tissues were taken for 3 years in 5 cities of Buenos Aires province. Tissue samples were analyzed by HPLC (fluorescence detector). Using the @Risk software the risk of consuming tissues with IVM residues above the Admitted Daily Intake (ADI) was evaluated. IVM residues were quantified in 87 (12.5%) samples (out of a total of 691). However, only 13 samples showed concentrations above the Codex MRL. Mean IVM concentrations (range) were 42.18(0.11–587.15), 31.66(2.96–283.33), 162.61(1.32–516.55), 22.78(1.51–65.40), 15.26(0.07–194.25) and 22.14(1.58–126.76) ppb for bovine meat, bovine fat, bovine liver, bovine kidney, pork meat, and pork fat, respectively. Fortunately, the probability of consuming bovine and porcine tissues with IVM residues above the ADI was nil. However, 1.88% of the samples showed IVM concentrations above the MRL, thus the implementation of residue surveillance programs guaranteeing consumer health is strongly recommended.

OA10.04

Spatio-temporal patterns in the sero-prevalence of *Taenia solium* cysticercosis of pigs in the southern highlands of Tanzania_ Dr. Mwemezi Kabululu_Tanzania Livestock Research Institute (TALIRI), Tanzania

Co-Authors - Dr. Bamidele Ogunro, Prof. Helena Ngowi, Pof. Maria Johansen

Taenia solium is among the major causes of epilepsy in sub-Saharan Africa, including Tanzania. Understanding geospatial distribution of the parasite is important to inform control strategies. This study assessed spatio-temporal trend in the *T. solium* infection in pigs from a rural area in south-western Tanzania.

Secondary data from a previous repeated cross-sectional study in Mbeya and Mbozi districts was used, involving sixteen villages, eight from each of the two districts. Serum samples were collected from 482, 460 and 421 pigs in 221, 196 and 139 households, respectively, at seven-month intervals. The samples were analysed for circulating antigens using Ag-ELISA. Geographic coordinates of the pig corrals adjacent to the households were also recorded using a hand-held device.

SatScan v9.7 was used to perform purely spatial analysis, based on the Bernoulli distribution model. Spatial clustering was assumed based on the relative risk of *T. solium* positivity. Statistical significance (set at 0.05) of the cluster was determined by comparing the log likelihood ratio with the null distribution derived by Monte Carlo simulations. The window with the highest log likelihood ratio was regarded as a primary cluster.

Results showed significant clustering of *T. solium* cases with the presence of a single cluster during each phase. The relative risk of *T. solium* infection among pigs within the primary cluster areas ranged from 3.0 to 6.7. The radii of the clusters expanded from 1.83 to 27.7 km before shrinking to 1.5 km; and location drifted from north to central and then southern part of the study area.

These findings suggest that with restricted resources, control measures in the area could be focused on pockets of high transmission rates. However, change in size and location of clusters suggest that to be effective, interventions should be instituted soon after clusters are identified.

22.08.23

Breakout 1: OA11 Vector Borne Infections in Companion Animals

OA11.01

Exploring the prevalence and genetic diversity of multiple zoonotic vector-borne diseases in pet dogs and cats of Hong Kong SAR; First molecular confirmation

_Sparagano, Prof. Olivier_City University of Hong Kong

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In recent years, the incidence of vector-borne diseases (VBDs) has increased throughout the globe. In particular, tick-borne diseases (*e.g.*, caused by *Ehrlichia canis*, *Ehrlichia ewingii*, *Anaplasma phagocytophilum*, *Anaplasma platys*, *Borrelia burgdorferi sensu stricto* (s.s.) and *Babesia gibsoni*) and mosquito-borne diseases (*e.g.*, caused by *Dirofilaria immitis*) diseases pose a burden on animal health. Nevertheless, there have been no studies undertaken on the occurrence of VBDs in pet dogs and cats in Hong Kong SAR. This study fills this gap, and is the first to determine the seroprevalence of major VBDs, such as those caused by *D. immitis*, *E. canis*, *E. ewingii*, *A. phagocytophilum*, *A. platys* and *B. burgdorferi s.s.*, in dogs and cats through commercially available SNAP 4Dx plus testing. Infection by all these pathogens and *Babesia sp.* was further assessed through PCR and DNA sequencing. A total of 224 blood samples were collected from domestic dogs (n = 159) and cats (n = 65) in Hong Kong SAR during summer 2022. Hematocrit and platelet counts were determined in each blood sample and other hematological parameters were assessed using an automatic hematology analyzer. All cat sera samples were negative for tested pathogens, but antibodies against some of the pathogens were detected in dog sera samples. Here, the highest figures were recorded for seroprevalence of *E. canis*/*E. ewingii* (10.69%), followed by *D. immitis* (5.67%), and *A. phagocytophilum*/*A. platys* (2.51%). No *B. burgdorferi s.s.* antibodies were detected in any of the dogs tested. Through molecular diagnostics, we detected the presence of *B. gibsoni* (3.77%), *E. canis* (3.14%), *D. immitis* (5.67%), and *A. phagocytophilum* (1.26%). Neighbor-Joining phylogenetic trees for vector-borne pathogens (*i.e.*, -genus *Anaplasma sp.*) showed 100% clustering to Japan, the USA and Germany, whereas -genus *Ehrlichia sp.* showed 100% clustering to China, Turkey, Cuba, and Greece. Similarly, genus *Babesia sp.* clustered 100% to India, Sri Lanka and Austria, while *D. immitis* clustered in Iraq, South Korea, Portugal, France, the USA and Italy. This study provides the first evidence on the occurrence of tick-borne pathogens in pet dogs in Hong Kong SAR. Based on these findings, it is recommended that appropriate screening should be undertaken in domestic dogs to evaluate the prevalence of these pathogens and promote the timely control of VBDs.

Keywords: *Anaplasma*, *Babesia*, *Ehrlichia*, *Dirofilaria immitis*, dogs, cats, risk factors, vector- borne pathogens, zoonotic.

OA11.02

Efficacy of a sarolaner/moxidectin/pyrantel (Simparica Trio®) for the prevention of *Thelazia callipaeda* eyeworm infection in dogs from endemic areas_Bezerra Santos, Dr. Marcos Antônio_University of Bari

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Thelazia callipaeda is a zoonotic vector borne nematode that infects the eyes of dogs, cats, wild carnivores, lagomorphs, and humans. This parasite is endemic in Europe and Asia, where human populations living in endemic areas are also at risk of acquiring the infection. Recently, cases of canine thelaziosis have been also reported in the United States. Here, we assessed the efficacy of a formulation containing sarolaner/moxidectin/pyrantel (Simparica Trio®) administered orally at monthly intervals, for the prevention of establishment of *T. callipaeda* infection in dogs. The study was conducted in endemic areas of Italy and France, where dogs (n = 125) were assigned into two groups consisting of a negative control group (G1; n = 62) in which animals were treated monthly with a control product (sarolaner; Simparica®), and a treatment group (G2; n = 63) in which animals were treated with Simparica Trio. Treatments were administered monthly from Day 0 to Day 150, or until study completion if the dog was found positive for *T. callipaeda*. Nine animals were withdrawn from the study (two that became positive at D30, and seven for reasons unrelated to eyeworm infection) resulting in 116 animals (n = 58 for G1; n = 58 for G2). In G1, 16 out of 58 animals (27.6%) were observed with eyeworms during the study and none of the animals from G2 were observed with eyeworms. Adult nematodes and larvae (L4) were recovered from the eyes of positive animals, counted, and morphologically and molecularly identified as *T. callipaeda*. Data herein presented demonstrated 100% efficacy (P<0.0001) of Simparica Trio for the prevention of *T. callipaeda* eyeworm infection in dogs. This control measure is crucial to reduce the risk of *T. callipaeda* infection in animals, including humans, inhabiting endemic areas.

Keywords: *Thelazia callipaeda*, Prevention, Dogs, Moxidectin, Simparica Trio, Zoonosis.

OA11.03

A report of canine testicular filariosis caused by *Dirofilaria repens*-like species from Georgia, USA_Lejeune, Dr. Manigandan_Cornell University

Co-Authors - Dr. Radhakrishna Para, Rebecca Young, Ms Holly White, Prof. Domenico Otranto

Dirofilaria species (subgenus *Nochtiella*) are known to cause subcutaneous dirofilariosis in dogs. *Dirofilaria repens* is the most known species which has been frequently reported in humans, being widely distributed in Europe and Asia. Conversely, this zoonotic parasite has never been reported in North America, with rare cases of this filarioid in the USA invariably associated with travel to endemic countries. In a few instances of infected shelter dogs, the unknown life history of hosts precluded researchers from authenticating the parasite's endemicity in North America. In this case study, we report a *D. repens*-like nematode, retrieved during neuter, from a one-year-old intact male Doberman pincher rescued from Georgia, USA. Prominent longitudinal cuticular ridges in the midbody confirmed the worm as *Dirofilaria (Nochtiella)* sp. Molecular analyses for 18S and 12S rRNA revealed 100% and 98.8% identity to the closest *D. repens* sequences in GenBank (MH981971 for 18S and AM779775 for 12S), respectively. However, the cox1 and

ITS1 had 96.1% and 92.6% similarity with *D. repens* (MT683122 for cox1 and OL616131 for ITS1). Intriguingly, the cox1 and ITS1 matched 99.61% with a *Dirofilaria* sp. reported from a human testicular filariasis case in Austria (cox1: GU474429 and ITS1: GU474430). Despite a 96.1% similarity for the cox1 gene with the closest *D. repens*, the corresponding protein sequence of Georgia isolate showed 100% identity with *D. repens* (CAO83038), suggesting the isolate could be *D. repens*. In contrast, a 92.6% match for ITS1 may indicate Georgia isolate as a closely related species within the *D. repens* taxonomical group. With only two cases of this unique *D. repens*-like species known so far, it is necessary to increase molecular surveillance to understand the range expansion of this zoonotic parasite.

OA11.04

***Rhipicephalus sanguineus* at the northern distribution edge: identification of ticks and detection of tick-borne pathogens in Hungarian dogs** _Modry, Prof. David_Biology Centre of CAS

Co-Authors - Dr. Paulina Maria Lesiczka, Prof. Luděk Žůrek, Dr. Farkas Szekely, Mr. Sajjad Ghodrati

The brown dog tick, *Rhipicephalus sanguineus sensu lato* (s.l.) is a complex of species with unsettled species concept. In the distribution of main lineages of ticks included in *R. sanguineus s. l.* (RS) was repeatedly discussed. Recently, the “tropical lineage” was recognized as *R. linnaei* while the “southeastern Europe” lineage is now *R. rutilus*. In Europe, RS has been considered mainly a Mediterranean tick species. In past decades, northward expansion is observed. “*R. sanguineus*” is known as a vector of a range of pathogens of medical and veterinary importance in the Mediterranean. In 2020-2021, a total of 1839 ticks were collected by veterinarians from Hungarian dogs and cats. All ticks were morphologically identified; 169 individuals as RS. The study aimed at genetic identification of RS, pathogens potentially transmitted by RS in this focus, and review of autochthonous distribution of pathogens transmitted by RS in Europe. In the Hungarian specimens, 16S rDNA, 12S rDNA, and Cox1 sequences clustered into a single haplotype, all belonging to *R. sanguineus s. str.* clade. This clade was well separated from *R. linnaei*, *R. rutilus* as well as from other species. All 169 ticks identified as *Rhipicephalus* were tested by nested PCR for *Anaplasma platys*, *Ehrlichia canis* (16S rRNA), *Babesia vogeli*, *Hepatozoon. canis* (18S rDNA), and *Rickettsia conorii* (ompA), all with negative result. However, sequencing of the amplicon from one dog revealed the presence of *A. phagocytophilum*. Our study confirmed persistence of RS population previously reported in 2016 and identified found ticks as *R. sanguineus s. str.* We did not find pathogens associated with RS. Further work is needed to investigate the “marginal” populations of “*R. sanguineus*” to monitor spread of pathogens transmitted by this tick species. Population of newly recognized species should be investigated to prove their vectorial competence for pathogens traditionally associated with brown dog ticks. Supported by Czech Science Foundation project 21-11661S; ticks were collected within “Protect-our-future-too” campaign.

OA11.05

Prevention of heartworm infection in dogs using a combination of moxidectin, imidacloprid and praziquantel: a longitudinal field study_ Dr. Filipe Dantas-Torres_Fiocruz, Brazil

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Abstract

The aim of the present study was to evaluate the effectiveness of a spot-on combination of moxidectin 3.5%, imidacloprid 10% and praziquantel 10% in the prevention of *Dirofilaria immitis* infection in dogs. For this purpose, a randomized and controlled clinical trial was conducted between August 2021 and October 2022, in the municipality of Goiana, state of Pernambuco, northeastern Brazil. Of the 213 dogs initially sampled (baseline), 68 (31.9%) were positive in the antigen test (SNAP 4Dx, Idexx) and/or in the microfilariae test (Knott test). On day 0, 140 dogs negative in both tests were randomly included in the treatment (n = 70) and control groups (n = 70). During the study 60 dogs (34 from treatment and 26 from control groups) were removed for different reasons. At the end of the study (day 360), 36 dogs from the treatment group and 44 from the control group were sampled and included in the calculation, resulting in an efficacy of 84.7% and a protection of 97.2% against *D. immitis* adults. Specifically, only one dog from the treatment group tested positive for antigens on day 360, being the same negative for microfilariae. On the other hand, eight dogs from the control group were positive for antigens and/or microfilariae, resulting in a significant difference in the number of positives between groups (Fisher's exact test, P=0.0367). Remarkably, the efficacy and protection for *D. immitis* microfilariae on day 360 was 100% (i.e., no dogs from the treatment group were positive for microfilariae), whereas three dogs from the control group were positive for microfilariae. In conclusion, the spot-on product based on a combination of moxidectin 3.5%, imidacloprid 10% and praziquantel 10% significantly reduced the risk of *D. immitis* infection in treated dogs as compared with untreated dogs, in a highly endemic area in northeastern Brazil.

Keywords: *Dirofilaria immitis*; heartworm; prevention; control; moxidectin

OA11.06

Efficacy and safety of Felpreva, a spot-on formulation for cats containing Emodepside, Praziquantel and Tigolaner against experimental infestation with the Australian paralysis tick (*Ixodes holocyclus*)_ Dr. Florian Roeber_Vetoquinol, Australia

Co-Author - Ms Chrissie Jackson, Dr Michael Chambers, Dr Veronica Smith, Dr Jane Hume, Dr Katrin Blazejak, Dr Norbert Mencke

The Australian paralysis tick *Ixodes holocyclus* continues to be a serious threat to companion animals along Australia's east coast. The tick produces a potent neurotoxin which causes a rapidly ascending flaccid paralysis, which if left untreated, can result in the death of the animal. There is currently only a limited number of products registered in Australia for the treatment and control of paralysis ticks in cats. Felpreva® is an effective spot-on combination containing emodepside, praziquantel and tigolaner. To investigate the therapeutic and long-term persistent efficacy of Felpreva® (2.04% w/v emodepside, 8.14% w/v praziquantel and 9.79% w/v tigolaner) against experimental infestation with *I. holocyclus* in cats, two studies were undertaken. Fifty cats were included in the studies on study Day -17. Cats were treated once on Day 0. Group 1 cats were treated with the placebo formulation and Group 2 cats were treated with Felpreva®. Cats were infested on Days -14 (tick carrying capacity test), 0, 28, 56, 70, 84 and 91 (weeks 4, 8, 10, 12 and 13). Ticks were counted on cats 24, 48 and 72 hours post-treatment and infestation, except during the tick carrying capacity test when they were counted approximately 72 hours post-infestation only. The 24 and 48 hour assessments were conducted without removing the ticks. The ticks were assessed, removed and discarded at the 72 hour assessment timepoints. Significant differences in ToL tick counts at ~24, ~48 and ~72 hours post infestation were observed between the treatment and control group. Differences were significant ($p < 0.05$ to < 0.001) in all instances. Treatment efficacies of 98.1 – 100% were observed ~72 hours post infestation through to 13 weeks (94 days) post-treatment. These results show that a single application

of Felpreva® provides effective treatment and control against induced infestation with paralysis ticks for 13 weeks.

OA11.07

Efficacy and speed-of-kill of Simparica Trio® against global ticks in dogs, and the relevance to tick-borne diseases _Geurden, Dr. Thomas_Zoetis

Co-Authors - Dr Raj Packianathan, Donghoon Bae, Karen Roth, Kristina Kryda, Andrew Hodge, Leen Van Brussel, Csilla Becskei, Dr Steve Maeder

Simparica Trio® (Zoetis) has been commercialized globally and multiple controlled studies were conducted to investigate its efficacy in the treatment and prevention of ticks in dogs. In each of these studies 16 dogs were randomly allocated to two groups and infested with the appropriate number of adult ticks on Days -2, 5, 12, 19, 26, 33. Dogs received a single treatment on Day 0 with either placebo or Simparica Trio that provided at least 1.2 mg sarolaner, 24 µg moxidectin and 5 mg pyrantel (as pamoate salt) per kg bodyweight. Ticks were counted at 48 or 72 hours after treatment and after each subsequent re-infestation, except in the speed-of-kill studies in which ticks were counted at earlier timepoints. Efficacy was calculated based on the percent reduction of mean live tick counts compared to the placebo group. No treatment-related adverse reactions were observed in any of these studies. In all, studies, the mean live tick counts for dogs treated with Simparica Trio were significantly lower ($P < 0.05$) than the control treated dogs at

each time point in each study. A single treatment at the minimal dose of Simparica Trio was highly efficacious in treating and preventing infestations of each of tick species from around the world for at least 28 days, and this high efficacy was further confirmed in field studies. For several global tick species known to be a vector for tick-borne diseases, a rapid speed-of-kill was demonstrated. The prevention of transmission of tick-borne diseases was demonstrated for *Ixodes scapularis* (Lyme disease) and *Dermacentor reticulatus* (Babesiosis) in validated disease models. These studies demonstrate that Simparica Trio is well tolerated and can be used globally in the treatment and prevention of tick infestations, has a fast speed-of-kill and aids in the prevention of disease transmission.

22.08.23

Breakout 2: OA12 Flea and Tick Control

OA12.01

Flea population growth by the numbers_Armstrong, Dr. Robert_Merck Animal Health

Co-Authors - Dr Michael Dryden, Dr Jason Drake, Dr Dorothy Normile

Fleas are highly fecund parasitic insects that rapidly colonize an infested premises causing irritation with hematophagous bites and spreading disease for companion animals and human residents. Precise flea population census of all life stages in an infested premises is not easy given adult motility on animal hosts and the preference of juvenile life stages including eggs, larvae and pupae to remain hidden deep in carpeting, flooring and furnishings. This study estimates flea life stage population growth using a mathematical model developed from laboratory and field measurements of flea fecundity and pet infestation. The infestation starts with four initial fleas and then every pet in the household gains an additional new 2 fleas per day in addition to the adult fleas resulting from household flea maturation. Survival from flea egg to adult is set at 10.8% based on a stable temperature of 24 °C and RH of 78%. Egg hatch is 60%; 20% of larvae survive; 90% of pupae emerge and daily adult mortality below the capped limit is 3%. Adult flea numbers infesting pets were capped at 5000 per dog and 100 per cat using recorded upper limits of flea adult counts on dogs and cats from flea control studies in an endemic area of Florida. Adults are in a 3:1 ratio of females to males; all females are fertile and initiate egg deposition within 24 hours after gaining a dog or cat host, depositing 10 eggs on day 1; 20 eggs on day 2; 30 eggs on day 3; and 50 eggs per day on day 4 and after. Model estimates are that flea populations peak ca 170 days following infestation initiation and the peak population plateaus with 0.05% adults; 32.95% eggs; 53.22% larvae and 13.78% pupae.

OA12.02

Tick identification and resistance of *Rhipicephalus microplus* to amitraz and ivermectin on livestock in Ecuadorian subtropical farms._Pérez-Otáñez, Ms.

Ximena_Universite´ Catholique de Louvain

Co-Authors - Ms. Valeria Paucar, Prof. Lenin Ron-Garrido, Ms. Linette Jácome, Mr. José Buitrón, Prof. Claude Saegerman, Prof. Richar Rodríguez-Hidalgo, Prof. Sophie O. Vanwambeke

Cattle ticks, mainly *Rhipicephalus microplus*, are the principal neglected ectoparasites for livestock in subtropical regions worldwide. These ticks affect meat and milk production, causing economic losses to cattle farmers. Ticks are also important vectors of pathogens, such as *Anaplasma* spp. and *Babesia* spp. Ecuador has a suitable climate for tick development. Acaricides are the main method of tick control; however, its misuse has led to resistance and multi- resistance development in ticks. The efficacy of acaricides has progressively decreased in Ecuador. This study aims to inventorise tick species found on cattle in Ecuador, and assess the presence of resistance to amitraz(Am), and ivermectin(Iv). The study focuses on Noroccidente de Pichincha over a two years period. We used the larval package test (PLT). Sixty-seven farms were sampled in 2020-2021. 97.01% (65/67) of farms had ticks on cattle. Four species were found: *R. microplus* 95.52%(64/67), *Amblyomma* spp. 1.49%(1/67), *Ixodes boliviensis* 1.49%(1/67), and *Ixodes montoyanus* 1.49%(1/67); the last two are considered accidental ticks for cattle. We found that 67.21% (41/61), and 57.38% (35/61) of *R. microplus* ticks were resistant to Am and Iv respectively in the first round of samples. In the second round of samples a year later, 59.57%(28/47) and

57.45%(27/47) of ticks were found resistant. We can conclude that our study demonstrates that tick resistance to acaricide is a widespread problem in Ecuadorian cattle farming for the two products examined. There was no significant decrease in resistance between the two years surveyed. There is broad scope to work with farmers to assess practices and improve them to insure continuing efficacy of active principles and the availability of acaricide control options for farmers.

Key words: pesticide, ticks, farms, cattle.

OA12.03

Functional and genetic comparison of *Ctenocephalides felis* and *Rhipicephalus sanguineus* isolates from different geographic regions _Heuer, Dr. Lea_MSD Animal Health Innovation GmbH

Co-Authors - Margaret Werr, Dr. Heike Williams, Hartmut Zoller, Eva Zschiesche, Dr. Claudia Plehn, Dr. Carolin Schneider

Currently available regulatory guidelines for EU approval of canine and feline ectoparasiticides require the conduct of dose confirmation studies with European isolates. This requirement may be redundant if there are no substantial differences between EU and non-EU laboratory parasite isolates. A project compared the sensitivity of multiple *Ctenocephalides felis* fleas and *Rhipicephalus sanguineus* ticks from different geographic locations to fluralaner functionally and by comparative sequence analysis. Isolates of *C. felis* sourced from Europe, USA and Australia and *R. sanguineus* isolates from Europe and USA were exposed to increasing fluralaner concentrations. After 48h of incubation, fleas and ticks were assessed by counting the number of dead, damaged and/or live individuals per fluralaner test concentration. Furthermore, comparative cDNA and genomic DNA sequence analysis of the predicted fluralaner binding sites in the GABA-gated chloride channel (GABAR) and glutamate-gated chloride channel (GluR) of the mentioned parasite isolates were performed, to detect potential alterations that may influence the fluralaner binding efficacy and dieldrin resistance. Predicted lethal and effective concentrations of fluralaner for the different flea isolates fell within a narrow range, as did the values for both tick isolates. No between-isolate differences were identified in the protein sequence of the most important fluralaner interaction sites of the European, US or Australian *C. felis* isolates, nor of the European or US isolates of *R. sanguineus*. These results indicate possible between-country interchangeability of in vivo results in dose confirmation studies for fluralaner-containing products. Thus, the data provide a justification for the potential reduction of the number of required dose confirmation studies for EU approval.

OA12.04

Baiting stray dogs in extraurban area: Innovative tools for an integrated control of echinococcosis_Ciccone, Dr. Elena_Department Of Medicine Veterinary And Animal Production, University Of Napoli Federico II

Co-Authors - Dr. Nicola Lattero, Dr. Antonio Bosco, Dr. Martina Nocerino, Dr. Paola Pepe, Dr. Lavinia Ciuca, Dr. Maria Paola Maurelli, Dr. Giuseppe Cringoli, Prof Laura Rinaldi

Cystic echinococcosis (CE) is an endemic zoonosis causing by the larval stage of *Echinococcus granulosus*, which presents worldwide distribution, especially in the Mediterranean countries. The CE control in a highly endemic area of southern Italy (Campania region) was based on a control program focused on the development of new procedures and tools that includes among the activities the treatment of sheepdogs. Therefore, the aim of this study was to implement the

anthelmintic treatment by extending it also to stray dogs in the proximity of CE positive sheep farms, by dropping baits with a drone in peri-pasture areas. Assessments were focused on the resistance of the baits over the time (at least 10 days) to different climatic conditions while preserving the palatability for dogs. Highly palatable chews laced with anthelmintic praziquantel were used as baits to perform this study. In addition, the integrity of the baits released by the drone from different heights was evaluated. Grazing areas were detected using GPS devices applied to sheep and sheepdogs, and a pilot baiting site was selected based on micro-epidemiology. Baits were placed in several arrangements and at different locations. The uptake by stray dogs was investigated using camera traps. The baits with double layer coverage showed the greatest resistance in the environment while preserving the palatability up to 10 days. In addition, the double layer coverage withstood heights of 30 meters dropped by drones on hard surfaces. In total 80% were eaten after 1 day, 15% after 2 days, 5% after 3 days. Most of the baits (95%) were consumed by stray dogs and the remaining (5%) were consumed by foxes, badger and wild boar. In conclusion, the baits tested are well suited in a control plan for the treatment of stray dogs present in the peri-pasture areas of CE positive sheep farms.

OA12.05

In vitro and in vivo characterization of resistance to commercial acaricides in field isolates of economically important ticks from East and West Africa_Evans, Mr.

Alec_Clinglobal

Co-Authors - Prof. Maxime Madder, Dr. Josephus Fourie

To increase their productivity and sustainability, small scale producers in Africa need access to affordable yield-enhancing inputs of which tick control is of paramount importance. Many acaricide classes have been in use for decades and treatment failure poses a major threat. Several factors may contribute to the establishment and spread of acaricide resistance and characterizing the extent of resistance in regionally applicable isolates is needed to provide relevant local advice. Larval packet tests following the FAO guidelines were performed on 23 strains of three tick species (*Rhipicephalus microplus*, *Rhipicephalus appendiculatus* and *Amblyomma variegatum*) isolated from six countries in East and West Africa. The results demonstrated widespread resistance to chlorfenvinphos and amitraz in all species and regions. Surprisingly, resistance to ivermectin was also demonstrated in *A. variegatum* and *R. appendiculatus* in West Africa. The efficacy of commercial acaricides against the four least susceptible isolates was further characterised in a series of nine in vivo, controlled GCP studies in cattle and goats. Cypermethrin, cymiazole and ivermectin were only partially effective against *R. microplus* with no residual efficacy. Flumethrin spray-on provided short-term control against *A. variegatum* after application directly to tick predilection sites whilst the pour-on formulation applied to goats showed residual efficacy up to one month. Therapeutic control was achieved against *R. appendiculatus* through the weekly spraying of flumethrin, amitraz or cymiazole and cypermethrin. Fipronil achieved moderate control of *R. microplus* and *R. appendiculatus* however the associated withdrawal restricts its practical application. Results from these analyses and the limited options for tick control available to livestock farmers in Africa are discussed in further detail. This highlights the need to focus on managing the use of available acaricides, identifying new classes or repurposing existing compounds as acaricides, prioritising vaccinology targeting ticks and improving our understanding of the impact and value of integrated tick management.

OA12.06

The comparative efficacy of single active macrocyclic lactone (ML) drenches with a novel dual active ML plus levamisole injectable drench assessed through the use of faecal egg count reduction tests in cattle from Australia._Oswin, Dr. Sally_Zoetis Australia

Co-Authors - Dr Matthew Playford, Mr Ashley George, Dr Raj Packianathan, Mr Andrew Hodge

The comparative efficacy of single active macrocyclic lactone (ML) drenches with a novel dual active ML plus levamisole injectable drench assessed through the use of faecal egg count reduction tests in cattle from Australia. Twenty-one faecal egg count reduction tests (FECRTs) were conducted in regionally diverse locations within Australia in weaner cattle aged 3-12 months of age where macrocyclic resistance was suspected or confirmed. Both dairy and beef breeds were represented. The drenches evaluated were single active MLs and a new combination injectable dual active drench was included in each study, containing both an ML (doramectin) and levamisole. A summary of the data from these 21 studies is presented. The average geometric mean (GM) and arithmetic mean (AM) efficacy of each of the single ML drenches used was below 95%, while that of the doramectin and levamisole combination was 99.7% (GM) and 99.3% (AM) respectively. Resistance to single active ML drenches was identified in all of the following nematode genera: *Cooperia*, *Haemonchus*, *Ostertagia*, *Trichostrongylus* and *Oesophagostomum*. Of the twenty-one studies conducted, efficacy below 95% of the ML (doramectin) and levamisole combination was identified in only one study in *Haemonchus* (GM and AM) and *Trichostrongylus* (AM) nematodes. This data indicates that ML resistance in young cattle in Australia is common and using a combination injectable drench containing both an ML (doramectin) and levamisole was highly effective on farms where ML resistance was established. In addition, a significant difference in efficacy was also identified when a common ML active was given according to label directions via injection versus pour-on application with the former being more efficacious.

22.08.23

Breakout 3: OA13 Molecular tools to detect resistance against nematodes

OA13.01

Role of nematode ABCB transporters and their regulation in anthelmintic

resistance_Lespine, Prof. Anne_INRAe Anne Lespine, Mélanie Albéric INTHERES, Université de Toulouse, INRAE, ENVT, 31027 Toulouse Cedex 3, France

Co-Author - Dr Mélanie Albéric

The multidrug resistance (MDR) transporter ABCB1 is involved in the pharmacokinetic and toxicokinetic of many xenobiotics, including ivermectin, an anthelmintic drug commonly used to treat parasitic diseases. Several ABCB gene homologs are present in parasitic nematodes infecting humans and animals and some of them have been associated with resistance to ivermectin, but their individual role remains to be elucidated. Being overexpressed in response to chemotherapy, notably to ivermectin-based treatment in nematodes, they lead to loss of drug efficacy by decreasing drug concentration at the target site. Using *Caenorhabditis elegans* as a nematode model, we decipherer structural and functional details of the transporters in parasites of interest. We developed complementary approaches to identify the role of individual transporter in drug transport and lipid accumulation, using imaging approaches. Overall, we identified a transcription factor regulating the expression of ABC transporters and lipid homeostasis genes as new original target to counter drug resistance. Increasing knowledge of the function of ABC transporters and their regulation is important for delaying the spread of anthelmintic resistance in helminths and to improve anthelmintic-based therapy.

OA13.02

In silico approach to screen phytochemicals active against 24 kDa excretory and

secretory protein of *Haemonchus contortus*_Sankar, Dr. Muthu_Indian Veterinary Research Institute

Co-Authors - Dr Vinay Kishor Tiwari, Dr C.S. Neethu, Dr Rajat Garg, Dr Subashish Bandyopadhyay, Dr K.A. Yashica

Much research was carried out on the utilization of excretory/secretory proteins (ESPs) of parasites as vaccine candidates against the haemonchosis prevention method. In order to maintain parasite viability inside the host during infection, ES proteins also serve as immunoregulators to control host immune responses. In recent years, ESPs of parasites are gaining attention as drug targets, as these proteins have great access to the drugs as they are released into the extracellular space. *Haemonchus contortus* is the most prevalent gastrointestinal nematode, and this parasite exhibits anthelmintic resistance to the available drugs. In our present study, we aimed to find the phytochemicals with anthelmintic actions against the ESP of *H. contortus* 24 kDa by in silico model. The homology modelling of the three-dimensional protein of 24 kDa of *H. contortus* was performed using the deduced amino acid sequence in the HHPred server. The three-dimensional model was refined in the DeepRefiner server and the refined model was validated in SAVES v6.0 server. The structure of 200 phytochemical compounds (ligands) of 27 commonly available plants was retrieved from PubChem for screening. The phytochemical compounds were evaluated for their physiochemical, and pharmacokinetic properties, drug likeness, and synthetic accessibility in the SwissADME web tool. Based on ADME scores, only 97 of 200 compounds were selected for the docking study. The site-specific and blind docking of 24 kDa and 97 phytochemicals was carried out in AutoDock 4.0. The

docking results showed that piperine and santonin had the lowest binding energy followed by fistulic acid, picrinine, rhein, nigellone, and guvacoline. Further, molecular dynamic simulation of the protein-ligand complexes would provide in the future a new effective anthelmintic drug from the available plant resources.

OA13.03

Pyrantel resistance in hookworms infecting dogs in Queensland, Australia _Abdullah, Dr. Swaid_The University of Queensland The University of Queensland, School of Veterinary Science, Gatton 4343, QLD, Australia 2CSIRO Agriculture and Food, Queensland Bioscience Precinct, St. Lucia, Brisbane, QLD 4067, Australia

Co-Authors - Professor Malcolm Jones, Dr Andrew Kotze

Hookworms are the most common intestinal parasites of dogs, treatment and control of these parasites mostly depends on regular deworming with anthelmintics. Pyrantel-based dewormers are relatively cheap and readily available option for dog owners. Pyrantel resistance in canine hookworms was first reported 15 years ago, however pyrantel-based dewormers are still used against hookworm infection in dogs. The present study was conducted to evaluate the efficacy of pyrantel against hookworms infecting dogs housed in a shelter facility in South-East Queensland, Australia. Ten dogs were examined using faecal egg count reduction test (FECRT). There was no reduction in FEC in any of the dogs following pyrantel treatment, with drug efficacies ranging from -0.9 % to -283.3 %. Given that these dogs originated from various sites across South-East Queensland, the study suggests that pyrantel resistance is widespread in this region, and hence this anthelmintic may not be a useful option for treatment of hookworm infections in dogs. The emergence of anthelmintic resistance in canine parasites poses a serious health risk not only to these animals but also to their owners and general public. The study highlights the need for further testing of efficacy of pyrantel and other anthelmintics against hookworm infections in dogs.

OA13.04

Comparison of ovine faecal strongyle egg counts from an accredited laboratory and a rapid, on-site parasite diagnostic system utilising a smartphone app and machine learning._O'Brien, Ms. Christiane_Micron Agritech

Co-Authors - Ms. Madeleine Bucki, Dr. Kerrie Ní Dhufaigh, Ms. Tara McElligott, Mr. Nigel Walshe, Mr. Andrew Weatherley

Conventional treatment for gastrointestinal parasitic worms (helminths) in grazing livestock often involves untargeted, metaphylactic blanket treatment of animals with anthelmintics. As a result, worm resistance to anthelmintic drugs has become a significant global issue. Faecal egg counts (FECs) are an important diagnostic test in combatting further anthelmintic resistance. FECs are labour-intensive, time-consuming and require trained personnel to process the samples and visually identify the parasite eggs. Consequently, the time between sample collection to results can take days. This study aimed to evaluate a rapid, on-site parasite diagnostic system utilising a smartphone app and machine learning and compare it to a traditional McMaster method performed by an accredited laboratory. A total of 105 ovine faecal samples were collected. Samples were homogenised and split equally between two containers. One container per sample was processed using the app-based system, the second container was sent to the laboratory. Briefly, 3 g of faeces were added to 42 mL of water (system) or saturated saline (laboratory). The mixture was then homogenised, strained and either centrifuged (system) or not

(laboratory) before analysis. Strongyle egg counts were conducted via video footage of samples by the system's machine learning (ML) and a trained technician (MT) and via McMaster slide by a laboratory technician (MM). Results were statistically analysed via GLM using SAS® (Version 9.4) software. The ratio of means was used to determine non-inferiority of the ML results compared to the MM results. Both of the system's egg counts (ML and MT) were higher ($p < 0.0001$) compared to those obtained from the laboratory (MM), and deemed non-inferior. There was no difference between the ML and MT counts. In conclusion, rapid, on-site diagnostic systems can help veterinarians to increase their testing capacity, perform on-farm testing and deliver faster and more targeted parasite treatment to combat anthelmintic resistance.

OA13.05

Widespread multi-drug resistance of strongyle nematodes in German sheep but not cattle as revealed by faecal egg count reduction tests and nemabiome deep sequencing data_ Dr. Jürgen Krücken_Freie Universität Berlin, Germany

Co-Authors - Dr. Stefan Fiedler, Dr. Fabian Horn, Dr. Christina Helm, Sabrina Ramünke, Tanja Bartmann, Alexandra Kahl, Dr. Ann Neubert, Dr. Wiebke Weiher, Ricarda Daher, Dr. Werner Terhalle, Dr. Alexandra Klabunde-Negatsch, Stephan Steuber, Georgvon Samson-Himmelstjerna, Paula Ehnert

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Anthelmintic resistance against all available drug classes is a worldwide problem in ruminants and multi-drug resistance poses a high risk for animal welfare. In the absence of recent data on the occurrence of anthelmintic resistance in German ruminants, the faecal egg count reduction test (FECRT) and deep amplicon sequencing (nemabiome) were used to examine the species composition and drug susceptibility status of strongyle populations. Efficacy of fenbendazole, ivermectin and moxidectin was evaluated using Mini-FLOTAC on eight, eight and twelve sheep farms, respectively. On eight and seven cattle farms susceptibility to fenbendazole and eprinomectin was investigated with FLOTAC. The FECR with 95% confidence intervals was calculated using hierarchical Bayesian modelling in eggCounts software. Internal-transcribed-spacer-2 sequences obtained by MiSeq sequencing were assigned to strongyle species using the nemabiome database and species compositions were calculated. Resistance was detected on 6/8 farms for fenbendazole, 6/8 farms for ivermectin, 3/12 farms for moxidectin and 3/8 farms for all three drugs. Nemabiome data before treatment identified *Teladorsagia circumcincta* (5.8-91.1% of the larvae) on 11/11 farms followed by *Trichostrongylus colubriformis* ($\leq 41.6\%$) and *Haemonchus contortus* ($\leq 42.1\%$) occurring on 10/11 farms. *Chabertia ovina* (51.5%) and *Oesophagostomum venulosum* (27.3%) represented the most abundant species on one farm each. Triple resistant *H. contortus* and *T. circumcincta* were found on three farms. Fenbendazole and ivermectin resistance were detected in *T. colubriformis*, *Trichostrongylus vitrinus*, *Trichostrongylus axei*, *Cooperia curticei* and *Cooperia fuelleborni*. In cattle, full efficacy of fenbendazole was found on all eight farms while eprinomectin resistance was detected on 1/7 farms. *Ostertagia ostertagi* and *Cooperia oncophora* were the most abundant species but on two farms *Bunostomum phlebotomum* and *T. axei* were the most abundant species, respectively. No post treatment nemabiome data were obtained. In sheep, parasite species with triple resistance occurred on multiple farms while in cattle anthelmintics remained mostly fully active.

OA13.06

ABC transporters in *Toxocara canis*: identification, expression and anthelmintic efflux activity_Jesudoss Chelladurai, Dr. Jeba_Kansas State University College Of Veterinary Medicine

Co-Authors - Dr. Katy Martin Ms. Pam Vardaxis, Theresa Quintana, Dr. Craig Reinemeyer, Dr. Paramasivan Vijayapalani, Dr. Alan Robertson, Dr. Matthew Brewer

Toxocara canis is a ubiquitous zoonotic nematode of dogs. The somatic larval stage of this parasite is tolerant to anthelmintic drugs of the macrocyclic lactone drug class. We have previously shown that the ATP binding cassette (ABC) transporter protein Tca-Pgp-11 expressed by the parasite is capable of interacting with and effluxing macrocyclic lactones and other ABCB1 interacting drugs. In this study, we investigated the repertoire of ABC transporter proteins in *T. canis*. We determined the sequences and performed phylogenetic analysis on ABC transporters encoded in the genome. We measured expression of 10 ABCB1 genes (P-glycoproteins) in adult and larval stages of *T. canis* using qPCR. We demonstrate that while macrocyclic lactones failed to significantly increase the expression of ABCB1 genes in *T. canis* larvae, expression of the genes was still evident. We also determined the localization of ABCB1 in adult and larval stages using colorimetric mRNA in situ hybridization (RNASCOPE). We measured the functional activity of ABCB1 proteins in larvae using a competitive transporter assay with a fluorescent substrate in the presence of ABCB1 interacting drugs and demonstrate that inhibitor drugs have a unique rank order of potency. Larval movement assays were also performed with macrocyclic lactones and inhibitor drugs. Taken together, data from this study increases our understanding of ABC transporters in the parasitic nematode *T. canis* and the role they play in anthelmintic tolerance.

22.08.23

Breakout 4: OA14 Alternative treatment for parasites of ruminants

OA14.01

First guide for the responsible and prudent use of anthelmintic chemicals to help control anthelmintic resistance in grazing livestock species by the World Organisation for Animal Health (founded as OIE)_Garcia Campos, Dr. Andres_World Organisation For Animal Health

Co-Authors - Dr. Mária Szabó, Professor Nick Sangster, Dr. Aimée Phillippi-Taylor, Dr. Anna O'Brien, Dr. Ellen Hart, Dr. Barbara Cyrus, Dr. Valentin Nicorescu, Dr. Nathalie Bridoux, Dr. Jacques Devos, Professor Christo Hilan, Professor Oubri Bassa Gbati, Dr. Patrick Vudriko, Dr. Rebecca Hibbard, Dr. ElisabethErlacher-Vindel, Dr. Javier Marcos Yugueros

Helminths of livestock may affect the health of animals and can significantly decrease the production of meat, milk and fibre, as well as negatively impact animal traction used for land cultivation, food security and public health. Anthelmintic chemicals are antiparasitic agents that are a vital tool in parasite control. However, resistance to anthelmintic chemicals has emerged globally and now poses a significant threat to animal health and productivity. Prudent use of chemical and non-chemical means to manage anthelmintic resistance is a global responsibility. The World Organisation for Animal Health (WOAH) set up an international expert group on antiparasitic resistance, who met several times virtually, producing a publication entitled 'Responsible and prudent use of anthelmintic chemicals to help control anthelmintic resistance in grazing livestock species'. This document promotes the prudent use of the few classes of anthelmintic chemicals that remain available for parasite control, with an emphasis on their use in food-producing ruminants. It also covers definitions, the global extent of anthelmintic resistance and causes of resistance, discussing moreover challenges that are central to resistance, such as the diagnosis of parasite infections and resistance, livestock parasite management to reduce resistance, and the regulation of pharmaceutical anthelmintic chemicals. Lastly, it identifies gaps in essential knowledge and skills, and highlights research and training needs. This is the first publication created by WOAH to address antiparasitic resistance, expanding our already extensive set of international standards on responsible and prudent use of antimicrobials, both in terrestrial and aquatic animals, to parasites of veterinary and public health importance.

OA14.02

The Migration Trap Assay: A high-throughput screening and diagnostic tool that can detect early resistance in *Haemonchus contortus*_Duguet, Dr. Thomas_Invenesis

Co-Authors - Mr. Fabrice Guégnard, Dr. Cédric Neveu, Dr. Lucien Rufener, Dr. Alexandre Vernudachi

Gastrointestinal nematodes such as *Haemonchus contortus* are developing increasing levels of resistance to the limited range of compounds available in the market, rendering most anthelmintics ineffective. For dairy farming of sheep and goats, only the macrocyclic lactone eprinomectin is allowed as it avoids waiting periods for milk. However, the urgent need for new compounds is not enough without a quick and low-cost way to diagnose susceptibility and resistance status on the field. To address this issue, a promising solution is the Migration Trap Assay (MTA) derived from the ALMA technology developed by the MNP research group at INRAE, France, and made available through Invenesis France, a custom research organization (CRO) specializing in phenotypic in vitro assays on endoparasites for compound screening. The MTA has shown high potential in differentiating a small proportion of resistant *H. contortus* L3

larvae (5% kokstad strain) from sensitive ones (95% weybridge strain). As a recent example, the MTA has precisely identified resistance factors to eprinomectin of 54.3 and 50.6 % before and after selection pressure in comparison to a sensitive (Weybridge) strain. The MTA is a highly versatile tool that can be used to study a range of endoparasites, including *Ascaridia galli* and *Parascaris equorum*, which are known to infest poultry and equines, respectively. By enabling research to identify resistance mechanisms and characterize field strains, the MTA offers a promising solution for the development of new anthelmintics and the optimization of existing treatments. This capability is crucial in the ongoing fight against parasitic infections and their impact on animal health and livestock production.

OA14.03

Microfluidic encapsulation of potent anthelmintic essential oil components for post-ruminal delivery to combat sheep haemonchosis_ Dr. Mohamed Abdelmoghny Ramadan Helal_National Research Centre, Egypt

Co-Authors - Dr. Ahmed M. Abdel-Gawad, Dr. Omnia M. Kandil, Dr. Marwa M. Khalifa, Dr. Gareth W.V. Cave, Dr. Hany M. Elsheikha

The anthelmintic effects of five pure essential oil constituents (geraniol, geranyl acetate, eugenol, methyl iso-eugenol, and linalool) were tested, using larval motility assay, on the third-stage larvae (L3s) of *Haemonchus contortus*. The geraniol, eugenol and linalool showed a strong inhibitory efficacy against larval motility of *H. contortus* comparable to positive control (20 mg/mL levamisole) within 24 hr ($p < 0.05$). The three oils also induced a considerable structural damage to L3s. Results of treatments were validated by quantitative fluorometric microplate-based assays using Sytox green, propidium iodide and C12-resazurin, which successfully discriminated live/dead larvae. The cytotoxicity of components on Madin–Darby Canine Kidney cells was evaluated using sulforhodamine-B (SRB) assay and no significant cytotoxic effect at concentrations $< 1\%$. The EOs were successfully encapsulated in the chitosan coated PLGA particles confirmed by FTIR, TGA and XRD results with encapsulation efficiencies of 95.14, 79.68 and 71.34% and loading capacities of 8.88, 8.38 and 5.65% in entrapped particles with eugenol, linalool and geraniol, respectively. Both uncoated and coated particles had a spherical uniform shape with a relatively smooth surface approximated sizes of 273-335 nm with narrow PDI (0.15-0.19). The chitosan coated particles were positively charged (24.7 ± 9.06) compared to the negative zeta potential of PLGA particles (-23.3 ± 5.01). The release profile of EOs from the particles showed an initial burst release phase followed by sustained release phase with significantly slower rates of release from coated particles than uncoated particles. The encapsulated oils in chitosan coated particles, up to concentrations of 5 $\mu\text{g/mL}$, is non cytotoxic and has no adverse effect cell growth, viability and cell membrane integrity compared to the free EOs and uncoated particles. Taken together, the results demonstrated that microfluidic encapsulation of potent anthelmintic EOs components in chitosan coated PLGA particles could be potential carrier for oral and post-ruminal delivery to combat the gastrointestinal nematodes.

OA14.04

Investigating the antiparasitic potential of biorefined red clover (*Trifolium pratense*) and identification of active compound(s)_Pan, Mr. Geng_University of Copenhagen

Co-Authors - Chao Liang, Dan Staerk, Mette Lübeck, Prof. Stig Thamsborg, Andrew Williams

Global livestock production faces challenges of environmental concerns and infectious disease prevention. In recent years, the process of biorefining plants for extraction of high-value feed protein has gained interest. Utilization of the side streams from this protein production would be highly valuable. We have assessed the potential antiparasitic properties of fibre pulp remaining after protein extraction of red clover (*Trifolium pratense*) and attempted to identify bioactive compounds. The current study was conducted to investigate the bioactivity of the biorefined red clover from two genetic lines, namely Callisto and Hammon. The grass material underwent a biorefining process, yielding a pulp fibre which was subsequently extracted with 70% acetone. The crude extract was tested with in vitro assay using the helminth, *Ascaris suum* as an antiparasitic model. To screen for bioactive compounds, compounds in the crude extract of the plant material were microfractionated by high-performance liquid chromatography (HPLC) and tested by in vitro assay. Afterward, the effective compounds were identified by nuclear magnetic resonance spectroscopy (NMR). Finally, liquid chromatography–mass spectrometry (LC-MS) was utilized to quantify changes in concentrations of effective compounds during the biorefining process. The crude extract of the pulp fiber of red clover showed more than 90% inhibition of *Ascaris suum* in vitro in the concentration of 1 mg/ml, 500 µl/ml, and 250 µl/ml. Among the compounds in the crude extracts, a flavonoid compound was found to have the most potent antiparasitic effect, and the concentration of this compound seemed to be boosted after the biorefining process. The biorefining process unleashed the antiparasitic potential of red clover, as evidenced by its increased antiparasitic effect. This effect is likely attributed to the increased levels of one specific compound. However, despite this observed activity, the mechanism of action of the flavonoid compound remains unknown. Thus, further investigations are needed to explore the possible interaction between the effective compound and the target worms.

OA14.05

In vitro anthelmintic effect and phytochemical screening of *Piper betel* on *Haemonchus contortus*_Dehuri, Dr. Manaswini_Odisha University Of Agriculture & Technology

Co-Authors - Dr Bijayendranath Mohanty, Dr Prasana Kumar Rath, Dr Balivada Deepika

The over burgeoning problem of anthelmintic resistance and environmental contamination has led to exploration of plant extracts as alternative means for control of *Haemonchus contortus*. The present study was carried out to determine the in vitro anthelmintic activity of *Piper betel* leaves and phytochemical analysis of plant extract. The ethanolic extract and its fractions (methanolic and chloroform) obtained by column chromatography of *P. betel* leaf extract in four different concentrations (10mg/ml -200mg/ml) were tested for adulticidal and ovicidal activity through Adult Motility Test (AMT) and Egg Hatch Test (EHT) respectively. There was significant ($p < 0.05$) inhibition of adult motility within 10 minutes of exposure. Adult motility assay showed a significant dose dependent motility with mean time of 9.6 ± 0.33 at highest concentration for ethanol extract while the other fractions recorded higher time. The ethanolic, methanolic fraction and chloroform fractions of *P. betel* leaf extract induced nearly cent percent inhibition of egg hatching ($99.64 \pm 0.36, 99.28 \pm 0.36$ and 98.20 ± 0.70 respectively) and IC 50 values were 0.62 mg/ml, 0.62 mg/ml and 0.76 mg/ml respectively at highest concentration. The scanning electron microscopy (SEM) micrographs showed changes in structure of adult *H. contortus* after

treatment with *P. betel* leaves. The major changes observed were the exfoliation of the tegument and wrinkled cuticle as compared to normal. The GCMS chromatogram of the leaf of *Piper betel* ethanolic extract analysis depicted the incidence of nine compounds of various chemical classes, the abundant amongst which were 3-Allyl-6-methoxyphenol (Eugenol) and hydroxychavicol.

OA14.06

Importance of nitroxynil for control of resistant liver fluke and barbers pole worm in Australian beef cattle_Durel, Dr. Luc_Virbac S.A.

Co-Author - Dr. Matthew Ball

Liver fluke (*Fasciola hepatica*) and/or Barbers Pole Worm (*Haemonchus placei*) are major constraints to efficient beef production in many regions of Australia. Triclabendazole has been important in liver fluke (LF) control (including immature stages), but resistance is now increasingly recognized. Macrocyclic lactones (MLs) have been relied upon as the main anthelmintic class to control Barbers Pole worm (BPW) for over two decades but resistance has become widespread. Nitroxynil is a unique narrow spectrum anthelmintic classified as a nitrophenolic compound. It was developed in the late 1960s but has not been used extensively in Australian cattle, making it useful against parasites overexposed to other actives. Nitroxynil is useful against the growing emergence of multidrug resistant LF and BPW. Used on its own nitroxynil is effective against adult and late immature LF (6–8 weeks old), *Haemonchus* spp., *Bunostomum* and *Oesophagostomum* spp. When nitroxynil and clorsulon are combined into one injection they provide a synergistic highly effective flukicide against both adult and early immature fluke (2 – 5 weeks old). A trial undertaken in NSW (Australia) involved infesting 42 beef cattle with LF strains known to be triclabendazole resistant. Oral triclabendazole had no efficacy against these LF while the nitroxynil 340g/L and 67g/L clorsulon combination (Nitrofluke®Injection, Virbac Australia) was 100% effective based on assessing fluke burdens 84 days after treatment at slaughter. A separate Faecal Egg Count Reduction Trial (FECRT) showed nitroxynil 340g/L, combined with clorsulon 67g/L and ivermectin 6.7g/L (Nitromec®Injection, Virbac Australia) had 100% efficacy against ML resistant BPW based on egg counts 14 days after treatment. Single active ivermectin was only 30% effective. Products containing nitroxynil alone or in combination provide another class of medicines with a unique mechanism of action for highly effective control of all stages of liver fluke and barbers pole worm.

22.08.23

Breakout 1: OA15 Ruminant parasites

OA15.01

Application of quantitative proteomics to discover biomarkers for tick resistance in cattle_Raza, Dr. Ali_The University of Queensland

Co-Authors - Prof Benjamin Schulz, Dr Amanda Nouwens, Dr Muhammad Noman Naseem, Mr Muhammad Kamran, Ms Emily Mantilla Valdivieso, Dr Edward Kerr, Dr Constantin Constantinoiu, Prof Nicholas Jonsson, Dr Peter James, Prof Ala Tabor

Breeding for tick resistance is a sustainable alternative to control cattle ticks due to widespread resistance to acaricidal drugs and the lack of a protective vaccine. The most accurate method used to identify tick resistance phenotype is the standard tick count, but this is labour-intensive and hazardous to the operator. Efficient genetic selection requires reliable phenotyping, such as biomarker(s), for accurately identifying tick-resistant cattle. Although breed-specific genes associated with tick resistance have been identified, the mechanisms behind tick resistance still needs further investigations. This study applied quantitative proteomics to examine the differential abundance of serum and skin proteomes using samples from naïve tick-resistant and -susceptible Brangus cattle at two-time points following tick exposure. The proteins were digested into peptides, followed by identification and quantification using sequential window acquisition of all theoretical fragment ion mass spectrometry. Resistant naïve cattle had a suite of proteins associated with immune response, blood coagulation and wound healing that were significantly (adjusted $P < 10^{-5}$) more abundant compared with susceptible naïve cattle, for example, complement factors (C3, C4, C4a), beta-2-glycoprotein-1, and fibrinogen. These findings were validated by identifying differences in the relative abundance of selected serum proteins with ELISA. The proteins showing a significantly different abundance in resistant cattle following early and prolonged tick exposures (compared to resistant naïve) were associated with immune response, blood coagulation, homeostasis, and wound healing. In contrast, susceptible cattle developed some of these responses after prolonged tick exposure. Resistant cattle were able to translocate immune-response related proteins towards the tick bite sites, which may prevent tick feeding. Physical barrier (skin integrity and wound healing) mechanisms and systemic immune responses are key contributors to resistance. Immune response-related proteins such as C4, C4a, AGP and CGN1 (naïve samples), CD14, GC and AGP (post-infestation) should be further investigated as potential biomarkers for tick resistance.

OA15.02

Efficacy of injectable eprinomectin (Eprecis® 20 mg/mL) for the treatment of larval *Oestrus ovis* infestation in naturally infected sheep_Sotiraki, Dr. Smaragda_Veterinary Research Institute Hellenic Agricultural Organisation (elgo-dimitra)

Co-Authors - Dr María Martínez Valladares, Dr Ourania Baka, Dr. Panagiota Ligda, Ms Laura Gonzalez, Dr Alexandros Stefanakis, DVM Thierry Pobel, Dr. Damien Achard, DVM Agathe Caruso-Vares

The larvae of the bot fly *Oestrus ovis* are obligate parasites of the nasal cavities and sinuses of small ruminants causing inflammation and mucous nasal discharge severely affecting the animals' productivity. The objective of this study was to evaluate the efficacy and the safety of injectable eprinomectin (Eprecis® 20mg/mL) against *O. ovis* larvae in naturally infested sheep. The study included two randomised, controlled, blinded trials conducted in Spain and in Greece. In each

country, 30 non-pregnant, non-lactating ewes previously exposed to at least one fly activity season, and originating from confirmed infected farms were enrolled. Inclusion criteria were a) to present signs compatible with *O. ovis* infection (i.e., nasal discharge, sneezing, snoring, respiratory distress, coughing), b) to not have received any parasiticide for at least 3 months, and c) to have general good health. After selection, the animals were transferred to each institute facilities and allocated, according to a pre-established random list stratified on age and clinical score, to a treated group receiving injectable eprinomectin subcutaneously at the dose of 0.2 mg eprinomectin/kg or to the control group receiving a saline solution. After treatment and for 14 days, the animals were clinically evaluated for signs of oestrosis. At D14, the animals were euthanized and necropsied to count the larvae and identify their developmental stage. L1 instar larvae were found in Spain and L2 and L3 in Greece. Treatment with injectable eprinomectin was highly effective against *O. ovis* larvae in comparison with the negative control group. Specifically, the percentage of efficacy was 96.4 % for the total larvae count and 96.2 % for L1 instar larvae and 100% for the total larvae count and for L2 and L3. Clinical signs were notably improved during the study in the animals treated with injectable eprinomectin. In both locations the treatment application demonstrated a very good safety.

OA15.03

Control of *Amblyomma sculptum* with *Metarhizium anisopliae* IBCB425 in the Roberto Burle Marx Park, São José dos Campos, São Paulo, Brazil – Into the third parasitic cycle. _Selbmann Sampaio, Dr. Paulo Henrique_ Instituto Biológico

Co-Authors - Dr. Rodrigo Giglioti, Dr. Paula Cristina Pereira Cabral, Ms. Júlia Salvador Marcondes, Mr. Erick José Nogueira de Andrade, Ms. Gisele Silva da Rocha, Mr. Theos Apolo Cordeiro da Silva, Ms. Vivian Letícia dos Santos Faria, Dr. Elisa Margarida Kovacs Farinha, Dr. Andréa Sundfeld, Dr. Guilherme Micai, Dr. Minoru Takahashi, Dr José Eduardo Marcondes de Almeida

The Roberto Burle Marx Park is an important leisure area in the Municipality of São José dos Campos, State of São Paulo, Brazil. A group of approximately 40 capybaras (*Hydrochoerus hydrochaeris*) resides there and sustains a population of *Amblyomma sculptum*, a three-host tick, vector of the Brazilian Spotted Fever, a disease caused by *Rickettsia rickettsii*. To mitigate risks, a control project started in September 2020, associating lawn management with the application of a wettable powder formulation of *Metarhizium anisopliae* IBCB425 (Ecometa, Toyobo). The fungus was sprayed monthly, at 2.0×10^{13} conidia/ha, from October to March, when the adult instars emerge. Ticks were monitored monthly with twelve attractive traps deployed on fixed points spread over 4.0 ha of gardens and lawns. Traps consisted of 0.7m \times 1.0m pieces of white non-woven fabric with approximately 200g dry ice placed on their center. After 40 minutes, traps were recovered and deposited directly in plastic containers closed with tulle and incubated at room temperature. After ten days, instars were counted, and live and dead individuals were recorded. Results from September 2020 to February 2023 were transformed into log₁₀ for approximation to the normal data distribution and were analyzed by the PROC MIXED procedure of the statistical package SAS, which included monitoring and condition (live or dead) and their interaction (monitoring \times condition) as fixed effects. Monitoring/month were used as repeated measures over time, and means were compared by Tukey's Test ($p < 0.05$). Reduction was significant for nymphs ($p < 0.001$) and adults ($p = 0.0419$). Fixed effects interaction was significant for nymphs ($p < 0.001$). No larvae were captured throughout the project. Most individuals trapped came from a single periferential point, where vegetation blocks treatment.

Despite the free roaming capybaras, which theoretically would replenish the tick stock, findings suggest IBCB425 has impeded parasitic development and reduced infestation to negligible levels.

OA15.04

World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for diagnosing anthelmintic resistance using the fecal egg count reduction test in ruminants, horses and swine_Nielsen, Dr. Martin_University of Kentucky

Co-Authors - Prof. Matthew Denwood, Prof. Stig Thamsborg, Dr. Paul Torgerson, Dr. John Gilleard, Dr Jozef Vercruyse, Prof. Bruno Levecke, Dr. Ray Kaplan

The fecal egg count reduction test (FECRT) remains the method of choice for establishing the efficacy of anthelmintic compounds in the field, including the diagnosis of anthelmintic resistance. In the new WAAVP guidelines, we 1) address the major issues relevant to experimental design, choice of faecal egg count (FEC) method, statistical analysis, and interpretation of the FECRT results, 2) make a series of general recommendations that are applicable across animal species, 3) provide separate guideline detail for cattle, small ruminants (sheep and goats), horses and pigs, and 4) provide overviews of the specific details required to conduct a FECRT for each of these host species. We provide two separate study design options for each animal species: a rigorous version that is intended for use in scientific studies, and a less demanding version intended for routine clinical use by veterinarians and livestock owners. Compared to the previous FECRT guidelines, four important differences are noted. First, it is now generally recommended to perform the FECRT based on pre- and post-treatment FEC of the same animals (paired study design), rather than on post-treatment FEC of both treated and untreated (control) animals (unpaired study design). Second, instead of requiring a minimum mean FEC (expressed in eggs per gram) of the group to be tested, we define a minimum total number of eggs to be counted before the application of a conversion factor to calculate the FEC. Third, we provide guidance for minimum group sizes. Finally, the thresholds for defining reduced efficacy are adapted and aligned to anthelmintic drug, parasite species and host, covering all major livestock species. In conclusion, the new guidelines provide improved methodology and standardization of the FECRT for livestock.

OA15.05

Immune responses of first grazing season heifers naturally exposed to *Dictyocaulus viviparus*._Roberts, Ms. Grisial_University Of Liverpool

Co-Authors - Dr John Graham-Brown, Dr Hannah Vineer, Prof Mark Viney

Dictyocaulus viviparus is a parasitic nematode of cattle. Infection (parasitic bronchopneumonia) causes coughing and respiratory distress with potential for permanent lung damage and death. Disease epidemiology is unpredictable, influenced by factors including farm management, metrology and herd immunity. *Dictyocaulus viviparus* ultimately induces strong protective immunity in cattle, including larval-specific responses which can be exploited through vaccination with live irradiated L3 orally to reduce infectious burden and disease severity. However, most studies of *D. viviparus* to-date have been performed under controlled experimental conditions, with natural infection less well understood. Thirty first grazing season Holstein dairy heifers were sampled from March to November 2022 on a farm in Wales, UK. Fifteen calves were vaccinated with a commercially anti-*D. viviparus* vaccine (Huskvac, MSD) ahead of spring turn-out, and 15 unvaccinated control animals. Calves were sampled monthly for blood and faeces in addition to pasture and bulk tank milk samples. Lungworm infection status was determined using the

Baermann technique and via diagnostic blood serum IgG ELISA (anti-*D. viviparus* major sperm protein), whilst pasture samples were used to determine levels of natural exposure. Larval pasture counts peaked in June to July, reaching ~ 5000 parasitic L3 per kg of dry matter. Peak lungworm infection was observed in November (based on seroconversion), with patent infection identified in 13 of 15 unvaccinated animals and 7 of 15 vaccinated animals. Additional investigations of *D. viviparus*-specific immune responses including in vitro cellular assays and antibody isotype responses are ongoing. We will present these data alongside our longitudinal observations of *D. viviparus* exposure and infection. This will provide insight into the immune responses associated with different exposure, infection and vaccine statuses under conditions of natural exposure, how these relate to on-farm control, disease epidemiology and, potentially, further avenues for investigation relating to diagnostic and/or vaccine candidates.

OA15.06

Is *Chorioptes texanus* to displace *Chorioptes bovis*? A note on the mites causing bovine chorioptic mange in central Europe_Rehbein, Dr. Steffen_Boehringer Ingelheim Vetmedica GmbH

Co-Authors - Sandra Mayr, Martin Visser

Chorioptic mange is the most common type of bovine mange in central Europe, mainly affecting housed dairy cattle. It has become a focus of parasite control measures after highly efficacious products with zero milk withdrawal became available. Bovine chorioptic mange was thought to be caused only by *Chorioptes bovis* mites until 1976 when *Chorioptes texanus* was described for the first time from mangy dairy cattle in Brazil. The first report of *C. texanus* in cattle in Europe came from Germany in 1999. Based on these findings and triggered by recent publications reporting the widespread occurrence of *C. texanus* in Japan including cases of co-infestation with *C. bovis*, local veterinarians, veterinary parasitologists and the company's technical service group were asked for skin scrapings from cattle, preferably dairy cows, clinically suspicious for chorioptic mange. Mites were isolated, cleared and microscopically identified on their morphology. Examination of scrapings collected in the years of 2019 to 2022 from cattle from Austria, France, Germany and Switzerland revealed *C. texanus* in 2/6 farms in Austria, 13/17 farms in France, 7/28 farms in Germany and 1/7 farms in Switzerland; *C. bovis* in 5/28 farms in Germany; and *Psoroptes ovis* in 2/17 farms in France. No co-infestations of *C. bovis* and *C. texanus* or *Chorioptes* species and *P. ovis* were observed. Occasionally *Bovicola bovis* and *Haematopinus eurytenuis* were identified in samples from Austria, France and Germany, and *Linognathus vituli* were isolated in scrapings from France and Germany. The records of *C. texanus* from Austria, France and Switzerland constitute first descriptions of this species in these countries. Although this investigation was conducted on an opportunistic basis and of small extent, results suggest a widespread occurrence of *C. texanus* in central Europe and may emphasize further studies on the presence of this mite as causative agent of bovine chorioptic mange.

Specific levels of IgA as biomarker for gastrointestinal nematode infections under natural conditions in different sheep breeds_Castilla Gómez de Agüero, Ms. Verónica_University Of León - Universidad De León

Co-Authors - Ms. Elora Valderas García, Ms. María Cambra Pellejà, Laura González del Palacio, Dr Rafael Balaña Fouce, Dr María Martínez Valladares

Gastrointestinal nematodes (GIN) are a major threat to health and welfare in small ruminants worldwide, causing important economic losses. Under experimental conditions, the infection caused by *Teladorsagia circumcincta*, one of the most prevalent GIN, is characterized by an increase of specific IgA levels in sheep with a resistant phenotype to the infection, resulting in low worm burdens in some breeds. The aim of this study is to determine if these associations are confirmed in naturally infected sheep from different breeds. For it, 589 ewes, 193 younger than 1 year old and 389 adult ewes, belonging to three breeds -Assaf, Castellana and Churra- which were under semi-extensive management, were studied. Faecal egg count (FEC) and specific IgA activity in serum, saliva and, nasal secretion samples against somatic L3 antigen of *T. circumcincta* (L3SE-Tc) and an antigenic recombinant fragment of the protein disulfide isomerase of *T. circumcincta* (PDI-Tc) were analyzed. FEC values for each breed were classified in four ranges to determine if it is a differential variable. The relationship between the FEC and IgA levels were evaluated by Spearman's rank correlation coefficient. FEC statistical analysis showed that breed was not a significant trait since it did not influence the level of infection among ewes sampled. In young ewes, antigen-specific IgA levels against L3SE-Tc were positive correlated with FEC in serum (Rho=0.297, p=0.00), saliva (Rho= 0.500; p=0.00) and nasal secretions (Rho=0.500; p= 0.00); against PDI-Tc only IgAs in nasal secretions showed a significant association (Rho=0.500; p=0.00) with FEC in young ewes. In adult sheep, IgA levels againstL3SE-Tcwas positive correlated in serum (Rho=0.123; p=0.00) and against PDI-Tc in nasal secretions (Rho=0.179; p=0.00). Our study showed that age has an important role in specific IgA levels under natural conditions of infection. Thus, IgA levels in nasal secretions could be proposed as a marker of GIN infection in young animals for further studies.

22.08.23

Breakout 2: OA16 Trematodes

OA16.01

The use of Cathepsin L1 (FhCL1) blood-tests ELISA in sentinel screening for liver fluke on sheep farms_Mc Evoy, Ms. Amanda_Teagasc

Co-Authors - Jesús López Corrales, Krystyna Cwiklinski, Richard Lawlor, Orla Keane, Amber Dorey, John Pius Dalton

Fasciola hepatica is a parasitic organism that poses a significant economic threat to the ruminant livestock industry worldwide. The disease, fasciolosis, can result in a range of symptoms including anaemia, weight loss and death, with the most severe symptoms attributed to early acute infection when the parasite is migrating through the liver. Early diagnosis and intervention are essential for the control and management of fasciolosis and to prevent major pathogenesis. However, the traditional gold standard method of detection, faecal egg counts (FEC), is limited to detecting chronic fasciolosis from 12 weeks post infection (wpi). Enzyme-linked immunosorbent assays (ELISA) using the *F. hepatica* cysteine peptidase FhCL1 on the other hand have been shown to detect pre- patent infection in serum from 3 to 4 wpi. In this study, sentinel lambs from three commercial farms in Ireland were monitored from September 2021 to March 2022 using both FEC and ELISA. All three farms showed a significant increase in the mean FhCL1 antibody levels and FEC over this time period, with a substantial rise in positive infection rates in January. ELISA screening, however, detected infection at least two months earlier than FEC. This suggests that the regular screening of sentinel lambs for *F. hepatica* seroconversion in a “test and treat” approach could mitigate the negative impact of early fasciolosis on flock health, welfare and productivity. In addition, we showed that whole blood samples taken on Whatman protein saver cards could substitute the conventional serum blood tubes in ELISA testing. These cards can be stored at room temperature for long periods of time and can be revisited at any time for ELISA. The adoption of these cards on farm would provide a simpler, cost-effective and eco-friendly alternative method for serological testing of sentinel lambs.

OA16.02

Evaluation of a recombinant *Fasciola hepatica* cathepsin L1 ELISA for Swiss horses_Eichenberger, Dr. Ramon M._Zurich University of Applied Sciences

Co-Authors - Dr Mary Sekiya, Prof. Grace Mulcahy, Prof Caroline F. Frey, Dr Philipp A. Kronenberg

Fasciola hepatica is a common trematode parasite of livestock in Ireland, Switzerland, and many other regions, causing significant economic losses and affecting animal welfare. Horses rarely develop patent liver fluke infection, even though liver damage can affect animal health. Therefore, *F. hepatica* infection in horses may be underreported. Recently, a serological test for the antibody detection has been reported based on recombinant parasite cathepsin L1 (FhCL1) [1]. Here, we optimized this test by evaluating different recombinant forms of FhCL1 combined with diagnostic epitope optimization. The test showed slightly improved diagnostic test characteristics by using the same set of abattoir-examined horses from Ireland, with a sensitivity of 65% (95% CI: 41 – 83.7) and specificity of 97.4% (95% CI: 93.1 – 99.2), respectively. Following treatment with triclabendazole, follow-up of 7 horses with a suspected *F. hepatica* infection showed, in addition to improved liver values, a drop in antibody titres, which fall below the test threshold after approx. 6 to 9 months. An evaluation of 561 horses from all over

Switzerland showed a seroprevalence of 2.3 %, without any obvious hotspots. These results indicate that *F. hepatica* might be a neglectable infection in Swiss horses. Nevertheless, few cases (especially in horses with liver disease of unknown reasons) demonstrate the need of reliable tests.

OA16.03

Development and evaluation of a colloidal gold immunofiltration assay for diagnosis of visceral schistosomosis in cattle_Mamane, Dr. Shivani_Department of Veterinary Parasitology, Madras Veterinary College, TANUVAS, Chennai.

Co-Authors - Dr. N. Jeyathilakan, Dr BR Latha, Dr TMA Senthilkumar

Visceral schistosomosis caused by *Schistosoma spindale* is an economically important chronic wasting snail-borne trematode infection which often goes undiagnosed in field condition due to lack of efficient diagnostic assays. In order to check the active visceral schistosomosis immunodiagnostic assay was standardized and evaluated for the detection of antibodies against *S. spindale* in cattle. The present study included development and evaluation of colloidal gold immunofiltration assay (CGIFA) and Enzyme linked Immunosorbent assay (ELISA) with conventionally prepared *S. spindale* excretory-secretory (ES) antigen using postmortem mesenteric examination as a reference standard. Statistical analysis was performed using Cochran Q test and kappa statistics which showed no significant difference between the developed tests and strong agreement with the gold standard test. CGIFA showed sensitivity, specificity and accuracy of 92.98 %, 96.74 % and 95.55 % whereas that of ELISA was 94.73%, 95.12% and 95% respectively. CGIFA was found to be simpler, rapid, effective and less expensive and fetched least expertise for interpretation of results. It can be used as portable diagnostic device for rapid diagnosis of *S. spindale* infection at field condition making it an ideal pen side diagnostic kit.

Keywords: Visceral schistosomosis, *Schistosoma spindale*, Immunodiagnostic assays, CGIFA, ELISA.

OA16.04

Geographical expansion of *Fasciola hepatica* (Linnaeus, 1758) in Brazil driven by changes in land use and cover: a disturbing One Health picture_Molento, Dr. Marcelo_Universidade Federal do Parana

Co-Authors - Dr Irineu Romero Neto, Dr Yara Brandao, Dr Thayany Almeidafv

Fasciolosis is a food-borne disease that affects humans and animals worldwide and is caused by *Fasciola* spp. Although *F. hepatica* is endemic in South America, its prevalence has been poorly reported despite the wide distribution of its intermediate host, *Pseudosuccinea columella*. This study aimed to analyze the prevalence of *F. hepatica* in cattle in Brazil over 18 years, based on data from the Ministry of Agriculture, as well as to examine changes in land use and cover. Data were available for 4,433 (80%) of 5,570 Brazilian cities (80%) and over 400 million livers for the period. Data showed an enormous geographical expansion of the disease, from 194 (4.4%) municipalities in 2002 to 747 (16.9%) in 2020, in all major biomes. This was due to the intense South-to-North cattle movement without proper animal inspection at state borders and the ability of the parasite to new environments. In the North region (Amazon Forest and Cerrado), there was an expansion of pasture areas with an increase of 13.4%, from 30.1 million hectares in 2002 to 40.4 million hectares in 2020. On the other hand, the Midwest, Southeast, and South (Pantanal, Atlantic Forest, and Pampa biomes) showed a reduction in pasture areas, mainly due

to the increase in soybean, corn, and sugar cane production. All regions showed a reduction in primary vegetation cover. We have also run the susceptible-infectious-recovered (SIR) model to estimate the transmission rate of *F. hepatica* in the different biomes. Fragile multinational biomes such as the Pantanal, Cerrado, and the Amazon basin have been widely used for livestock and other harmful activities, which has led to desertification and reduced biodiversity. The spread of Fasciolosis can be considered a public health problem in South America, especially in critical areas such as indigenous and protected lands, affecting vulnerable populations and with unknown consequences.

OA16.05

Comparison of Mini-FLOTAC, Flukefinder and sedimentation techniques for detection and quantification of *Fasciola hepatica* and *Calicophoron daubneyi* eggs using spiked and naturally infected bovine faecal samples_Ciuca, Dr. Lavinia_1Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, Naples, Italy

Co-Authors - Dr. Antonio Bosco, Dr. Maria Paola Maurelli, Dr. Paola Vitiello, Dr. Giuseppe Cringoli, Dr. Joaquin M. Prada, Prof Laura Rinaldi

Fasciolosis (*Fasciola hepatica*) and paramphistomosis (*Calicophoron daubneyi*) are two important infections of ruminants. *Calicophoron daubneyi* is the predominant species in Europe and its prevalence increased in the last 10-15 years. Given the importance of reliable tools for liver and rumen fluke diagnosis in ruminants, the aim of this study was to evaluate the diagnostic performance of the Mini-FLOTAC (MF), Flukefinder (FF) and Sedimentation (SED) techniques for the detection and quantification of *F. hepatica* and *C. daubneyi* eggs using spiked and naturally infected cattle faecal samples. Briefly, negative bovine faecal samples were artificially spiked with either *F. hepatica* or with *C. daubneyi* eggs to achieve different egg count levels: 10, 50, and 100 eggs per gram (EPG) of faeces. Moreover, ten naturally infected cattle farms from southern Italy with either *F. hepatica* and/or *C. daubneyi* were selected. For each farm, the samples were analysed individually only with MF technique and as pools using MF, FF and SED techniques. Bayesian Latent Class Analysis (LCA) was used to estimate sensitivity and the accuracy of the predicted intensity of infection, as well as the prevalence in the naturally infected farms. The outcome of the present study showed that the highest number of eggs (*F. hepatica* and *C. daubneyi*) recovered was obtained with MF, followed by FF and SED in spiked infected samples at 50 and 100 EPG, while at lower infection levels of 10 EPG, FF gave the best results. Moreover, the sensitivity for all the techniques included in the study was estimated at above 90% at infection levels above 20 EPG for both *F. hepatica* and *C. daubneyi* eggs. However, MF was the most accurate of the three techniques evaluated to estimate fluke infection intensity. In conclusion, optimization and standardization of techniques are needed to improve the FEC of fluke eggs.

OA16.06

Co-culture with HepG2 spheroids spurs in vitro growth and development of the infective stages of the helminth pathogen *Fasciola hepatica*_Calvani, Dr. Nichola_University Of Galway

Co-Authors - Mrs Emma McDermott, Dr Richard Lalor, Dr Carolina De Marco Verissimo, Dr Mahshid Dehkordi, Kerry Thompson, Dr Howard Fearnhead, Professor John Dalton, Ms Aiste Vitkauskaitė

The helminth parasite *Fasciola hepatica* is a significant cause of animal and human morbidity worldwide. Part of the difficulty in developing new chemotherapeutics and vaccines for the control of fasciolosis lies in our inability to culture and propagate juvenile worms in vitro. Several

laboratories maintain *F. hepatica* short-term in simple media, but these are usually for the purpose of collecting excretory/secretory (E/S) products containing molecules important in parasite host interaction, rather than for biological studies. Here we show that the infective stage of the parasite, the newly excysted juvenile (NEJ), exhibit significant growth and development in vitro when co-cultured with spheroids derived from HepG2 cells, a human non-tumorigenic liver cell line with high proliferation rates and epithelial-like morphology. We investigated parasite development using antibody probes against two major NEJ proteases, FhCL1 and FhCL3, and by scanning electron microscopy (SEM). Parasites grown in the presence of HepG2 spheroids exhibit not only a rapid increase in size (length and width) but also extensive development of the gut caecum, musculature, and surface sensory system. Parasites were observed regularly interacting with the spheroids, sometimes invading the tissue, and moving between or tangentially to them indicating the importance of tactile stimuli. There was also evidence of parasites 'grazing' on the peripheral cells of the spheroids. We propose that the methodology developed here mimic in vivo parasite host liver interactions, greatly improving our ability to investigate and understand *F. hepatica*-host biology with future prospects for the development of new parasite control methods, such as vaccines and anthelmintic drugs.

22.08.23

Breakout 3: OA17 Parasite vaccines

OA17.01

Interactions between gut microbiome and nematode parasites in sheep_Lanusse, Dr. Carlos_Conicet, Argentina

Co-Authors - Matias Faraco, Prof. Dr. Ignacio Alvarez, Dr. Mercedes Lloberas, Patricia Cardozo, Lucila Moriones, Paula Dominguez, Dr. Fernanda Imperiale, Dr. Juan Pedro Liron

Traditionally, research in host-helminthic interactions has focused on the host's immune system and the parasite. Over the last decade, the development of the next generation DNA sequencing tools allow us to add a third player, the host gut microbiome. The aim of the present work was to investigate in vivo, the interaction between sheep gastrointestinal (GI) parasites and the gut microbiome. In this line, we used 15 naturally infected sheep in order to explore how the nemabiome and gut microbiome compositions influenced each other before and after treatment with monepantel (MNP) and the broad-spectrum antibiotic oxytetracycline (OXT). We wait for microbiome stabilization and 17 days post treatment, abomasum and colon content and worm samples were taken. As we expected, adult parasites were not observed in the GI tract of the MNP treated animals, with the exception of *Oesophagostomum* in colon. High-throughput Illumina sequencing of the bacterial 16S rRNA V3-V4 region showed that the abomasum relative abundance of the methanogenic archaeobacteria and cyanobacteria changes in the MNP treated animals ($\log_2FC= 2.09$, $P_{adj}=0.045$; $\log_2FC=-1.39$, $P_{adj}=0.046$, respectively). No statistical differences between groups were observed in the colon bacteria content. OXT treatment did not affect the parasite content of the animal GI tract. To conclude, the results here presented showed that the lack of parasites or the direct effect of the MNP, impact in the microbial composition of the sheep abomasum, particularly in the Archaea content, rumen microorganisms that produce methane, a gas that has been implicated in global warming.

OA17.02

The development of an Australian *Tritrichomonas foetus* inactivated vaccine._Tabor, Prof. Alicja_The University Of Queensland

Co-Authors - Dr Gry Boe-Hansen, Prof Michael McGowan, Dr Kieren McCosker, Mr Tony Cavallaro

In North Australian extensively grazed beef herds, losses from confirmed pregnancy to weaning are typically in the order of 5 to 15 percent and are estimated to cost the industry between \$60-\$AUD100 million a year. A recent abattoir survey undertaken across this northern industry indicated that one in 10 culled bulls were infected with *Tritrichomonas foetus* using qPCR. This prompted the development of an Australian strain as a new vaccine for trichomoniasis for development. After developing methods to grow pure cultures, establish viable storage conditions, and preliminary pilot trial was planned. Bulls were vaccinated with two doses one month apart and challenged preputially with live *T. foetus* parasites. A preliminary trial demonstrated 67% efficacy using old cull bulls. Future trials will employ younger bulls and challenge the bulls more frequently to mimic field challenge conditions. This research was supported by Meat & Livestock Australia and the Queensland Government.

The translationally controlled tumor protein (TCTP) of *Babesia bovis* participates in the establishment of an acute infection and contains B-cell epitopes that induce neutralizing antibodies _Mosqueda, Prof. Juan_Autonomous University Of Queretaro

Co-Authors - MSc Chyntia Perez-Almeida, PhD Diego Hernandez-Silva, MSc Edwin Hernandez-Esau, Professor Jose Gomez-Soto, Professor Carlos Vega y Munguia, Professor Urso Davila-Montero, Professor Masahito Asada, Professor Shin-Ichiro Kawazu

Babesia bovis causes a disease characterized by high virulence and mortality. It has been proposed that Apicomplexa parasites produce proteins that suppress the immune response. Tumor translationally controlled protein (TCTP) is a multifunctional protein; one of its extracellular functions is the regulation of the immune response. It has been postulated that it interferes with the immune response by blocking the interaction of the host's TCTP with its receptor, preventing the activation and proliferation of B cells and therefore the activation of the immune response. This protein has not been characterized in *B. bovis*. The aims of this work were to characterize the TCTP of *B. bovis*, to evaluate its participation in the establishment of the infection and to determine the capacity of specific antibodies to neutralize the invasion of the parasite. The complete sequence of the TCTP gene was amplified by PCR in different *B. bovis* isolates, and each one was cloned and sequenced. A 100% amino acid identity was observed in all sequences obtained. Bioinformatics tools were used to predict the secondary structure, which showed the characteristic alpha helix. Three peptides containing predicted B-cell epitopes were designed and synthesized. They were used to immunize rabbits and cattle. Transcription was assessed using mRNA from intraerythrocytic parasites using an RT-PCR protocol. Expression was confirmed by confocal microscopy using specific rabbit antiserum. Subsequently, four *Bos taurus* steers were immunized 3 times every three weeks with the mixture of peptides emulsified with Montanide ISA 71 VG adjuvant while another four steers were immunized only with the adjuvant. Twenty-four days after the last immunization, the animals were challenged with a virulent strain of *B. bovis* and underwent a daily physical examination for 15 days. Less severe clinical signs were observed in animals immunized with the TCTP peptides. A lower amount of total antibodies was observed in the serum of the animals of the control group after the challenge, in comparison with the total antibodies in the serum of the animals immunized with the peptides, indicating an interference in the bovine immune response caused by the TCTP of *B. bovis*. A neutralization assay was carried out using an in vitro culture of *B. bovis*. A percentage inhibition of 32-24% was observed using sera from cattle immunized with the TCTP peptide mixture. It is concluded that *B. bovis* has a tctp gene that is transcribed and expressed in intraerythrocytic stages, and that the protein contains conserved peptides with B-cell epitopes that induce neutralizing antibodies in immunized cattle and, finally, *B. bovis* TCTP has a role in the establishment of infection. Funded by UAQ-FONDEC (FNV-2020-06), USDA-ARS (59-2090-1-001-F), The Japan Society for the Promotion of Science and the National Research Center for Protozoan Diseases.

OA17.05

Assessing vaccine candidates for *Onchocerca volvulus* and their associated immune responses using a bovine – *Onchocerca ochengi* natural infection model_ Dr. John Graham-Brown, University of Liverpool, UK

Co-Authors - Dr Tessa Walsh Dr Lisa Luu, Dr Germanus Soh Bah, Dr Ndode Herman Okah-Nnane, Mr David Ekale, Dr Rene Billingwe Ayiseh, Prof Darrick Carter, Dr Sean Gray, Dr Vincent Tanya, Dr Sara Lustigman, Professor Benjamin Makepeace

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Introduction

Human onchocerciasis is a neglected tropical disease affecting 21 million people in sub-Saharan Africa caused by the filarial nematode *Onchocerca volvulus*. Attempts at disease elimination via mass administration of the microfilaricidal drug ivermectin have seen marked but limited success, indicating a need for additional intervention tools including vaccines. Here, we use a bovine *Onchocerca ochengi* natural infection model to assess the efficacy of a recombinant vaccine for human onchocerciasis.

Methods

Immunologically naïve calves (n=30) were recruited for trial of a recombinant fusion protein (Fus1) derived from the *O. volvulus* antigen candidates 103 and RAL-2 formulated in Montanide ISA 201 VG. Fifteen calves received immunisation, with the remaining 15 animals receiving adjuvant only controls. Primary and booster immunisations were administered at an interval of 4 weeks. Four weeks post-immunisation animals were transferred to a site with natural *O. ochengi* transmission for a period of 24 months. Animals received a further booster immunisation 6 months post-exposure. During both immunisation and exposure periods animals were sampled routinely for clotted serum and peripheral blood leucocyte responses, with infection status monitored post-exposure through regular enumeration of adult female worm nodules and skin snips to determine microfilarial burdens. Samples collected for immunological investigations were subjected to an array of assays including serum antibody ELISA and peripheral blood leucocyte cultures with subsequent analyses via transcriptomics, cytokine ELISAs, ELISpots and FACS to determine T- and B-cell response profiles.

Results and discussion

Following immunisation, all vaccinated calves showed strong antigen-specific serum IgG responses to both Fus1 and its constituent parts, inducing consistent IgG2 responses. Antibody titres progressively waned over the subsequent exposure period, but remained elevated above those of unvaccinated control animals. A rapid increase in antigen-specific serum IgG (including IgG2) was also detected in vaccinated animals after the second booster immunisation, indicating effective memory B-cell responses. Further data analysis relating to the immune responses and possible protective effects of vaccination against natural challenge with *O. ochengi* is ongoing, with preliminary results expected by August 2023.

OA17.06

Reduced faecal egg output after vaccination with a recombinant *Ostertagia ostertagi* glycoprotein in cattle_Claerebout, Prof. Edwin_Ghent University

Co-Authors - Laurens Zwanenburg, Myrna Bunte, Sanaz Mohktari, Prof. Ruud Wilbers, Dr. Angela van Diepen, Prof. Cornelis Hokke, Prof. Peter Geldhof

The abomasal nematode *Ostertagia ostertagi* is a major pathogen of grazing cattle in temperate climate regions. Because of emerging anthelmintic resistance, vaccination could be a future alternative for worm control in cattle. Vaccination with an activation-associated secreted protein (ASP), purified from adult worms, and Quil A adjuvant has repeatedly shown to result in a significant reduction (40-74%) of faecal egg counts after experimental *O. ostertagi* challenge infections in calves. However, vaccination with recombinant ASP proteins was unsuccessful. Recently it was discovered that mucosal antibodies of calves vaccinated with the protective native ASP were directed against core alpha 1,3 fucosylated N-glycans on the ASP glycoprotein, which were not recognised by antibodies from calves vaccinated with unprotective *Pichia* recombinants. Recombinant alpha 1,3 core fucosylated ASP was therefore expressed in tobacco plants, *Nicotiana benthamiana*, and tested in two vaccination experiments. In both vaccine trials, helminth-naïve calves were immunised three times with three weeks interval with ASP and Quil A adjuvant or Quil A alone (control group), followed by a trickle infection of 25,000 *Ostertagia* L3 larvae. In Trial 1, calves were vaccinated with either the native purified ASP (n=7), the recombinant *Nicotiana*-expressed ASP (n=8) or Quil A adjuvant only (n=7). In Trial 2, calves were vaccinated with the recombinant ASP (n=12) or Quil A only (n=12). In Trial 1, arithmetic mean faecal egg counts were reduced by 57% and 45% after vaccination with the native and recombinant ASP, respectively (NS). In Trial 2, mean faecal egg counts were reduced by 39% in the vaccinated animals ($P < 0.05$). Worm burdens were not significantly reduced in the vaccinated animals, which is in line with the outcome of previous studies using the same infection model. An impact on worm burden is expected after a full grazing season, as a result of reduced egg shedding on pasture.

OA17.07

Comparative analysis of secretome of low and high passaged *Theileria annulata* infected bovine lymphocytes_ Dr. Azhahianambi Palavesam

Co-Authors - Dr K Govindan, Dr N Madan, Dr. Chinnaiyan Soundararajan, Dr G Tirumurugaan K

Translational Research Platform for Veterinary Biologicals, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram, Chennai- 600051, India *Theileria annulata* is a tick-transmitted parasite that causes tropical bovine theileriosis. In vitro passage of the *T. annulata* schizont infected bovine lymphocytes lead to attenuation of the parasite and serve as a vaccine candidate. To unravel attenuation markers, the secreted proteome of low and high passage of *T. annulata* infected lymphocytes were analysed. The cell free supernatant of *T. annulata* schizont infected lymphocytes were collected from 55th and 240th passages. The secreted proteome was analysed using Orbitrap-HR-LC/MS and data was compared with the UniProt database. Bovine and parasite proteins such as serotransferrin, beta-2-microglobulin, alpha-2-HS-glycoprotein, actin cytoplasmic 1, profilin, MMP-9, ubiquitin ribosomal fusion protein, signal peptide containing protein, histone H4, 60S ribosomal protein L24, tubulin alpha chain, and 14-3-3 protein were the proteins with high coverage in the 55th passage. Serotransferrin, beta-2-microglobulin, alpha-1-acid glycoprotein, alpha-2-HS-

glycoprotein, serpin family A member 1, interferon α inducible protein, DBP, EF-hand domain-containing protein, histone H4, 60S ribosomal protein L24, triosephosphate isomerase, COesterase domain-containing protein, and protein-tyrosine-phosphatase were the proteins with high coverage in the 240th passage. MMP-9, one of the major virulence factors of *T. annulata*, ubiquitin ribosomal fusion protein and 14-3-3 protein could not be detected in the higher passage level. Keywords: *Theileria annulata*, secretome, proteome, virulence factors, passage

22.08.23

Breakout 4: OA18 Novel diagnostics I

OA18.01

Eprinomectin-resistant *Haemonchus contortus* in collective summer pastures: spread the word, not the worm. _Jouffroy, Ms. Sophie_Ecole Nationale Vétérinaire De

Toulouse/ceva/inrae

Co-Authors - Elisa Giraud, Clara Girard, Ms. Julie Petermann, Arthur Bureau, Dr Anne Lespine, Pr Philippe Jacquet

Mountainous summer pastures have long been considered an opportunity for a break from parasite infection in dairy sheep. However, within the last four years, the rapid onset of eprinomectin resistance in *Haemonchus contortus* in dairy sheep herds in the French western area of the Pyrénées range was mainly observed in herds transhumant for an average of 3 months in high altitude summer pastures. Demonstration in 2021 of eprinomectin-resistant *Haemonchus contortus* in a transhumant herd of basco-bearnais sheep motivated Fecal Egg Count Reduction Tests (FECRT) in the 3 other herds using the same collective pasture from 2018 to 2021. Tests were conducted with injectable eprinomectin at doses of 0.2mg/kg as recommended. GIN species present pre and post treatment were identified using rtPCR and morphological identification of larvae. In the first transhumant herd included, FECR value was 55% [CI: 8; 85]. This result was anticipated, as eprinomectin treatments were administered regularly 2 to 3 times per lactation for the last couple years. However, in the herd that has been and still is mixed with the initial herd in collective pastures, eprinomectin had only been used once. The -53% FECR [-315; 27] for eprinomectin therefore came as a surprise to the farmer. Identification of *H. contortus* post-treatment was in agreement with the symptoms present at the time of test, but was a shock in a herd that had so far never dealt with severe haemonchosis. On 2 other farms, FECR values were 60% [-52; 95] and 99% [97; 100]. Interestingly, despite low post-treatment mean FECs (50 and 5 EPG respectively), a small number of *H. contortus* larvae were present post-treatment in these farms. These results underline the potential transfer of resistant gastro-intestinal nematodes from one herd to the next, exerting farmers to add these to the list of collectively managed sanitary issues in summer pastures.

OA18.02

Detection and culling of superspreaders can help to control bovine

besnoitiosis_Petermann, Ms. Julie_National Veterinary School of Toulouse

Co-Authors - Dr Christelle Grisez, Mr Christophe Lacz, Mr Jean-Pierre Alzieu, Pr Philippe Jacquet

Bovine besnoitiosis, caused by *Besnoitia besnoiti*, is an emerging disease in Europe. As no vaccine nor efficient treatment are available, the control of this disease depends mainly on control of introductions into a *Besnoitia*-free herd and on detection and culling of seropositive animals in infected farms. This latter control strategy is very difficult to implement in herds showing high serological prevalences. In this field study, the interests and limits of detection and culling of superspreaders individuals were evaluated. Thirteen cattle farms, with high besnoitiosis seroprevalence, were enrolled in the study. All individuals above six months of age were tested in ELISA. Skin samples at the tail base were performed in all seropositive animals and analysed by Real Time PCR. A recommendation of rapid culling of individuals with Cq values under 36 (considered as superspreaders) was provided to the farmers. ELISA tests on previously negative

animals of the herd were done every year during two or three years to monitor newly infections. Among 863 seropositive cattle, 218 individuals (25.4%) showed *B. besnoiti* DNA in their skin sample and among them, 104 (12%) showed Cq values below 36. The proportion of superspreaders varied from farm to farm (5 to 32.6%). In four farms, recommendations were not followed by the farmers and serological prevalences increased strongly in the next 12 months. When superspreaders were removed rapidly (in six farms), the serological conversions decreased and clinical cases disappeared in one or two years. However, when this removal was delayed or partial (3 farms), new infections happened and some clinical cases were recorded. The detection and rapid culling of superspreaders in a *B. besnoiti* infected farm can significantly reduce the number of new infections and could permit the eradication of this parasite from a heavily infected farm.

OA18.03

Comparing 18s rDNA, COI and ITS1 metabarcoding for bovine *Eimeria* species diversity. Parimiseti, Dr. Venkateswara Rao_University of Calgary, Dept. of Comparative biology and experimental medicine

Co-Authors - Dr Elizabeth Redman, Dr Manigandan Lejeune, Ms Holly White, Dr. Berit Bangoura, Ms Nicollete Shaw, Ms Shawna Zhu, Ms Rebecca Chen, Dr. Roy Lewis, Dr. John S Gilleard

Bovine coccidiosis is an economically important disease caused by the gastrointestinal protozoa *Eimeria* species. Routine diagnosis by morphological identification of oocysts is inconsistent and error prone for overlapping species, hence we are developing metabarcoding approaches for reliable identification of bovine *Eimeria*. Reference sequences for 18s rDNA, COI mtDNA and ITS1 rDNA are available for sixteen bovine *Eimeria* species including three cryptic species in GenBank. The sixteen *Eimeria* species were grouped into 8 morphotypes based on their difficulty in discriminating by morphology. Morphotype 2 or ZECA group has the more challenging species *E. zuernii*, *E. ellipsoidalis*, *E. cylindrica* and *E. alabamensis* and morphotype 8 included undefined type1, type2 and type3. Nine morphologically validated samples each classified by three independent technicians were compared and *E. ellipsoidalis*, *E. alabamensis* and *E. cylindrica* showed high variation among the three readers. Another morphologically validated clinical sample set by single technician (n = 115) was PCR amplified and deep sequenced for short variable regions of 18s rDNA, COI mtDNA and full ITS1 using the Miseq illumina platform. In addition, 18s rDNA full gene was sequenced for the same samples using the PacBio sequel II platform. The raw short read and long read data were quality filtered through corresponding DADA2 bioinformatic pipelines to generate amplicon sequence variants (ASVs) and species classified by BLAST. There was high agreement ($R_c > 0.7$) between the three molecular classifications species proportions except for *E. alabamensis* (COI vs 18s, COI vs ITS) and *E. auburnensis*. ITS1 classified thirteen bovine *Eimeria* including undefined type 1. The samples classified as undefined type 1 using ITS1 assigned to unclassified *Eimeria* by 18s rDNA full gene using BLAST-97 (97% threshold species identity). Also, fifteen ASVs assigned by ITS1 as undefined type 1 grouped monophyletically.

OA18.04

Identification and phylogenetic analysis of forensically significant *Chrysomya bezziana* and *C. megacephala* based on cytochrome oxidase I and II genes in Tamil Nadu, India

_Soundararajan, Dr. Chinnaiyan_Tamil Nadu Veterinary and Animal Sciences University

Co-Authors - Dr. Krishnamoorthy Rajeshkumar, Dr K.P Prabhu, Arumugam Komathi, V.R Harini

Forensic scientists can estimate the postmortem interval and cause of death by studying the insect population. Adult flies, maggots and pupae were collected from a dog and Indian wild boar (*Sus scrofa cristatus*) during post mortem examination at the Vadalur village, Coimbatore district and Coonoor forest range, The Nilgiris, Tamil Nadu, India. The collected maggots were stored in 70% alcohol for molecular study. The collected maggots were identified as by stereozoom microscope. DNA was isolated from the maggots of the *Chrysomya bezziana* and *C. megacephala*. PCR was employed for the specific target of mt COI and mt COII regions. The phylogenetic analysis was employed using both neighbour-joining and maximum likelihood methods. The sequence mt COII of 628 (*C. bezziana*) and 559 (*C. megacephala*) base pairs were analysed by the BLASTn programme, NCBI. One isolate from *C. bezziana* (Thailand) was also used as an outgroup in order to distinguish the evolutionary relationship between *C. megacephala* and *C. bezziana*. The phylogenetic analysis was employed using MEGA 11.0 software. The Collected maggot sample from dog from the province of Tamil Nadu was assigned accession numbers as OP723514. The Collected blow fly sample from wild boar from the province of Tamil Nadu was assigned accession numbers as OP697871. Interestingly, this was the first mt COII report of *C. megacephala* in India.

Keywords: *Chrysomya bezziana* - *Chrysomya megacephala* – Molecular identification – dogs - Wild Indian boar – Tamil Nadu – India

OA18.05

A novel system for anti-parasitic drug delivery_Maier, Prof. Alexander G._Australian National University

Co-Authors - Mr. Blake Curtis, Mr. Patrick Yates, Associate Professor Giel van Dooren, Professor Malcolm McLeod, Prof. Alexander G. Maier, Dr. Merryn Fraser

Many parasites rely on the uptake of host nutrients for their survival. Interference with the nutrient uptake mechanism can lead to the death of the parasite and hence it is a popular approach to drug design. We were wondering whether it would also be possible to exploit the essential uptake of lipids for a more efficient delivery of existing and experimental anti-parasitic drugs. Using the apicomplexan malaria parasite *Plasmodium falciparum* as a model system, we tested various fluorescently labelled lipids for the efficient uptake into parasites. We then replaced the fluorophores with anti-malarial compounds. The efficacy of these lipid-drug conjugates was then tested in various assays and dose-response experiments against different life-cycle stages of *P. falciparum*. We further explored efficacy against other protozoan parasites such as *Toxoplasma*, *Eimeria* and *Leishmania*. Coupling of existing or novel anti-parasitic drugs increased the efficacy 4 - 20-fold compared to uncoupled drugs in all life-cycle stages tested. At the same time a significant decrease in toxicity against host cells was observed. The conjugates also showed beneficial effects on drug-resistant parasite lines compared to the drug alone. Our novel drug delivery system improves efficacy and reduces toxicity of a wide range of existing and novel

drugs. It is applicable to a wide range of parasitic diseases and might provide an avenue to counteract drug resistance.

OA18.06

Quantitative detection of *Theileria haneyi* infections in South Africa_Bhoora,
Dr.Raksha_University of Pretoria

Co-Author - Dr Nicola Collins, Ms Tshenolo Mbaba

Genetically diverse *Theileria equi* and *Babesia caballi* parasite genotypes have compromised the molecular and serological detection of equine piroplasmiasis globally, and this has been further complicated by the recent description of *Theileria haneyi* in the USA. *Theileria haneyi* was recently reported to occur in South African horses infected with *T. equi* genotype C. Differentiation between *T. equi* and *T. haneyi* is accomplished either by nested PCR assays targeting the ema-1 gene of *T. equi* and a *T. haneyi*-specific gene, or a ThEMA-11 indirect ELISA based on a protein exclusive to *T. haneyi*. We developed a quantitative real-time PCR assay targeting the ema-11 gene for the detection of *T. haneyi* infections in horses from South Africa. Field samples were screened for the presence of *T. haneyi* using established molecular genotyping and nested PCR assays. The reference *T. haneyi* Eagles Pass ema-11 gene sequence was used to design primers to amplify and sequence the ema-11 gene from *T. haneyi*-positive field samples. An alignment of the ema-11 gene sequences obtained in this study was used to design a *T. haneyi*-specific TaqMan minor groove binder (MGB™) real-time PCR assay. The diagnostic efficacy of the assay was evaluated by screening field samples. The assay was able to detect *T. haneyi* and, in conjunction with a *T. equi*-specific real-time PCR, to distinguish between *T. haneyi* and *T. equi* infections. The *T. haneyi*-specific real-time PCR assay could be used as a complementary diagnostic assay, together with the indirect ThEMA11 ELISA for importing and exporting horses.

23.08.23

Breakout 1: OA19 Flies and fly control

OA19.01

A novel clade of bat-associated *Bartonella* detected in the bat fly *Leptocyclopodia ferrari* (Diptera: Nycteribiidae) parasitizing *Cynopterus brachyotis* (Chiroptera: Pteropodidae)

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Bartonellosis are emerging infectious diseases that are common in humans and animals worldwide. Several *Bartonella* species associated with companion animals such as *Bartonella henselae* and *B. rochalimae* are species with zoonotic implications and have become a global concern. Other *Bartonella* species associated with wild animals, however, remain underappreciated particularly in the developing regions of the world. To explore further on this neglected bacterial agent, *Leptocyclopodia ferrari* (Nycteribiidae) bat flies collected from *Cynopterus brachyotis* (Pteropodidae), an endemic fruit bat species in Southeast Asia, were molecularly examined for the presence of *Bartonella*. Both 16S–23S ribosomal RNA intergenic spacer region and citrate synthase gene sequences exhibited less than 95 % similarity to all previously reported *Bartonella* spp. Further phylogenetic analysis revealed a novel clade of this *Bartonella* sp. with high bootstrap support. The vectorial capacity of bat flies in transmitting this novel pathogen merits further investigation.

OA19.02

Providing better understanding of climate and Environmental drivers of sand fly borne diseases – the climos Project_Berriatua, Prof. Eduardo_University of Murcia

Co-Authors - Dr Manos Athanatos, Dr Suzana Blesic, Dr Giogia Bongiorno, Prof Remi Charrel, Prof Orin Courtenai, Dr Maya Davidovich-Cohen, Prof Jerome Depaquit, Dr Vit Dvorak, Dr Ozge Erisoz, Dr Federica Ferraro, Dr Maria Maia, Dr Valentina Foglia Manzillo, Dr Nenad Gligoric, Dr Vladan Gligorijevic, Dr Diana Guardado, Dr Gordon Hamilton, Dr Nils Hempelmann, Dr Tally Hatzakis, Dr Vladimir Iovic, Dr Edwin Kniha, Dr Lahor Orshan, Prof Yusuf Ozbel, Dr Slomit Paz, Dr Florence Robert-Gangneux, Dr Jovana Sadlova, Dr Luis Samaniego, Dr Daniel San Martin, Dr Tugba Guler Sonmez, Dr Frank Van Langevelde, Prof Petr Volf, Dr David Wright, Dr Carla Maia

Over the last two decades, four successive research consortia (EDEN, EDENext, VBORNET, and VectorNet) aimed at improving knowledge, surveillance, and control of vector-borne diseases in Europe and neighbouring countries. Among these, phlebotomine sandfly-borne diseases, including leishmaniasis and phleboviruses represent a significant public health and veterinary concern. CLIMOS (Climate Monitoring and Decision Support Framework for Sand Fly-borne Diseases Detection and Mitigation with Cost-benefit and Climate-policy Measures) aims to complement and build on previous efforts to conduct innovative and applied research on the impact of climate change on sand fly density and the transmission of sandfly-borne diseases. The consortium brings together 29 partners across 16 countries including researchers, healthcare and veterinary practitioners, technology platform designers and at-risk communities. Novel technologies to monitor and minimise human contact with sand flies will be developed, and climatic, environmental, demographic, and other epidemiologic drivers of sand fly density

and sand fly-borne infections will be identified in longitudinal studies across Europe and neighbouring countries. Available and newly collected data and input from partner public health ministries will feed into epidemiological-climatic predictive mathematical models to develop an early warning system for public use. Funding: Study is co-funded by European Commission grant 101057690 and UKRI grants 10038150 and 10039289.

Keywords: Big Data; Copernicus; *Leishmania*; Phlebotomine Sand Flies; Phlebovirus

OA19.03

Updates on the blood source preferences and pathogen load in two sand fly species (Diptera: Psychodidae) in Romania_Pop, Dr. Cristina Daniela_USAMV Cluj-Napoca

Co-Authors - Dr. Angela Monica Ionică, Prof. Andrei Daniel Mihalca

Blood feeding preferences of sand fly species on vertebrate hosts are risk indicators of potential new foci of diseases affecting humans and animals. In the present study, the sand fly females of *Phlebotomus perfiliewi* and *P. neglectus*, captured in Romania between 2013 and 2021, were molecularly assessed. *Leishmania infantum* presence was evaluated by real-time PCR assay, Toscana virus (TOSV), and Sand fly fever Sicilian virus (SFSV) RNA and the blood source preference by different PCR assays. All sand flies tested negative to the pathogen presence. Regarding the source of the blood meal, 52/74 (70.3%) sequences were obtained in total, 32/52 (61.5%) for *Ph. neglectus* and 20/52 (38.5%) for *P. perfiliewi*. In *Ph. neglectus*, 1/32 (3.1%) fed on *Apodemus agrarius*, 7/32 (21.9%) on *Cervus elaphus*, 7/32 (21.9%) on *Homo sapiens*, 3/32 (9.4%) on *Lepus europaeus*, 11/32 (34.4%) on *Bos taurus*, 2/32 (6.2%) on *Capreolus capreolus* and 1/32 (3.1%) on *Sus scrofa*. In *Ph. perfiliewi*, 1/20 (5.0%) fed on *Bos taurus*, 1/20 (5.0%) on *Ovis aries*, 1/20 (5.0%) on *Gallus gallus*, 16/20 (80.0%) on *Equus caballus* and 1/20 (5.0%) on *Homo sapiens*. The various domestic and wild vertebrate species may indicate the opportunistic feeding behavior of the sand fly species increasing the risk of disease transmission of public health concern. It appears that the local sand fly vector populations are not carrying so far pathogen species, but the results cannot be generalized to the entire Romanian territory, nor to all sand fly species present in Romania. More studies are necessary for a better understanding of each sand fly species feeding behavior and pathogen load when assessing the possible risk transmission of human and animal diseases.

OA19.04

Potential distribution of *Chrysomya* flies in India based on bioclimatic variables through a MaxEnt modelling approach_Bhangale, Dr. Gajendra_Maharashtra Animal and Fishery Sciences University Nagpur

Co-Author - Dr Babasaheb Narladkar

The flies of *Chrysomya* genus are of immense veterinary and public health importance due to their ability to cause blow fly myiasis and also an important fly in forensic entomology in human as well as veterinary jurisprudence. The present study attempted to delineate the potential habitat suitability of geographical areas for spread and distribution of *Chrysomya* sp. flies in India. For this the potential habitat distribution of *Chrysomya* was modelled using algorithm in Maxent, a maximum entropy modelling program. The climate niche for *Chrysomya* was developed based on the relationship between presence only data for 61 locations and a reference climate data downloaded from WorldClim. The predictions from Maxent were mapped in QGIS, an open source computer aided tool for mapping and analyzing spatial data. The established model in Maxent gave a reasonable estimate of *Chrysomya* spp. range with better discrimination of suitable and unsuitable areas for its occurrence under the current climatic conditions. This was

evident from the highest value of AUC i.e. 0.716 and 0.815 for training and test data, respectively. A Jackknife test for variable importance indicated that Bio6 (Minimum temperature of coldest month) with highest gain value was the most important abiotic factor influencing the potential habitat distribution of *Chrysomya* flies followed by Bio1 (Annual mean temperature) and Bio4 (Temperature seasonality). The modelled habitat distribution of *Chrysomya* spp. will be a useful guide for researchers on the potential distribution of a variety of *Chrysomya* flies and identified areas with a high potential risk of blow fly myiasis cases on susceptible animal population.

OA19.05

Molecular identification of *Culicoides* species and host preference blood meal in the African horse sickness outbreak-affected area in Hua Hin district, Prachuap Khiri Khan province, Thailand_Kamyinkird, Dr. Ketsarin_Department of parasitology, Faculty of Veterinary Medicine, Kasetsart University

Co-Authors - Miss Suchada Choocherd, Mr Wissanuwat Chimnoi, Miss Nutsuda Klinkeaw, Miss Chanya Kengradonkij, Miss Pornkamol Phoosangwalthong, Dr. Nipa Thammasonthijarern, Dr. Khampee Pattanatanang, Dr. Tawin Inpankaew, Dr. Jumnongjit Phasuk, Dr. Burin Nimsuphan

African horse sickness (AHS) was reported as an outbreak in Thailand in 2020. Hematophagous arthropods, known as *Culicoides*, are the suspected vector responsible for AHS transmission. Horses in Hua Hin district, Prachuap Khiri Khan province, Thailand, were affected and died from AHS in 2020. However, the potential *Culicoides* species and its host preference blood meal in the affected areas are unknown. To investigate the potential transmitted vectors of AHS, *Culicoides* were collected using light traps placed near horse stables. Six horse farms including five farms with AHS history and one farm without AHS history were included in this study. Morphological and molecular identification of *Culicoides* species was performed. Polymerase chain reaction (PCR) targeting cytochrome b oxidase I (COXI) gene for confirmation of *Culicoides* species, identification of prepronocceptin (PNO) gene for host preference blood meal, and bidirectional sequencing were conducted. Consequently, 1,008 female *Culicoides* were collected with 708 and 300 female *Culicoides* captured at positions A and B at a distance of <2 and >5 m from the horse, respectively. Fourteen *Culicoides* species identified by morphology were noted, including *C. oxystoma* (71.92%), *C. imicola* (20.44%), *C. actoni* (2.28%), *C. flavipunctatus* (1.98%), *C. asiana* (0.99%), *C. peregrinus* (0.60%), *C. huffi* (0.60%), *C. s. brevitaris* (0.40%), *Culicoides innoxius* (0.30%), *C. histrio* (0.30%), *C. minimus* (0.10%), and *C. geminus* (0.10%). PCR detection of the *Culicoides* COXI gene has confirmed *Culicoides* species in 24 of 26 DNA samples. PCR targeting PNO gene revealed that the *Culicoides* collected in this study have consumed *Equus caballus* (86.25%), *Canis lupus familiaris* (6.25%), *Sus scrofa* (3.75%), and *Homo sapiens* (3.75%) for their blood meal. Human blood was identified from two samples of *C. oxystoma* and a sample of *C. imicola*. Three dominant species including *C. oxystoma*, *C. actoni*, and *C. imicola* were reported in the Hua Hin area to prefer consuming horse blood. Moreover, *C. oxystoma*, *C. imicola*, and *C. brevitaris* have consumed canine blood. This study revealed the species of *Culicoides*, the potential vector that has possibly transmitted AHS in Hua Hin district, Thailand during the outbreak.

Keywords: *Culicoides*, African horse sickness, blood meal analysis, host preference blood feeding.

Pyrethroid resistance in stable flies (*Stomoxys calcitrans*) in Germany: Phenotypic characterization and genotype quantification using deep amplicon sequencing_Daher, Ms. Ricarda_Federal Office of Consumer Protection and Food Safety (BVL))

Co-Authors - Dr. Stefan Fiedler, Dr. Burkhard Bauer, Dr. Wiebke Weiher, Francis Mutebi, Prof. Dr. Ard. M. Nijhof, Dr. Stephan Steuber, Prof. Dr. Peter-Henning Clausen, Dr. Jürgen Krücken

Stable flies (*Stomoxys calcitrans*) are obligate blood feeders with serious impact on livestock health and productivity. Decades-long use of pyrethroids for pest control has resulted in selection of resistance. In *S. calcitrans*, pyrethroid resistance has so far been associated with the genetic markers *kdr* (L1014F) and *kdr-his* (L1014H) but not with *super-kdr* (M918T) in the voltage-sensitive sodium channel (*vssc*) gene. This study aimed to characterize phenotype/genotype relationships and determine genotype frequencies in German stable fly populations by deep amplicon sequencing. Four stable fly field isolates and one susceptible reference strain (MSD) were reared in the laboratory and LD50-values for deltamethrin were determined by topical application. Phenotypically tested individuals were genotyped using allele-specific PCRs. The field isolates were phenotypically resistant to deltamethrin with LD50 values of 18.04, 19.58, 40.46 and 46.98 ng deltamethrin/fly, 47.5 to 123.6-fold higher than for MSD strain with 0.38 ng/fly. PCRs detected *kdr* and *kdr-his* genotypes in all field isolates, with significant ($p < 0.05$) higher odds to survive treatment already in heterozygous individuals compared to wild-type flies, and stronger effects of *kdr* than *kdr-his* on the resistance phenotype. In 2019, stable flies were collected from 65 dairy farms in different areas of Germany. Flies were pooled per farm before deep amplicon sequencing of the *vssc* gene. Results showed presence of *kdr* and *kdr-his* variants on all farms with up to 55 % of *kdr* and 85 % of *kdr-his* alleles on individual farms. Phylogenetic analysis suggested at least two independent origins of *kdr* and *kdr-his* variants. In 19/65 pools, *super-kdr* alleles in stable flies (frequency 0.6-9.5 %) were detected. Collectively, these data show a correlation of *kdr* genotypes with resistance phenotypes, widespread occurrence of resistant genotypes on German dairy farms and the first report of the *super-kdr* genotype in *Stomoxys* spp.

23.08.23

Breakout 2: OA20 Zoonoses and One Health I

OA20.01

Reptiles as paratenic host and sentinel for *Angiostrongylus cantonensis* in Macaronesian island Tenerife. _Anettová, Ms. Lucia_Masaryk University

Co-Authors - Mr Vojtech Baláž, Ms Elena Izquierdo-Rodriguez, Ms Anna Šipková, Mr Radovan Coufal, Prof Pilar Foronda, Prof David Modry

The rat lungworm, *Angiostrongylus cantonensis* is a zoonotic metastrongyloid nematode considered an emerging pathogen. It originates in South-East Asia but managed to spread globally into tropics and subtropics. Macaronesia, in particular island of Tenerife, represents an infection focus close to Europe. Reptiles are known to serve as paratenic hosts and can be a source of infection for humans and animals. Endemic lizards *Gallotia galloti* are a dominant part of terrestrial fauna native to Macaronesia; our recent study confirmed these saurians to serve as paratenic hosts of *A. cantonensis* and other metastrongylids in Tenerife. Herein, we are reporting results of a follow-up study addressing a potential of these abundant lizards to serve as a sentinel for mapping of the geographic distribution of *A. cantonensis*. In 2022, 129 lizards were captured alive using fall traps in 9 localities across the island. Spontaneous caudal autotomy was induced in captured lizards which were subsequently released. Collected tail muscle samples were examined for the presence of *A. cantonensis* DNA by a species-specific qPCR assay and the amount of the DNA was quantified using standard curve; 31 out of 129 samples tested positive for *A. cantonensis* DNA by qPCR; the parasite's presence was confirmed from all the localities, with prevalence ranging from 4.55% (South) to 63.64% (Northeast). The amount of the *A. cantonensis* larvae differs significantly ($F_{3,27} = 5.5235$; $p < 0.01$) among areas. The main outcome of this study was proving that tail muscles obtained using the caudal autotomy of lacertid lizards can be used for intravital detection of *A. cantonensis* and other metastrongylids, i.e., parasites that use lizards as paratenic hosts. This study also proved that *A. cantonensis* is present also in arid habitats of the South of Tenerife, where it was not confirmed before. Supported by project of Czech Science foundation No. 22-26136S.

OA20.02

Epidemiology of *Leptospira* spp. from environment and animal reservoirs in Peninsular Malaysia_Mohd Zain, Prof. Siti Nursheena_Universiti Malaya

Leptospirosis in Malaysia is regarded as an emerging disease with a dramatic increase in the number of reported cases over the last decade caused by pathogenic spirochetes of the genus *Leptospira*. These bacteria colonize the kidneys of reservoir animals and contaminate the environment through the excretion of urine. Human transmission occurs either directly from exposure to contaminated urine or infected tissues, or indirectly via contact with contaminated soil or water. Therefore, the objective of this study was to determine the circulating species in the host reservoirs (rat, dog, cat, swine) and environment contamination (water and soils). Urine and kidney samples of 657 rodents, 150 dogs, 50 cats and 81 swine were screened with 8.9% rats, 7.3% dogs and 6.2% swine positive for the pathogenic bacteria. Positive samples were detected in 25% of the environmental samples collected. Microscopic agglutination test (MAT) resulted with 4 serogroups identified among the zoonotic samples however none from the environment. Molecular characterization of the isolates using PFGE showed a high diversity among the environmental strains (8 profiles) while only 5 different patterns were generated among zoonotic

and clinical strains using Not I enzyme. Six antimicrobial agents (doxycycline, penicillin G, trimethoprim, ampicillin, chloramphenicol and sulfomethaxazole) were tested for antimicrobial susceptibility with all isolates resistant to trimethoprim, chloramphenicol and sulfomethaxazole. Doxycycline, ampicillin and penicillin G were effective to all the clinical and zoonotic isolates except to the environmental isolates.

OA20.03

Tracing the origins of *Echinococcus multilocularis* in Hokkaido, Japan: genetic validation of a historical hypothesis_Hayashi, Mr. Naoki_Hokkaido University

Co-Authors - Dr. Ryo Nakao, Dr. Yuma Ohari, Dr. Takao Irie, Dr. Hirokazu Kouguchi, Dr. Elisha Chatanga, Dr. Wessam Mohamed, Dr. Mohamed Moustafa, Dr. Gohta Kinoshita, Dr. Munehiro Okamoto, Dr. Kinpei Yagi, Dr. Nariaki Nonaka

Echinococcus multilocularis is a species of tapeworm in the family Taeniidae, causing a life-threatening zoonotic disease, alveolar echinococcosis (AE) in humans. The parasite is currently distributed throughout Hokkaido, the northernmost prefecture of Japan, where approximately 20 AE cases are reported per year. Historical records suggested that human activities in the modern era, such as translocations of infected animals, have triggered the introduction of *E. multilocularis* into Hokkaido from St. Lawrence Island in the USA. In order to validate this hypothesis, we constructed complete mitogenomes from 66 parasite specimens, consisting of 60 from Hokkaido, 5 from St. Lawrence Island, and 1 from Europe, by amplicon-based sequencing. The genetic analysis, including haplotype networks and phylogeographic analysis, was performed based on the mitochondrial coding sequences. The network analysis revealed that the parasite population in Hokkaido was divided into two haplogroups. A major haplogroup exhibited a star-like network, suggesting population expansion following a bottleneck event. Comparisons with data from other endemic areas showed that the ancestral haplotype of the major haplogroup was identical to that of St. Lawrence Island, supporting the most probable transmission route suggested by historical descriptions. In contrast, the minor haplogroup was closely related to those detected in Sichuan, China. The phylogeographic analysis yielded two strongly supported diffusion routes to Hokkaido from St. Lawrence Island and Sichuan, over 3,000 km across the ocean. These results imply that complex and inadvertent animal translocations in the modern era have caused multiple introductions of *E. multilocularis*. This study highlights the anthropogenic impacts of introducing the deadly parasite *E. multilocularis* and has significant implications for the further spread of this parasite, which may be caused by global animal translocations.

OA20.04

Alternative drugs for the control of the strongyloidiasis in humans and animals_Martínez-Valladares, Dr. María_University of Leon

Co-Authors - Lara Linnemann, Jennifer Antwi-Ekwuruke, Elora Valderas García, Minka Breloer

Strongyloidiasis is a zoonotic disease caused in humans by *Strongyloides stercoralis*, with different animal species such as dogs, cats and non-human primates as reservoirs. Ivermectin (IVM) is the treatment of choice in humans, although albendazole at repeated doses has also been recommended; in animals, these treatments, together with febendazole, are the most widely used, although there is no clear indication for the treatment of strongyloidiasis. In this context, the objective of this study was to test 13 commercial drugs (benzimidazoles, macrocyclic lactones, levamisole and pyrantel pamoate) and 33 newly synthesized compounds (benzimidazole, amino alcohol and diamine derivatives) against *S. ratti* larvae as alternative treatments for animals and

humans. The methods used were the Larval Motility Test (LMT) and the Larval Migration Inhibition Test (LMIT). Those compounds with activities higher than 90% in the initial screening at 50 μM were selected to determine their half maximal effective concentration (EC₅₀). The EC₅₀ for IVM was 2.47 μM and 1.21 μM by the LMIT and LMT, respectively, however other commercial drugs showed greater in vitro efficacies; by the LMIT: 0.14 μM for levamisole, 0.48 μM for pyrantel pamoate, 0.81-1.54 μM for doramectin, 1.80 μM for abamectin and 2.36 μM for moxidectin; by the LMT: 0.12 μM for levamisole, 0.60 μM for moxidectin and 0.67 μM for abamectin. Regarding the newly synthesized compounds 12 of them showed greater or similar EC₅₀ values than IVM: 1 benzimidazole (1.93 μM), 6 amino alcohol (1.29 - 2.34 μM) and 5 diamine (1.41 - 2.47 μM) derivatives compounds. Study funded by 'Stopping Transmission Of intestinal Parasites (STOP)' project (EDCTP2 programme supported by the European Union; RIA2017NCT-1845-STOP) and 'Agencia Estatal de Investigación, Ministerio de Ciencia e Innovación' (PID2020-119035RB-I00, Spain).

OA20.05

Zoonotic microorganisms and parasites associated to reptiles in the markets of Marrakech, Morocco_Mendoza Roldan, Prof. Jairo Alfonso_University of Bari

Co-Authors - Viviane Noll Louzada-Flores, Dr. Nouha Lekouch, Intissar Khouchfi, Giada Annoscia, Prof. Andrea Zatelli, Prof. Frédéric Beugnet, Prof. Julia Walochnik, Prof. Domenico Otranto

The markets of Marrakech, known in Arabic as souks, are places where many species of reptiles are sold for medicinal, magic purposes, as pet or used for snake charming. This exceptional epidemiological context has never been studied, considering the interactions between humans, reptiles, and zoonotic pathogens. Thus, the objective of this study was to screen blood and feces of reptiles kept in the markets of Marrakech for parasites and other pathogens in order to assess the risk of zoonotic transmission. Owned reptiles (n= 118), belonging to vendors or snake charmers, were examined, and sampled. DNA was extracted and molecular screening (cPCR, nPCR, qPCR, dqPCR) was performed to identify microorganisms and parasites (i.e., *Anaplasma/Ehrlichia* spp., *Rickettsia* spp., *Borrelia burgdorferi sensu lato*, *Coxiella burnetii*, *Babesia/Theileria* spp., *Cryptosporidium* spp., *Giardia* spp., *Leishmania* spp., Cestoda). Overall, 34 reptiles (28.9%) were positive for at least one pathogen. *Anaplasma* spp. were detected from blood of four snakes, being two Montpellier snakes positive for *A. phagocytophilum*, while *Rickettsia* spp. were detected in one Mediterranean chameleon and four puff adders. Moreover, *Leishmania tarentolae* was molecularly detected in a Mediterranean chameleon and a Montpellier snake. On the other hand, the *cox1* gene generated a large number of sequences for nematodes, cestodes, fungi and bacteria. For example, *Proteus vulgaris* was identified from a Mediterranean chameleon. *Cryptosporidium* spp. nPCR yielded a positive sample (i.e., *Cryptosporidium* sp. apodemus genotype I) from a Moroccan worm lizard, as well as for pathogenic bacteria such as *Pseudomonas aeruginosa* in an Egyptian cobra, and *Morganella morganii* from a puff adder. Results from this study demonstrate the risk of zoonotic transmission of microorganisms and parasites from reptiles that are kept in the souks of Marrakech (Morocco), being in direct and straight contact with humans.

Keywords: Snakes; Reptiles; Snake charmers; pathogens; zoonoses; souks

Practices facilitating the spread of zoonotic soil-transmitted helminths: Perspective from the pet owners_Rayulu, Prof. (Dr.) V. Chengalva_Sri Venkateswara Veterinary University

Co-Authors - Dr. V. Gnani Charitha, Prof. P. Malakondaiah, Prof. K. Aswani Kumar, Prof A. Jagadeesh Babu

Animal origin soil-transmitted helminths are known for establishing *larval migrans* in humans. Soil-transmitted helminths (STHs) come under the saprozoontic category and infection to humans is by hand-to-mouth contact or by skin-penetration with parasitic forms (eggs/larvae) that are prevailing in soil/hair coat of pets. A questionnaire-based survey was designed to analyze the facts associated with STH's of zoonotic potential in Andhra Pradesh, India. The survey intends to convene details about owner associated factors, animal-related factors, knowledge/awareness and management practices. Data was collected over a period of 10 months and about 1047 pet owners were surveyed. A total of 366 (34.9%) responses were retrieved. Most pet owners in the study preferred adopting young (1-2 years) male dogs (61.74%) over females; pure breeds (78.4%) over local non-descriptive breeds. Owners lacking sound educational backdrop were poor in maintaining personal hygiene and more than half of the respondents (63.7%) were unaware of parasites being transmitted through soil. Pertaining to the child and pet animal association, 67.7% of individuals owning pet animals were having one or more children below 15 years of age. Data appertaining to dog's defecating sites and risk zones for children gaining access to soil revealed that the most contaminated sites were school playgrounds (40.9%) followed by backyards and under tree open areas. In the study, preferences of dog owners towards breed, sex, and age along with poor dog management and personal hygiene practices predispose them to a greater risk of zoonoses.

Keywords: Questionnaire survey, Soil-Transmitted Helminths, Practices, Awareness, Zoonotic Potential.

23.08.23

Breakout 3: OA21 Wildlife and Aquatic parasites

OA21.01

Respiratory and cardio-vascular parasites infecting carnivores in Algeria: preliminary results_Deak, Dr. Georgiana_University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca

Co-Authors - Noureddine Mechouck, Angela Monica Ionica, Zihad Bouslama, Andrei Daniel Mihalca

Algeria, the largest African country is home to 21 carnivorous mammal species, including canids, felids, and mustelids, which are distributed mainly in the northern part of the country. Due to their specific diet, ecology and behaviour, carnivores are exposed to a large range of parasites, including metastrongyloid lungworms such as *Angiostrongylus vasorum*, *Crenosoma vulpis*, *Aelurostrongylus abstrusus*, *Troglostrongylus brevior* and *Eucoleus aerophilus* or the filarioid *Dirofilaria immitis*. To present, there are no data about the occurrence and diversity of cardio-respiratory parasites infecting carnivores in Algeria. Considering the large number of carnivore species and the veterinary importance of these parasites, the aim of our study was to investigate the diversity of pulmonary and cardio- respiratory parasites infecting carnivores in Algeria. A total of 26 carcasses comprising 1 *Vulpes vulpes* (3.84%), 3 *Canis aureus* (11.5%), 13 *Vulpis zerda* (50%), 5 *Canis familiaris* (19.2%), 3 *Felis catus* (11.5%), and 1 *Herpestes ichneumon* (3.84%) were collected and examined by complete parasitological necropsy complemented by histopathological examination. In addition, blood and fecal samples from alive 33 *Canis familiaris* were tested using Knott's test and the Baermann method, respectively. Detected parasites were morphologically identified and molecularly characterized to species level. The only examined red fox was co-infected with *C. vulpis* and *E. aerophilus* (3.8%) and one jackal was infected with *A. vasorum* (3.8%). Among the examined alive domestic dogs, no respiratory or cardiovascular parasites were detected, besides 5 that presented a positive larvoscopy for *Strongyloides* spp. larvae (15.1%) and one that had a positive Knott test for *Acanthocheilonema* spp. (3%). No other parasites were yet identified. This study reports for the first time the presence of *C. vulpis* and *E. aerophilus* in the African continent and infection by *A. vasorum* in Algeria. Carnivores are important reservoirs and spreaders of parasitic diseases highlighting the need for further large-scale studies in Africa.

Keywords: Algeria, carnivores, lungworms, parasites.

OA21.02

Into the large ears: nematodes, mites, and bacteria associated with otitis externa in Asian elephants (*Elephas maximus*)_Bezerra Santos, Dr. Marcos Antônio_University of Bari

Co-Authors - Dr. Jairo Alfonso Mendoza-Roldan, Dr. Peter M. DiGeronimo, Dr. Erica Ward, Dr. Bruce Noden, Dr. Francesca De Luca, Dr. Elena Fanelli, Dr. Domenico Valenzano, Dr. Riccardo Paolo Lia, Prof. Domenico Otranto

The Asian elephant (*Elephas maximus*) is an endangered species that harbors several parasites. Among the ectoparasites, ear mites of the genus *Loxanoetus* have the potential to cause external otitis, which may also be associated with other microorganisms. In this study we assessed the relationship among ear mites, nematodes, yeast, bacterial rods, and cocci affecting the ears of Asian captive elephants in Thailand. Ear swabs were individually collected from both ears of legally owned elephants (n = 64), and samples were microscopically examined for the presence

of mites, nematodes, yeast, bacterial rods, cocci, and host cells. Mites of the species *Loxanoetus lenae* were present in 43.8% (n = 28/64) of the animals (19 animals with mites in one ear and nine in both). Nematodes of the genus *Panagrolaimus* were detected in 23.4% (n = 15/64) of the animals (ten with nematodes in one ear and five with nematodes bilaterally). The detection of nematodes in both ears was significantly associated with the presence of mites in both adult elephants (Fisher's exact test, P = 0.0278) and in females (Fisher's exact test, P = 0.0107). In addition, the detection of higher categorical burdens of nematodes was also significantly associated with the presence of mites (Fisher's exact test, P = 0.0234) and epithelial cells (Fisher's exact test, P = 0.0108), and marginally for bacterial cocci (Fisher's exact test, P = 0.0499). Data herein obtained suggest that the presence of *L. lenae* mites in the ear canal is significantly associated with the occurrence of other microorganisms such as soil nematodes, bacteria and yeasts, as a potential effect of increased dustbathing behavior in infested animals. Overall, this would represent a paradigmatic example of a parasite infestation affecting animal behavior.

Keywords: Elephants, mites, nematodes, external otitis, bacteria, yeasts, dustbathing.

OA21.03

Morphological and molecular detection of *Mansonella* sp. and associated *Wolbachia* endosymbionts in ring-tailed coatis (*Nasua nasua*) in urban areas from Midwestern Brazil_Perles, Dr. Livia_Unesp

Co-Authors - Prof. Domenico Otranto, Dr. Wanessa Teixeira Gomes Barreto, Dr. Gabriel Carvalho de Macedo, Dr. Riccardo Paolo Lia, Dr. Jairo Alfonso Mendoza-Roldan, Dr. Heitor Miraglia Herrera, Dr. Marcos Rogerio Andre

Coatis (Procyonidae, *Nasua nasua*) are carnivorous well adapted to anthropized environments, acting as reservoirs hosts for many arthropod-borne zoonotic pathogens. To investigate the diversity of filarial nematodes, blood samples (n= 100 animals) were obtained from two urban areas in Midwestern Brazil. Blood smears were stained with Diff-Quick and microfilariae were analyzed using morphological keys. DNA was extracted from blood and were submitted to PCR assays based on the *cox1*, *hsp70* and *myoHC* genes for nematodes and 16S rRNA for *Wolbachia*. Third-three percent of the samples presented at least one microfilaria, characterized by a serpentine shape without sheath, measuring $190.5 \pm 14.4 \mu\text{m}$ in length and $3.29 \pm 0.28 \mu\text{m}$ width; anterior end was rounded with short head space and presented cephalic space with scattered fine nuclei. The tail was long, slender, pointed with nuclei to the end. Based on the above, microfilariae were morphologically identified as belonging to the genus *Mansonella*. Twenty-five *cox1* sequences were obtained showing 89% nucleotide identity with *M. ozzardi*. Phylogenetic analyses clustered the obtained *cox1* sequences within *Mansonella* spp. clade. Sequences from both *myoHC* and two *hsp70* genes showed 99.8% nucleotide identity with *Mansonella* sp. and clustered into a clade within *Mansonella* sp. previously detected in coatis from Paraná state, Brazil. Two blood samples were positive for *Wolbachia*, with a 99% nucleotide identity with *Wolbachia* previously detected in *M. perstans*, *M. ozzardi* and *M. atelensis* and ectoparasites (*Pseudolynchia*, *Melophagus* and *Cimex*). The study showed a high occurrence of *Mansonella* sp. in the coati population examined, suggesting that this animal species play a role as reservoirs of a novel, yet to be described, species within the Onchocercidae family.

Key-Words: Onchocercidae, *Mansonella*, Procyonidae, *Wolbachia* sp.

OA21.04

Helminth community of loggerhead sea turtles, *Caretta caretta* in a neritic feeding ground in the Mediterranean Sea_Marchiori, Dr. Erica_Dept. Animal Medicine, Production and Health, University of Padova

Co-Authors - Prof. Rudi Cassini, Dr. Cinzia Centelleghes, Prof. Federica Marcer

The opportunistic diet of loggerhead sea turtles *Caretta caretta*, their long life-span and migratory behavior contribute to shaping the diversity of the gastrointestinal helminthic community. Higher infection levels are expected in turtles in their neritic ontogenic stage; higher diversity of the component community is also typical of neritic grounds, being most sea turtle specialist parasites heteroxenous nematodes and trematodes likely exploiting benthic organisms as intermediate hosts. In the period 2009-2022, 152 stranded dead loggerhead sea turtles were collected along the coasts of a neritic feeding ground (NW Adriatic Sea) and submitted to necropsy. Carapace length was used to define the pelagic/transitional (<30cm) or neritic (30cm) ontogenic stage. Parasites collected through washing and filtering digestive tract content were counted and identified morphologically with keys in literature. To describe parasite population structure, prevalence (P) and mean abundance (mA) were calculated for each taxon. Their difference between ontogenic stages was also tested. Component community structure was described by relative abundance (RA), species richness, importance index (I), and Shannon-Wiener's diversity index (H). Prevalence for gastrointestinal helminthic infection was 78.3% (95% C.I.: 71.7-84.8%); overall, 6704 helminths from 9 taxa were recovered, all sea turtle-specialists, mostly belonging to the Digenea (7 genera). Component community structure was similar to that of other neritic areas, with the trematode *Rhytidodes gelatinosus* (P=39.7; mA=9.4) and the nematode *Sulcascaris sulcata* (P=25.4%, mA=6.6) having higher prevalence and abundance. The ubiquitous trematode *Enodiotrema* sp. was significantly (p=0.02) more frequent in turtles at pelagic/transitional stage (P=35.0%) than in larger ones (P=14.4%), confirming its lower importance (I) within the helminthic community structure than in pelagic environments. The high value of diversity (H=2.01) encountered exceeds those found in similar neritic grounds. This study confirms the influence of the different ecosystems exploited by *C. caretta* in the two stages of its life cycle in shaping its helminth community.

OA21.05

Gastrointestinal parasites in non-human primates in zoological gardens in Northern Italy_Marchiori, Dr. Erica_Dept. Animal Medicine, Production and Health, University of Padova

Co-Authors - Dr. Giorgia Dotto, Dr. Cinzia Tessarin, Mrs Laura Voltan, Mrs Lucia Bono, Miss Silvia Minato, Prof. Federica Marcer

Gastrointestinal parasitic infections may affect health status of exotic animals in captivity. Data on the parasitofauna of non-human primates (NHP) housed in Italian zoological gardens are limited. For this reason, prevalence of gastrointestinal parasites in NHP living in two zoological gardens of Northern Italy were evaluated. In 2021 an overall number of 96 faecal pools were collected seasonally from 26 groups of NHP. Mini-Flotac method was applied to fecal samples to detect gastrointestinal helminthiases, while detection of the protists *Cryptosporidium* spp., *Blastocystis* sp. and *Giardia intestinalis* was performed targeting SSU rRNA through nested PCR and real-time PCR. *Giardia intestinalis* assemblage was studied by sequencing β giardine or TPI gene. Molecular typing of *Blastocystis* isolates was carried out by nucleotide sequence analysis of SSU rRNA gene. Twenty-two out of 96 examined fecal pools (22.9%) were positive for one or more

helminth eggs, including mainly Trichurid, Capillariid and Strongylid. All samples were negative for *Cryptosporidium* spp., while 16/26 (61.5%) animals were positive for *G. intestinalis* at real-time PCR in one (n=6) or more (n=10) samplings; sequences obtained from 6 animals revealed all of them to be assemblage B. *Blastocystis* sp. was detected in 22/26 of the NHP (84.6%) and belonged to three Subtypes (ST 4, 5 and 8). The detection of gastrointestinal parasites without overt clinical signs of disease is suggestive of subclinical infection. The molecular characterization of *Trichuris* eggs could be useful to ascribe them to the correct species, considering the high genetic heterogeneity of this genus in NHP. The presence of *Giardia* assemblage B and potentially zoonotic subtypes of *Blastocystis* confirm that NHP are potential reservoirs for zoonotic pathogens, but further genetic characterization of *G. duodenalis* subtypes may help in disclosing the origin of the isolates and cross- transmission between enclosures.

23.08.23

Breakout 4: OA22 Zoonoses and One Health II

OA22.01

Occurrence of commensal intestinal protist, *Blastocystis* sp., in a gut-healthy human population and their animals in the Czech Republic Lhotská, Ms. Zuzana_Biology Center, Institute of Parasitology, The Czech Academy of Sciences, České Budějovice, Czechia

Co-Authors - Milan Jirků, Oldřiška Kadlecová, Kristýna Brožová, Dagmar Jirsová, Christen Rune Stensvold, Martin Kolísko, Barbora Pafčo, Vladislav Ilík, Kateřina Jirků Pomajbíková

Blastocystis sp. is a widespread intestinal protist colonizing the human intestine and its prevalence varies between low- and high-income countries. Its role in the human gut ecosystem is still unclear as knowledge of its epidemiology and factors affecting gut colonization remains incomplete. To date, very few studies have addressed the question of whether there is an association between the incidence of *Blastocystis* in humans in high-income countries and contact with pet and farm animals. In this study, we specifically focused on whether this protist is transmitted between humans and animals and whether animal contact or lifestyle has an impact on the composition of the gut microbiome. This study provides data on the prevalence and subtype diversity of the gut protist *Blastocystis* sp. and its subtypes in a gut-healthy human population and animals with which the subjects were in frequent contact. A total of 288 stool samples were obtained from asymptomatic individuals across the wide age-range and 136 samples from animals. *Blastocystis* sp. was detected in samples from both human and non-human hosts by PCR and its subtypes determined based on the obtained sequences and its phylogenetic analyzes. In humans, the overall prevalence was 24% and eight subtypes were found; in animals, the prevalence was 10%, and only five subtypes were detected. A higher incidence of *Blastocystis* sp. was observed in individuals in frequent contact with farm animals and travellers outside of Europe. Moreover, we also performed NGS analyzes of the diversity of the gut bacteriome on selected samples. Based on our results, it also appears that living in a village and the presence or absence of *Blastocystis* has an effect on the composition of the gut microbiome.

OA22.02

A survey of the occurrence of *Giardia intestinalis* in the gut-healthy human population Brožová, Ms. Kristýna_Biology Center, Institute of Parasitology, The Czech Academy of Sciences

Co-Authors - Zuzana Lhotská, Oldřiška Kadlecová, Klára J. Petrželková, Milan Jirků, David Modrý, Kateřina Jirků Pomajbíková

Giardia intestinalis is one of the most common gastrointestinal protists worldwide. *Giardia* infection in humans can be asymptomatic or cause disease called giardiasis. *Giardia* is mainly detected in patients with clinical giardiasis. However, there is limited information on the prevalence of *G. intestinalis* in the population of gut-healthy, asymptomatic individuals in high-income countries. In our study we compared conventional PCR (cPCR) and real-time PCR (qPCR) to detect *G. intestinalis* in asymptomatic volunteers and their animals (pets and livestock) with which they were in close contact in the Czech Republic. We tested three genes for cPCR for triosephosphate isomerase, beta-giardin, and the small ribosomal subunit. Conventional PCR showed insufficient sensitivity for detection of weak colonization in an asymptomatic population of humans and their animals. Therefore, qPCR was used to detect *G. intestinalis* in human and animal samples. Using qPCR, we examined 431 samples (296 humans and 135 animals) and

found 7% prevalence of *G. intestinalis* in asymptomatic humans (22/296) and 19% positive samples of animals (25/135). We also found out an effect of the specific factors (living locality, contact with animals, traveling, age and gender) on the distribution of *G. intestinalis* in human samples. Regarding the specific factors, only contact with animals was found to influence the prevalence of *G. intestinalis*. We detected assemblages only in five positive samples because there is a general problem with the sensitivity and specificity of currently available molecular protocols for several genes for detection of *G. intestinalis*, and obtaining sequences by cPCR is only possible for moderately to strongly positive samples. To better understand the molecular epidemiology and biology of *G. intestinalis*, more appropriate tools for genotyping in samples with weak colonization in asymptomatic individuals need to be found and optimized.

OA22.03

***Taenia solium* cysticercosis and taeniosis reporting challenges in the current medical and veterinary disease reporting systems in Tanzania.** _Mlowe, Dr. Fredy_Ileje District Council

Co-Authors - Professor James Mlangwa, Dr. Ernatus Mkupasi, Professor Andrea Winkler, Mr. Anthony Nyerere, Dr. Ayubu Churi, Professor Helena Ngowi, Professor Esron Karimuribo

Taenia solium cysticercosis and taeniosis (TSCT) are two forms of a zoonotic disease caused by *T. solium* tapeworm. Towards promotion of a one-health approach to the control of TSCT, the present study aimed at assessing the TSCT reporting in the medical and veterinary sectors in Tanzania. A cross-sectional study was conducted between January and April 2020 in Babati and Mbulu districts (northern Tanzania), Kongwa district (central Tanzania), Mbinga and Nyasa districts (southern Tanzania) and Zonal Veterinary Centers in Iringa region (southern Tanzania) and Arusha region (northern Tanzania). A questionnaire survey was administered to 154 officers in charge (OsIC) of primary healthcare facilities (PHFs) and 110 meat inspectors (MIs) to collect quantitative data. Key informant interviews were conducted to 16 medical and 17 veterinary officers from level one healthcare facilities and district livestock offices respectively up to their respective ministerial level. It was found that *T. solium* taeniosis and neurocysticercosis (NCC) were not specifically reported in the current medical disease surveillance system. The livestock disease reporting system accommodated porcine cysticercosis (PCC) reporting. Nevertheless, approximately 77.3% (n = 110) of the MIs admitted inadequate facilitation in livestock diseases surveillance system that hindered efficient reporting of PCC among other diseases. In addition, all MIs admitted that disease reports submitted were incomplete, submitted late (73.3%, n=110) or not submitted at all (88.8%, n=110). It is concluded that the medical diseases reporting system did not provide for specific reporting of neither taeniosis nor NCC. The livestock diseases reporting system provided for PCC reporting. However, inadequate facilitation of the general livestock diseases reporting system hindered efficient PCC reporting. A one health approach in strengthening the medical and veterinary disease reporting systems for efficient TSCT reporting is recommended for evidence based and successful disease control strategies.

OA22.04

Diagnosis of cystic echinococcosis in buffaloes using lateral flow through (LFT) assay_
Prof. Sangaran Arumugam_TANUVAS, India

Co-Authors - Prof S Arunkumar, Prof T.M.A Senthilkumar

Cystic echinococcosis is regarded as one of the important zoonotic parasitic disease which is of public health importance and economic significance. Hydatid cyst fluid antigen (HCFA) was prepared for screening the sera samples of buffaloes collected in the study. The HCFA was utilized for developing the LFT and the technique was standardized. To assess the efficacy of LFT, standard enzyme linked immune sorbent assay (ELISA) was performed. Upon comparison, with ELISA, the LFT detected 64 sera samples as positive and 86 sera samples as negative with a sensitivity and specificity of 81.9 and 93.58 per cent respectively. The kappa analysis of the LFT revealed that the result of the developed technique is in substantial agreement with that of ELISA.

OA22.05

Bacterial Microbiota associated with *Rhipicephalus sanguineus sensu lato* (Acari: Ixodidae) ticks from Tamil Nadu_ Dr.A. Aruljothi_TANUVAS, India

Co-Authors - Dr. Bhaskaran Ravi Latha, Dr Arunkumar S, Dr Senthilkumar T.M.A, Dr Balagangathara thilakar M

Close relationships between ticks and microbial communities are important for tick fitness and pathogen colonization and transmission. *Rhipicephalus sanguineus sensu lato*, distributed widely can carry and transmit various pathogens and pose serious damages to public health and economics. However, little is known about the broader array of microbial communities and symbionts in *Rhipicephalus sanguineus sensu lato* ticks under natural conditions. In the present study, the composition of bacterial communities associated with *Rhipicephalus sanguineus sensu lato* ticks from Tamil Nadu was investigated. In this study 600 semi engorged ticks were collected from 3 different agro climatical zones such as north, west and south zones of Tamil Nadu. All the ticks were identified as *Rhipicephalus sanguineus sensu lato* by using morphological keys and by molecular identification. All the ticks were dissected out to separate the midgut, ovary and salivary glands and the DNA was extracted and stored at - 20 °C. PCR amplification was carried out to obtain a 689bp and 691bp fragment of the COM1 gene and wsp gene for identification of Coxiella like endo symbionts and Wolbachia sps. respectively. Next generation sequencing revealed presence of endosymbionts such as Proteobacteria, Gamma proteobacteria, alpha proteobacteria, Corynebacterium sps, Pseudomonas sps, Coxiella sps, Clostridium sps, Staphylococcus sps, Stenotrophomonas sps, Firmicutes sps, Achromobacter sps etc. Elucidating the relationships between tick

microorganisms as well as tick symbiont interactions will provide invaluable information, which may aid in the future development of arthropod pest and vector-borne pathogen transmission control strategies.

Keywords: *Rhipicephalus sanguineus* sensu lato, microbiomes, *Coxiella* like endo symbionts, *Rickettsia* like endo symbionts

24.08.23

Breakout 1: OA23 Protozoan Parasites

KL

Diagnosis of bovine tropical theileriosis_Dr. Sunil W. Kolte_Maharashtra Animal and Fishery Sciences University, India

Tick-borne diseases harm bovines and seriously impact milk yield breeding, resulting in an economic burden on farmers and those dealing with dairy industries. Theileriosis is a haemoprotozoan parasitic infection caused by *Theileria annulata* in tropical and subtropical regions. Theileriosis commonly occurs in high-yielding exotic breeds and crossbreeds like Jersey and Holstein Friesian (HF). Reduced milk production and mortality make theileriosis an economically significant disease. We cannot break the chain because typical clinical symptoms are not observed when animals are infected with subacute or chronic infections. Blood smear examination and PCR are the two methods that can detect theilerial disease. In this study, we have targeted the unique antigen *Theileria annulata* cysteine protease (TaCP) which plays an essential role in the pathogenesis of theileriosis. In the present study, we have synthesized recombinant TaCP antigen through a bacterial expression system and generated monoclonal antibodies against TaCP using hybridoma technology. This is followed by the standardization of sandwich assay. We have screened 810 bovine samples from different regions of Vidarbha and Maharashtra. Sandwich ELISA was compared with blood smear examination and PCR and indirect ELISA. We observed 63.77 %, 97.60 %, 80.70% 89.76% sensitivity and 91.36 %, 94.18 %, 90.64% and 93.16 % specificity of blood smear examination, PCR, Indirect ELISA and Sandwich ELISA respectively. This newly developed Sandwich ELISA can be further explored to detect theileriosis in cattle.

OA23.01

The Key Molecular Biological Characteristics between *Trypanosoma musculi* and *Trypanosoma lewisi*_ Prof. Zhao-Rong Lu_Sun Yat-Sen University, China

Trypanosoma musculi, the mouse trypanosome and *T. lewisi*, the rat trypanosome have been known for many decades. Interestingly, these two trypanosomes have been recognized as a host specific trypanosome. In fact, *T. musculi* cannot infect rat while *T. lewisi* can't infect mouse either. Current evidence indicated that although they have similar morphology and life cycle, they show significant difference in molecular bases. At least a dozen of cases of human infected with *T. lewisi* were reported, while none of human infected with *T. musculi* was recorded so far as we known. Evidence demonstrated that *T. lewisi* similar to *T. gambiense*, the pathogen of human African sleeping sickness is resistance to the lysis by normal human serum or APOL-1. However, normal human serum can lyse *T. musculi* both in vitro and in vivo. Due to the obvious differences between these two rodent trypanosomes, we will discuss their main biological characteristics including their genomes and kinetoplast DNA (kDNA, similar to the mitochondrial DNA in other eukaryotes) with details. (Work was supported by National Natural Sciences Foundation of China, No: 31720103918)

OA23.02

Identification of a novel host-parasite interaction in *Theileria annulata*_Srivastava, Dr. Anand_National Institute of Animal Biotechnology, Hyderabad

Co-Authors - Ms Prajana Parimita Kar, Dr. Kota Arun Kumar, Dr. Prasanna Babu Araveti

Theileria annulata infection in cattle leads to a disease known as theileriosis. It is an intracellular parasite that belongs to the phylum Apicomplexa and is transmitted by ticks of *Hyalomma* species. The infective forms of this parasite in cattle are sporozoites that are introduced into the host when the infected ticks take a blood meal. The sporozoites selectively invade bovine B cells, macrophages, or monocytes, leading to their cellular transformation. However, the selective killing of parasite in the parasite-infected cell leads to the loss of its cancerous phenotype, suggesting that parasite factors play an essential role in the transformation of the host cell. The parasite factors involved in the host cell transformation are not well explored. In pursuit of this, we revisited the probable secretome of the parasite and, following a stringent downscaling criterion, *Theileria* prohibitin (TaPHB-1) was identified as one of the factors secreted into the host cells. Interestingly, in infected cells, TaPHB-1 localized both on the parasite surface and in the host cytoplasm, and independent approaches such as coimmunoprecipitation, yeast two-hybrid screening (Y2H), and liquid chromatography-tandem mass spectrometry (LCMS/MS) confirmed RuvB-like AAA ATPase 1 (RUVBL-1) as one of its interacting partners. Further, the *T. annulata* infection does not affect the localization of bovine prohibitin. Mitigating the expression of bovine RUVBL-1 precluded the translocation of TaPHB-1 in the host cell cytoplasm without affecting the host cell viability. Taken together, we report for the first time a unique interaction of TaPHB-1 with bovine RUVBL-1 that is likely needed to cause cancer-like hallmarks during theileriosis.

OA23.03

***Tritrichomonas foetus* long read genome sequencing methods.** _Tabor, Prof. Alicja_The University of Queensland

Co-Authors - Dr Gry Boe-Hansen, Prof Michael McGowan, Dr Kieren McCosker, Mr Tony Cavallaro

Bovine trichomonosis has been found to be highly prevalent in North Australian extensively grazed beef herds. Three Australian strains of *Tritrichomonas foetus* were sequenced using long read sequencing Oxford Nanopore Technologies (ONT) to determine the conservation among Australian isolates to support vaccine development. A lab strain, and two field isolates from the Northern Territory and Queensland respectively were selected for sequencing. DNA extraction processes were modified to avoid cytosolic nuclease activity to yield high quality long DNA strands. The total lengths varied from 104.7, 110 and 111.7 Mbp with 368, 194, and 226 contigs respectively. These genome assemblies have improved previous *T. foetus* and *Trichomonas vaginalis* genomes with 10 to 100- fold less contigs assembled from our data. Preliminary genome comparisons with *T. foetus* Strain K (reference genome) showed that the Australian genomes did not have or only partially had copies of Iron only hydrogenase large subunit C terminal domain protein. One Australian strain had a longer NADP-reducing hydrogenase subunit HndC and the Australian lab strain lacked a copy of the glycerol- 3-phosphatase gene. The two field isolates had a genomic similarity of 99.2%. We report a significant improvement on *T. foetus* genome assemblies using ONT long read sequencing. This research was supported by Meat & Livestock Australia and the Queensland Government.

The potential of *Rhipicephalus microplus* as a vector of *Ehrlichia ruminantium* in West Africa_Biguezoton, Dr. Abel_Centre International De Recherche-d'eveloppement Sur L'elevage En Zone Subhumide (cirades)

Co-Authors - Dr Mireille Some, Dr Frédéric Stachurski, Dr Christine Chevillon

Heartwater, or cowdriosis, is a virulent tick-borne rickettsial disease of ruminants caused by *Ehrlichia ruminantium*, biologically transmitted by *Amblyomma* species (*A. variegatum* in West Africa). In West Africa, this bacterium was recently reported to naturally infect the invasive cattle tick, *Rhipicephalus microplus* (Rm) through trans-ovarian transmission from replete adult females to offspring. A 'sheep-tick-sheep' cycle was set up to determine whether feeding the progeny of these ticks on naïve sheep could lead to infection, and to compare clinical outcomes resulting from this transmission with those observed following infection by the natural *A. variegatum* (Av) vector. Using local strains of ticks and of *E. ruminantium* (BK242), we recorded, using the PCR technique, the presence of bacterial DNA in ticks (larvae for Av and females for Rm) engorged on sheep inoculated by BK242-infected blood. The bacterial DNA was also detected in the next stages of the lifecycle of *R. microplus* (eggs and larvae), and in sheep infested either by those *R. microplus* larvae or by *A. variegatum* nymphs moulted from larvae engorged on blood-inoculated sheep. Bacterial infection in these sheep was demonstrated by detecting antibodies to *E. ruminantium* using the MAP1-B ELISA and by isolation of the bacterium on cell culture from blood. The sequences of PCS20 gene detected in ticks and sheep were identical to that of the BK242 strain. Our results confirm that *R. microplus* can acquire and transmit *E. ruminantium* to the next stage. However, this transmission resulted in a mild subclinical disease whereas severe clinical disease was observed in sheep infested by *A. variegatum* infected nymphs, suggesting differences in the tick/bacteria relationship.

24.08.23

Breakout 2: OA24 Education in Veterinary parasitology

OA24.01

Adoption of virtual reality as an intrinsically motivating learning tool in parasitology_Preston, Dr. Sarah_Federation University Australia

Co-Authors - Dr Damien Whitburn, Mr Evan Dekker

Engagement of students in course content can be challenging. Immersive Virtual Reality (iVR) is a gamification teaching tool that has been utilised to increase engagement through experiential learning pedagogy. In this study we tested if tertiary parasitology students' experience would be enhanced through adoption of iVR as an intrinsically motivating learning tool. To evaluate this adoption, a custom experience was delivered to 102 science students from two universities, with feedback assessed using the Hedonic-Motivation-System- Adoption-Model (HMSAM). A survey tool, factor analysis and structural equation modelling was undertaken to evaluate response data testing relationships between constructs of the HMSAM. Respondents from both universities consisted of similar ages (85% and 77% between 18-25), similar "no-previous-exposure-to-VR" (65% and 71%) but considerable differences in gender (49/51% cf 33/66% male/female ratio). There was no significant difference in average response values for constructs except for Perceived Usefulness (5.2±1.3 cf 5.8±1.2, p<0.001). Model fit values proved HMSAM was appropriate for measuring student's hedonistic and utilitarian perspectives of the iVR experience with perceived ease of use, perceived usefulness, joy, ability to control, immersion levels and intention to use reflecting significant positive relationships. However, differing from similar studies, the curiosity construct was not found useful in this context.

OA24.02

Are Australian veterinarians prepared to diagnose and treat canine monocytic ehrlichiosis?_Oskam, Dr. Charlotte_Harry Butler Institute, Murdoch University

Co-Authors - Matthew Fox, Em. Professor Peter Irwin, Professor Rebecca Traub, Dr Amanda Barbosa, Harry Davis

In 2020, the exotic tick-borne disease, Canine Monocytic Ehrlichiosis (CME), was first reported in dogs in the Kimberley region of Western Australia. In response to the incursion of this serious disease, we surveyed Australian veterinarians to determine if they were prepared to diagnose and treat CME, with the hypothesis that these professionals are not prepared. An observational cross-sectional study was designed to assess the level of knowledge about CME and preparedness of Australian veterinarians to manage CME cases. A survey was directed to Australian veterinarians in clinical and non-practicing roles, as well as to veterinary students, between September 2021 and March 2022. The survey included questions about tick life-cycle and bacterial characteristics, clinical signs, diagnosis, and treatment of CME, as well as information regarding the implications of its introduction to Australia. Nine questions pertaining to knowledge of *Ehrlichia canis* and CME were assessed, with a pass mark of 70%. A total of 213 participants responded, which included 55 students, 36 veterinarians who have been in clinical practice for under five years, and 122 veterinarians who have been practicing for more than five years. Veterinarians with less than five years of clinical experience had a lower test of score (58.75%) when compared to veterinarians with more than five years' experience (68.54%). The student group scored 60.31%, while the national average was 66.05%. This is less than the pass mark of 70% and it can be concluded therefore that Australian veterinarians are currently not

prepared to diagnose and treat CME. Veterinarians in the Northern Territory scored higher than any other state with a mean score of 77.2% which correlates with their increased exposure to and experience with CME cases. Overall, these results identify a need for further education associated CME, as well as other exotic diseases with significant economic and welfare impacts.

OA24.03

Isolation of endo- and ectoparasites from zoological gardens for a forty-year period in Peninsular Malaysia. _Subramaniam, Prof. Vellayan_Universiti Teknologi Mara (uitm)

Endo- and ectoparasites isolated from Zoo Negara Malaysia are reviewed during 1980–2023 period. The parasites were collected since 1980 from zoo animals in Malaysia. The zoo animals mentioned in this paper were donated, purchased or exchanged to Zoo Negara Malaysia. Literature records of parasites from zoo animals in Malaysia were reviewed, resulting in a list of 59 species belonging to 14 different groups. Parasites obtained in this paper include blood nematodes (*Brugia pahangi*, *Cardiofilaria pavlovskyi*, *Dirofilaria immitis*, *D. repens*, *Loxodontofilaria asiatica*, *Setaria cervi*, *Thelezia* sp.), eye nematodes (*Oxispura mansonii*, *Cardiofilaria pawloskyi*, *Ceratospira* sp.), intestinal nematodes (*Ascaridia columbae*, *Contracaecum micropapillatum*, *Heterakis gallinarum*, *Syngamus trachea*, *Trichuris trichuria*, *Ascaridia* sp.), blood protozoa (*Babesia bovis*, *Plasmodium knowlesi*, *Trypanosoma evansi*, *Lankesterella* sp.), intestinal protozoa (*Balantidium coli*, *Blastocystis hominis*, *Cepedea dimidiata*, *Chilomastix mesmili*, *Entamoeba blattae*, *E. coli*, *E. histiolytica*, *E. ranarum*, *Giardia lamblia*, *Iodamoeba butschili*, *Nyctethorus cordiformis*, *Opalina ranarum*, *Tritrichomonas* sp.), tissue protozoa (*Sarcocystis nesbitii*, *S. singaporensis*, *S. zuoi*, *Sarcocystis* sp.), cestodes (*Raillietina celebensis*, *R. echinobothrida*, *R. microscolecina*, *Variolopsis* sp.), trematodes (*Echinostoma revolutum*, *Eurythrema pancreaticum*, *Paramphistomum epiclitum*, *Pfenderius papillatus*, *Fasciolopsis* sp., *Brachylaimidae*), acanthocephalans (*Centrotychidae*), fungi (*Aspergillus fumigatus*, *Cryptococcus neoformans*), pentastomes (*Armillifer moniliformis*), ticks (*Amblyomma helvolum*, *A. varanense*, *Haemaphysalis nadchatrami*, *Rhipicephalus microplus*, *R. sanguineus*, *Amblyomma* sp., *Haemaphysalis* sp.), mites (*Ophionyssus natricis*), leeches (*Hirudinaria manillensis*), parasitic flies (*Cephalopina titillator*, *Chrysomya bezziana*, *Gastrophilus ternicinctus*, *Gyrostigma pavesii*), and lice (*Haematomyzus elephantis*). The infection and infestation of the above parasites have resulted in the death of important zoo specimens. Recommendations for preventive actions and better care are given in this paper.

Keywords: Endoparasites; Ectoparasites; Zoo Animals; Peninsular Malaysia

OA24.04

WAAVP Education Sub-Committee: challenges and opportunities in Veterinary Parasitology_ Dr. Georgiana Deak_University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania

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Subtheme: Education in veterinary parasitology

Abstract

One of the missions of the WAAVP is “to encourage research in veterinary parasitology and to promote exchange of information and material between individuals and organizations interested in this field”. The Education, Training and Career Development Sub-Committee (EdCom) of the World Association for the Advancement of Veterinary Parasitology (WAAVP) was established in November 2021 aiming to promote our discipline worldwide by providing training courses and other opportunities principally, but not exclusively, for WAAVP members, students, academics and veterinarians with a specific interest in parasites of animals. Members of the sub-committee comprises volunteer veterinary parasitologists based in different continents and from both academia and industry. The remit of the EdCom include i) promoting WAAVP residency program in veterinary parasitology for young researchers from low- and middle-income countries, ii) organizing online courses and webinars, iii) organizing WAAVP-accredited training courses, iv) arrangement of engagement with universities for advancement of teaching veterinary parasitology, v) planning for joint education initiatives with organizations, institutions, scientific societies and foundations. Tangible first actions of the EdCom rules for the organization of training courses under the WAAVP have been established. Members of the EdCom intend to offer a voice to young researchers, to support their aspirations, to help/assist them by travel grants, specific scholarships and research grants, and to provide them moral and financial support for becoming successful and highly effective parasitologists

OA24.05

26 years of WAAVP African Foundation Scholarships (1997-2023): Next generation of veterinary parasitologists are unstoppable! _Krecek, Prof. Rosina_Independent Scholar

Co-Authors - Professor Annemarie Oldewage, Professor Barend Penzhorn, Professor Roger Prichard, Professor Stephen Were, Professor Isaac Phiri, Mrs Audra Wilburn Richburg

In 1997, the World Association for the Advancement of Veterinary Parasitology (WAAVP) African Foundation (AF) established a successful endowment which continues today. Travel scholarships are awarded to next-generation (NG) African veterinary parasitologists to present their research findings at WAAVP Conferences. To date, this Foundation has reviewed applications from 219 NG veterinary parasitologists from 25 African countries and awarded scholarships to 95 from 16 countries to travel and present their research at WAAVP Conferences in Europe, Asia, South America, North America, and Australasia. The WAAVP AF supports deserving awardees presenting their research to an international audience to bring recognition to their institutions, countries, academic fields, and to develop global networks. A 2020 peer-reviewed scientific journal publication reported that between 1999 and 2018, these

awardees published 808 articles in journals indexed by NCBI PubMed.gov and Web of Science. Of these, 124 were published in African journals. Since 2019, awardees published a further 212 papers, and 13 appeared in African journals. This total output is 1020 publications. The WAAVP AF is unique in supporting NG African veterinary parasitologists. This initiative is even more critical now in 2023 and for the future of Africa. Many previous awardees continue to promote their own graduate and postdoctoral students evidenced by an excellent output of research articles in peer-reviewed scientific journals. The WAAVP AF attributes its 26 years of success to being financially well invested in South Africa, a dedicated WAAVP AF Committee, a supportive WAAVP Executive Committee, and awardees committed to identify innovative and novel solutions to address parasitic diseases that plague animal and human health. The WAAVP AF promises for the foreseeable future to support accomplished veterinary parasitologists from across Africa.

OA24.05

Parasitology Summer Course (ParSCo) in Italy: local learning with international impacts_ Dr. Nicole Szafranski

Co-Authors - Dr. Jairo Alfonso Mendoza-Roldan, Dr. Marcos Antonio Bezerra-Santos, Dr. Livia Perles, Dr. Emanuele Brianti, Dr. Filipe Dantas-Torres, Prof. Domenico Otranto

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Subtheme: Education in veterinary parasitology

Abstract:

Parasitology Summer Course (ParSCo) is a weeklong intensive residency course organized by the Department of Veterinary Medicine of the University of Bari (Italy), accredited by the European Veterinary Parasitology College and the World Association for the Advancement of Veterinary Parasitology. ParSCo aims at providing veterinary parasitologists and graduate students with a combination of didactic lessons and practical training. The course focuses on parasitic pathogens and their arthropod vectors encompassing a wide variety of parasites of medical and veterinary importance within the Mediterranean Basin. The 2022 ParSCo course took place in Lipari of the Aeolian Islands north of Sicily allowing for the added uniqueness of studying these diseases within an isolated island environment.

ParSCo is divided into oral lectures (35.3%) and practical-based activities (64.7%) led by an international team of scientists and veterinarians working in veterinary parasitology. Lecture topics included arthropod vectors (e.g., ticks and sand flies) and their respective pathogens (e.g., *Leishmania* spp., *Dirofilaria* spp., *Thelazia callipaeda*, and tick-borne pathogens) as well as other parasites of domestic and wildlife species common in the Mediterranean region with special attention given to the reptilian populations of the Aeolian islands. The hands-on activities included arthropod collection methods, arthropod dissection, taxonomic identification of collected ecto- and endoparasites, and various diagnostic techniques. Fourteen participants

representing five continents with various parasitological backgrounds attended with a full sponsorship provided for one attendee from a lower income country. The real-world applicability and international reach of the last eight editions of this summer course has resulted in more than 100 attendees since 2012, thus promoting new research partnerships and collaborative projects to develop, resulting in over 30 scientific articles. In an increasingly interconnected world where parasitic pathogens and vectors continue to

spread to new regions, training opportunities such as ParSCo are key to preparing effective global veterinary parasitologists.

24.08.23

Breakout 3: OA25 Miscellaneous Parasites

OA25.01

In-vivo efficacy of chalcones functionalized nanoparticles on Plasmodium berghei ANKA infected mice model and their mode of action_ Dr. Shweta Sinha_Postgraduate Institute of Medical Education and Research, India

Co-Authors - Prof. Rakesh Sehgal, Prof. Uma Nahar Saikia

Introduction: Critical studies based on nanotechnology for the development of antimalarial drugs are aimed to solve key problems in malaria treatment, such as disease severity, reduced level of drug toxicity, interruption of transmission of Plasmodium sp, increased efficacy of drugs, and mainly focussed on combating multidrug resistance. In the present study the potentials of chalcones functionalized nanoparticles were assessed on Plasmodium berghei in vivo.

Material and Methods: The synthesis of nanoparticle and three nanoconjugates were done through standard chemical method followed by their characterization through UV-Vis Spectrophotometer, TEM, etc.. Most potent synthesised nanoconjugates screened from in vitro antimalarial drug sensitivity assay were chosen for in vivo study. For antimalarial activity Peters' 4-day suppressive test against P. berghei Anka infection was used.

Additional parameters such as, Rosetting frequency, Morphological & Histopathological examination, Survival assay was done for detail features. To study mechanism of action oxidative stress was measured in each groups of mice by measuring, nitric oxide, reduced glutathione and lipid peroxidation in the serum and liver or spleen tissue homogenates. For further clarity cytochrome c oxidase gene expression using RT-qPCR was checked in each treatment groups. The results were analysed using ANOVA followed by post-hoc tests. Values of $p < 0.05$ was considered as significant.

Results: Percentage suppression was also significantly reduced in all treatment group as compared to infected control groups. The rosette frequency was significantly reduced ($p < 0.05$) in CQ- treated and nano-conjugated group. The detailed histopathological features of liver and spleen sections shall be discussed while presentation in all allocated groups. Augmented levels of oxidative biochemical and gene expression confirms the role of oxidative pathway.

Conclusion: These chalcones functionalized nanoparticles provides a basis for designing

more such conjugates that may enhance the antimalarial activity and could be used for combating drug resistance Malaria.

Keywords: Plasmodium, Nanotechnology, Drug Resistance, RT-qPCR

OA25.02

Biosynthesis, characterization and anthelmintic activity of silver nanoparticles of *Clerodendrum infortunatum* isolate_Majumdar, Ms. Rima_Cooch Behar Panchanan Barma University

Co-Author - Dr. Pradip Kumar Kar

Over the past few decades, the green synthesis of nanoparticles has gained importance for their therapeutic efficacy and eco-friendly nature. Integrating green chemistry principles into multidisciplinary nanoscience research has paved the way for developing environmentally benign and sustainable methods for synthesizing gold and silver nanoparticles. In the present study, the flowers obtained from *Clerodendrum infortunatum* (L.), belonging to the family Verbenaceae, have been used for biosynthesizing silver nanoparticles (AgNPs) to evaluate the anthelmintic potential. UV-Vis spectroscopy, XRD, FTIR and TEM analyses were performed to ascertain the formation of AgNPs. *Clerodendrum*-derived AgNP (CLE-AgNP) has significantly affected the normal physiological functions of the poultry parasite *Raillietina* spp., a menace to the livestock industry. Our study manifests that CLE-AgNPs cause considerable distortion of the surface tegument of this cestode parasite leading to changes in the host-parasite interface. The histochemical localization studies of the tegument-associated enzymes viz. AcPase, AlkPase, ATPase and 5'-Nu, exposed to the drug, showed a substantial activity decline, thus establishing the anthelmintic potential of the CLE-AgNPs. The significant loss of activity of neuronal components such as NSE and ChE indicates the role of this green synthesized AgNP as an anthelmintic. Pharmacodynamic studies have also shown that it does not adversely affect the host when administered at the same molar dose.

OA25.03

Measuring electron transport chain function for biological and drug discovery against apicomplexan parasites. _Ramesh, Mr. SaiShyam_Australian National University

Co-Author - Dr. Alexander Maier

The mitochondrial electron transport chain (ETC) performs several biological functions such as a). generation of cellular ATP through oxidative phosphorylation, b). serving as an electron sink for metabolic pathways such as de novo pyrimidine biosynthesis and c). maintaining mitochondrial membrane potential. Proper functioning of mitochondrial ETC is necessary for the growth and survival of the apicomplexan protozoan parasites like *Babesia*, *Eimeria*, *Toxoplasma* and *Plasmodium*. The mitochondrial ETC of these protozoan parasites is an attractive target for antiparasitic drugs due to its highly distinct nature from the vertebrate host ETC. For example, atovaquone, which is used to treat *Toxoplasma* and *Plasmodium* infections, is highly selective for the parasite ETC. With increased treatment failure due to the spread of anti-parasitic drug resistance, it is important to look beyond current available drugs. However, to characterise the functions of novel protozoan protein and identify novel ETC inhibitors, we require a scalable assay to assess the ETC function. Here, we have used *P. falciparum* parasites as a model organism, to describe a methodology to assess the ETC function by measuring the oxygen consumption rate (OCR) using a Seahorse XFe96 extracellular flux analyzer. The method can be amenable to

screen compound libraries for identifying different ETC inhibitors, to locate the target of those inhibitors within the protozoan ETC and to characterise individual complexes of the ETC. We have successfully used this method to demonstrate the impact of novel drugs on the ETC of *P. falciparum*.

OA25.05

Drug exposure induces gene expression changes in ivermectin-resistant *Teladorsagia circumcincta* _Keane, Dr. Orla_Teagasc

Co-Authors - Dr Jason D. Keegan, Dr. Barbara Good, Dr. Paul Cormican

Anthelmintic resistance is now widespread in gastrointestinal nematodes (GIN) of livestock. However, the molecular mechanisms of resistance remain to be fully elucidated. In order to shed light on the genes and pathways activated by GIN in response to drug exposure, gene expression in resistant worms, exposed or unexposed to the anthelmintic ivermectin (IVM) was compared. Sheep infected with IVM-resistant *Teladorsagia circumcincta* were treated or not with IVM; the following day adult male worms were recovered from the abomasum and snap frozen. RNA was subsequently extracted from individual worms, cDNA prepared, amplified and used to generate sequencing libraries, which were pooled and sequenced on the HiSeq2500 with 100 bp paired-end reads. Sequence reads from all samples (n = 18) were used to generate a de novo transcriptome. Reads for each individual sample were subsequently aligned to this transcriptome. Genes differentially expressed (DE) between resistant worms exposed or not exposed to IVM were identified and Gene Ontology (GO) terms enriched among the DE genes were also detected. A large number of DE genes were detected between drug exposed and unexposed *T. circumcincta*. GO terms related to metalloendopeptidase activity, eating behaviour and pharyngeal pumping were significantly enriched among the genes more highly expressed in IVM-exposed worms, indicating that upregulation of genes involved in feeding, a process inhibited by IVM, may facilitate worm survival. GO terms related to ribosome, translation, metabolic processes and DNA replication were enriched among the genes more lowly expressed in worms exposed to IVM, possibly due to the diversion of resources from normal cellular processes to detoxification.

OA25.06

Epidemiological study of cephalopinosi in the United Arab Emirates_ Dr. Bojan Gajic_UAE University, UAE

Co-Authors - Dr. Moneeb Qablan, Dr. Layaly Hamdan, Prof. Tamara Ilic, Dr.

Temesgen Sulyman

Cephalopina titillator, the camel nasal bot fly, is a dipteran species causing obligatory nasopharyngeal myiasis in camels. Most common clinical symptoms of cephalopinosi comprise loss of appetite, nasal discharge, sneezing, snorting, coughing and breathing difficulties. Larval instars of the camel bot fly damage host nasal and pharyngeal mucosa, resulting in congested mucous membranes, mild hemorrhage, abundant mucous secretion and development of multiple small granulomas at the site of larval attachment. Additionally, infestation affects the camels' general wellbeing resulting in diminished milk and meat production as well as impairing the physical performance of racing animals. Prevalence of cephalopinosi in dromedary camels from some African and Asian countries is high varying from 52.3% to 82.6%. However, while there is a lot of data from neighboring countries, no studies have been conducted on *C. titillator* in the United Arab Emirates. Therefore, the aim of our study was to determine the prevalence, larval burden and seasonal dynamics of cephalopinosi in UAE camels.

From January to May 2023, the heads of 397 dromedary camels of different sex and age were collected from the local abattoirs and inspected for the presence of *C. titillator* larval stages. In total, 24.7% of the animals were positive, with the maximum of 67 larvae detected per animal. Infestation peak was observed in January, with constant decrease in the following months. During the study period, only L2 and L3 larval instars were recovered.

Our results show that *C. titillator* is highly prevalent in dromedary camels in the UAE. However, a long-term study is needed to elucidate the parasite seasonal dynamics and identify the most optimal timing to apply preventive and control measures.

24.08.23

Breakout 4: OA26 Avian Parasites

OA26.01

Long-term efficacy of a drug-loaded polymeric device on lice-infested laying hens_Lanusse, Dr. Carlos_Conicet, Argentina

Co-Authors - Ms. Paula Dominguez, M.Vet Jose Rodriguez, Dr Marcelo Del Hoyo, M.Vet Patricio Vidondo, Dr. Carlos Lanusse, Prof. Luis Alvarez

Lice infection is an important health problem in aviculture, especially in free-ranged laying hens. However, few specific treatments intended to lice control in aviculture are available. The goal of the current work was to evaluate the long-term efficacy of a drug-loaded polymeric device in lice-infested laying hens. Since lice are obligate parasites, the medicated device was challenged in different groups of lice-infested hens. For that, a cypermethrin/deltamethrin-based polymeric device (ring shape) was placed in the shank of laying hens (n= 6, Group A) infected with lice (*Goniocotes gallinae*), which shared the pen with other infected non-treated hens (n= 40). Lice were counted pre-treatment, and at different times post-treatment including 1, 4, 7, 14, 21, and 30 days. At this time, ring polymeric devices were removed and placed in a different group (Group B) of lice-infested laying hens. A similar scheme of counting lice to that described for Group A was carried out in Group B. After the month of treatment, the rings were placed in a new group (Group C) of lice-infested hens, and the lice count was repeated as described. Overall, the control of the lice-killing effect of the device was monitored for 3 months. No systemic or local adverse effects were observed in treated animals. The device reached 100% efficacy at 1-day post-treatment in Group A and at 4 days post-treatment in Groups B and C. In all groups, the animals remained free of lice until the time the ring was removed to be placed in a new group of infested hens. Three months after continuous use of the device, all treated animals (Group C) were kept free of lice. The results obtained show that the medicated device was effective for the long-term control (three months) of lice in laying hens.

OA26.02

In vitro comparative evaluation of oocysticidal and sporulation inhibition effect of essential oils of *Trachyspermum ammi* and *Cymbopogon flexuosus* against *Eimeria tenella*_ Dr. Aditi Kalita_TANUVAS, India

Medicinal plant like *Trachyspermum ammi* and *Cymbopogon flexuosus* have been shown to possess many therapeutic properties against animal and human diseases including chicken coccidiosis. Chemical anticoccidial drug resistant has been increased in recent days due to which identification of molecule is need of the hour. This study was aimed to evaluate the in vitro comparative effect of essential oils (Eos) of *Trachyspermum ammi* and *Cymbopogon flexuosus* against *Eimeria tenella* oocysts. Essential oil from both *Trachyspermum ammi* and *Cymbopogon flexuosus* were obtained through hydro-distillation using Clevenger apparatus at 80° C for 1-2 hours. For in vitro trials, 20, 30 and 40 per cent of working concentration were prepared from essential oil and ethanol mixture (1:5 ratio stock solution). Prepared working solution of different concentration were tested against *Eimeria tenella* oocysts. Oocysticidal activity was evaluated by either destruction or sporulation inhibition of the oocysts. The results of the study revealed that 45.0 ± 0.25 , 43.0 ± 0.18 and 27.0 ± 0.29 per cent of the sporulated oocysts and 27.8 ± 0.18 , 12.0 ± 0.19 and 59.0 ± 0.55 per cent of unsporulated oocysts were damaged in 20, 30 and 40 per cent concentration of *Trachyspermum ammi* essential oil, whereas *Cymbopogon flexuosus* found to

have caused damaged 25 ± 0.36 , 35.6 ± 0.16 and 20.0 ± 0.41 per cent of sporulated oocysts and 11.8 ± 0.20 , 16.5 ± 0.25 and 32.0 ± 0.08 unsporulated oocysts in 20, 30 and 40 per cent essential oil concentration respectively. In positive control group (1 % Sodium hypochlorite), 78.0 ± 0.03 and 17.3 ± 0.21 per cent sporulated and unsporulated oocysts respectively were found damaged. While 81.31 ± 0.06 and 80 ± 0.03 per cent oocysts sporulated and did not show any damage in the negative control groups (50 % ethanol and water). Highest sporulation inhibition of 59.0 ± 0.55 per cent was recorded in the 40 per cent *Trachyspermum ammi* Eos group compared to *Cymbopogon flexuosus* Eos

OA26.03

Phytogetic feed additive as an alternative method to control poultry red mites (*Dermanyssus gallinae*, De Geer, 1778) _Bosnjak-Neumüller, Dr. Jasna_PATENT CO.

Co-Authors - Dr. Dajana Todorović, Dr. Larisa Ilijin, Dr. Vesna Perić-Mataruga, Dr. Nikola Delić, Dr. Aleksandar S Stanojković, Dr. Marko Pajić, Dr. Slobodan Knežević, Dr. Jog Raj, Dr. Marko Vasiljević, Prof. Olivier Sparagano

The aim of this research was to evaluate a phytogetic feed additive (PFA), RIDofMITE® (PATENT CO. DOO), in managing a naturally occurring poultry red mite (PRM), *Dermanyssus gallinae*, the infestation of laying hens in a cage system. In the trial farm, 52 weeks old, 1,560 laying hens (ISA Brown line) were housed for 45 days. The mites were gathered using uniform AVIVET traps. The traps were left for 2 days and collected on days -12, -5. and 0 before treatment (control period), and on days 2, 5, 8, 11, 16, and 23. and 30 after treatment (trial period) when hens started to consume PFA, at a dose of 500g/ton. After storing the PRM mites at -80°C for 48 hours, the number of blood-fed adults was counted. Egg production and the general health of the hens were monitored daily. Normal distribution of data was tested following the Kolmogorov–Smirnov test, while the Kruskal-Wallis analysis of variance and Mann-Whitney U test (post hoc) were used to evaluate the impact of PFA on the population of PRM ($P < 0.05$). Significant differences in the number of blood-fed adults were observed between traps collected 12 days (417.00 ± 107.36) and 5 (1229.55 ± 259.19) ($P < 0.05$) days before the administration of PFA and between Day 12 before the trial and Day 0 (1121.25 ± 18)1.60 of the trial ($P < 0.01$). The number of blood-fed adults was lower in all traps set after PFA inclusion in the feed compared to their number on day 0 of the trial, but the difference was only significant on day 15. (282.30 ± 78.15 ; $P < 0.01$), 23. (299.20 ± 69.97 ; $P < 0.001$) and 30. (245.65 ± 68.71 ; $P < 0.001$). Tested PFA, in this study conditions, controls PRM presence.

OA26.04

Assessment of *Eimeria* oocysts load in production animal fecal samples examined at the Cornell Animal Health Diagnostic Center, Ithaca, NY, USA_Frye, Dr. Elisha_Cornell Animal Health Diagnostic Center

Co-Authors - Ms Holly White, Dr Manigandan Lejeune

Coccidiosis is a common protozoan disease of production animals characterized by diarrhea, tenesmus, dehydration, and death. The most pathogenic coccidia of economic significance is *Eimeria bovis* and *E. zuernii* in cattle; *E. ovinoidalis* in sheep; and *E. ninakohlyakimovae* and *E. caprina* in goats. A retrospective analysis of fecal flotation tests ($n=14,726$) performed at the Cornell Animal Health Diagnostic Center during 2019-2022 was carried out to assess coccidia load [oocysts per gram (OPG)] and to determine frequency count for pathogenic *Eimeria* species. Of 6,014 bovine samples examined, *Eimeria* oocysts were detected in 2,247 (37.4%). A load of <100

OPG was recorded in 1,499 cattle (66.7%) and >10K OPG in 80 cattle (3.6%) of 2,247 infected. *Eimeria bovis* and *E. zuernii* were observed in 1,390 (61.9%) and 337 (15.0%) of 2,247 positive bovine cases, respectively. Additionally, diarrheic feces were noted in 441 *E. bovis* (31.7% of 1,390) and 150 *E. zuernii* (44.5% of 337) positive cases. Of 3,099 ovine samples, *Eimeria* oocysts were detected in 2,461 (79.4%). Less than 100 OPG was found in 1,431 (58.1%) and >10K in 63 (2.6%) of 2,461 infected sheep. *Eimeria ovinoidalis* was found in 43.3% of 2,461 oocyst-positive sheep. Of 5,613 caprine samples, 4,951 (88.2%) had *Eimeria* oocysts. A load of <100 OPG was detected in 1,844 (37.2%), 100-9,999 OPG in 2,874 goats (58%), and >10K in 233 (4.7%) of 4,951 infected goats. *Eimeria caprina* and *E. ninakohlyakimovae* were found in 1,227 (24.8%) and 1,860 (37.6%) of the 4,951 positive goats, respectively. In summary, *Eimeria* oocysts were detected in 37.4% of cattle, 79.4% of sheep, and 88.2% of goats examined. Loose feces poorly correlated with pathogenic cattle *Eimeria* detection. Most sheep and cattle had OPG <100, while most goats had 100-9,999 OPG. Clinical assessment coupled with OPG determination must form the basis for treating coccidiosis.

OA26.05

Prevalence of Duck Parasites in Telangana State, India_ Prof. M. Udayakumar_PVNRTVU, India

Co-Author - Professor Sreenivasa Murthy

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A study conducted on the prevalence of duck helminth parasites in four districts of Telangana state, India based on necropsy and coprological examinations revealed an overall prevalence of 86.82 % (303/349) of parasitic infections of which, mixed and single infections were 77.65 % and 9.17 %, respectively. The incidence of trematodes was highest (80.52 %) followed by cestodes (35.24 %) and nematodes (12.32 %). Nine trematode species viz., *Echinostoma paralum* (74.21 %), *E. revolutum* (28.94 %), *Hypoderaeum conoideum* (15.19 %), *Prosthogonimus cuneatus* (3.72 %), *P. ovatus* (29.23 %), *Philopthalmus* spp. (30.37 %), *Notocotylus* spp. (24.93 %), *Catantropis verrucosa* (9.74 %) and *Tracheophilus cymbius* (0.57 %), two cestode species viz., *Hymenolepis* spp. (28.94 %) and *Fimbriaria fasciolaris* (22.35 %) and a single nematode *Tetrameres* spp (12.32 %) were recovered from necropsied ducks. A single species of louse viz., *Menacanthus stramineus* was found in ducks over 4 months of age.

The parasitic incidence was highest in summer (95.42 %) followed by winter (82.95 %) and rainy (79.78 %) seasons. Prevalence of trematodes, cestodes and nematodes in summer, winter and rainy seasons were 89.31 %, 79.84 % and 68.54 %; 24.33 %, 41.86 % and 41.57 % and 14.50 %, 10.08 % and 12.36 %, respectively. Significantly ($P \leq 0.05$), high prevalence was recorded in 0-2 months (92.37 %) of ducks compared to those of 2-4 months (83.63 %) and 4 months- 2 years (82.98 %) age group of ducks. Incidence of trematodes (87.79 %) was highest in 0-2 months age group followed by cestodes (23.66 %). Similarly the incidence of trematodes (80.12 %) was highest followed by cestodes (35.09 %) and nematodes (4.68 %) in 2-4 months aged ducks. Whereas in 4 months - 2 years age group, the incidence of nematodes (74.47 %) was highest followed by cestode (68.09 %) and trematode (61.70 %) infections. Sex wise studies indicated a non-significant higher prevalence of duck parasites in females (88.40 %) than in male (85.12 %) ducks. Among the helminths, the trematode, cestode and nematode parasites in male and female ducks were 80.95 %, 32.74 % and 8.33 % and 80.11 % , 37.57 % and 16.02 %, respectively.

Key words: Duck, helminth, parasite, prevalence

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24.08.23

Breakout 1: OA27 Canine & Feline Parasites

OA27.01

Molecular survey on non-apicomplexan vector-borne pathogens in domestic and wild felids in Romania_ Dr. Luciana-Catalina Rus (Panait)_University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

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Abstract:

The epidemiology of vector-borne pathogens is constantly evolving, with a recent increase in global prevalence and a larger geographic spread. However, information about non-apicomplexan vector-borne agents circulating in felids in Romania is limited, the present study aiming to update the current knowledge by investigating their presence and distribution. Overall, 421 blood and tissue samples (371 domestic cats, 34 wild cats, and 6 Eurasian lynx) were molecularly screened for spotted fever group rickettsiae, *Bartonella* spp., *Ehrlichia* spp., *Anaplasma phagocytophilum*, *Borrelia burgdorferi sensu lato*, *Francisella tularensis*, *Dirofilaria immitis* and *D. repens*. In domestic cats, the overall positivity was 19.4% for *A. phagocytophilum*, 1.1% for *E. canis* and 0.8% for *Bartonella* spp. (*B. henselae* in two cats and *B. clarridgeiae* in one cat). No coinfections were detected. The only pathogen found in wild felids was *A. phagocytophilum*, with an infection rate of 50%. *E. canis*, *B. henselae*, *B. clarridgeiae* and *A. phagocytophilum* are molecularly described for the first time in domestic cats in Romania. However, more studies are needed into

the epidemiology, clinical significance and the possible involvement of various arthropod vectors in their transmission.

Keywords: vector-borne pathogens, domestic cats, wild felids, PCR, Romania

OA27.02

Prevalence, risk factors and clinical features of *Hepatozoon* spp. infections in domestic cats from the Mediterranean basin_Carbonara, Ms. Mariaelisa_University of Bari

Co-Authors - Prof. Roberta Iatta, Dr. Giovanni Sgroi, Prof. Elias Papadopoulos, Dr. Clara Lima, Prof. Emilie Bouhsira, Prof. Guadalupe Miró, Prof. Gad Baneth, Prof. Domenico Otranto

Domestic cats with outdoor lifestyle are highly exposed to arthropod vectors as well as to many pathogens they may transmit. Although tick-borne diseases (TBDs) are well documented in dogs, data in cats are still scant and usually characterized by a non-specific clinical picture. In this study we evaluated the prevalence of apicomplexan parasite infections, associated risk factors, clinical signs and clinicopathological findings in domestic feline populations of six Mediterranean countries. Between September 2019 and August 2021, blood and serum samples were collected from cats living in France, Greece, Israel, Italy, Portugal and Spain (100 per country; n = 600), along with animal information (i.e., age, sex, breed, housing conditions and geographical origin), clinical status and laboratory parameters. Cats were grouped by age as kittens (up to 1-year), young (between 1- 6 years), mature (between 7-10 years) and senior (older than 10 years). Blood samples were tested for the detection of *Hepatozoon* spp. and piroplasmids by conventional PCR targeting the 18S rRNA gene. Overall, a 14.5% prevalence of *Hepatozoon* spp. infection was recorded, with a higher occurrence in cats from Greece (30%) and Portugal (23%), followed by Spain (15%), Israel (15%) and France (4%); cats from Italy scored negative. *Hepatozoon felis* was identified in 86 animals (14.3%), with three different sequence types, whereas *H. silvestris* in one shelter cat from Portugal (0.2%). No piroplasmid DNA was amplified. The risk of *Hepatozoon* spp. infection was linked to geographical origin, housing condition and age. No significant correlation was found with any clinical manifestation, while increased serum ALT activity was the only laboratory abnormality associated with the infection. Data suggest that domestic cats from the Mediterranean basin, mainly shelter or free roaming and young animals, are highly infected with *H. felis* and only occasionally with *H. silvestris*, with subclinical or mild clinical signs.

Keywords: cats; *Hepatozoon felis*; *H. silvestris*; clinical-pathological findings

OA27.03

The “Chicão” project: a community-based initiative toward controlling canine vector-borne diseases_De Abreu Teles, Dr. Pedro Paulo_University of Bari

Co-Author - Dr. Guilherme Ribeiro Valle, Dr. Filipe Dantas-Torres, Dr. Jennifer Ottino, Dr. Marcos Antonio Bezerra-Santos, Dr. Jairo Alfonso Mendoza-Roldan, Prof. Domenico Otranto, Dr. Vitor Márcio Ribeiro

Canine vector-borne diseases (CVBDs) represent a major threat to animal health, especially in poor socio-economic contexts in tropical and subtropical regions, where the access to veterinary services is scarce. With the objective of controlling CVBDs in a community with limited access to veterinary services, a multisectoral initiative (“Projeto Chicão”) was implemented from 2011 in São Joaquim de Bicas municipality (20°02'56”S, 44°16'26”W), Minas Gerais state, south-eastern Brazil. During the past 12 years, a team of volunteers including veterinarians, students, researchers and citizens, provided monthly assistance in many fields to the local population and their dogs. The initiative was organized by the Pontifical Catholic University of Minas Gerais and based on five main pillars: 1. Clinical care (i.e., reception, sorting, anamnesis, clinical evaluation, sample collection, treatment and control of infectious diseases); 2. Field diagnostic laboratory; 3. Health education for the community (i.e., lectures for adults and games for children); 4.

Collaboration and fundraising campaign to cover costs of the project; and 5. Research (i.e., epidemiological and clinical data analysis). The main CVBDs diagnosed combining both serology and blood PCR were anaplasmosis, babesiosis, ehrlichiosis and leishmaniosis. Data obtained were presented to the local community and owners to increase awareness about CVBDs and the importance of their prevention. This multisectoral initiative may represent a bridge between research and control of CVBDs, allowing the translation of locally acquired scientific information into practices that could improve the health and welfare of dogs and their owners in deprived communities.

Keywords: Vector borne diseases; Zoonoses; One health; arthropod control.

OA27.04

Does *Hepatozoon canis*, which is common in African wild dogs (*Lyacon pictus*), negatively impact populations of this endangered canid?_ Penzhorn, Prof.

Barend_University of Pretoria, South Africa

Co-Authors - Dr Edward C Netherlands, Ms Carlie Stroebel, Prof Louis H du Preez, Ms Ntji Shabangu, Prof Paul Tshepo Matjila, Dr Ockert Louis van Schalkwyk

Barend L. Penzhorn, Faculty of Veterinary Science, University of Pretoria, South Africa

With numbers declining, African wild dogs (*Lyacon pictus*) or 'painted wolves' are an endangered species. As such, any pathogens that could potentially negatively impact African wild dog populations are worthy of scrutiny. There are several published reports of *Hepatozoon* from African wild dog populations. Since these reports were based on examination of blood smears by microscopy, specific identity of the *Hepatozoon* gamonts seen could not be confirmed. In a general health survey of African wild dogs in the Kruger National Park, South Africa, blood specimens (n = 75) collected from 54 individuals were examined by molecular characterisation for the presence of *Hepatozoon* spp. At first sampling, specimens from 42 of 54 individuals (77.7%) were positive, based on the primer set HepF300 and HepR900. Twenty individuals were resampled between 51 and 69 days after first sampling; one of these was resampled twice. Samples from six individuals that had tested negative previously now reacted positive. Assuming that all 54 individuals were still alive, the prevalence had therefore increased to 48 individuals infected, or 88.8%. Resultant 18S rDNA sequences isolated from these specimens share a 99% similarity to *Hepatozoon canis* sensu strictu. Phylogenetic analysis placed the *Hepatozoon* sp. from African wild dogs within the *H. canis* cluster, which includes *Hepatozoon* from other canid and tick hosts. *Hepatozoon* schizonts can cause severe lesions in various tissues, including muscles, especially in immunocompromised individuals. African wild dogs are sedentary only when denning to raise pups; otherwise, they are nomadic and constantly on the move. It is postulated that severely affected individuals would not survive during the nomadic phase.

OA27.05

Prevalence and risk factors associated with ectoparasites of owned dogs in two Caribbean islands _Charles, Dr. Roxanne_The University of The West Indies

Co-Authors - Ms Samantha Cooper, Ms Tashira Graham, Ms La Shontae Shepherd, Mr Andy Williams, Dr. Reda Mohamed

Dogs are known to host a number of ectoparasites of veterinary and public health significance. A cross-sectional survey was conducted on two Caribbean islands to determine the prevalence and factors associated with ectoparasites of pet and working dogs. A total of 275 dogs

(Trinidad=137; Jamaica=138) were screened for ectoparasites and questionnaires were completed to obtain information on location, age, sex, breed and management practices by owners/handlers. A total of 38 dogs (27.7%) from Trinidad and 28 dogs (20.3%) from Jamaica were infested with at least one species of ticks, fleas or mites. *Rhipicephalus sanguineus* was the most prevalent tick in Trinidad (16%) and Jamaica (14.5%) while *Ixodes* spp. was found only in Jamaica (0.7%). *Ctenocephalides felis* was more prevalent in Trinidad dogs (22.6%) compared to Jamaica (4.3%), and *Pulex irritans* was found on one dog (0.7%) in Trinidad. The ear mite, *Otodectes cynotis* was detected on a dog from Jamaica. Parasite prevalence was significantly higher in pet and intact female dogs on both islands and outdoor dogs in Jamaica only ($p < 0.05$). Apart from causing direct harm to their hosts, the arthropods detected in this study are known vectors of pathogens of veterinary and public health importance. As such, client education on the treatment and control of ectoparasites on both islands is warranted.

OA27.06

Do physical attributes of topical formulations impact how cat owners choose antiparasitic treatments?__Wright, Dr. Andrea_Zoetis

Co-Authors - Kennedy Mwacalimba, George Vasliakos, Satoshi Matsumoto, Yasuho Onai, Tatsuya Suzuki, Danielle Riley, Ashely Enstone

Topical formulation physical attributes may impact cat owner preference for different antiparasitic treatments. Physical attributes may also be a barrier for compliance/adherence to administration. The purpose of this study was to compare the physical qualities (stickiness, drying time and usability) of commercially available cat spot-on formulations of Revolution Plus® (0.5ml-1.0ml), Revolution® (0.75ml), Broadline® (0.9ml), and Frontline Plus® (0.5ml). Three healthy cats with no abnormalities in coat or skin condition were administered each formulation by body weight per label instructions. Each attribute was evaluated, and photos taken after 5min, 10min, 15min, 30min, 1hr, 4hrs, 6hrs, 24hrs and 48hrs to visually assess and score drying time. Viscosity (millipascal-seconds) and volatile matter were also measured (Institute of Food and Environmental Hygiene, Inc. Japan). In a follow up qualitative assessment, product profiles based on formulation labels and time-series photos were developed to serve as interview stimuli with owners with experience in administration of spot-on formulations. One-hour qualitative interviews were conducted with 8 cat owners in 4 countries (Australia UK, Greece, and Canada). Transcripts were analyzed thematically to identify product attributes most important to owners. The laboratory study demonstrated that Revolution Plus and Revolution were less sticky and dried faster than Broadline and Frontline Plus. Broadline viscosity was 28.8 mPa.s, followed by Frontline Plus (16.0 mPa.s), Revolution Plus (3.19 mPa.s) and Revolution (2.91 mPa.s). The usability scored equally for Revolution Plus and Revolution. Overall, three key overarching themes emerged as important concepts for pet owners when considering the ease of use of topical formulations: physical properties, negative precautions following administration and the overall ease of use. The results suggest that physical attributes (drying time, oiliness, volume, risk of creating a mess and appearance at application site) of spot-on formulations may influence cat owner preference for topical formulations and their compliance and adherence to administration.

24.08.23

Breakout 2: OA28 Novel Diagnostics II

OA28.01

The revised WAAVP anthelmintic efficacy guidelines. _Geurden, Dr. Thomas_Zoetis

Co-Authors - Emily Smith, Jozef Vercruysse, Tom Yazwinski, David Burden, Frederic Beugnet, Steffen Rehbein, Martin Nielsen

For several decades, the WAAVP (World Association for the Advancement of Veterinary Parasitology) has issued guidelines to assist in the planning, conduct and interpretation of studies designed to assess anthelmintic efficacy based on parasite counts or diagnosis of infection. These WAAVP guidelines have been recently revised and continue in the provision of science-based and internationally harmonized recommendations for the efficacy assessment of anthelmintic drugs in food-producing and companion animals, including recommendations for the evaluation of faecal egg count reduction in field studies, in consideration of the new WAAVP guidelines for faecal egg count reduction testing. A new general anthelmintic efficacy guidelines document was published and outlines the general principles of anthelmintic efficacy evaluation which are applicable to all host animal species and types of studies (dosage determination, dosage confirmation and field studies). This general guideline was complemented by species-specific guidelines, which provide updated and detailed recommendations for the individual host species and their specific parasites. The revised guidelines include an assessment of scientific and technological advances since previous guideline versions. Scientific advances inherent to the determination of anthelmintic efficacy that were considered during the current review, but were not addressed in the revised guidelines, are discussed in a separately published WAAVP reflection paper. The revised WAAVP anthelmintic guidelines no longer provide guidance for evaluation of generic anthelmintic drugs nor do they provide regulatory guidance, as this is the remit of the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) anthelmintic guidelines. The most relevant changes will be discussed along with any proposed changes considered yet not maintained.

OA28.02

WAAVP Guidelines: Update on Progress and Discussion_Fernandez, Dr.

Silvina_CONICET / National University of Central Buenos Aires Province

Co-Authors - Dr. Thomas Geurden, Dr Barry Hosking, Dr Ray Kaplan, Dr Emilie Bouhsira, Dr Anja Joachim, Dr Jozef Vercruysse, Prof. Edwin Claerebout, Dr Livio Costa-Júnior, Dr Maggie Fisher

Following the lively discussion in the last WAAVP Conference in Dublin, the WAAVP Guideline Subcommittee (GLSC) has been very active. Suggestions made at the last conference have been considered and the outcomes will be shared. There has been a number of changes in membership of the GLSC, with several new members joining and Standard Operating Procedures have been put in place for internal processes. In addition to considerable progress on the review and updating of older guidelines, most notably the general anthelmintic and ectoparasiticide guidelines, work has begun on new guidelines including assessment of anthelmintics against liver flukes and vaccines as well as a review of the nomenclature guideline. This presentation will begin with a brief overview of considerations given to suggestions, work undertaken in the past two years, future plans and then the floor will be opened for discussion.

OA28.03

Characterization of the cytochrome P450 oxidoreductase in *Rhipicephalus microplus* ticks as a potential drug target_Sparagano, Prof. Olivier_City University of Hong Kong

Co-Authors - Mr. Adeyinka Fadahunsi, Mr Christopher Kumm, Dr Kirsty Graham, Dr Adalberto Perez de Leon, Dr Felix Guerrero, Prof. Olivier Sparagano, Dr Robert Finn, Mr. Adeyinka Fadahunsi.

Management of the cattle tick, *Rhipicephalus microplus*, presents a challenge because some populations of this cosmopolitan and economically important ectoparasite are resistant to multiple classes of acaricides. Cytochrome P450 oxidoreductase (CPR) is part of the cytochrome P450 (CYP450) monooxygenases that are involved in metabolic resistance by their ability to detoxify acaricides. Inhibiting CPR, the sole redox partner that transfers electrons to CYP450s, could overcome this type of metabolic resistance. This report represents the biochemical characterization of a CPR from ticks. Recombinant CPR of *R. microplus* (RmCPR), minus its N-terminal transmembrane domain, was produced in a bacterial expression system and subjected to biochemical analyses. RmCPR displayed a characteristic dual flavin oxidoreductase spectrum. Incubation with nicotinamide adenine dinucleotide phosphate (NADPH) lead to an increase in absorbance between 500 and 600 nm with a corresponding appearance of a peak absorbance at 340–350 nm indicating functional transfer of electrons between NADPH and the bound flavin cofactors. Using the pseudoredux partner, kinetic parameters for both cytochrome c and NADPH binding were calculated as $26.6 \pm 11.4 \mu\text{M}$ and $7.03 \pm 1.8 \mu\text{M}$, respectively. The turnover, K_{cat} , for RmCPR for cytochrome c was calculated as 0.08 s^{-1} which is significantly lower than the CPR homologues of other species. IC₅₀ (Half maximal Inhibitory Concentration) values obtained for the adenosine analogues 2', 5' ADP, 2'- AMP, NADP⁺ and the reductase inhibitor diphenyliodonium were: 140, 82.2, 24.5, and 75.3 μM , respectively. Biochemically, RmCPR resembles CPRs of hematophagous arthropods more so than mammalian CPRs. These findings highlight the potential of RmCPR as a target for the rational design of safer and potent acaricides against *R. microplus*.

OA28.04

Morphological and molecular characterization of *Fasciola hepatica* and *Fasciola gigantica* phenotypes from co-endemic localities in Mpumalanga and KwaZulu-Natal provinces of South Africa_Malatji, Dr. Mokgadi Pulane_University of Kwazulu-natal

Co-Authors - Ms Sayurika Haridwal, Prof Samson Mukaratirwa

Fasciolosis is a food- and water-borne disease caused by digenean trematode species, *Fasciola hepatica* and *F. gigantica*. They are widely distributed and infect a wide range of definitive hosts, causing enormous economic loss due to reduced productivity in domestic ruminants. The two species are co-endemic in KwaZulu-Natal and Mpumalanga provinces of South Africa. This study aimed characterizing *Fasciola* spp. collected from cattle slaughtered at abattoirs located in the two provinces where two species are endemic. Seventy-one liver fluke specimens were collected from cattle abattoirs in Enhlazeni and Nelspruit in Mpumalanga province, Pietermaritzburg in KwaZulu-Natal province, and Zimbabwe. *Fasciola gigantica* specimen was confirmed as the only species occurring in Zimbabwe, and used as control in the morphological and molecular assessment of the collected specimens. Of the 71 specimens collected, 37 were classified as *F. hepatica*, 12 as *F. gigantica* and 22 as *Fasciola* spp. using morphological characters. Of these species, 11 of 37 *F. hepatica* and 6 of 22 *Fasciola* spp. were aspermic or having very scanty sperm. *Fasciola* spp. specimens consisting of 15 spermatid liver flukes and 5 aspermic liver flukes are randomly chosen used for molecular identification.

Analysis of the 15 spermatic specimens confirmed the presence of *F. hepatica* (n = 9) and *F. gigantica* (n = 6) using the CO1 marker and as *F. hepatica* (n = 4), *F. gigantica* (n = 7) using the ITS-1/5.8S/ITS-2 marker. The remaining 4 aspermic flukes (one did not resolve) morphologically identified as *F. hepatica* were confirmed them as *F. hepatica* (n = 4) by both CO1 and ITS-1/5.8S/ITS-2. Phylogenetic analysis based on both CO1 and ITS-1/5.8S/ITS-2 showed that *F. hepatica* species formed a moderately supported monophyletic clade with *F. gigantica*. Their ancestral history was further confirmed by haplotype network, which formed novel haplotypes that corresponded with the structure of the phylogenetic tree. Results from this study showed that morphological characters alone have limitations in distinguishing *F. hepatica* and *F. gigantica*, and accentuate the importance of complementing morphological techniques by molecular techniques, especially in endemic areas where the two species are co-endemic.

Keywords: *Fasciola* species, Morphometrics, Co-endemic, South Africa, ITS-1/5.8S/ITS-2, CO1.

OA28.05

Molecular characterization of the interplay between *Fasciola hepatica* juveniles and laminin as a mechanism to adhere to and break through the host intestinal wall_González-Miguel, Dr. Javier_IRNASA-CSIC

Co-Authors - María Torres-Valle, Ms. Marta López-García, David Becerro-Recio, Mar Siles-Lucas, Dr. Javier González-Miguel

The trematode *Fasciola hepatica* is the main causative agent of fasciolosis, a zoonotic disease of growing veterinary and public health concern. *F. hepatica* metacercariae are ingested by the host and excyst in the intestine thereby releasing the newly excysted juveniles (FhNEJ), which break through the gut wall and migrate towards the major biliary ducts. Since blocking *F. hepatica* development is particularly challenging after crossing of the intestinal wall, targeting this first step of intra-mammalian migration might result in increased therapeutic success rates. The intestinal extracellular matrix (ECM) is constituted by a network of structural proteins, including laminin (LM) and fibronectin (FN), that provides mechanical support while acting as physical barrier against intestinal pathogens. These infectious agents have evolved multiple mechanisms to subvert the intestinal ECM by expressing proteins that bind to and degrade a variety of ECM components, mechanisms that remain largely unexplored in FhNEJ. Here, we employed ELISA and immunofluorescent assays to test for the presence of LM- and FN-binding proteins on a tegument-enriched antigenic fraction of FhNEJ, and further determined their identity by two-dimensional electrophoresis coupled to mass spectrometry analysis. Additionally, we performed enzymatic assays that revealed for the first time the capability of the juvenile-specific cathepsin L3 to degrade LM, and that LM degradation by FhNEJ proteins is further potentiated in the presence of host plasminogen. Finally, a proteomic analysis showed that the interaction with LM triggers protein changes on FhNEJ that may be relevant for parasite growth and adaptation inside the mammalian host. Altogether, our study provides valuable insights into the mechanisms employed by FhNEJ to cross the intestinal wall, which may lead to the identification of targetable candidates for the development of more effective control strategies against fasciolosis. Funding: RTI2018-093463-J-I00 by MCIU, AEI and FEDER, and CLU-2019-05 and CL-EI-2021-01 by JCYL and European Union ERDF.

Assessment of antibody response in Balb/c mice experimentally infected with *Toxocara canis* using two recombinant (rTc-CTL-1 and rTES-120) antigens _Kavitha, Dr.

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Co-Authors - Dr C. Sreekumar, Dr B.R. Latha, Dr A. Mangala Gowri

Two recombinant *Toxocara canis* excretory and secretory antigens viz. rTc-CTL-1 and rTES-120 from larvae were developed by amplifying, cloning and expression of respective genes in *Escherichia coli*. Indirect ELISA was performed to detect anti-*Toxocara* IgG antibodies in experimentally infected Balb/c mice with *T. canis* using two recombinant antigens. The mice were divided into 3 groups (n=8); Group I, infected with 100 eggs; Group II, infected with 1000 eggs and Group III, uninfected control mice. The mice were bled at 0, 7, 14, 28, 45, 60, 90, 120 and 150 days post infection (dpi). The rTc-CTL-1 antigen based ELISA showed the antibody response in both the infected groups were increased from 7 dpi, reached maximum at 28 dpi, then gradually declined that was maintained up to 150 dpi. The rTES-120 antigen based ELISA detected antibody only at 28 dpi with a maximum at 60 dpi, then moderately declined but observed up to 150 dpi. The antibody response of group II mice was significantly higher than the group I mice throughout the observation period compared to control group (P<0.01). Statistical analysis showed a highly significant difference in the antibody response between the group I and group II mice from 14 to 150 dpi with rTc-CTL-1 ELISA and from 28 to 150 dpi with rTES-120 ELISA (P<0.01). It was concluded that rTc-CTL-1 ELISA detect antibody in early infections compared to rTES-120 ELISA and also the antibody response was directly proportional to the dosage of infective eggs.

Keywords: *Toxocara canis*, rTc-CTL-1, rTES-120 antigens, Balb/c mice, ELISA, Antibody response.

24.08.23

Breakout 3: OA29 Parasite Control

OA29.01

Pharmacokinetic and field efficacy evaluations of combined anthelmintic treatments to optimize control of resistant nematodes in cattle_ Dr. Candela Canton_CIVETAN, Argentina

Co-Authors - Mr. Chris Miller, PhD Tania Waghorn, Dr. Laura Ceballos, Ms. Paula Dominguez, Dr. Lucila Canton, Dr. Miguel Buffarini, Dr. Carlos Lanusse, Dr. Dave Leathwick, Dr. Luis Alvarez

A pharmaco-parasitological assessment of two different nematocidal combinations of a macrocyclic lactone and a benzimidazole anthelmintic were performed in 3 cattle farms with a multi-resistant field scenario (A and B in Argentina and C in New Zealand). Ivermectin (IVM) + ricobendazole (RBZ) both given subcutaneously and abamectin (ABA)+oxfendazole (OXF) both given orally were assessed in calves infected with gastrointestinal nematodes resistant to both chemical families. No adverse pharmacokinetic (PK) interactions were observed after each combined treatment, with no differences in PK parameters ($P>0.05$) observed between the single-drug and the combined-based strategies. The initial efficacies were 40% (IVM), 64% (RBZ) and 90% (IVM+RBZ) (Farm A), and 54% (IVM), 84% (RBZ) and 98% (IVM-RBZ) (Farm B). After repeated annual use (over 5 years) of the same combination on both farms, the efficacy of the combined treatment decreased to 83% and 93% on Farm A and B, respectively. To optimize drug activity against those highly bi-resistant nematode populations, oral combined treatments were assayed. On Farm C, the *Cooperia* spp. were resistant to OXF, and the *Ostertagia* spp. were resistant to both ABA and OXF. In this context, the combined treatment only achieved an 84% efficacy against *Ostertagia* spp. The presence of multiple resistant *Ostertagia* spp. determined a reduced efficacy of the combined treatment. In contrast, on Farms A and B the *Ostertagia* spp. were only resistant to OXF and both anthelmintics failed to control *Cooperia* spp. Under this scenario, the combination was the only treatment that achieved 97-100% efficacy against all genera, indicating an additive effect. Overall, anthelmintic combinations can be useful to optimize the control of resistant gastrointestinal nematodes of cattle. However, its rational use should be strongly supported by pre-treatment diagnosis and considering the epidemiological situation of each individual farm especially the importance of refugia in maintaining effectiveness of the combinations

OA29.02

Albendazole resistance in *Fasciola hepatica*: surveillance in different areas of Spain and Argentine_ Alvarez, Prof. Luis_CIVETAN

Co-Authors - Dr. Juan Pedro Lirón, Dr. Candela Canton, Ms. Verónica Castilla Gómez de Agüero, Ms. Elora Valderas García, Dr. Marcela Larroza, Ms. Paula Soler, Dr. Carlos Lanusse, Prof. Luis Alvarez, Dr. María Martínez Valladares

Triclabendazole is the worldwide most used compound to control *Fasciola hepatica* and reports on drug resistance are abundant. parasite. Although the flukicidal use of albendazole (ABZ) is not as widespread, ABZ-resistance reports in liver flukes have increased in recent years. The work reported here describes a survey of the susceptibility/resistant status of different *F. hepatica* isolates to ABZ in two geographic regions of Spain and Argentine, using the egg hatch test (EHT). *F. hepatica* eggs were obtained from the gall bladder or faeces of natural infected cattle or

sheep from different geographical areas of northern Spain (Castilla y León, Galicia and Asturias) and east (Litoral) and south (Patagonia) of Argentine. Fluke eggs were incubated at 25 °C in darkness for a 12 h period with ABZ (0.5 nmol/mL). Untreated eggs served as control. After incubation, all eggs were gently washed to facilitate drug removal, and kept in darkness at 25 °C for 15 days. After this period, eggs were exposed to light for 2 h to stimulate the hatching of miracidia. Hatched and unhatched (undeveloped) eggs were evaluated using an optical microscope and the ovicidal activity, expressed as a percentage, was calculated. A total of 42 (Spain) and 28 (Argentine) *F. hepatica* isolates were assessed for ABZ-resistance. After the EHT, 4 (9%) isolates from Spain results ABZ-resistant. The EHT could be successfully performed in 28 Argentinian isolates, resulting 75% of them resistant to ABZ. The high prevalence of ABZ-resistance in *F. hepatica* observed in Argentina can be explained by the widespread use of this flukicidal drug instead of triclabendazole. Oppositely, the low ABZ-resistance observed in the isolates collected from Spain may be related to the common use of a variety of alternative flukicidal drugs, such as closantel, nitroxylnil and/or clorsulon.

OA29.03

Building a community for sustainable management of livestock tick control and acaricide resistance mitigation_Lempereur, Dr. Laetitia_Fao

Co-Author - Dr. Lenaig Halos

A community of practice on acaricide resistance management of livestock ticks was recently created which now has over 300 members across sectors, disciplines and geographical areas. This community welcomes members to learn from each other, co-create new knowledge, and tap into the practical experience of a large community of practice for the advancement of the sustainable management of livestock tick control and acaricide resistance mitigation. This community is logistically supported by a virtual platform (<https://virtual-learning-center.fao.org/mod/page/view.php?id=7392>) for information exchange, resource consultation, dialogue between its members or seminar organization. The creation of this community is currently enabling the development of new guidelines on sustainable management of livestock tick control and acaricide resistance mitigation at global level. These guidelines cover various complementary themes such as, diagnostic of acaricide resistance, integrated ticks and acaricide resistance management, regulation and access to acaricide products, research and innovation and communication and implementation, for which guidance is provided notably via recommendations, decision tree etc., with the ultimate aim of supporting the development and implementation of intervention programs or concrete actions by the various stakeholders. Building a global community on complex issues such as tick control requires the effort to be open to diversity and cooperation as implied by the one health approach. Although, it's well outweighed by the benefits of creating opportunities for commonly agreed strategy which will be carried by a broad community to ensure its acceptance, communication and implementation.

OA29.04

First occurrence of triclabendazole resistance in *Fasciola hepatica* on German sheep farms_ Dr. Jürgen Krücken_Freie Universität Berlin, Germany

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Abstract

Fasciola hepatica causes severe economic losses in sheep production and treatment options are limited. Triclabendazole is the only compound with activity against juvenile flukes and has been widely used for decades. However, data about the susceptibility of *F. hepatica* populations to anthelmintics in Germany are lacking. This study aimed to describe the current situation of *F. hepatica* infections in German sheep flocks using coproscopical examinations and to evaluate the efficacy of triclabendazole in a field study conducted from 2020-2022. An initial screening on 71 sheep farms, many of them with a known history of fasciolosis, revealed frequencies of *F. hepatica* at individual sheep and farm level of 14.5% and 35.2%, respectively. In addition, eggs of rumen flukes were found at a frequency of 3.6% in sheep and on 15.5% of all farms. Due to low egg shedding intensity, faecal egg count reduction (FECR) tests were only conducted on eleven farms including 3-53 sheep/farm. Individual faecal samples were collected before and two weeks after treatment to evaluate the faecal egg count (FEC) reduction using the FLUKEFINDER® or a modified FLUKEFINDER® method. On all farms a coproantigen reduction test was conducted in parallel. Lacking efficacy of triclabendazole was shown in one farm associated with a high number of animal losses due to acute fasciolosis. On this farm, the in ovo miracidium development test for *Fasciola* was also performed showing a high ovicidal activity of albendazole in vitro while closantel was effective in vivo. Adequate efficacy of triclabendazole was observed on all other farms. In conclusion, triclabendazole resistance of *F. hepatica* does not appear to be a widespread problem on German sheep farms yet but can have dramatic impact on animal health in individual flocks

OA29.05

Resistance pattern to avermectins and milbemycins in current strains of Australian cattle nematodes_ Dr. Matthew Ball_Virbac Animal Health, Australia

Co-Author - Dr Wynne Gibbison

Drench resistance to single active drench molecules is widespread in Australian cattle nematodes. A data set of 20 Australian cattle Faecal Egg Count Reduction Tests (FECRTs) and five total worm counts has been collated from independent cattle advisors, government agencies, universities and Virbac studies conducted between 2018 and 2021. The overall mean efficacy for single active drenches in these evaluations was 72%, but there was significant variation between farms and between active ingredients. There are differences in resistance patterns between both worm species and drench molecules. Haemonchus and Cooperia resistance to macrocyclic lactones (ML) is common. Levamisole is generally still effective against these worms. MLs are performing adequately against Ostertagia on most farms but resistance has been detected and protection periods are likely shortened. Levamisole is not always reliable against Ostertagia. More data is needed to assess Ostertagia resistance. The milbemycin molecule, moxidectin, may have some potency advantages against ML-resistant Haemonchus, but not Cooperia. Total worm count data obtained post-mortem from cattle infected with field worm strains is the most reliable way to assess drench effectiveness, but is not practical for on-farm use. FECRT in cattle need individual counts using 15 cattle per group and ideally a control group because, in some situations, comparing Day 14 results for treated cattle to Day 14 results for untreated control cattle may be more useful than comparing Day 14 and Day 0 results for treated cattle. Haemonchus is more production-limiting than Cooperia. Haemonchus drench resistance is a more significant problem for the cattle industry. It is recommended that combination (more than one active against the same parasite in pre-mixed formulation) or concurrent (more than one active administered on the same day) drenches be the default position for treatment of Australian cattle nematodes.

Screening of newly synthesized benzimidazole compounds with fasciolicidal properties_Valderas-García, Dr. Elora_University of Manchester

Co-Authors - Javier Díaz García, Verónica Castilla Gómez de Agüero, Laura González del Palacio, Marta Ruiz Somacarrera, Dr Esther del Olmo, Dr Rafael Balaña Fouce, Dr María Martínez Valladares

Fasciola hepatica is a zoonotic food-borne disease that affects both humans and animals worldwide. Its impact on farm profits has been calculated at about \$3 billion a year, whereas in humans, up to 17 million people in 51 countries are estimated to be infected. In the absence of an effective vaccine, control strategies rely on chemotherapy, with triclabendazole being the only drug effective against all stages. In addition, the emergence of anthelmintic resistance reaffirms the urgent need to find new therapeutic drugs. Therefore, this study evaluated the in vitro fasciolicidal activity of 70 newly synthesized benzimidazole derivatives. The first screening was performed by measuring the ovicidal activity of each compound at dose of 50 μM . Subsequently, the activity of the most active compounds was assessed at 10 μM . Four hit compounds (BZD31, BZD46, BZD56 and BZD59) and four commercial drugs (triclabendazole, albendazole sulfoxide, clorsulon and oxfendazol) were then selected to carry out in vitro tests at 5 μM . Their activity was also evaluated in a resistant isolate from Argentina, which had previously shown a 58% survival rate in sheep treated with albendazole at therapeutic doses. At 5 μM , all hit compounds showed activities over 70% in the susceptible isolate of the parasite and only BZD31 displayed an activity over 50% in the resistant isolate, with no commercial drug reaching this value. The activity of the four compounds, together with albendazole sulfoxide, was also evaluated against adult flukes at 10 μM . In this case, BZD59 was the only compound producing a significant motility reduction after 72 hours of incubation. In conclusion, two hit compounds (BZD31 and BZD59) identified in this work could be potential candidates in the discovery of new fasciolicidal drugs. Further studies are warranted. Study funded by PID2020- 119035RB-I00 (Agencia Estatal de Investigación, Ministerio de Ciencia e Innovación, Spain).

24.08.23

Breakout 4: OA30 Tools for evaluating intestinal parasites of dogs and cats

OA30.01

Assessment of voltage-gated calcium channel beta subunit gene of *Dipylidium caninum* as a diagnostic DNA marker for the early detection of praziquantel resistance in dogs_

Dr. Ranju Manoj_Cornell University, USA

Co-Authors - Rebecca Young, Danielle Maguire, Dr. Guilherme Gomes Verocai, Dr. Dwight D. Bowman, Dr Manigandan Lejeune

Dipylidium caninum is a common, zoonotic cestode of companion animals. Effective treatment relies on cestocidal isoquinolone drugs, including praziquantel (PZQ); however recent reports suggest drug resistance for *D. caninum* among canine population in North America. Though pharmacodynamics is unknown, the consensus is that PZQ disrupts voltage-gated calcium channel (VGCC) leading to worm paralysis and death. This study aimed to assess the feasibility of using VGCC beta subunit gene as a molecular diagnostic marker for PZQ resistance. *Dipylidium caninum* samples were obtained from infected dogs (n=5) and cats (n=2) either submitted to AHDC parasitology lab or solicited from pet owners. Characterization of isolates as 'susceptible' or 'resistant' was based on the treatment history. A specimen from each animal was PCR-amplified using published primers for VGCC and Sanger sequenced. Curated *D. caninum* sequences from resistant isolates obtained in this study were compared with the reference genome for the species (isolate LGH-2015; QEYB01001179), and with the resistant isolates (Colorado & Michigan) from a published study. A nine-nucleotide deletion (position 709724-709732) was consistently detected in all resistant dog isolates from this study and were identical to Colorado and Michigan isolates. In contrast, PZQ susceptible cat isolates were devoid of that nine-nucleotide deletion. Interestingly, cat VGCC sequence distinctly differed (88% similarity) from that of the *D. caninum* reference genome, wherein a twenty-nucleotide addition and other polymorphisms were consistent. This clearly supports the current notion that *D. caninum* in cats may be a distinct species. A shortcoming of our study was that representative PZQ susceptible dog isolates were not included, but this will be later addressed. Nonetheless, development of PCR-based diagnostic test targeting genetic mutations enables early detection of PZQ resistance and aids with appropriate treatment decision to control *D. caninum* infection in pet animals

OA30.02

Clinical safety of a novel antiparasitic product for cats, and effectiveness of this spot on association of fipronil, moxidectin and praziquantel against nematodes, cestodes, fleas, otodectic and notoedric mange, and biting lice_Cruz, Dr. Breno Cayeiro_Ourofino Saúde Animal Ltda.

Co-Authors - Dr. Igor Renan Honorato Gatto, Ms. Milenni Garcia Michels, Ms. Maira Neto Zampier Farias de Souza, Patricia Chiba Tagava, Dr. Ferdinando Nielsen de Almeida, Juliana de Moraes Intriери, Dr. Diefrey Ribeiro Campos, Dr. Fábio Barbour Scott

The dissemination of antiparasitic resistance underlies a constant need for new products: either new molecules or new combinations and/or dosages of established compounds. Macrocyclic lactones, endectocides, revolutionized the market with their excellent effectiveness and, above all, the convenience offered by a single application controlling a wide range of endoparasites and ectoparasites. This concept became the basis for new antiparasitic combinations: broadening the spectrum of products, and combating existing resistance and delaying its future emergence,

making it more difficult for parasites to escape selection pressure, by employing several modes of action simultaneously. For treatment and prophylaxis of parasites in pets, whenever possible, combinations of actives in a single product are the preferred alternative, providing combined effects and fewer treatments. Thus, Ourofino Saúde Animal Ltda. developed an antiparasitic formulation based on Fipronil, Moxidectin and Praziquantel, administered in a single topical (spot on) use on the back of animals, at doses of 0.3 mL for cats up to 2.5 kg and 0.9 mL for cats weighing 2.6 to 7.5 kg. A complete package of pivotal studies, all following established reference guidelines (WAAVP, VICH, EMA), in compliance with good clinical practices and principles of ethics and animal welfare, confirmed this product's safety in kittens, from 30 days of age onwards, and its efficacy against the most relevant feline parasites. In two distinct geographic regions, its anthelmintic efficacy was proven against *Ancylostoma* spp. (>98%); *Toxocara* spp. (>96%) and *Dipylidium caninum* (100%). In cats artificially infested with *Ctenocephalides felis felis*, average effectiveness of 94.58% was obtained (>90% up to 49 days post-treatment). When treating cats naturally infested with these fleas, mean efficacy of 98.25% was obtained. In cats affected by mange, naturally infested with *Otodectes cynotis* or *Notoedres cati*, and also cats suffering with *Felicola subrostratus* lice, 100% effectiveness was sustained for at least 30 days.

Keywords: *Ancylostoma* spp.; *Ctenocephalides felis felis*; *Dipylidium caninum*; Efficacy; *Felicola subrostratus*; *Notoedres cati*; *Otodectes cynotis*; *Toxocara* spp.

OA30.03

The sublethal concentration of chlorine improves the cytotoxicity of *Acanthamoeba castellanii*_Bahrami, Dr. Somayeh_Shahid Chamran University of Ahvaz

Co-Authors - Dr Zeinab Asadi, Professor Mehdi Zarei, Professor Hossein Hamidinejat, Professor Fiona L. Henriquez

Chlorine is a common disinfection agent commonly added to public water facilities and supplies. In this study, we examined the cytopathic and phagocytic properties of *Acanthamoeba castellanii* trophozoites following exposure to sublethal concentrations of chlorine. Two hours of exposure to five ppm hypochlorite calcium was considered the sublethal concentration for *A. castellanii* trophozoites. Assays on cytotoxicity and adhesion in RAW 264.7 macrophages, osmosensitivity, and thermotolerance were conducted to determine whether treated trophozoites were more pathogenic than untreated ones. The phagocytic characteristics of treated cells were evaluated by assessing bacterial uptake. Antioxidant activity and oxidative stress biomarkers were compared in treated and untreated trophozoites. We also determined the mRNA expression of the genes for mannose-binding protein (MBP), cysteine protease 3 (CP3), and serine endopeptidase (SEP). Trophozoites treated with chlorine exhibited more extensive cytopathological effects, resulting in the detachment of macrophage monolayers. Treated trophozoites could not grow at high temperatures (43 °C). Besides, they showed osmotolerance to 0.5 M D-mannitol but not to 1 M. In comparison with untreated cells, chlorine-treated cells showed higher bacterial uptake rates. In treated and untreated cells, glutathione and glutathione/glutathione disulfide ratios were significantly different as a result of chlorine treatment, antioxidant enzyme activities, total antioxidant capacity, and malondialdehyde levels increased significantly. In chlorine-treated trophozoites, virulence genes were upregulated. Chlorine can form resistance and virulent amoebae if it is not used at a proper concentration and exposure time.