WAAVP

30th Conference of the World Association for the Advancement of Veterinary Parasitology

CURITIBA 2025

CURITIBA - BRAZIL

ABSTRACT BOOK

August 17th - 21st 2025

Viasoft Experience

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Room 1
Symposium

Antiparasitic Guidelines - Limitations and Future Directions

Anja Joachim (WAAVP Guidelines Subcommittee, University of Veterinary Medicine, Austria), Angela Di Cesare (WAAVP Guidelines Subcommittee, University of Teramo, Italy), Ray Kaplan (WAAVP Guidelines Subcommittee, St. George's University, Grenada), Edwin Claerebout (WAAVP Guidelines Subcommittee, Ghent University, Belgium), Livio M. Costa Júnior (WAAVP Guidelines Subcommittee, Federal University of Maranhão, Brazil), Thomas Geurden (WAAVP Guidelines Subcommittee, Zoetis, Belgium), Silvina Fernández (WAAVP Guidelines Subcommittee, CONICET / National University of Central Buenos Aires Province, Argentina)

The revision and creation for guidelines requires two prerequisites, (a) the topic should attract sufficient interest to be helpful under the current research and development topics in veterinary parasitology; b) the scientific community, specifically the WAAVP members, should be able to provide sufficient expertise in the field to put together a team of qualified authors to write a revision or a new guideline, as well as a team of qualified reviewers to comment on the manuscript. In this context, this symposium will cover three topics: (1) a general introduction on objectives and process of developing the WAAVP Guidelines (Silvina Fernández); (2) companion animal guidelines, what we have and what we need (Anja Joachim and Angela Di Cesare); and (3) implementing the new FECRT guideline, from principles to practical application (Ray Kaplan). Objectives and process of developing the WAAVP Guidelines: The process of creating the WAAVP guidelines will be explained, highlighting the intrinsic steps and the most common difficulties encountered. Companion Animal Guidelines, what we have and what we need: the evaluation of efficacy of anticoccidials in dogs, parasiticides against vector-borne pathogens in dogs and cats, and anthelmintics for dogs and cats have been the subject of recent guidelines. However, the guideline for evaluating the efficacy of ectoparasiticides for companion animals should be revised next because the spectra of compounds and target parasites have greatly increased since the last edition in 2013, necessitating adaptations of the guideline. Implementing the new WAAVP FECRT Guideline, from principles to practical application: The guideline for diagnosing anthelmintic resistance using the faecal egg count reduction test in farm animals was published in 2023, since then enquiries about its use in the farm practice as well as in research have been received, thus prompting this presentation to highlight the practical application of this much needed guideline. Suggestions on revisions or new guidelines with other hosts and other parasites can be suggested and are welcome for discussion in the WAAVP Guidelines Subcommittee.

Room 2
Oral Communication

Molecular and Microbiome-Based Approaches for Tick Control

Session Chair: Patricia Gôlo (Federal Rural University of Rio de Janeiro, Brazil)

Exploring bacterial microbiota and immune system in *Amblyomma* sp. ticks: implications for rickettsial acquisition and tick fitness

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Amblyomma sculptum and *Amblyomma aureolatum* are known vectors of *Rickettsia rickettsii*, the causative agent of Brazilian spotted fever. *Amblyomma aureolatum* shows high susceptibility to *R. rickettsii* and harbors a dense microbial population in its midgut, predominantly composed of bacteria from the *Francisella* genus. In contrast, *A. sculptum* has a nearly sterile midgut, which correlates to its low susceptibility to infection. In the current study, we analyzed the microbiota of additional organs of *A. sculptum* and *A. aureolatum* exposed or not to *R. rickettsii*. As previously observed in the midgut, lower bacterial loads were detected in the ovaries and salivary glands of *A. sculptum* compared to *A. aureolatum*. The bacterial composition was analyzed through high-throughput sequencing of the V3-V4 hypervariable regions of the bacterial 16S rRNA gene. *Francisella* was the predominant genus in all organs of nonexposed *A. aureolatum*, but its proportions were higher in the salivary glands and in the ovaries. *Amblyomma aureolatum* engorged females were treated with tetracycline, resulting in a significant reduction of the bacterial loads in the eggs. In addition, the levels of antimicrobial peptide transcripts were lower in the eggs of antibiotic-treated females. Descendant larvae from antibiotic treated females showed a higher rickettsial acquisition. The antibiotic treatment also had a negative effect on tick development, decreasing the percentage of molt from larvae to nymph. Altogether, these results indicate that the resident microbiota in *A. aureolatum* ticks may play a protection role against *R. rickettsii* infection as well as in the tick development, pointing microbiota as a potential tool to control of tick-borne diseases.

Investigations on the microbiome of *Rhipicephalus australis*

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Ticks act as vectors for many pathogens affecting both humans and animals. For example, the cattle tick can transmit various pathogens

such as *Anaplasma* and *Babesia* spp., resulting in significant economic losses to the global cattle industry. Despite its relevance, our understanding of the cattle tick's microbiome, particularly in Australia, is limited. This study aimed to characterise the microbiome of different life stages of *Rhipicephalus australis*, including larvae, nymphs and adults, by targeting the V3-V4 regions of the 16S rRNA gene. Specimens collected from cattle farms in Queensland in 2024 were decontaminated using a bleach solution and preserved in 70% ethanol before being subjected to DNA extraction and PCR. Sequencing of the libraries was performed using the Illumina NextSeqTM 1000 platform. Paired-end FastQ reads were uploaded in the QIIME2 environment for subsequent quality filtering and analysis using cutadapt, DADA2, VSEARCH, and BLASTn. Preliminary analyses revealed the presence of several bacterial taxa, including *Arsenophonus*, *Acinetobacter*, *Coxiella*, *Coxiella*-like endosymbionts, *Stenotrophomonas*, *Lactococcus*, *Morganella*, *Salmonella*, *Staphylococcus*, *Providencia* and other bacteria mainly belonging to the families *Coxiellaceae, Enterobacteriaceae, Moraxellaceae, Staphylococcaceae* and *Streptococcaceae*. The data obtained from this study will provide valuable insights into the microbiome of *R. australis* and support developing of improved control strategies.

Immunogenic properties of trypsin inhibitor-like (TIL) from *Rhipicephalus microplus* and their peptide derivatives as vaccine candidates against ticks

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Rhipicephalus microplus is an ectoparasite that affects animal and human health. It is resistant to chemical acaricides, requiring alternative control strategies like immunological control via vaccines. This study aimed to characterize the inhibitory activities of trypsin inhibitor-like (TIL) previously identified in tick saliva and identify potential B-cell epitopes for an anti-tick vaccine. *In-silico* analyses of five TIL protein sequences of *R. microplus* were performed, including structures, domains and phylogenies. Recombinant proteins were obtained using a mammalian cell system and were used to immunize rabbits. Antigenic peptides were synthesized based on predicted B-cell epitopes (RmTIL-7-pept 01 and RmTIL-7-pept 02) and used for rabbit immunizations. The neutralizations of TIL proteins by antibodies were assessed using an enzymatic assay. BLAST analyses for selected five TIL sequences of *R. microplus* showed similarity with various protein sequences of *R. microplus* and *Rhipicephalus sanguineus*. Aligment of the sequences of TIL-4 and TIL-7 revealed 93.83% similarity. In enzyme assays, purified IgG (at 20 µg) neutralized the inhibitory activities of TIL-4 and TIL-7, with neutralization levels of 69.3% and 64.4%, respectively. Moreover, cross-reactivity was observed, with TIL-4 IgG neutralizing 79.42% of TIL-7, while TIL-7 IgG neutralized 65.28% of TIL-4 inhibitory activity. Among two peptides, only the antibody RmTIL-7-pept 01 neutralized the inhibitory activity of TIL-7 by 17.31% at the same concentration. Based on these activities, the TIL-7 protein and its derived peptide were selected for vaccination in rabbits. The rabbits will be challenged with *R. sanguineus* and the serum will be analyzed by dot blot, western blot

and ELISA. This study identified the immunogenic potential, enzyme inhibition activities and potential B-cell epitopes of *R. microplus* TIL proteins, providing valuable data for effective anti-tick vaccines.

Characterization of the core bacterial community of *Rhipicephalus microplus* from Brazil

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The cattle tick *Rhipicephalus microplus* is one of the main sanitary and economic challenges in Brazilian livestock farming. Due to increasing resistance to chemical acaricides and their environmental repercussions, investigating the tick-associated microbiota—particularly the core microbiota, defined as the bacterial taxa consistently found across individuals—emerges as a promising research area. Characterizing the core microbiota can provide insights into microbial ecological interactions and may inform future studies aiming to develop sustainable tick control strategies. Although international efforts have advanced in this field, studies applying next-generation sequencing (NGS) to the microbiota of *R. microplus* are still scarce in Brazil. To contribute to this knowledge gap, we characterized the core bacterial community in 20 engorged female ticks collected in April 2024 from the APTA Experimental Farm (São Paulo, Brazil). DNA was extracted, the V4 region of 16S rRNA gene was amplified, and sequencing was performed using an amplicon-based NGS approach. Bioinformatics analyses and taxonomic classification were conducted using QIIME 2 tools at West Chester University (USA). The results revealed a conserved core microbiota comprising the families Ruminococcaceae, Lachnospiraceae, Coxiellaceae, Helicobacteraceae, and Oscillospiraceae, with a predominance of the genera *Faecalibacterium*, *Coxiella*, *Lachnoclostridium*, and *Helicobacter*. These findings are consistent with previous reports, reinforcing the existence of a stable core microbial community in *R. microplus*. Although further functional studies are needed to determine the biological roles of these bacterial taxa, our study establishes a foundation for future investigations on the ecological relevance of the core microbiota and its potential applications in sustainable tick management.

Exploring miRNA functions in the salivary glands of the soft tick *Ornithodoros moubata*

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MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by inhibiting or degrading messenger RNA (mRNA). In ticks, salivary miRNAs have been proposed to play a crucial role in modulating host–vector interactions during blood feeding. We previously identified miRNAs in the saliva of *Ornithodoros moubata* (Om), the main vector of African Swine Fever and tick-borne relapsing fever in Southeastern Africa. Our findings suggested that miRNAs may influence host immune responses, gene expression and vascular development. However, their function within *Ornithodoros* tick physiology remains unclear. To explore the role of salivary miRNAs in regulating physiological processes in the salivary glands (SG) of Om ticks. Using our previously saliva miRNA dataset and the miRBase database, we identified 86 conserved mature miRNAs. The presence of four saliva miRNAs—miR-375, miR-252b, miR-1 and miR-279—was confirmed in the SG via qPCR. We predicted the 3'UTR of the Om sialotranscriptome and conducted miRNA target predictions. To assess the functional role of specific miRNAs, three candidates—miR-375, miR-252b and miR-1—were silenced using antagomirs in female Om ticks. Effective miRNA knockdown in SG was confirmed via qPCR, and phenotypic effects on tick biology were assessed by feeding antagomir-treated females on rabbits. miRNA target prediction analysis yielded 3,901 potential targets in Om SG, primarily related to carbohydrate metabolism and cellular organization. After antagomir knockdown, successful silencing in SG was confirmed. Six predicted target genes were analysed, revealing Metis1 as a likely target of miR-252b, with its expression significantly upregulated following miRNA suppression. These findings highlight the regulatory role of miRNAs in tick SG physiology and offer new perspectives on tick-host interactions, potentially contributing to the development of innovative tick control strategies.

Expanding knowledge of the Bm05br protein as an anti-*Rhipicephalus linnaei* and *Rhipicephalus microplus* antigen

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Ticks are hematophagous arthropods that transmit pathogens to animals and humans. In the search for new anti-tick alternatives, the identification of immunoprotective proteins is an effective strategy for the control of these arthropods. Bm05br (Brazil *Rhipicephalus microplus* protein 05) is a protein with an unknown function identified in the saliva of *R. microplus*. Characterizing the immunological potential of the Bm05br protein as a candidate antigen for a tick vaccine. *Escherichia coli* strain BL21 (DE3) was used for expression of Bm05br. Rabbits were immunized with this protein and the challenge was performed using the *Rhipicephalus linnaei* strain. Artificial feeding experiments were performed using the *R. microplus* Porto Alegre strain. Sera were analyzed by ELISA. Organs, tissues and saliva were collected from *R. linnaei* for antigen analysis. The parameters evaluated (feeding, oviposition and larval hatching) in immunization with rBm05br did not show significant differences between the groups. Anti-Bm05br antibodies recognized the homologous protein Rs05br (Brazil protein 05 of *R. linnaei*) in different developmental sta-

ges, organs and saliva of *R. linnaei.* The evaluated parameters (weight gain, egg mass laying and larval hatching rate) under artificial feeding were not statistically significant. rBm05br did not induce a protective response against infestation in *R. linnaei.* However, the sequence and antigenic analysis between Bm05br and Rs05br suggested that *R. linnaei* could serve as a tick model for cross-protection studies. Although rBm05br did not affect feeding or reproduction of the *R. linnaei*, Bm05br cannot be completely ruled out as a possible antigen against other tick species, and future research should explore new vaccine formulations and animal immunization models.

Plenary Oral Communication

One Health Perspectives on Parasites in Wildlife and Aquatic Environments

Preliminary report on the prevalence of myxosporean parasite *Kudoa* spp. (Myxozoa: Multivalvulida) in fish for consumption from Portugal

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Kudoa is a genus of parasitic myxosporean that mainly infect the muscles of marine and estuarine fish, causing significant economic impacts on the fishing and aquaculture industries. Some species within the genus produce proteolytic enzymes that degrade the muscle post-mortem, turning the flesh into a slimy liquid, known as “jelly muscle” or “soft flesh”, and can render fish unappetizing and unsuitable for sale. While most *Kudoa* species are harmless to humans, certain species have been linked to foodborne illnesses when infected fish is eaten raw. The aim of this work was to stablish the prevalence of *Kudoa* spp. in fishery products collected from 3 first sale establishments in Portugal during two times of the year (summer and winter). Two muscle samples (anterior and posterior) were collected from each fish and subsequently macerated in 0,9% NaCl solution, filtered and analysed. The identification of positive samples was based on the presence of myxospores by morphological analysis under an optical microscope. The prevalence of *Kudoa* spp. varied between 95 and 100% in Atlantic horse mackerel (*Trachurus trachurus*), between 0 and 40% in Atlantic chub mackerel (*Scomber colias*) and between 0 and 25% in Atlantic mackerel (*Scomber scombrus*). Additionally, *Kudoa* myxospores were detected in 20% of the European pilchards (*Sardina pilchardus*) analysed. No macroscopic alterations were detected. These preliminary results confirmed the presence of *Kudoa* spp. in fishery products for human consumption. The infection of fish with spores of *Kudoa* spp. can be observed and diagnosed microscopically or can be suspected macroscopically if, occasionally, changes in the texture or colour of the muscle are observed. Consequently, withdrawal of the fish from the supply chain and subsequent economic losses may occur and most importantly

public health concerns may arise because of its zoonotic potential.

Detection of zoonotic protozoa in free-ranging raccoons (*Procyon lotor*) from aquaculture zones in Saxony (Germany): A One Health perspective

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The raccoon (*Procyon lotor*) is an invasive alien species (IAS) in Europe, originating from the Americas. It is widely distributed in Germany and is considered a threat as a potential reservoir of zoonotic pathogens. The Upper Lusatian Heath and Pond Landscape in Saxony, Europe's largest economically managed pond landscape, hosts numerous aquacultures where raccoons are prevalent. Fecal samples from 104 raccoons, obtained between 2020 and 2022, were collected from six pond farming areas in Saxony, Germany. Antigen-positive samples underwent DNA extraction, purification, and PCR amplification targeting genes specific to *Giardia* (*gdh*, *tpi*, *bg*) and *Cryptosporidium* (SSU rRNA, *gp60*), followed by sequencing. Statistical analysis revealed relations between infection status and variables such as location, water source, fishery, and raccoon demographics. *Giardia duodenalis* sub-assemblage BIV, was detected in 25% of samples. Significant associations were observed with aquaculture companies ($P = 0.046$) and fecal score ($P = 0.037$). *Cryptosporidium* sp. skunk genotype was identified in 1.9% of samples, alongside sequences resembling *Cryptosporidium bovis*, *Cryptosporidium andersoni*, *Cryptosporidium parvum*, and *Cryptosporidium* sp. goose genotype I. Juvenile raccoons showed significantly higher infection rates with *Cryptosporidium* than adults ($P = 0.032$). This study highlights raccoons' role as potential carriers of zoonotic pathogens in Saxony's pond landscapes. The observed presence of *Giardia duodenalis* sub-assemblage BIV and *Cryptosporidium* genotypes suggests possible health risks, particularly in fishing areas where raccoons are frequently encountered. These findings support the need for targeted monitoring and control efforts in aquaculture settings to mitigate pathogen transmission.

Detection of *Neospora caninum* in South American Fur Seals (*Arctocephalus australis*) on the Southern Coast of Santa Catarina, Brazil

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Neospora caninum is an obligate intracellular protozoan of the phylum Apicomplexa with worldwide distribution. It infects a wide range of homeothermic hosts and causes, mostly, reproductive problems in cattle. Antibodies against *N. caninum* have been detected in pinnipeds, including sea lions (*Zalophus californianus*), seals (*Phoca vitulina, Phoca hispida, Erignathus barbatus*), and walruses (*Odobenus rosmarus*), indicating a broad host range. The present study investigated the presence of *N. caninum* in southern fur seals (*Arctocephalus australis*), by molecular analysis. Four deceased *A. australis* were detected and collected by the Santos Basin Beach Monitoring Project - Section 1 (ABIO license n. 640/2015). During necropsy, a pool of tissues (heart, lung, liver, brain, and skeletal muscle) from each animal was subjected to DNA extraction using a commercial kit (DNeasy Blood & Tissue Kit, Qiagen, Germany) and analyzed by real-time PCR (qPCR) for parasite detection. *N. caninum* DNA was detected in three of the four animals. The presence of this parasite in the marine ecosystem is intriguing, considering that canids, the definitive hosts, shed few oocysts into the environment. It is possible that, like in *Toxoplasma gondii* cycle, *N. caninum* oocysts reach the marine ecosystem via wastewater runoff and are bioaccumulated by fish, cephalopods, and lobsters, the main components of the fur seal diet. Reports of *N. caninum* infection in marine animals are scarce, highlighting the need for further studies to elucidate the epidemiology of this protozoan in aquatic environments.

Captive lemurs (*Lemur catta*) as hosts of *Leishmania infantum* and other zoonotic pathogens

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Safari-style zoological parks provide visitors the opportunity to interact closely with wildlife. However, these interactions raise concerns about potential zoonotic risks and exposure, as well as animal health and welfare. This study investigated the presence of zoonotic pathogens in ring-tailed lemurs (*Lemur catta*) and the occurrence of *Leishmania* spp. in phlebotomine sand flies collected from a zoological park in southern Italy. Blood (n = 30) and feces (n = 29) were obtained from lemurs during routine health checks. Seventy-two sand flies were trapped near lemur enclosures and preserved in ethanol. Fecal samples underwent copro-para-

sitological methods (e.g., mini-FLOTAC®, Baermann technique), while molecular assays were performed on blood, feces, and sand flies (e.g., cPCR, qPCR) targeting different pathogens. Additionally, blood samples were screened serologically using SNAP® Leish 4Dx® kits to detected *Leishmania infantum*, *Anaplasma* spp., *Ehrlichia* spp., and *Dirofilaria immitis*. Two lemurs (6.66%) tested positive for *L. infantum* by SNAP® Leish 4Dx®, with one confirmed by qPCR. Among the sand flies, 71 were identified as *Phlebotomus perniciosus* and one as *Sergentomyia minuta*, with a female *P. perniciosus* positive for *L. infantum* in qPCR. Molecular tests also detected *Giardia duodenalis* (44.82% assemblage B; 6.66% sub-assemblage BIV), *Entamoeba coli* (27.58%), and *Blastocystis* sp. subtype 8 (6.66%). The detection of *L. infantum* in both lemurs and *P. perniciosus* emphasizes the need for effective monitoring protocols in endemic areas. Furthermore, the identification of *G. duodenalis* (assemblage BIV) and *Blastocystis* sp. (ST8) raises concerns about contamination in enclosures and food sources, highlighting the importance of maintaining strict sanitary protocols measure to avoid the infection risks for animals, zookeepers and visitors.

Q fever in Brazil: evaluation of the exposure of small mammals to *Coxiella burnetii* in the state of São Paulo

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Coxiella burnetii, the etiological agent of Q fever, is distributed worldwide and plays an important role in human and animal health. In the peridomestic cycle, the involvement of ruminants (cattle, sheep, and goats) is well-known. However, in the enzootic cycle, the involvement of wild animals has been poorly studied, with small mammals (order Rodentia and Didelphimorphia) being susceptible hosts of *C. burnetii* and possible carriers in the natural environment. The objective of this study was to evaluate the natural infection by *Coxiella burnetii* in small rodents collected in anthropized (state of São Paulo) and natural (Pantanal) areas. For this purpose, sera from small mammals were tested by indirect immunofluorescence assay (IFA) with phase I (strain At12) and phase II (Nine Mile strain) antigens of *C. burnetii*, which correspond to the chronic and acute phases of *C. burnetii* infection, respectively. Extracted DNA from small mammals' blood, liver, lungs, and spleen were molecularly tested by qPCR directed to the IS1111 gene of *Coxiella* spp. Out of 302 small mammals tested by serology, 4 (1.32%) reacted to phase I and 1 (0.33%) to phase II, from 4 different cities in São Paulo. None of the organs tested (554) yielded *Coxiella* DNA by molecular analysis. It is concluded that small mammals do not play a significant role in the maintenance of C. burnetii in the studies areas, acting only as accidental hosts with a low potential for transmission to the human population, justified by the low infestation rate by ectoparasites (ticks) and a shorter lifespan compared to other mammals.

Free-living xenarthrans mammals with high rates of vector-borne pathogens and zoonotic infections in Brazil

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Xenarthrans correspond to a group of species exclusively from the American continent, including animals belonging to the orders Pilosa (anteaters and sloths) and Cingulata (armadillos). Between 2008 and 2014, blood samples were collected from 24 free-living Xenarthra mammals across three Brazilian biomes, the Amazon rainforest, Pantanal and the Cerrado, spanning four states: Amapá, Mato Grosso, Mato Grosso do Sul and Minas Gerais. Of these, 54.2% (13/24) were *Myrmecophaga tridactyla*, 29.2% (7/24) were *Tamandua tetradactyla,* 12.5% (3/24) were *Euphractus sexcinctus*, and 4.1% (1/24) were a rescued *Choloepus didactylus*. In this study, we investigated the occurrence of vector-borne pathogens in wild xenarthrans. Granulocytic/platelet Anaplasmataceae was found in 8.4% (2/24) of the samples, monocytic Anaplasmataceae in 4.2% (1/24) of the animals. Specific primers to *Anaplasma marginale* showed 16.6% (2/24) of positive animals, hemotropic *Mycoplasma* spp. in 4.2% (1/24), piroplasms in 70.8% (17/24), kinetoplastids in 75% (18/24) and *Trypanosoma evansi* in 12,5% (3/24). All animals tested negative for *T. vivax* and *T. cruzi/T. rangeli* in specific PCR reactions. Co-infections occurred in 66.7% of the positive samples. Sequencing revealed that hemotropic *Mycoplasma* sp., which are closely related to hemoplasmas previously described in xenarthrans; *Babesia* sp., which are closely related to *B. bovis, B. bigemina* and *B. ovata;* and *Leishmania* sp., which are closely related to *Leishmania braziliensis.* Our results showed a high occurrence of vector-borne pathogens in anteaters, armadillos and sloths, including zoonotic species, which may pose a risk to animal and human public health.

Mechanisms of drug action and drug resistance in animal trypanosomiasis: Differences between *Trypanosoma* species.

Harry De Koning (University of Glasgow, UK), Marzuq Ungogo (University of Glasgow, UK)

Animal trypanosomiasis is a complex of diseases, spread from South America through Africa to Asia and the Philippines. It is known as surra, dourine, nagana and mal de calderas among other names. It is caused by *Trypanosoma* species from three different subgenera, including *T. brucei*, *T. evansi*, *T. vivax*, *T. congolense*, *T. equiperdum*, with different (or no) vectors, hosts, tropisms etc. Yet, the resulting differences in pharmacology have been poorly investigated. Considering the few drugs available, the most efficient use of them must be made, for cure, prophylaxis and to avoid resistance. Here, we present a broad investigation of the differential effects of veterinary trypanocides on the various trypanosome species, document the biochemical causes thereof, as well as the exact drug resistance mechanisms for diamidine (e.g. diminazene (Berenil)) and arsenical (melarsomine) drugs. We also present a thorough investigation into the mechanism of action, cross-resistance profile and resistance mechanism of quinapyramine (Antrycide) in *T. evansi* and *T. equiperdum*, which has previously been reported to induce cross-resistance to isometami-

dium (Samorin) and diminazene in some trypanosome species and consequently taken out of use in sub-Sharan Africa. The identification of drug resistance markers enables the molecular monitoring of the spread of such resistance.

Liver Fluke Symposium

Towards Precision Parasite Control - In the Lab

Session Chairs: Nichola E. D. Calvani (The University of Sydney, Australia) and Marcelo Molento (Federal University of Paraná, Brazil)

Native and recombinant vaccine efficacy against *Fasciola hepatica* in small ruminants

Amanda McEvoy (Teagasc), Jesús Lopez Corrales (University of Galway, Ireland), Saoirse Ellis (Teagasc), Orla Keane (Teagasc), Krystyna Cwiklinski (University of Liverpool, England), John P Dalton (University of Galway, Ireland), Richard Lalor (University of Galway, Ireland)

Fasciola hepatica is an economically significant parasite that adversely affects livestock health and productivity worldwide. With growing resistance to anthelmintic drugs, there is an urgent need for alternative and sustainable control strategies. Vaccination is a promising solution, however no effective vaccine is currently available for *F. hepatica* infections. This study investigated vaccine strategies using native and recombinant antigens derived from adult fluke excretory/secretory (ES) products, fractionated by size exclusion chromatography. Proteomic analysis identified the predominant proteins within each native fraction, which were recombinantly expressed and formulated into vaccine cocktails according to their fraction (G1: rFhHDM, rFhLAP, rFhPrx; G2: rFhGAPDH, rFhGPI, rFhEnolase, rFhFBA; G3: rFhCL1, rFhCL2, rFhCys-1, rFhKunitz-1, rFhSOD-1, rFhTrx). Sheep were immunised with the native and recombinant fractions formulated in Montanide adjuvant and then experimentally or naturally challenged with *F. hepatica* metacercariae. Vaccination elicited robust proliferative cellular responses upon restimulation of peripheral PBMCs in both the native and recombinant groups. However, antibody profiles varied depending on antigen composition. While no significant reductions in parasite burden or faecal egg counts were observed between vaccinated and control groups, sheep immunised with a recombinant cocktail enriched in glycolytic enzymes (G2) exhibited greater weight gain compared to the controls. These findings demonstrate that recombinant antigen-based vaccination could improve host resilience under fluke challenge without reducing overall parasite burden, supporting the potential role for immunisation as part of an integrated control strategy of inducing resilience for fasciolosis in ruminants.

Development of a qPCR assay and a "Trematiome" deep amplicon sequencing for fluke species differentiation in the UK livestock

Umer Chaudhry (Long Island University College of Veterinary Medicine (LIU-CVM), USA)

Fasciola spp. are a significant economic threat to ruminant production worldwide. Traditional diagnostic methods rely on egg sedimentation from faeces, a time-consuming methodology lacking sensitivity and specificity. This study aimed to develop and validate two diagnostic methods: firstly, qPCR for accurate identification of *Fasciola* spp., and secondly, a "Trema-

biome," deep amplicon sequencing technique for identifying fluke species important to global ruminant health from faecal egg DNA. To detect *Fasciola* spp., primers targeting mitochondrial DNA were repurposed to develop a SYBR Green qPCR diagnostic. For the detection of UK relevant fluke species, the "Tremabiome," approach was developed. Specifically, a reference sequence library and taxonomy file were generated for 21 fluke species, enabling species sequence read separation and extracting amplicon sequence variants (ASVs). To validate the "Tremabiome" approach faecal samples were collected from cattle and sheep across the UK. Fluke eggs were isolated by sedimentation, with diagnosis confirmed using microscopy, qPCR and "Tremabiome" approach. qPCR demonstrated high sensitivity, detecting *F. hepatica* DNA (19.2 fg) and *F. gigantica* (6.4fg), with no cross--amplification with other flukes. "Tremabiome" was able to detect as few as five *F. hepatica* and *Calicophoron daubneyi* eggs, identifying mixed infections. High levels of co-infection (14.4%) of *F. hepatica* and *C. daubneyi* were observed in faecal samples, followed by single infections with *C. daubneyi* (12.6%) and *F. hepatica* (3.2%). Data analysis identified 55 and 32 ASVs for *F. hepatica* and *C. daubneyi*, respectively, with phylogenetic clustering within their respective species clades.

Phenotyping *Fasciola hepatica* populations for albendazole sensitivity by *in vitro* egg hatch test.

Emily Herschell-Kelly (University of Liverpool, United Kingdom), Laura Ceballos (CIVETAN, CONICET, Argentina), Laura Maté (CIVETAN, CONICET, Argentina), Luis Ignacio Alvarez (CIVETAN, CONICET, Argentina), Jane Hodgkinson (University of Liverpool, United Kingdom)

The liver fluke *Fasciola hepatica* is a major cause of morbidity and mortality in ruminant livestock in many parts of the world and infection results in substantial economic losses to the sector. Intensive use of highly effective triclabendazole (>99% efficacy) to control the impact of *F. hepatica* infection has resulted in widespread resistance. Alternative anthelmintics, like the adulticide albendazole (ABZ) (≥80% efficacy), are often employed to reduce transmission. This study reports *in vivo* controlled efficacy tests (CET) and *in vitro* egg hatch test (EHT) evaluation of ABZ sensitivity for two *F. hepatica* isolates, one from Argentina (Ag) and one from Sweden (E). For each isolate CET: Sheep (n= 6) were infected with ~200 metacercariae, three were treated at 12 weeks post-infection with ABZ (7.5mg/kg), three were left untreated, total worm burden was calculated at necropsy 14 days post-treatment. A ≥80% reduction in CET = ABZ susceptible. EHT: Five x 200 eggs were exposed to ABZ (5, 0.5, 0.05 µM) or MeOH only. Ovicidal activity (%OA) at the discriminating dose (0.5µM) and statistical difference in egg development rate was calculated (pairwise t-test). Isolates were identified as: a) resistant = %OA ≤40%, no statistical difference, b) susceptible = %OA ≥70%, statistically significant difference, c) equivocal = between %OA >40% and <70%. For isolate Ag, CET = 57.65% reduction in worm burden in ABZ+ sheep compared to untreated controls (ABZ- mean = 85 ± 21.8 SD, ABZ+ mean = 36 ± 7.94 SD, p = 0.047), indicating ABZ resistance. In EHT at the 0.5µM discriminating dose, % OA = 45.6% ± 1.96 SD and 41.2% ± 1.5 SD development rate (p = 0.01) indicating they fell in the equivocal range. For isolate E CET = 89.23% reduction in worm bur-

den (ABZ- mean = 97.5 ± 6.36 SD, ABZ+ mean = 10.5 ± 9.19 SD, p = 0.012), indicating ABZ susceptibility. In EHT %OA = 74.0% ± 3.5 SD at 0.5 µM, ($p < 0.001$) and development rate of 19.6% ± 3.5 SD ($p< 0.05$), indicating ABZ susceptibility. The EHT can reliably screen *F. hepatica* populations for their susceptibility to ABZ, but it may only detect ABZ resistance in highly resistant populations. However, it is a valuable screening method for phenotyping parasites for molecular studies on signatures of ABZ resistance.

Fasciola hepatica exploits the host fibrinolytic system to facilitate trans-intestinal migration

Judit Serrat (Institute of Natural Resources and Agrobiology of Salamanca, Spain), Marta López-García (Institute of Natural Resources and Agrobiology of Salamanca, Spain), María Torres-Valle (Institute of Natural Resources and Agrobiology of Salamanca, Spain), María Teresa Ruiz-Campillo (Universidad de Córdoba, Spain), Verónica Molina-Hernández (Universidad de Córdoba, Spain), Mar Siles-Lucas (Institute of Natural Resources and Agrobiology of Salamanca, Spain), Javier González-Miguel (Institute of Natural Resources and Agrobiology of Salamanca, Spain)

After excysting in the host's duodenum, newly excysted juveniles of *Fasciola hepatica* (FhNEJ) migrate to the intrahepatic biliary ducts. Understanding this migratory process is essential for developing more effective strategies to control fasciolosis. One key factor that may contribute to this process is plasmin, the active form of plasminogen (PLG) and a central enzyme in the host's fibrinolytic system. This broad-spectrum protease is commonly exploited by pathogens to facilitate tissue invasion, highlighting its potential role in the pathogenesis of fasciolosis. The aim of this study is to determine whether FhNEJ exploit the host fibrinolytic system as a mechanism to facilitate trans-intestinal migration. FhNEJ were obtained *in vitro*, and their ability to bind PLG and generate plasmin was assessed using ELISA and chromogenic assays. PLG-binding proteins in FhNEJ were identified via two-dimensional electrophoresis followed by mass spectrometry. To further explore the role of FhNEJ-induced plasmin generation in parasite migration, we used a co-culture system with host intestinal cells exposed to FhNEJ, and conducted experimental infections in mice treated with a fibrinolysis inhibitor. FhNEJ express proteins capable of binding PLG and promoting its activation into plasmin. Co-stimulation of host intestinal cells with FhNEJ and PLG significantly increased plasmin activity, which in turn enhanced collagen degradation and the secretion of PLG activators. Proteomic analysis of the stimulated cells revealed differential expression of proteins associated with cell adhesion, migration, and immune evasion. Moreover, mice with inhibited fibrinolysis showed reduced parasite migration during early infection. These findings highlight the important role of the host fibrinolytic system in facilitating the dissemination of FhNEJ and provide valuable insights into host-parasite interactions during the initial stages of fasciolosis.

Uncovering the adult _Fasciola hepatica_ glycoproteome: Distinct N- and O-glycan features of the mature stage

Carolina De Marco Verissimo (University of Galway, Ireland), Krystyna Cwiklinski (University of Liverpool, UK), Jonas Nilsson (University of Gothenburg, Gothenburg, Sweden), Ekaterina Mirgorodskaya (University of Go-

thenburg, Sweden), Chunsheng Jin (University of Gothenburg, Sweden), Tadgh Kilbane (University of Galway, Ireland), John P. Dalton (University of Galway, Ireland)

We recently published a comprehensive glycoproteomic study of *Fasciola hepatica* newly excysted juveniles (NEJs), revealing a highly heterogeneous glycosylation profile across 123 glycoproteins, 71 of which are secreted into the host environment. Given the dynamic and non-template-driven nature of glycosylation, and its crucial role in host-parasite interactions, developmental stage-specific changes in protein glycosylation are likely essential for parasite survival within different host niches, such as the gut and liver (NEJs) and the bile ducts (adults). In this study, we characterize the glycoproteome of adult *F. hepatica* using integrated glycomics and glycoproteomics approaches on somatic and excretory/secretory (ES) extracts. We identified 244 glycoproteins (44 in the ES fraction) and mapped 478 glycopeptides with their associated glycans. Similar to NEJs, adult worms exhibit extensive glycan heterogeneity generated by 1,914 N- and O-glycoforms. NEJs and adults co-express 82 glycoproteins, although with strikingly different glycan profiles. The present study expands the liver fluke glycan repertoire to 121 N- and 22 O-glycan structures. Some NEJ-specific features, such as pentosylated O-glycans, are absent in adults. In contrast, adult-specific N-glycans bear unique modifications with phosphate, glucuronic acid, and phosphoethanolamine, features rare or inexistent among helminths. Additionally, phosphocholine-containing N- and O-glycans were observed in adult glycoproteins. While the functions of these anionic and zwitterionic glycans remain unknown, their stage-specific expression suggests developmental regulation and therapeutic potential. Many identified glycoproteins are linked to tissue invasion, immune evasion, and virulence, making them promising candidates for further functional studies and vaccine development.

Room 2
Oral Communication

Innovative Strategies and Molecular Targets for the Development of Anti-Tick Vaccines
Session Chair: Naftaly Githaka (International Livestock Research Institute- ILRI, Kenya)

Experimental refinements in *Rhipicephalus microplus* challenge models to support anti-tick vaccine development

Alec Lewis Evans (GALVmed Nairobi, Kenya), Maxime Madder (Clinglobal, Mauritius), Julian Liebenberg (Clinvet, South Africa)

While guidelines for one-host tick challenge protocols have long been applied to assess synthetic acaricides, the prolonged efficacy of novel compounds and the complexity of evaluating anti-tick vaccines necessitate a review of existing methodologies. This study optimised one-host tick challenge models for vaccine efficacy studies with the goal of enhancing statistical power to detect treatment effects. We analysed data from controlled lab trials to identify key variables hypothesised to influence host susceptibility and tick retention and compared these against data from published vaccine/challenge studies. The influence of these variables, namely gender, body weight, and tick burden, on *R. microplus* development, was subsequently verified in a controlled lab trial. Findings informed a model validation study evaluating: (i) the effect of an initial infestation on subsequent

host-tick susceptibility, (ii) the influence of randomisation strategies based on animal lD, body weight, or tick count on variability, and (iii) optimal group sizes for statistical reliability. Bulls consistently exhibited significantly higher tick counts than cows. Tick count variability significantly increased after a second infestation and resting periods between infestations did not affect susceptibility. lnfestation jackets did not improve tick retention but reduced inter-animal variability. Applying these insights in a model validation study guided the identification of optimal randomisation variables and group sizes based on expected vaccine efficacy. Randomisation on body weight reduces variability after a subsequent challenge but does not reliably replicate in-field conditions. Considering an expected reduction in tick counts of 55% and a CV% of 40, a group size of 8 animals would be required to identify significance whilst a vaccine efficacy of >70% would require <8 animals irrespective of CV%.

Development and validation of a *Rhipicephalus microplus* challenge model specifically for use to evaluate anti-tick vaccines in cattle

Julian Edmund Liebenberg (Clinvet South Africa)

Regulatory authorities along with veterinary pharmaceutical companies must ensure that potential anti-tick vaccines are fit for purpose and achieve acceptable, consistent efficaciousness when used appropriately. Controlled challenge studies utilising robust and repeatable methodology and complying with relevant guidelines is of utmost importance to evaluate the potential efficaciousness of interventions. This is of particular importance in the field of anti-tick vaccines where guidelines are limited, rendering the comparison of results from different trials challenging. The objective of this study was to validate a *Rhipicephalus microplus* challenge model for evaluating anti-tick vaccine efficacy. lmportant parameters related to geographic location and the host, as defined based on previous studies, were specifically considered in the design of the model. The other major objectives were to investigate the effect of different randomisation procedures and to determine the optimal sample size at which robust statistical results could be achieved. The model was evaluated and compared between two clinical research sites in different geographic locations under Good Clinical Practice. Procedures were standardised at both sites to determine the effect of study location. Cattle were infested with approximately 3 000 *R. microplus* larvae on Day -28 and Days 56 and 58 at each site. Daily tick collections, counts and weighing were performed following each infestation period. Descriptive statistics were calculated for total and mean tick counts and weights for each assessment period (first vs last tick collections). Groups were compared using ANOVAs with group effect on mean tick count and weight. A significant difference was observed in tick counts and weights between study locations and assessment period while no statistical differences were observed between randomisation procedures or group sizes. The study highlighted the most important parameters to consider when designing future one host tick challenge models to evaluate the efficacy of vaccines.

A novel recombinant Australian cattle tick vaccine

Hannah V Siddle (The University of Queensland, Australia), Mikayla Crouch (The University of Queensland, Australia), Nimitha Ramachandran (The University of Queensland, Australia), Alicja E Tabor (The University of Queensland, Australia)

Cattle ticks and tick-borne diseases impact global agriculture in tropical and sub-tropical regions at $US22-30b annually. The burden is impacted by climate changes increasing the geographic range and increasing resistance to acaricides impacting the need for the development of alternative control methods. Previous commercial vaccines based on Bm86 have limited distribution due to either poor efficacies due to Bm86 protein variations or the requirement for more than one vaccine boost per year in countries reliant on extensive grazing mustering only once a year. A novel vaccine has been developed against *Rhipicephalus australis* (Australian cattle tick) using a reverse vaccinology approach to identify candidates. Candidates were filtered using data from in vitro tick feeding with sera from resistant cattle and the mixed screening of approximately 20 candidates in cattle resulting in the final selection of two candidate antigens. Two vaccine trials have been conducted to assess efficacy in *Bos taurus* cattle, Trial 1 (n=6/group) and Trial 2 (n=10/group). Both trial protocols involved vaccination followed by a 28-day boost and larval challenge (10,000) at one month and 6 months post boost. The efficacy of the vaccines was determined using tick numbers, egg weights per tick, and percent larval emergence. Results of Trial 1 show a combined efficacy of 86% ($p<0.05$) and a reduction of tick fecundity. There was evidence of vaccination boosting under natural field challenge conditions between the two artificial challenges demonstrated by increasing efficacies at 83% and 90%, respectively. The preliminary results of Trial 2 indicate a reduction in tick numbers in vaccinated animals using a higher dose of the two candidate proteins (students t-test $p=0.05$). The results highlight the potential of tick vaccines as a tool for control, with ongoing work interrogating the immune responses of cattle to the vaccine.

Rhipicephalus microplus voraxin-alpha contains B-cell epitopes that reduce ticks' biological fitness in immunized cattle

Juan Mosqueda (Universidad Autonoma de Queretaro, Mexico), Daniel Gustavo López-Díaz (Universidad Autonoma de Queretaro, Mexico), María Martina Esperanza Pérez-Soria (Universidad Autonoma de Queretaro, Mexico), José Rodrigo Morales-García (Universidad Autonoma de Queretaro, Mexico), Rafael Jiménez-Ocampo (Campo Experimental Valle del Guadiana-INIFAP, Mexico), Gabriela Aguilar-Tipacamú (Universidad Autonoma de Queretaro, Mexico), Massaro W. Ueti (Animal Diseases Research Unit, USDA-ARS, USA)

Rhipicephalus microplus ticks are a serious pest of cattle in tropical and subtropical regions, mainly due to the losses they cause by reducing meat and milk production, as well as causing hide damage, in addition to their role as vectors of babesiosis and anaplasmosis. Integrated tick control must include the use of anti-tick vaccines to reduce tick populations and mitigate the ecological impact associated to the extensive use of acaricides. To develop improved vaccines, evaluation of new tick antigens is essential. Voraxin, mainly described as a testes-derived protein, is transferred from males to females during copulation, and it is crucial for stimulating engorgement in female ticks and for

the development of their organs. In this study, *R. microplus* voraxin-alpha (voraxin-α) was amplified and sequenced, four peptides with predicted B-cell epitopes were designed and their immunogenic properties were evaluated. Each peptide was mixed with a commercial adjuvant and inoculated into two cattle from a tick-free region to corroborate if they were truly immunogenic. The antibody responses to each peptide were assessed using indirect ELISA. Two peptides were immunogenic and were selected for further testing involving immunization and experimental infestation with 10,000 larvae and two cattle per evaluated peptide. Immunization with peptide 3 reduced tick survival in 17.54%, oviposition in 13.84% and egg hatching in 22.16%, while peptide 4 impaired oviposition in 17.74% and egg hatching in 18.14%. No effect on weight was observed. In conclusion, ticks fed on cattle producing specific antibodies against voraxin-α Bcell epitopes, have a reduced survival, oviposition and fertility, which are important biological parameters, related to tick fitness. Vaccine trials are required to evaluate this antigen as a vaccine candidate against *R. microplus*.

Characterization of Scoloptoxin SSD14, Insitol monophosphatase and Neprilysin as anti--tick vaccines

Shafi Ullah (Universidade Federal do Rio Grande do Sul, Brazil), Luis Fernando Parizi (Universidade Federal do Rio Grande do Sul, Brazil), Abid Ali (Abdul Wali Khan University Mardan, Pakistan), Itabajara da Silva Vaz Junior (Universidade Federal do Rio Grande do Sul, Brazil)

Ticks are parasitic arthropods, responsible for vectoring tick-borne pathogens to animals and humans. Globally, synthetic acaricides are used for controlling tick infestations. Herein, Scoloptoxin SSD14, Insitol monophosphatase and Neprilysin antigens were immunologically characterized as anti-tick vaccine. Using bioinformatics tools, three *Rhipicephalus microplus* proteins (Scoloptoxin SSD14, Insitol monophosphatase and Neprilysin) were selected as a vaccine candidates based on physiochemical analyses and epitope prediction. The DNA fragments encoding these proteins were ligated into PET 32+ plasmids, inserted into *Escherichia coli* BL-21 star for expression. Rabbits were subcutaneously immunized using 100 µg of each purified protein emulsified in oil adjuvant, and the control group received only adjuvant. The bioinformatic analyses of these proteins revealed the presence of several immunogenic epitopes which could be useful in a vaccine against ticks. Following vaccination with these antigens, the Scoloptoxin SSD14 statistically decreased adult female egg laying up to 16%, and egg fertility decreased by 37%, with overall vaccine efficacy of 47%. Insitol monophosphatase statistically reduced the number of adult females up to 23%, egg laying in adult females up to 11%, and larval hatching decreased by 40%, counting for 59% total vaccine efficacy. Neprilysin statistically reduced the nymph numbers to 40%, larval hatching up to 41%, with overall vaccine efficacy of 64%. These results show the potential of these antigens in anti-tick vaccine development.

Biological Control of One-Host-Ticks by Anti-Tick Bm86 Immunomodulator

Suman M Mahan (Veterinary Medicine Research & Development, Ruminant Biologics Zoetis Inc. USA)

One-Host-Ticks also known as Cattle Fever Ticks or Blue Ticks consist primarily of 4 main

species: *Rhipicephalus (Boophilus) microplus, R. annulatus, R. decoloratus* and *R. australis*. The control of these ticks is economically important for cattle producers globally as they cause significant losses from morbidity and mortality due to blood loss and transmission of infections such as Anaplasmosis and Babesiosis. In addition, they have the unique ability of developing resistance to the current acaricides. An alternative that is sustainable, environmentally friendly control strategy is needed for control of these ticks. Zoetis Inc. has developed an anti-tick immunological product which can be used for control of *Rhipicephalus microplus, R. annulatus, R. decoloratus* and *R. australis* in regions of the world where these One-Host-Ticks exist. The Bm86 Immunomodulator is a unique two-dose product because of its novel formulation and contains a unique proprietary adjuvant. The Bm86 Immunomodulator induces a robust immune response activating both humoral and cell-mediated immunity. The Bm86 Immunomodulator induces a prolonged duration of immunity which has a strong anti-tick effect impacting the ability of One-Host-Ticks to complete their life cycle (repletion, fecundity, and fertility). These data will be presented. The Bm86 Immunomodulator is safe for use in young calves, and all types of cattle including pregnant cattle and can be incorporated into tick control programs.

Plenary Oral Communication

Leishmaniasis and Sand Fly: Epidemiology, Animal hosts, Vector Surveillance, and Control

Phlebotomine sand fly surveillance across Europe and neighbouring countries in 2023 conducted within the CLIMOS project

Carla Alexandra Soares Maia (Universidade Nova de Lisboa, Portugal), Erisoz Kasap Ozge (Hacettepe University, Turkey), Adam Katja (University of Primorska, Slovenia), Alcover Magdalena (Universitat de Barcelona, Spain), Arserim Suha Kenan (Ege University, Turkey), Bañuls Anne-Laure (University of Montpellier, France), Barandika Jesús (Basque Institute for Agricultural Research and Development (NEIKER), Spain), Basseville François (University of Montpellier, France), Bernardini Ilaria (Istituto Superiore di Sanità, Italy), Berriatua Eduardo (University of Murcia, Spain), Bianchi Riccardo (Istituto Superiore di Sanità, Italy), Bisia Marina (Benaki Phytopathological Institute, Greece), Bongiorno Gioia (Istituto Superiore di Sanità, Italy), Bouhsira Emilie (University of Toulouse, France), Cevidanes Miranda Aitor (Basque Institute for Agricultural Research and Development (NEIKER), Spain), Cuadrado-Matias Raúl (Spanish Game & Wildlife Research Institute, Spain), Dardé Marie-Laure (Institute of Epidemiology and Tropical Neurology, OmegaHealth, France), Delaunay Pascal (University of Nice Côte d'Azur, France), Delacour Sara (University of Zaragoza, Spain), Depaquit Jerome (University of Reims Champagne-Ardenne, France), Díaz-Sáez Victoriano (University of Granada, Spain), Falchi Alessandra (University of Aix-Marseille, University of Corsica, France), Favennec Loïc (University of Rouen Normandy, France), Fernández Guillerm (University of Zaragoza, Spain), Fisa Roser (Universitat de Barcelona, Spain), Robert-Gangneux Florence (Université de Rennes, France), Ivovic Vladimir (University of Primorska, Slovenia), Izri

Arezki (Avicenne Hospital, France), Jiménez Maribel (Collaborative Biomedical Research Center in Infectious Diseases, Spain), Kirstein Oscar (Israeli Ministry of Health, Israel), Kniha Edwin (24Medical University of Vienna, Austria), La Russa Francesco (Istituto Zooprofilatico Sperimentale della Sicilia, Italy), Liénard Emmanuel (University of Toulouse, France), Lucientes Javier (University of Zaragoza, Spain), Mangiapelo Claudia (Istituto Superiore di Sanità, Italy), Maroon-Maroon Inés (Collaborative Biomedical Research Center in Infectious Diseases, Spain), Martin-Sánchez Joaquina (University of Granada, Spain), Martinez-Barciela Yasmina (Universidade de Vigo, Spain), Mathieu Bruno (University of Strasbourg, France), Mekarnia Nalia (University of Reims Champagne-Ardenne, France), Mercier Aurélien (Institute of Epidemiology and Tropical Neurology, OmegaHealth, France), Mhaidi Idris (University of Montpellier, France), Michaelakis Antonios (Benaki Phytopathological Institute, Greece), Molina Ricardo (Collaborative Biomedical Research Center in Infectious Diseases, Spain), Morales-Yuste Manuel (University of Granada, Spain), Ozbel Yusuf (Ege University, Turkey), Paz Adolfo (University of Santiago de Compostela, Spain), Pekagirbas Metion (Adnan Menderes University, Turkey), Pérez-Cutillas Pedro (University of Murcia, Spain), Platzgummer Katharina (Medical University of Vienna, Austria), Polina Alejandro (Universidade de Vigo, Spain), Pomares Christelle (University of Nice Côte d'Azur, France), Prudhomme Jorian (Université de Rennes, France), Randrianambinintsoa Fano (University of Reims Champagne-Ardenne, France), Risueño José (University of Murcia, Spain), Roca-Geronès Xavier (Universitat de Barcelona, Spain), Ruiz-Fons Francisco (Spanish Game & Wildlife Research Institute, Spain), Sánchez Rita (University of Santiago de Compostela, Spain), Sevila Julie (University of Aix-Marseille, University of Corsica, France), Toz Seray (Ege University, Turkey), Yetismis Kardelen (Adnan Menderes University, Turkey), Dvorak Vit (Charles University, Czech Republic)

Sand fly-borne diseases, including leishmaniasis and phleboviruses, represent a major public health and veterinary concern. CLIMOS project aims to characterize the climatic, environmental, demographic, and epidemiological characteristics associated with the presence and abundance of sand flies at different geographic scales across Europe and neighbouring countries. To conduct active sand fly field surveillance across Europe and neighbouring countries during the sand fly season of 2023. Standardized sampling with CDC light traps was conducted in Portugal, Spain, France, Italy, Slovenia, Croatia, Germany, Austria, Czech Republic, Turkey and Israel between April-November in 2023. A total of 117 sampling sites were surveyed with an overall trapping effort of 3790 trap/nights for temporal data collections. Additional sand fly bycatch data were collected through the IDAlert project, which sampled 70 sites in Greece using BG traps. Sand flies were collected in all surveyed countries, except in the Czech Republic. More than 70,000 sand flies, belonging to 22 species of the genera *Phlebotomus* and *Sergentomyia* were recorded. Results confirm that the beginning and end of the sand fly season varied between the different locations, with species density following either a unimodal or bimodal seasonal pattern. This data, together with the results of sand fly-borne pathogens screening, will feed into epidemiological-climatic predictive mathematical models to help develop an early warning system to improve surveillance and

control of sand fly and transmitted pathogens in Europe and neighbouring countries.

Leishmania vs _Sauroleishmania_: new perspectives in the control of leishmaniasis

Jairo Alfonso Mendoza Roldan (University of Bari, Italy), Domenico Otranto (University of Bari, Italy)

While most species within the subgenus *Leishmania* are regarded as pathogenic to mammals, including humans, those in the subgenus *Sauroleishmania* are considered non-pathogenic (except for *Leishmania adleri*) and associated to Squamata reptiles. However, in areas such as the Mediterranean basin, both subgenera occur in sympatry and interactions within vertebrate hosts and vectors have been recently reported. For example, in canine leishmaniosis (CanL) endemic areas of Mediterranean, *Leishmania infantum* (Leishmania) occur in sympatry with the non-pathogenic *Leishmania tarentolae* (*Sauroleishmania*), being the latter vectored by the herpetophilic sand flies *Sergentomyia minuta*. Indeed, molecular diagnostics and serological positivity to *L. tarentolae* in humans and dogs, as well as *L. infantum* in synanthropic reptiles have spurred interest in these interactions. In these epidemiological contexts, reptiles, sand flies, mammals, including humans, living in the same environments, can be exposed to both species of *Leishmania*. Nonetheless, new isolates are warranted to fully unravel the life cycle of *Sauroleishmania* in reptiles as well as in mammals. Likewise, apart from the standardization of novel and highly specific molecular diagnostics, isolation of pathogenic *L. infantum* from reptiles is necessary to understand the role of these animals as reservoirs of mammalian leishmaniases. Moreover, recent studies have demonstrated that *L. tarentolae* could have applicability as a surrogate of pathogenic *Leishmania* species, being a suitable candidate for a vaccine against human and canine leishmaniases, exploiting its immunological cross-reactivity with other *Leishmania* species. Importantly, the non-pathogenic nature of *L. tarentolae* in dogs was recently confirmed, with a possible shift to Th1 phenotype after derived macrophages infection, as demonstrated by the expression of IFN-gamma. Therefore, *L. tarentolae* has demonstrated to have a great potential as immune-prophylaxis/immune-therapy against *Leishmania* infections in dogs and humans.

Canine leishmaniasis in central and southern Tunisia: focus on different _Leishmania_ species.

Lilia Zribi (Institut Pasteur de Tunis, Tunisia)

Tunisia represents the perfect example of a Mediterranean Country where different *Leishmania* species may express their infectivity causing Visceral leishmaniasis (VL) by *Leishmania* (*L.*) *infantum*, Zoonotic Cutaneous Leishmaniasis (ZCL) by *L. major* and chronic cutaneous leishmaniasis (CCL) by *L. tropica*. The recent detection of *L. major* in two dogs living in Tunisia confirms how this animal may host both visceral and cutaneous *Leishmania* species. The present study reports the results of 4 field surveys performed in central and southern districts of Tunisia: Zaghouan (ZA); Kairouan (2 surveys, K1 and K2); Tataouine (TA), to assess the prevalence of *Leishmania* species in dog. One hundred and sixteen dogs were enrolled. Blood, lymph node and skin samples were collected with the owner consent. Sixty -two dogs were enrolled during 2024 (K2, n= 32and TA,

n= 30), fifty-four dogs were sampled during 2021 (ZA, n=32) and 2022 (K1, n= 22) but never investigated before. In total 218 biological samples were analyzed by qPCR (kDNA), end-point PCR (ITS-1) and nested-PCR (SSUrRNA). The purified positive PCR products were sequenced. All dogs were classified as asymptomatic or with mild clinical signs, not specifically attributable to Canine Leishmaniosis (CanL) due to the presence of fleas and tick infestation. Thirtyfive dogs tested positive (30.2%), the most to *L. infantum* (29/116, 25%). Extremely high prevalence was found in K2 (23/32, 71.8%) compared with previous K1 (7/22, 31.8%). One dog (K1) was positive to *L. tropica,* the first detection of this species in Tunisia, while one dog (ZA) confirmed the presence of *L. major*. Interestingly, we found for the first time a dog positive to *L. infantum* in TA, an arid area where no VL cases have been previously recorded. This study pointed out the high circulation of *L. infantum* in central Tunisia and underlines as the dog can host all the 3 *Leishmania* species present in this country.

Phlebotomine sand flies from Somalia: species diversity and distribution

Kassim Abdullahi Jimale (University of Bari, Italy), Marcos Antonio Bezerra-Santos (University of Bari, Italy), Jeilani Bussuri Mio (Somali National University, Somalia), Said Ali Ibrahim (Somali National University, Somalia), Juweria Abdulkadir Mohamoud (Somali National University, Somalia), Mohamed Adam Mahamud (National Public Health Reference laboratory, Somalia), Domenico Otranto (University of Bari, Italy)

Phlebotomine sand flies are vectors of protozoa of the genus *Leishmania*, which affect different animal species, including humans. Somalia is considered an endemic country to visceral leishmaniasis (VL), mainly caused by *Leishmania donovani*. However, data on sand fly diversity in the country remain limited due to lack of survailance. Here we assessed the sand fly fauna of Somalia, to identify potential vectors of *Leishmania* spp. Samples were collected from four ecological sites (i.e., predomestic, cave, farmland and termitehills) across three districts in southern-central Somalia (i.e., Beledweyne, Balad, and Mogadishu). Insects were trapped at leat twice a month using CDC light traps and sticky papers soaked in sesame oil, from December 2024 to February 2025. Sand flies were mounted using Hoyer's medium and thereafter morphologically identified to species level. Overall, 362 sand fly specimens were collected and identified as *Phlebotomus alexandri* (*n* = 13; 3.6%), *Phlebotomus sergenti* (*n* = 10; 2.8%), *Phlebotomus bergeroti* (*n* = 4; 1.1%), *Sergentomyia clydei* (*n* = 172; 47.5%), *Sergentomyia antennata* (*n* = 78; 21.5%), *Sergentomyia squamipleuris* (*n* = 34; 9.4%), *Sergentomyia africana* group (*n* = 19; 5.2%), *Sergentomyia schwetzi* (*n* = 12; 3.3%), and *Sergentomyia* spp. (*n* = 20; 5.5%). Sand flies were found in all surveyed districs and ecological sites, with predomestic areas and caves having the highest abundance (i.e., *n* = 165, 45.5%; *n* = 113, 32.2%, respectively) compared to farmland (*n* = 75; 20.5%) and termitehills (*n* = 9; 2.5%). This study provide data on phlebotomine sand fly species diversity in southern-central Somalia where no similar entomological data have been published. Proven or suspected vectors of both zoonotic and anthroponotic cutaneous leismaniases (i.e., *P. alexandri, P. sergenti* and *P. bergeroti*) where herein identified, advocating further monitoring and control of these vectors in these endemic areas.

Circulating immune complexes as biomarker in CanL: protein composition related to disease stage.

Jerónimo Carnés (R&D Allery & Immunology Unit. LETI Pharma S.L.U.), Nuria Parody (R&D Allery & Immunology Unit. LETI Pharma S.L.U.), Gemma Navarro (R&D Allery & Immunology Unit. LETI Pharma S.L.U.), Cristina Cacheiro-Llaguno (R&D Allery & Immunology Unit. LETI Pharma S.L.U.)

Canine visceral leishmaniasis (CanL) is a global zoonosis caused by *Leishmania infantum* potentially fatal for dogs. Susceptible dogs develop an uncontrolled Th2-immune response characterized by high parasite-specific antibody titers. Such response leads to the formation of circulating immune complexes (CIC) composed by aggregates of *Leishmania* proteins and anti-*Leishmania* IgG and IgM. Gradual CIC deposition in tissues is responsible of clinical manifestations of CanL, including renal pathology. A *Leishmania*-specific method to isolate and measure CIC in serum samples was described. The predictive value of CIC to disease progression was evidenced by a positive correlation between CIC levels and several parameters associated to different CanL stages. To characterize the protein composition of CIC in sick animals in different stages of CanL disease and its relation to the clinical and immune status. Sera from 24 dogs (UCM Veterinary Hospital, Madrid) classified according to LeishVet (stage-I, stage-II, stage-III/IV) were obtained from blood samples (n=8/group). CIC were isolated and quantified by *Leishmania*-specific-PEG-ELISA. Isolated CIC were digested with trypsin to obtain peptides labeled with TMT. Peptides were fractionated by Pierce High pH Reversed-Phase Peptide Fractionation. Identification was performed with uniprot-canis-lupus-familiaris database using PEAKSv11.5. Quantitative analysis was developed by RP-LC-MS/MS (PEAKSv11.5 "Reporter Ion Quantification TMT", "Quantifications") to calculate overall ratio from the total intensity of labeled quantifiable peptides. To identify the differentially expressed proteins upregulated in the different disease stages, a cluster and a heatmap representation were done using pheatmap Rpackage (V1.0.12) in R (V4.3.3). Pathway enrichment analysis was done with enrich GO function from the cluster Profiler Rpackage. The analysis of CIC samples identified 196 differentially expressed proteins. Five different protein clusters were defined (G1up, G2up, G3up, G1,2up and G2,3up) for pathway enrichment and GO terms analysis ($p<0.05$). The results confirmed that CIC protein composition is stage-specific. Protein composition of CIC is specific for CanL stage and reflects the immune status of the host stage. CIC is a promising biomarker in CanL useful for diagnosis, treatment monitoring and vaccine efficacy control.

Evaluation of the impact of combination of LETIFEND® vaccine + SCALIBOR® collar on canine leishmaniosis prevalence in field condition

Valentina Foglia Manzillo (Dept of Veterinary Medicine and Animal Production Napoli, Italy), Gaetano Oliva (Dept of Veterinary Medicine and Animal Production Napoli, Italy), Germano Castelli (National Reference Centre for Leishmaniosis - IZS Palermo - Italy), Federica Bruno (National Reference Centre for Leishmaniosis, Italy), Roberto Rosenthal (DVM Practitioner, Italy), Serena Montagnaro (Dept of Veterinary Medicine and Animal Pro-

duction, Italy), Ines Balestrino (Dept of Veterinary Medicine and Animal Production, Italy), Liliana Colombo (MSD Animal Health), Manuela Gizzarelli (Dept of Veterinary Medicine and Animal Production, Italy)

Canine leishmaniosis (CanL) is endemic in Italy with an estimated median prevalence of 17.7%. Preventive strategies play a pivotal role in disease control. This study evaluated the impact of LETIFEND® vaccines and SCALIBOR® collars used together on Favignana Island, Sicily, Italy. Dogs (n=154) with owner consent underwent clinical evaluation, blood sampling, and conjunctival swabs to determine their CanL and co-infection status using serology, qPCR, and SNAP® 4Dx® Plus testing. The CanL positivity rate was 12.3% (19 dogs). Dogs testing positive for any pathogen were excluded. Negative dogs (n=108) were vaccinated with LETIFEND® and provided with SCALIBOR®. Sixty-two dogs were evaluated every 6 months for a period of 2 years. Forty-six dogs failed to attend the subsequent evaluations and did not complete the study for reasons unrelated to *Leishmania* infection. Each follow-up included clinical examination, rapid Leishmania test, SNAP® *Leish*® Test, and conjunctival swab qPCR. Serum samples were stored for retrospective IFAT analysis. After one-year, negative dogs received a booster vaccination and collar replacement. At the end of the study, 2 dogs tested positive by SNAP® *Leish*® test and showed clinical signs. CanL morbidity was 3.22% (2/62; 95% CI 0.00–7.22). Retrospective IFAT analysis identified 9 low-positive dogs not detected by the rapid test. All these dogs remained asymptomatic and showed no clinicopathological alterations throughout the study. The estimated cumulative incidence was 14.5% (SE 8.77; 95% CI 5.7–23.3), while the incidence density, calculated as the number of new cases/total time at risk (months), was 0.61%. No adverse reactions were reported. The study demonstrates efficacy and safety of the LETIFEND® and SCALIBOR® combination in preventing clinical leishmaniosis in dogs over 2 transmission seasons. Re-vaccination of seropositive dogs did not lead to disease progression.

Usefulness of volatile organic compounds for the repellence and attraction of arthropod vectors

Marcos Antonio Bezerra Santos (University of Bari, Italy), Domenico Otranto (University of Bari, Italy)

Volatile organic compounds (VOCs) are chemicals emitted as products of cell metabolism, reflecting the physiological and pathological conditions of living organisms. Several VOCs are produced by vertebrate hosts; however, only a relatively small number influence the behavior of arthropod vectors, and these are defined as 'allelochemicals', with an attractant (kairomones) or repellent (allomones) effect. In this context, kairomones can serve as selective tools for studying population abundance, monitoring invasive species, surveillance of vector-borne pathogens, and predicting pathogen outbreaks. Additionally, the repellent effect of allomones represents a potential tool for controlling arthropod vectors. Indeed, VOCs play a crucial role as olfactory cues for arthropods of medical and veterinary importance (e.g., mosquitoes, sand flies, and ticks), influencing their behavioral choices such as host preference and the selection of oviposition sites for gravid females. Moreover, deadly vector-borne pathogens such as *Plasmodium falciparum* and *Leishmania infantum* have been suggested to manipulate the

VOC profile of host cells, making them more attractive to mosquitoes and sand fly vectors, respectively. In this regard, studies on VOCs have demonstrated their potential usefullness as attractants or repellents for mosquitoes, sand flies and ticks, as well as for the diagnosis of vector-borne diseases (VBDs), such as malaria and leishmaniasis. Here, we provide an overview of the scientific data available on VOCs, focusing on their role in studying the hostseeking behavior of arthropod vectors, and their potential as attractants, repellents, or tools for the early diagnosis of VBDs.

16:00 – 17:30

Room 1
Symposium

Liver Fluke Symposium

Towards Precision Parasite Control - In the Field

Session Chairs: Grace Mulcahy (University College Dublin, Ireland) and Javier González-Miguel (Institute of Natural Resources and Agrobiology of Salamanca, Spain)

Fascioliasis in Peru: Current Status with a One Health Approach

Pedro Luis Ortiz Oblitas (Universidad Nacional de Cajamarca, Perú), César A. Murga-Moreno (Universidad Nacional de Cajamarca, Perú), Cristian Hobán (Universidad Nacional de Cajamarca, Perú), David Ruiz-Pérez (Universidad Nacional de Cajamarca, Perú), Dayana M. Terrones-Cerna (Universidad Nacional de Cajamarca, Perú), Ana M. Fernández-Sánchez (Universidad Nacional de Cajamarca, Perú), Jhover Díaz-Muñoz (Universidad Nacional de Cajamarca, Perú), Sandra Quispe (Universidad Nacional de Cajamarca, Perú), Fabiano Cruzado-Chávez (Universidad Nacional de Cajamarca, Perú), José Aliaga-Tambo (Universidad Nacional de Cajamarca, Perú), Fernanda Cruzado-Chugden (Universidad Nacional de Cajamarca, Perú), Angie Chávez-Pérez (Universidad Nacional de Cajamarca, Perú), Alejandra Hoyos-Sangay (Universidad Nacional de Cajamarca, Perú), Miguel M. Cabada (The University of Texas Medical Branch, United States)

Fascioliasis is a neglected parasitic disease caused by the trematode *Fasciola* spp. In South America, *F. hepatica* affects people in poor rural Andean communities, generating significant economic losses in livestock farming. The objective of this study was to describe the epidemiology of fascioliasis in the Cajamarca province of Peru. Between March 2023 and January 2025, we tested humans, livestock, and snails for fascioliasis as part of a one-health study in Peru. Subjects of both sexes, 3 years and older, and livestock and Lymnaeid snails present in their farms were included. Stool samples from 1249 humans, 1254 cattle, 562 sheep, 163 swine and 514 snails were collected. Human stool was tested using rapid sedimentation and Kato-Katz microscopy tests. Livestock stool was tested by rapid sedimentation microscopy and snails by microscopy. *F. hepatica* eggs were detected in 110 humans (8.8% ± 1.5), 77 (70.0%) were children (3-17 years), 33 (30.0%) were adults; and 68 (61.8%) were females. *F. hepatica* eggs were detected in 424 cattle (33.8% ± 2.6), 214 sheep (38.1% ± 4.0) and 17 swine (10.4% ± 4.7). The larval forms of *F. hepatica* were detected in 65 (12.6% ± 2.9) snail specimens. Ninety six humans with fascioliasis were treated with two doses of triclabendazole at 10 mg/kg of body weight

after ingesting a meal and separated 24 hours. To date, only 40 subjects have completed the 90-day follow-up and 12 (30.0%) stopped passing eggs in the stool. Twenty five school aged children and 3 adults have failed to respond. The prevalence of fascioliasis among human, livestock, and snails was high in the Cajamarca province. The proximity of definitive and intermediate hosts in the farms facilitates the maintenance of *Fasciola*'s life cycle and the decreasing effectiveness of triclabendazole threatens control efforts.

Fasciola hepatica in sheep in the United States: a prevalence survey suggests expansion of the range to the Great Lakes region

Adriano Vatta (Louisiana State University, USA), Adriano Vatta, Brooke Delcambre, Alyson Wiedenheft, Matthew Branan, Elisa Preston, Collin Hayes, Peter Thompson, Moara Rodgers, Joan Burke, Natalie Urie

Fasciola hepatica, the common liver fluke, has been shown to be endemic in the Pacific Northwest and Gulf Coast. To provide updated information on *F. hepatica* prevalence, as part of the National Animal Health Monitoring System Sheep 2024 study (OMB Control Number 0579-0488), selected farms from the top 30 sheep-producing states voluntarily participated in fecal sample collection from April to December 2024. On each farm, feces were collected from at least 8 sheep and pooled to make one composite sample. The samples were examined for the presence of eggs by fecal sedimentation (FLUKEFINDER®, Soda Springs, ID) and *F. hepatica* antigen in feces by indirect sandwich ELISA (BIO K 201 – Monoscreen AbELISA *Fasciola hepatica*, Bio-X Diagnostics S.A., Rochefort, Belgium). Percentages presented below were weighted to account for the survey design of the study. Samples were collected from 62, 111, 118, and 47 operations in the western, central, northeastern, and southeastern regions, respectively. At the national level, 6.7 ± 1.6 % of operations had sheep that tested positive for *F. hepatica* by either or both tests. At the regional level, the corresponding percentages were 10.1 ± 4.2 % in the western region, 8.9 ± 3.2 % in the central region, 5.4 ± 2.3 % in the northeast region, and 2.4 ± 2.3 % in the southeast region ($P = 0.411$). This is the first report of *F. hepatica* in the northeast region. The percentage of operations with a positive test by at least one method was 9.9 ± 4.9 % for farms in Wisconsin, Indiana, and Michigan. At least one sample tested positive from Iowa. These results provide an update to the known distribution of *F. hepatica* and should guide the veterinary community in looking for this parasite in areas where it was not thought to occur.

Working towards the implementation of a Fasciola hepatica prediction tool for Dutch dairy farms

Lonneke Nijhuis (Utrecht University, Netherlands), Manon M.C. Holstege (Research & Development, Royal GD, Netherlands), Adriaan F.G. Antonis (Utrecht University, Netherlands), Deborah C.K. van Doorn (Utrecht University, Netherlands; Research & Development, Royal GD, Netherlands), Jaap A. Wagenaar (Utrecht University, Netherlands), Gerdien van Schaik (Utrecht University, Netherlands; Research & Development, Royal GD, Netherlands)

Infections with *Fasciola hepatica* in dairy cattle often lead to chronic illness and associated production losses. In the Netherlands, groundwater levels in peat meadow areas will increase

to prevent CO2 emission, which will probably increase infection rates. With limited treatment options due to triclabendazole resistance, more sustainable management practices are urgently needed. This study aimed to identify associations between weather conditions and *F. hepatica* infections in Dutch dairy herds. This will enhance the understanding of the transmission dynamics, and will support the development of a prediction tool to identify herds at risk of infection. From 2018 till 2023 bulk tank milk samples were collected from 2660 Dutch dairy farms, though not all farms participated for the entire study period. *F. hepatica* antibody levels were measured yearly in October using ELISA. For each farm, monthly averages of rainfall, temperature, radiation and relative soil moisture, annual warm days, soil type and herd size were obtained. Logistic regression analyses were performed retrospectively using generalized estimating equations, with continuous variables analysed as quartiles and data also stratified by soil type. The analyses show that higher monthly temperatures (Dec: ORQ1-Q4 2.94, $p < 0.001$) and rainfall (Nov: ORQ1-Q4 2.33, $p < 0.001$) at the end of the previous grazing season were associated with an increased likelihood of having antibody levels indicative of a *F. hepatica* infection. Similarly, a higher annual number of warm days was positively associated (ORQ1-Q4 1.76, $p < 0.01$). The odds of having an infection are higher for farms on peat (OR 1.69, $p < 0.001$) and heavy clay soils (OR 1.75, $p < 0.001$) compared to those on sandy soil. Rainfall and temperature during specific periods are strongly associated with *F. hepatica* infections in Dutch dairy herds, supporting the development of a prediction tool for high-risk areas. To predict infections more accurately at farm level, future analyses could be improved by incorporating farm management factors and changes in *F. hepatica* antibody levels during the season. Ultimately, improved surveillance should lead to more sustainable management of *F. hepatica* infections on dairy farms.

Beyond Suspicion: Confirming the prevalence and occurrence of drug-resistant *Fasciola hepatica* in sheep, goats and cattle in the Southern Tablelands of NSW, Australia

Chelsie Uthayakumar (The University of Sydney, Australia), Hayley Martinez DeCristi (The University of Sydney, Australia), Emily Kate Francis (The University of Sydney, Australia), Roger Willoughby (Gunning Ag & Water Solutions), Shannon Taylor (The University of Sydney, Australia), Nichola Eliza Davies Calvani (The University of Sydney, Australia)

In Australia, *Fasciola hepatica* (liver fluke) is the 13th most important cause of losses to the sheep meat industry. First detected in Australia in 1995, resistance to the frontline drug, Triclabendazole (TBZ) is now present worldwide. In 2023, livestock producers from the NSW Southern Tablelands raised concerns over a 230% increase in liver fluke due to suspected drug resistance. To confirm or deny these suspicions as the cause of drug failure, we evaluated the prevalence and drug susceptibility of *F. hepatica* on eight farms. Nine mobs (seven sheep, one goat, one cattle) were split into three treatment groups of 15 animals/group and were administered either TBZ, AVOMEC DUEL/Albendazole (ABZ) (sheep/goats), or water. Treatments were administered according to individual animal weights, with faecal samples, animal weight, and body condition score collected on Day 0 and 21. Our results showed evidence of TBZ resistance (89%-92% efficacy) on one sheep property. Interestingly, TBZ susceptibility (97%-98%

efficacy) but ABZ resistance (77%-79% efficacy) was detected on the goat property, marking the first report of ABZ resistance in goats. Our results reinforce the increasing threat of drug resistance, highlighting the need for ongoing surveillance and development of alternative control options.

Mapping the discrepancy between farmer perception of liver fluke infection risk areas and actual *G. truncatula* habitats.

Rhys Jones (Aberystwyth University, UK) Rhys Jones, Gwen Rees, Serian Evans, Chelsea Davis, Hefin Williams, Chris Smith, Russ Morphew, Peter Brophy, Manod Williams

Liver fluke is amongst the most detrimental pathogens of livestock globally. Anthelmintic resistance seriously hamper efforts to control fluke efficiently, and so there is an urgent need for widespread uptake of non-chemical control strategies where land or grazing management strategies impairs the fluke lifecycle which involves an intermediate host (*G. truncatula*). However, it is unclear how well farmers can identify risk areas for targeted action to reduce fluke infection risk on their farms. The objective of this study was to evaluate farmer perception and understanding of fluke infection risk areas and to identify discrepancies between the types of areas perceived to be risk areas and those where *G. truncatula* snails were found. Qualitative interviews were conducted with 16 Welsh farmers who also identified perceived fluke risk areas on maps, before a physical and eDNA survey of their farmland was undertaken to identify risk areas. Major uncertainty was conveyed by farmers regarding the location of fluke infection risk areas on their farm as well as to general fluke prevalence in their livestock and their confidence in applying control strategies. Discrepancies were found when comparing areas identified as risk areas by farmers and actual *G. truncatula* habitats (Kappa agreement = -0.05 (95% CI -0.25 – 0.14). Farmers were significantly more likely to identify ponds as risk areas ($p = 0.03$), however, *G. truncatula* snails were less likely to be found in such habitats ($p = 0.02$). Farmers also associated fluke risk with acidic and high organic matter soils, however, these soil conditions were negatively associated with *G. truncatula* presence ($P < 0.05$). Results suggests that farmers have limited ability to identify key fluke risk areas to apply land and grazing management strategies to limit fluke infections in their livestock. As such further guidance and support needs be offered to better inform of and design non-chemical control of liver fluke.

Co-desigining national research priorities for fasciolosis: Lessons from Australia

Nichola Eliza Davies Calvani (The University of Sydney, Australia), Neil D. Young (The University of Melbourne, Australia)

In March 2025, a national fasciolosis workshop was held at the University of Melbourne, bringing together researchers, industry, and government stakeholders to co-design Australia's future research agenda for *Fasciola hepatica*. For the first time in several decades, all major research groups with active fasciolosis funding participated alongside representatives from Meat & Livestock Australia, Dairy Australia, Zoetis, Virbac, and the New South Wales Department of Primary Industries. Structured around four core sessions; (1) industry and government perspectives, (2) on-farm research, (3) fundamental science, and (4) an open

discussion on priorities, the workshop facilitated alignment across the research–practice interface. While this event was only a starting point, several collaborative projects are now in development, guided by a shared recognition that research impact is maximised when producers and stakeholders are involved from the outset. Key areas identified for investment included: national-scale epidemiology, improved stewardship of current anthelmintics, and robust economic modelling to support decision-making and producer adoption. These themes, which are grounded in end-user needs, form the basis of a multi-institutional approach designed to ensure coordinated, scalable outcomes. This talk will present key decisions from the workshop and outline plans for a follow-up two-day meeting in 2026. We warmly invite the international parasitology community to join us as we revisit progress, reaffirm priorities, and co-design the next phase of collaborative research in fasciolosis.

Room 2
Round Table

Best Practices and Innovation in Cattle Tick Control

Session Chair: Shyma Kunhipurayil (Bihar Animal Sciences University, India)

Innovative Pharmacological Approaches to cattle tick Control

Adrian Lifschitz (UNCPBA-CICPBA-CONICET, Argentina)

Control of *Rhipicephalus microplus* in livestock largely depends on synthetic acaricides. However, widespread and improper use has led to resistance across nearly all active ingredients. The discovery of new compounds in veterinary medicine is slow and complex. In fact, only one novel acaricide—fluralaner—has been introduced after more than 25 years. Once administered, acaricides must be released from their formulation, reach effective concentrations at the target site, and penetrate the tick (pharmacokinetics) before exerting its action (pharmacodynamics). Therefore, the choice of administration route and formulation is critical for maximizing efficacy. A clear understanding of the pharmacokinetic behavior of each drug is essential for treatment success. In many regions, plunge dips remain central to tick control. Maintaining effective concentration in the dip solution is crucial for achieving high efficacy. Systemic-acting compounds have become key tools for controlling ticks. Notable examples include macrocyclic lactones (ivermectin, abamectin, doramectin, eprinomectin, and moxidectin), the chitin synthesis inhibitor fluazuron, and fluralaner—a recently developed isoxazoline with a novel mechanism of action. Each of these drugs has distinct pharmacokinetic profiles that influence their duration, efficacy, and role in resistance development. The introduction of new molecules like fluralaner offers a promising tool for integrated tick management but requires responsible use to preserve its effectiveness and ensure long-term control.

From lab to field: bioassays as decision-making tools for acaricide application

Guilherme Klafke (Instituto de Pesquisas Veterinarias Desiderio Finamor, Brazil), Priscila Teixeira Ferreira (Instituto de Pesquisas Veterinarias Desiderio Finamor, Brazil), Ana Luiza Loch (Instituto de Pesquisas Veterinarias Desiderio Finamor, Brazil), Rovaina Laureano Doyle (Instituto de Pesquisas Veterinarias De-

siderio Finamor, Brazil), Jose Reck (Instituto de Pesquisas Veterinarias Desiderio Finamor, Brazil)

The use of bioassays to evaluate acaricide efficacy remains the most effective tool for managing acaricide resistance and improving cattle tick (*Rhipicephalus microplus*) control strategies in the field. Bioassays support early detection of resistance, guiding decisions on product rotation, dosage, and strategic adjustments. Accurate predictions of field efficacy also promote more rational and sustainable acaricide use, reducing unnecessary treatments, environmental impact, and associated costs. However, challenges remain, including the standardization and harmonization of methods across laboratories, the difficulty of replicating field conditions in lab settings, and the interpretation of results in complex, multifactorial scenarios involving host, environment, and management variables. In this study, we compared laboratory bioassay results with field efficacy data for the systemic acaricides ivermectin and fluazuron on six cattle farms in Rio Grande do Sul state, southern Brazil. The larval immersion test for ivermectin accurately predicted susceptibility levels across all farms. One farm classified as susceptible in the bioassay (resistance ratio, RR = 1.22) showed 95% field efficacy 21 days after treatment with injectable ivermectin. Four farms with moderate RR values (2.06–2.29) exhibited field efficacies between 83% and 87%, while the highest RR (6.67) was associated with only 50% efficacy. For fluazuron, the adult immersion test using a discriminating dose showed that the three tick populations with low in vitro efficacy (<80%) had poor field performance (0–66%) using the pour-on formulation. Conversely, the three populations with high in vitro efficacy (88–94%) showed field efficacies exceeding 96%. In conclusion, our results show that laboratory bioassays are reliable tools and should be routinely used for designing effective and sustainable tick control strategies.

Protecting the innovation: Integrated control strategies that can delay the emergence of acaricide resistance in cattle ticks (*Rhipicephalus microplus*)

Nick N. Jonsson (University of Glasgow, UK)

Bringing novel acaricides to the market requires a huge economic investment, the scale of which demands protection. Although many distinct strategies exist to bring new products to the market, veterinary pharmaceutical companies have a limited period of complete protection for new products and need to balance the competing requirements of maximising the return on investment during this period of protection, and maximising the sustainability of their products in the field. Although it has proven impossible to accurately predict when a product will begin to lose efficacy in the field due to genetic resistance in *Rhipicephalus microplus*, it is reasonable to assume that it will do so, likely within a decade or two of introduction. It is also reasonable to assume that the interval during which resistance is not a problem can be extended by applying best practice (techniques or processes that have been proven to reliably lead to desired outcomes). There can be more than one best practice for any given problem or process and for cattle tick control, the lack of robust scientific evidence in some important areas means that recommendations are based partly on the literature and partly on the authors' collective experience in the field. Three principles of sustainability can be applied for best practice in cattle tick control: 1) ensuring

environmental and human safety; 2) enhancing the production of high-quality, high welfare animal products; and 3) maintaining the financial viability of cattle farms. We propose five points as the basis of an approach to these three sustainability principles: 1) Treat with the right product; 2) Treat using the right dose; 3) Treat the right animals; 4) Treat at the right time; 5) Use non-acaricidal methods. Each of these is addressed in the presentation. Applying these points is a sound basis for integrated control of ticks, that can be expected to extend the effective lifespan of new acaricides.

Plenary Round Table

Taxonomy and Phylogeny of Trypanosomatidae: Linking Diversity to Disease

Canine leishmaniasis in the Americas: from etiology to zoonotic importance

Filipe Dantas-Torres (Instituto Aggeu Magalhães, Fiocruz, Brazil)

Canine leishmaniasis is a widespread disease on the American continent, with cases reported from Uruguay to the USA and Canada. While numerous *Leishmania* spp. have been reported in dogs in this region, *Leishmania infantum* and *Leishmania braziliensis* are the most common etiological agents of canine leishmaniasis from a continental perspective. Other species may also be locally prevalent in dogs from some countries, including *Leishmania panamensis* in Colombia. The participation of dogs in the transmission cycle of various *Leishmania* spp. has long been speculated. Still, evidence indicates that their role as reservoirs of species other than *L. infantum* is minor. Various native wildlife (e.g., small rodents, marsupials, sloths, and monkeys) are, in fact, the primary hosts of *Leishmania* spp. in the American continent. Considering the clinical importance of canine leishmaniasis and the limited treatment availability in the Americas, preventive measures are key to mitigating the risk of infection in uninfected dogs. This may also minimize the role of infected dogs as reservoirs, which is pivotal to reducing the risk of infection in humans and other susceptible animals, including cats.

August 19 - 2025
09:00 – 10:00

August 19 - 2025
9:00 – 10:00

Plenary
Plenary Session

Smart Agriculture: AI Applications in Small Ruminant Health and Pasture Monitoring

Thomas H. Terrill (Fort Valley State University), Aftab Siddique (Fort Valley State University), Ajit K. Mahapatra (Fort Valley State University), Sudhanshu S. Panda (University of North Georgia, USA), Eric R. Morgan (Queen's University, UK), Andres A. Pech-Cervantes (Prairie View A & M University, USA), Jan A. van Wyk (University of Pretoria, South Africa)

Innovations in artificial intelligence (AI) and sensor technologies are rapidly advancing animal and plant health management. At Fort Valley State University, researchers are applying machine learning (ML) to develop fast, non-invasive, and scalable tools for diagnosing parasitic infections, monitoring anemia, and identifying beneficial forage species in small ruminants. AI-driven systems have been used to analyze physiological data from bioelectrical impedance analysis (BIA), automate anemia detection from blood image patterns, and replace labor-intensive FAMACHA© scoring with image-based classification using convolutional neural networks (CNNs). These models improve diagnostic precision, reduce subjectivity, and are adaptable to mobile applications suitable for use in low-resource settings. In parallel, AI-powered image recognition tools have enabled accurate detection of anti-parasitic forage plants such as sericea lespedeza (Lespedeza cuneata) within mixed field vegetation, improving pasture management. Integration of remote sensing data, including satellite climate information and RFID-based behavioral tracking, enhances the potential for predictive modeling of parasite outbreaks and forage growth across diverse environments. These technologies collectively aim to support precision livestock care, reduce treatment errors, and promote sustainable farming. International collaborations strengthen the reach of these innovations, offering new opportunities for veterinary training, farmer engagement, and global agricultural resilience.

10:30 – 12:00

Room 1
Symposium

TroCCAP-ESCCAP-CAPC-Joint Symposium
Session chair: Filipe Dantas-Torres (Fundação Oswaldo Cruz, Brazil)

Companion animal parasite control in the tropics

Filipe Dantas-Torres (Instituto Aggeu Magalhães, Fiocruz, Brazil; Tropical Council for Companion Animal Parasites)
The Tropical Council for Companion Animal Parasites Ltd. (TroCCAP) is a not-for-profit organization whose mission is to independently inform, guide, and make best-practice recommendations for the diagnosis, treatment, and control of companion animal parasites in the tropics and sub-tropics, to protect animal and human health. In line with this primary mission, TroCCAP recently developed guidelines for diagnosing, treating, and controlling feline and canine parasites in the tropics. The development of these guidelines required unique and complex considerations to be addressed, often inapplicable to developed nations. Much

of the tropics encompasses middle-to-low-income countries in which poor standards of environmental hygiene and large populations of stray dogs and cats coexist. In these regions, a range of parasites poses a high risk to companion animals, which may place their owners at risk of acquiring parasitic zoonoses. These considerations led to the development of unique recommendations with regard, for example, to deworming and endoparasite testing intervals for the control of both global and 'region-specific' parasites in the tropics. Moreover, the off- or extra-label use of drugs for the treatment and control of parasitic infections is common practice in many tropical countries, and many generic products lack manufacturers' information on efficacy, safety, and quality control. Recommendations and advice concerning the use of such drugs and protocols are also addressed in these guidelines. Creating these guidelines is an essential first step towards improving the education of veterinarians, specifically regarding best practices for the diagnosis, treatment, and control of canine and feline parasites in the tropics.

Room 2
Oral Communication

Innovative Strategies and Challenges in the Control of Parasites in Birds and Swine

Associated risk factors and phylogenetic diversity of poultry coccidiosis across three agroclimatic zones of Jammu and Kashmir, North India

Aiman Khursheed (Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, India), Anish Yadav (Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, India), Shafiya Imtiaz Rafiqui (Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, India), Anand Kushwaha (Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, India), Vikas Yadav (Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, India), Rajesh Godara (Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, India), Sanku Borkataki (Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, India), Shilpa Sood (Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, India), Vikrant Soodan (Guru Angad Dev Veterinary and Animal Science University, India), Rajesh Katoch (Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, India)

Coccidiosis is a prevalent and economically impactful enteric disease affecting poultry, primarily caused by various *Eimeria* species. In India coccidiosis is arguably the most costly poultry disease resulting in yearly losses of around £447.01 million as a direct outcome of disease on poultry and its management. This study decisively employed conventional, computational, and molecular methods and recorded 59.89% poultry farms positive for coccidiosis across three agroclimatic zones in the Union Territory of Jammu and Kashmir, North India. Importantly, unorganized poultry farms showed significantly higher infection rates compared to organized farms, with the highest prevalence recorded during the monsoon season, followed by post-monsoon, winter, and summer. The subtropical zone clearly demonstrated a higher incidence of infection compared to both the intermediate and temperate zones. Using morphometric analysis through COCCIMORPH software, se-

ven species of Eimeria: *E. tenella, E. necatrix, E. mitis, E. acervulina, E. maxima, E. praecox,* and *E. brunetti* were identified, with *E. tenella* emerging as the most predominant species. Molecular characterization was effectively conducted using ITS-1, resulting in the generation of eighteen sequences. The comparison of ITS-1 sequences of different isolates of Jammu generated in the present study with those available in public domain of NCBI revealed that four isolates of *E. tenella* i.e. Jammu (MZ983636.1), Kathua (OL989151.1), Poonch (OL505606.1) and Udhampur (OL505603.1) showed relatedness closely to identity with Egypt (JQ061004.1) and South Africa (MK404742.1) ITS-1 sequences whereas, *E. tenella* of Srinagar isolate was closely related to South Africa (MK404743.1). The comparison of *E. mitis* ITS-1 sequences of five isolates showed a phylogenetic relatedness with China (GQ1573626.1) and Australia (AF446063.1). South Africa ITS-1 (MN727043.1; MN7270441.1) sequences showed phylogenetic closeness with Jammu (OK011553.1) and Udhampur (OL957472.1) isolates of *E. acervulina* while as Srinagar (OL695840.1) isolate was closely related to South Africa (MN727046.1). The three isolates of *E. maxima* showed a phylogenetic relatedness with the sequences of South Korea (HQ338947.1; HQ386733.1). The Jammu (OK030703.1) isolate of *E. praecox* was observed to be closely related to sequences submitted by UK (LN609943.1) and China (JN022589.1) while *E. necatrix* of Srinagar (OM501135.1) isolate was phylogenetically close with the sequences of Nigeria (LT549033.1) and Kerala (MW353998.1). This study elucidates that risk factors established for poultry coccidiosis will help in understanding disease dynamics in Jammu and Kashmir and consequently allows professionals and policy makers to design targeted prevention and control strategies. Additionally, data generated through phylogenetic study will help in developing preventive measures in form of vaccine development using genetic resource available, ultimately supporting the health and productivity of poultry.

Evaluation of the *E. acervulina* and *E. maxima* scoring system in anticoccidial sensitivity tests (AST's)

Brecht Maertens (Poulpharm, Belgium), Maarten De Gussem (Poulpharm, Belgium)

Coccidiosis is one of the most economically devastating diseases of poultry industry. Losses exceed six billion US dollars annually due to a low productivity, mortality and high costs on prophylaxis and treatment programs. The infestation can be caused by several *Eimeria* species, for which Johnson and Reid have published an intestinal scoring system in 1970. On this day, this lesion scoring system is still used as the golden standard in both field practises as in experimental conditions such as anticoccidial sensitivity studies (AST's), which are used to determine the efficacy of anticoccidials in the prevention of *Eimeria* infections in chickens. During these AST studies, parasitological parameters as intestinal lesions and oocyst shedding are monitored as a primary parameter according to regulation authorities, while zootechnical parameters as daily weight gain (DWG) are included as secondary parameters. Yet often, the correlation between the intestinal lesions score and DWG are not clear. Therefore, an evaluation of the scoring system for the two most relevant species included in AST's, *E. acervulina* and *E. maxima*, was performed at different challenge dosses. Our findings indicate the importance and higher relevance of DWG observations compared to the induction of in-

testinal lesions. Furthermore for *E. maxima* it was confirmed that the scoring system has limitations, which makes it difficult to routinely obtain high scores and even more difficult to determine potential efficacy of anticoccidial drugs, if efficacy is evaluated on reduction of lesions. To overcome this problem we suggest an additional parameter, caudal mid-intestinal content consistency, and have developed an adjustment to the scoring system. Finally this adjusted scoring system was compared to the original system during an AST with nine commercially available anticoccidials and was found to have a stronger correlation with the effect on DWG compared to the original system.

Comparative efficacy of the *Cysticercus cellulosae* excretory/secretory and somatic antigens using Dot ELISA in pigs

Sangaran Arumugam (Tamil Nadu Veterinary and Animal Sciences University, India), Abirami S (Tamil Nadu Veterinary and Animal Sciences University, India), Sreekumar Chirukandoth (Tamil Nadu Veterinary and Animal Sciences University, India), Porteen, K (Tamil Nadu Veterinary and Animal Sciences University, India)

Porcine cysticercosis is an important cause of economic loss in developing countries due to the presence of the cysticerci in various tissues of the pig, meant for meat consumption. In endemic areas across the world, *Taenia solium* infection in human beings is associated with poverty, open defecation and easy access of scavenging pigs to human faeces in the environment. However, there is a paucity of ante mortem diagnosis of this disease in pigs for which immunoassays will be of immense help. Pigs slaughtered at local slaughter houses, organized and unorganized pig farms in and around Chennai, India were inspected for the presence of *C.cellulosae* cysticerci. The E/S antigens were prepared by *in vitro* culture of the cysticerci whereas the somatic antigen was prepared by homogenizing the cysticerci and the antigens were purified. Blood from 175 pigs were collected and the sera separated for use in Dot ELISA. Using both the purified E/S and somatic antigens, out of the 175 sera samples of pigs screened by Dot ELISA, 46 (E/S antigen) and 29 (somatic antigen) sera samples were found positive respectively. The study assessed the diagnostic efficacy of the use of both E/S antigen and somatic antigen in Dot ELISA for porcine cysticercosis and it was inferred that the E/S antigen was found to be a better antigen.

Parasite zoonotic in pigs and their producers: associating scientific research and rural extension

Camila Souza Carvalho Class (Universidade Federal Fluminense, Brazil), Ingrid da Silva Reis (Universidade Federal Fluminense, Brazil), Ana Luiza Soares de Araujo (Universidade Federal Fluminense, Brazil), Laís Lisboa Corrêa (Universidade Federal Fluminense, Brazil), Breno Torres da Silva (Universidade Federal Fluminense, Brazil), Renan de Souza ferreira (Centro Universitário Serra dos Órgãos, Brazil), Pedro Mendes de Souza (Universidade Federal de Juiz de Fora, Brazil), Fabiana Batalha Knackfuss (Universidade do Grande Rio, Brazil), Roberto Júnio Pedroso Dias (Universidade Federal de Juiz de Fora, Brazil), Alynne da Silva Barbosa (Universidade Federal Fluminense, Brazil)

Brazil stands out in pig production, with properties characterized as industrial and family-run. In these properties, parasitic diseases represent obstacles to production. In addition, there are zoonotic parasites, such as *Balantioides coli*, being the pig the main reservoir. This study aimed to estimate the prevalence, risk factors inherent to gastrointestinal parasites in pigs, identify molecularly *B. coli* in the feces of pigs and their producers, and to mediate information about the parasites for producers. Visits were made to 15 pig farms in the Rio de Janeiro and Minas Gerais states. Feces were collected from 1,148 pigs and 47 from producers of these animals. Forms were applied, and extension activities were conducted. These samples were subjected to direct examination, sedimentation, flotation, FLOTAC techniques and molecular tools for research on *B. coli*. Parasites were detected in 69.9% of the pigs, with emphasis on Ciliophora group (50%), coccidia (37.3%), *Trichuris suis* (19.7%) and strongyles (19%). Statistical differences were obtained when comparing the helminth egg count values between the properties. Several risk factors were associated with the frequency of parasites in pigs, such as the arrangement of drinkers on the floors and the type of antiparasitic provided for the pigs. Molecular evidence of the *B. coli* was found in the feces of pigs from all farms by Sanger and Metabarcoding sequencing and in 13 producers by Sanger and in two producers by Metabarcoding. Variants of *B. coli* A and B were characterized, with the latter being the most identified. Among the extension activities, those that stood out were: “Happy Pig and Sad Pig”, a dynamic of self-recognition of management and “Correction of homework” to remember the mediated information. The results generated highlighted the need for the creation of programs that provide assistance and training in the farms to invest in the control of these parasites, valuing animal welfare and producer health.

Toltrazuril resistance in *Cystoisospora suis* – detection and genetic background

Anja Joachim (University of Veterinary Medicine Vienna, Austria), Bärbel Ruttkowski (University of Veterinary Medicine Vienna, Austria), Teresa Cruz Bustos (University of Veterinary Medicine Vienna, Austria)

Cystoisospora suis, the cause of neonatal porcine coccidiosis, poses a challenge to pig production due to the emergence of reduced efficacy of toltrazuril (TLZ). In Europe, the majority of intensively reared piglets receive metaphylactic TLZ, but the parasite is still highly prevalent, raising doubts about sustained efficacy. Determining the resistance status of the strain Holl-1 by experimental infection in comparison to the susceptible strain Wien-1 led to the development of an *in vitro* merozoite development test (MDA) for investigations into the TLZ resistance of field isolates, and revealed genetic differences between the two strains of C. *suis*. The MDA showed reduced efficacy of TLZ in two other field isolates from Europe (30-50% compared to >95%) but has the significant disadvantage that it requires a sufficient number of clean, sporulated and vital oocysts from field samples. Comparative genomic and transcriptomic analyses of defined C. suis isolates can identify resistance markers. Using WGS and RNASeq, we studied strains Holl-1 and Wien-1, analyzing nuclear and mitochondrial (mt) genomes and differential gene expression (DEG). Variable expression and mutations in host-cell invasion genes suggest adaptive changes. Mutations and differential expression of

retrotransposon-linked genes indicate potential genomic rearrangements under TLZ-induced selection, and mt transcriptome analysis revealed downregulation of COI I, III, and Cytb mRNA levels in the resistant strain upon toltrazuril exposure, linking these to potential drug resistance mechanisms. Further research into putative markers for resistance or susceptibility is needed to develop new diagnostic strategies optimizing coccidiosis control in pigs, and to focus on resistance-breaking novel compounds for treatment.

A novel vaccine approach for controlling *Ornithonyssus bursa* in Poultry

Alvaro Gustavo de Jesus Torres (Universidade Federal de Minas Gerais, Brazil), Geralda Gabriele da Silva (Universidade Federal de Minas Gerais, Brazil), Diogo Fonseca Soares Pereira (Universidade Federal de Minas Gerais, Brazil), Maykelin Fuentes Zaldivar (Universidade Federal de Minas Gerais, Brazil), Lucilene Aparecida Resende (Universidade Federal de Minas Gerais, Brazil), Wanessa Moreira Goes (Universidade Federal de Minas Gerais, Brazil), André Tetzl Costa (Universidade Federal de Minas Gerais, Brazil), Lorena Lopes Ferreira (Universidade Federal de Minas Gerais, Brazil), Ricardo Nascimento Araujo (Universidade Federal de Minas Gerais, Brazil), Reysla Maria da Silveira Mariano (Universidade Federal de Minas Gerais, Brazil), Walderez Ornelas Dutra (Universidade Federal de Minas Gerais, Brazil), Denise Da Silveira Lemos (Universidade Federal de Minas Gerais, Brazil), Rodolfo Cordeiro Giunchetti (Universidade Federal de Minas Gerais, Brazil)

Hematophagous mite infestations cause significant economic losses in poultry farming due to increased mortality, reduced egg production, and the costs of acaricide-based control strategies. Vaccination is a promising alternative, as it does not induce resistance, avoids environmental contamination, and requires no withdrawal period before slaughter. Previous studies on *Dermanyssus gallinae* have shown that immunized birds develop IgY responses, suggesting that similar approaches could be effective against *Ornithonyssus bursa*, the tropical fowl mite. This study aimed to develop the first immunobiological against *O. bursa*. A preliminary clinical trial was conducted on *Gallus gallus domesticus* (laying hens), involving three groups (n = 4 per group): a control group receiving three placebo doses, an adjuvant group receiving adjuvant plus placebo, and a vaccine group receiving a formulation containing *O. bursa* antigens, adjuvant, and placebo. Doses were administered at 14-day intervals (T0, T14, T28), and 21 days after the final dose (TF), the animals were challenged with mite infestation. The health of the animals was monitored, with no adverse effects observed. Mites fed for 12 hours before being collected and placed individually in well plates for assessment of survival, oviposition, and fecundity at 0h, 24h, 48h, 72h, 96h, and 120h post-feeding. Vaccine efficacy was calculated based on mite mortality and reproductive impairment, with the vaccinated group achieving 99.99% efficacy. The vaccine demonstrated safety, immunogenicity and elicited a high rate of protection. The next studies will be conducted on a poultry farm to validate the vaccine formulation.

Phylogenetic analysis of a Schistosomatidae species from three Brazilian tanagers (*Ramphocelus bresilia* Linnaeus, 1766) in a German zoo

Nora-Rachelle Döscher (Freie Universität Berlin, Germany), Christoph Schulze (Landeslabor Berlin-Brandenburg, Germany), Andreas Ochs (Berlin Zoological Garden, Germany), Melanie Göldner Landeslabor (Berlin-Brandenburg, Germany), Georg von Samson-Himmelstjerna (Freie Universität Berlin, Germany), Jürgen Krücken (Freie Universität Berlin, Germany)

Avian schistosomes infect mainly aquatic birds and show a high species diversity even in temperate zones such as Central Europe. A meta-analysis revealed prevalence estimates between 13% (Asia) and 43% (America) of schistosomes in aquatic birds. Their indirect life cycle involves aquatic snails as intermediate hosts. We present three cases of schistosomosis in Brazilian tangares (*Ramphocelus bresilia*) in a German zoo and phylogenetic analysis for species identification. Pathohistological examination of three Brazilian tanagers that died at the Berlin Zoo suddenly without previous clinical symptoms; PCR and sequencing of 18S, cytochrome oxidase I (COI I) and ITS1-5.8S-ITS2 of Schistosomatidae eggs from internal organs followed by generating phylogenetic trees based on COI I and partial ITS1-5.8S-ITS2 sequences. Massive hematogenous spread of schistosome eggs, particularly to liver, lung, intestines and kidney was observed in pathohistological examinations. No adult flukes were found. However, the vascular network of the Vena portae was not routinely sampled. Available sequences in GenBank were not sufficient for a precise species identification of the parasites. Phylogenetic analysis using a maximum-likelihood approach revealed that they belong to the subfamily Gigantobilharziinae. Brazilian tanagers have not yet been described as hosts of Schistosomatidae. It is possible that the parasites detected in the present study are a previously undescribed species, or one without sequences currently available in GenBank. As the three birds hatched in different zoos in Germany and were relocated to Berlin Zoo, the infection definitley occurred in Germany, most probable at Berlin Zoo. The infection route and the involved intermediate hosts have not yet been identified. However, zoos with a broad spectrum of animals in small habitats could facilitate the transmission from resevoir hosts to non-native species.

Plenary Oral Communication

Digital Innovation and Decision Support in Parasite Diagnosis and Control
Session chair: Fiona Kenyon (Moredun Research Institute, UK)

From microscope to machine: A comparison of traditional microscopy with image analysis devices for faecal egg counts of gastrointestinal nematode eggs in ovine samples

Amanda McEvoy (Teagasc), Padraig O'Boyle (Teagasc), Saoirse Ellis (Teagasc), John P. Dalton (University of Galway, Ireland), Michael Parkinson (University of Galway, Ireland), Orla Keane (Teagasc), Cynthia Machín (University de Las Palmas de Gran Canaria, Spain)

Faecal egg counts (FEC) are a critical diagnostic tool for detecting gastrointestinal nematode (GIN) infections in livestock, guiding treatment decisions and monitoring anthelmintic drug efficacy. Recently, machine learning (ML) methods have been developed to facilitate the detection and enumeration of parasite eggs in livestock. Despite increasing use by veterinarians and farmers, limited published data exis-

ts regarding the technical characteristics and performance of these new methods relative to traditional diagnostic techniques. This study evaluated the performance of traditional manual methods (McMaster and Mini-FLOTAC) with new ML diagnostic tools (FECPAKG2, Micron and OvaCyte) using lambs naturally infected with GIN. Faeces were collected from 41 lambs, homogenised, and split into two aliquots for examination by each method. The techniques were performed according to their respective standard protocols, with the Strongyle egg count recorded either by trained staff (McMaster and Mini-FLOTAC) or automatically by the device (FECPAKG2, Micron and OvaCyte). As McMaster is the most widely used diagnostic method, it served as the reference method for comparison. Based on replicate aliquots, all methods showed high concordance correlation coefficients (>0.9). Mini-FLOTAC and Micron displayed similar concordance to McMaster (≥0.98) whereas OvaCyte and FECPAKG2 were significantly lower than McMaster (P<0.001). Strongyle egg counts were compared using ANOVA with Dunnet's post-hoc test used to compare FEC methods to McMaster. No significant difference between McMaster and Mini-FLOTAC (P=0.26) or FECPAKG2 (P=0.81) was found. However, Micron reported significantly higher eggs per gram (EPG) (P<0.0001), while OvaCyte reported significantly lower EPG compared to McMaster (P=0.0002). This study highlights key differences between traditional and ML methods, underscoring the need for continued refinement and standardised validation of new diagnostic tools.

FEC Check: Development of a decision support tool to aid interpretation of gastrointestinal nematode faecal egg counts in sheep

Eilidh Geddes (Moredun Research Institute, United Kingdom), Andrew Duncan (University of the Highlands and Islands, United Kingdom), Kate Lamont (Scotland's Rural College (SRUC), United Kingdom), Jade M. Duncan (Moredun Research Institute, United Kingdom), Neil Sargison (University of Edinburgh, United Kingdom), Fiona Kenyon (Moredun Research Institute, United Kingdom), David J. Bartley (Moredun Research Institute, United Kingdom), Lynsey A. Melville (Moredun Research Institute, United Kingdom)

Gastrointestinal nematodes (GINs) are a cause of significant production losses in livestock. With increasing prevalence of anthelmintic resistance and increasing pressure to reduce the environmental impact of anthelmintic usage, sustainable and effective parasite control is essential. Faecal egg counts (FECs) are a simple tool for sheep producers to monitor GIN challenge, and are being increasingly used to guide treatment timings, estimate pasture contamination and test anthelmintic efficacy. However, interpretation can be challenging. Develop a free web-based application with stakeholders to support the effective interpretation of FECs. A decision support tool (DST) was developed using a 'traffic light'-style gradient of potential clinical impact of FECs, providing farmers with a visual representation of the clinical importance of their results. This was accompanied with resources to aid in the collection and processing of samples and guidance to support effective decision-making. Four focus groups were conducted with farmers, livestock advisors and vets to examine the barriers to FEC uptake and provide feedback on the prototype tool. Uptake of final DST in the first 12-months post-launch was evaluated. The tool was trialled by17 farmers, and 16 vets/advisors. Most farmers (87%)

currently used FECs on their farm but agreed that the level of interpretation and clinical guidance provided with the results varied substantially between test providers. The DST was well received at all focus groups, with simplicity and ease of use identified as key principles to drive uptake. In the 12-months post-launch, the final DST had 1916 users. The final DST represents a timely resource to improve the interpretation of FECs results reporting for farmers and other stakeholders. The initial uptake observed within the first year since launch is promising for the wider adoption of evidence-based parasite management.

ParaDiag: A novel ai-driven tool for parasite identification in veterinary practice

Loic Antoine (Boehringer Ingelheim, France), Frederic Beugnet (Boehringer Ingelheim, France), Domenico Otranto (Bari Veterinary Faculty, Italy), Elias Papadopoulos (Aristotle University of Thessaloniki, Greece), Jacques Guillot (Parasitology department, France), Luis cardoso (University of Trás-os-Montes e Alto Douro, Portugal), Reuben Sunli Kumar Shari (Universiti Putra Malaysia, Malaysia), Piyanan Thaweethawonsawat (Chulalongkorn university, Thailand), Magalie René-martelet (VetAgro Sup, France), Camille Ganblin (Boehringer Ingelheim, France), Do Yew Tan (Boehringer Ingelheim, France)

Artificial intelligence is transforming veterinary care by enabling rapid, precise data analysis, supporting early, and more accurate diagnostic. ParaDiag is an innovative and cost-effective service designed to support veterinarians in parasite identification. It leverages existing clinic microscope and veterinarian's smartphone without requiring investment in new devices. Powered by advanced image recognition with deep-learning algorithms, it aims to identify major parasites of dogs and cats with a mean Average Precision (mAP) of more than 90%. It includes Ascarids (roundworms), Diphyllobothriidae (broad tapeworms), *Dipylidium* (Flea tapeworm), Ancylostomatidae (hookworms), *Strongyloides* (Threadworms), Taeniidae (Taenid tapeworms), Trichurids (Whipworms & *Capillaria*), *Giardia*, Coccidia, and Metastrongylids (lungworm larvae). The model has been trained on a dataset of more than 7000 ethically sourced images from partnering with 7 veterinary parasitology departments located in Europe and Asia to recognize and identify all major parasites visible in dogs and cats fecal samples. Future developments will expand the model capabilities to recognize blood and external parasites, as well as parasites of other hosts than dogs and cats, i.e. cattle, sheep, goat, horse, swine, and rabbit. ParaDiag should be available as an App synchronized with a desktop version to facilitate integration with the numerous Practice Management systems available. The tool will generate customizable PDF report to support pet owner awareness on parasites and their consequences. By facilitating parasites identification, ParaDiag aims to support veterinarians in transitioning from treating most pets to adopting more regular examinations of faeces and blood, as recommended in recent guidelines from ESCCAP and TROCCAP. This shift aligns with the growing emphasis on preventive healthcare and early detection, ensuring better outcomes for pets and a more proactive approach to parasite management.

Five-point check system implementation in goat smallholders from Malawi: a machine learning approach to identify highly infected animals

Javier Ventura-Cordero (Autonomous University of Campeche), Paul M. Airs (Queen's University Belfast), Eric R. Morgan (Queen's University Belfast), Andrews C. L. Safalaoh (Lilongwe University of Agriculture and Natural Resources), Andrew Cooke (University of Lincoln), Michael R.F. Lee (Harper Adams University), Taro Takahashi (Rothamsted Research), Jan van Wyk (University of Pretoria)

Goats are crucial in central Malawi as assets for the livelihoods of smallholders and a buffer against food insecurity. Many factors impact productivity, but gastrointestinal nematodes are a major limitation to smallholdings. The study aimed to identify factors that drive high faecal egg counts in goats naturally infected using a machine learning approach. Two hundred female Indigenous goats from 2 to >4 years old were included. Indicators such as physiological status, age, five-point check score, farm number, location and season were recorded fortnightly. Load burden was individually determined using faecal egg count (FEC). Conditional inference trees were used to determine the significant variables, leading goats with high faecal egg counts (> Q3 of the dataset) to be classified as high parasitised goats and those with faecal egg counts < Q3 as low parasitised. The performance of the algorithms was estimated using accuracy, recall, precision and F1 score. The dataset was split into 70% training and 30% testing. The algorithm identified three significant variables: season, body condition score and FAMACHA (P<0.001). The quartile three threshold was 600 eggs per gram of faeces (EPG). During the dry season, 100% of the goats were classified as low parasitised. However, 78% of goats showing >2 BCS and FAMACHA 1 and 2 were classified as low parasitised. The performance of the best algorithm is an accuracy of 0.86, a recall of 0.51, a precision of 0.15 and an F1 score of 23%. It is noteworthy that deworming management in goats may not be necessary during the dry season. FAMACHA and BCS are part of the five-point check system, and performing this methodology monthly could help identify animals with low FEC among goat smallholders in Malawi.

Using machine learning and advanced bioassays to discover novel tick repellents and acaricides

Marnix Vlot (TropIQ Health Sciences), Jennifer Wei (Google Research), Martijn Vos (TropIQ Health Sciences), Carlos Ruiz (Washington University), Rob Henderson (TropIQ Health Sciences), Jessica Konijnenburg (TropIQ Health Sciences), Jeff Riffell (Washington University), Koen Dechering (TropIQ Health Sciences)

Current products for control of veterinary ectoparasites are centered around a very limited set of mechanisms of actions and pharmacophores. We set out to discover novel antiparasitics using a multidisciplinary approach that leveraged machine learning, computer vision, and advanced laboratory automation. We digitized thousands of records of repellency and acaricide activity for training of predictive graph neural networks (GNNs). Model predictions were verified in automated bioassays against a variety of arthropods and through live imaging of mosquito brain activity. As a result, we established a computational representation for odor at the molecular level that can predict behavioral responses in a wide variety of organisms. This approach allowed us to identify more than 100 highly effective arthropod repellents, including compounds outperforming market

standards. Live imaging of calcium signaling in mosquito brains revealed distinct signaling pathways for structurally diverse compounds. Computer vision-based analysis of tick movement provided insights into the mechanisms of action of repellents, distinguishing between contact irritants and spatial repellents. In an adaptation of the approach, we trained GNNs on acaracide data. Robotic arms and custom--designed 3D-printed components enabled high-throughput assays to evaluate predicted acaricidal activity following contact or systemic exposure. The results revealed a 6% hit rate on predicted acaricides from a chemical diversity screen. These compounds killed *Ixodes ricinus* ticks within 24 hours at an ED50 of 2.5 µg/cm2 and form the basis for further refinement of the GNN models and hit optimization. The combined data demonstrate how a data-driven approach can significantly enhance the speed and efficiency of small molecule discovery in ectoparasite research.

Buffalo Fly Counting: Comparing Visual Scoring Methods with Object Counting Digital Platforms

Anthony Feez (University of Queensland, Australia), Nigel Perkins (University of Queensland, Australia), Benjamin Wood (University of Queensland, Australia), Swaid Abdullah (University of Queensland, Australia)

Buffalo fly (*Haematobia irritans exigua*) is consistently recognised for its significant impact on cattle health, welfare, and production. Buffalo fly (BF) infestation was recently ranked number one among the endemic diseases for the red meat industry by Meat and Livestock Australia (MLA). Buffalo fly counting is essential but inherently challenging, as it requires estimating the number of frequently moving flies on a constantly agitated host. Estimation of fly numbers on animals has been done using different counting techniques such as visual estimates, photographs, recorded video, and infrared thermography. Accurate estimates of BF numbers on an animal are essential for several reasons including, treatment efficacy evaluation, phenotyping animal susceptibility to breed resistant cattle, and establishing economic threshold levels for the instigation of effective integrated pest management (IPM) protocols. The current study uses digital images of BF-infested cattle and compares the visual scoring method against an object counting digital platform. The two techniques were compared for consistency and agreement among four assessors. The statistical analysis was performed using packages in R program. The reliability of repeated counts for consistency and agreement were assessed using repeatability (r) and reproducibility (R) analyses. The results showed 99% consistency in fly counts within each assessor and 99% agreement in counts between assessors. Our study indicates that a digital counting platform can provide a useful and reliable count of BF numbers. From these findings a practical tool to assess BF infestation on cattle could be developed.

From paddocks to pixels: A statewide look at worm resistance

Emily Kate Francis (The University of Sydney, Australia), Analise McDonald (The University of Sydney, Australia), Crystal Elliot (The University of Sydney, Australia), Olivia Kelly (The University of Sydney, Australia), Mark Edward Westman (Elizabeth Macarthur Agricultural Institute, New South Wales Department of Primary Industries, Australia), Janina McKay-

-Demeler (Elizabeth Macarthur Agricultural Institute, New South Wales Department of Primary Industries, Australia), Jan Šlapeta (The University of Sydney, Australia)

Australia's livestock producers are navigating an increasingly complex worm control landscape, with rising levels of anthelmintic resistance threatening the sustainability of grazing systems. While molecular diagnostics have transformed our ability to detect resistance, their value depends on delivering results in a form that is accessible, timely, and meaningful to end users. We present the WormResistance-Monitor (WoRM) Dashboard: a first-of-its-kind, interactive platform that integrates four years of surveillance data from over 500 livestock properties across New South Wales. Using mixed amplicon metabarcoding and targeted SNP detection, the dashboard visualises gastrointestinal nematode species and resistance to levamisole and benzimidazole across sheep and cattle populations. Users can explore trends by postcode, host species, nematode, resistance marker, and frequency threshold. This longitudinal dataset reveals the regional extent and persistence of resistance, offering practical insights to guide more targeted and sustainable parasite control. By bridging the gap between laboratory diagnostics and farm decision-making, this open-access tool marks a step-change in applied parasitology and highlights the value of statewide, collaborative molecular surveillance.

14:00 – 15:30

Room 1
Oral Communication

Animal leishmaniosis, by LeishVet and Brasileish

Session chair: Filipe Dantas-Torres (FIOCRUZ, Brazil)

Domestic dogs as sentinels for the epidemiological scenario: the example of Italy and Tunisia

Gaetano Oliva (University of Naples Federico II, Italy)

Infected dogs are considered the main domestic animal reservoir of *Leishmania infantum,* also if some human dermotropic zymodemes are not common in dogs. Canine leishmaniosis (CanL) affects millions of dogs in Europe and north Africa where it is endemic. Many factors as climatic changes that contribute to colonization and establishment of sand fly vectors, and increased burden of infected canine hosts have contributed to the spread and occurrence of CanL in areas where it was not previously recorded. In this new scenario, the canine leishmaniosis prevalence remains one of the factors that can account the occurrence of human cases. Southern Italy is historically considered highly endemic for both human VL and CanL, while till 30 years ago autochthonous CanL was considered absent or rarely occurring in northern regions of continental Italy. Recent surveys have confirmed the presence of new cases and the continuous increase of canine seroprevalence in areas where the presence of CanL was sporadic. In these continental regions, dozens of new municipalities are

now considered endemic. In some new established foci, the canine seroprevalence may reach values like southern Italy. Sand fly trapping confirmed the presence of vectors, mainly *P. perniciosus*. Interestingly, new positive municipalities are also recorded in the easternmost regions bordering nonendemic territories of Slovenia. Tunisia is a Mediterranean Country where different *Leishmania* species (*L. infantum; L. major; L. tropica*) may express their infectivity. Four recent canine field surveys performed in central and southern districts of Tunisia, evidenced the increase of *L. infantum* prevalence in central regions, the detection of all 3 *Leishmania* species in dogs and the first evidence of *L. infantum* infection in dog, in an arid area endemic for cutaneous *Leishmania* species.

Animal leishmaniosis in Israel: a multispecies reality

Gad Baneth (The Hebrew University, Israel)

Leishmaniosis affects humans and animals in the Middle East and it involves infection with several species of *Leishmania* in Israel. To describe the current status of *Leishmania* sp. infection in animals in Israel. Animals were surveyed by serology and PCR. Four species of *Leishmania* have been reported in Israel, *Leishmania major* which causes cutaneous leishmaniasis mainly in the south of Israel, and infects humans, rodents, dogs and cats; *Leishmania tropica* that causes cutaneous disease in humans in central and northern Israel, its main reservoir host is the rock hyrax, and it infects also rodents, dogs, wildlife canids and cats; *Leishmania infantum* which causes visceral or cutaneous disease in humans and also infects dogs, wild canids, horses, cats and rodents, and *Leishmania donovani* which was recently described to cause cutaneous disease in people in southern Israel and may involve hares as its reservoir host. Different sand fly species transmit the various *Leishmania* spp., with *Phlebotomus papatasi* responsible for the transmission of *L. major*; *Ph. sergenti* and *Ph. arabicus* transmit *L. tropica*; *Ph. alexandri* transmits *L. donovani*; and *Ph. perfiliewi galilaeus*, *Ph. syriacus*, and *Ph. Tobbi* are the putative vectors of *L. infantum*. All species and forms of the disease in Israel appear to be zoonotic and have animal reservoirs. While cutaneous leishmaiosis caused by *L. major* and *L. tropica* is the most frequent form of the disease in humans in Israel, *L. infantum* infection in considerably more frequent in dogs, where only rare clinical cases of *L. major* and *L. tropica* infections are detected. Israel is a meeting point of continents, climates, vector sand fly spp. and *Leishmania* spp. and despite the small size of this country there is large diversity of *Leishmania* life cycles and habitats.

Non vectorial transmission of *Leishmania infantum* and consequences: an example from France

Patrick Bourdeau (Nantes University, France), Emma Monge (Nantes University, France), Florian Carrez (Nantes University, France)

Parasites of the genus *Leishmania* are adapted to the group of sand flies, which constitute their biological hosts and vectors. Other modes of transmission have gradually been described, particularly in dogs (*Leishmania infantum):* venereal, vertical transmission from bitch to her puppies, blood transfusion, and dog-to-dog transmission (particularly in groups).

In France, the impact of canine leishmaniosis has increased not in terms of prevalence but in terms of its spread within the territory where it was historically located in the southeast.

Indirect information on possible non-vectorial transmission in France from surveys conducted in veterinary clinics are presented. Two national surveys, each covering five years, were conducted in 2011 then 2017 by sending questionnaires to veterinary clinics providing information on clinicalcanine leishmaniosis based on coded questions. The questions potentially related to a non-vectorial transmission were: the existence of autochthonous cases in the area of veterinary practice, multiple cases in breeding facilities, cases in several dogs from the same litter, and cases in the female dog and her puppies. The results were analyzed department by department (n=95) or at the national level. The two studies collected 1335 and 650 responses, respectively, representing 23.2% and 11.6% of veterinary clinics in France and covering the entire territory. Some general aspects have already been presented previously. Autochthonous cases were observed in the 32 departments of the enzootic zone, and 23 non-enzootic (10 in 2011; 16 in 2017), including 10 vector-free departments. In 2017, 19.2% of clinics reported infections distributed in 23 enzootic departments or 7 non-enzootic departments. In 21 enzootic departments and 6 others, cases involving multiple dogs from the same family or female dogs and their offspring were recognized. The percentages of veterinary clinics that faced these situations by comparing 2011 to 2017 at National level were respectively 4.8 to 5.8% for cases in females and their progeny and 5.2 to 7.4% for dogs of the same litter. From these data and the analysis of epidemiological situations it is possible to present a global cartography, through the field experience of veterinary profession in France, suggesting extension and evolution of non-vectorial transmission of canine Leishmaniosis.

New biomarkers in the diagnosis of Canine Leishmaniosis

Guadalupe Miró Corrales (Universidad Complutense de Madrid, Spain)

Leishmania infantum (syn. *L. chagasi*) is the most prevalent protozoa species infecting dogs and several animal species (including people). It is highly endemic in the Mediterranean Basin, Brazil and other South American countries. In areas of canine leishmaniosis (CanL), infections caused by *Leishmania infantum* are not synonymous with clinical disease, the percentage of sick dogs is only the “tip of the iceberg” because there is a high percentage of dogs with subclinical infection. Interpretation of the results in the diagnosis of canine leishmaniosis (CanL) is essential because these two types of patients are clinically healthy infected dogs and sick dogs. The key to diagnosis is to properly differentiate these two patients with the help of the results obtained. When presented with a dog with compatible clinical signs with CanL carrying out a quantitative serology by IFAT or ELISA to quantify the humoral response to get a concrete antibody titration is the first step. Serology, however, is an indirect diagnosis and the only way to demonstrate the infection is by evidence of the parasite from lymph nodes and /or bone marrow aspirates where finding amastigotes inside macrophages confirms *Leishmania* infection. If cytology is negative and our suspicion is strong of CanL, the best way of increasing sensitivity is to carry out a molecular diagnosis to detect parasite DNA by conventional PCR, nested PCR, or quantitative PCR. Bone marrow, lymph node, spleen or skin are the most sensitive tissues for PCR diagnosis; while blood, buffy coat and urine significantly reduce the sensitivity of the molecular

diagnosis. Then it is important to run nonspecific tests to assess the general condition of the patient and the possible organic impact of the parasite. CBC, biochemistry panel, urinalysis with UPC, and protein electrophoresis are the most common biomarkers used. Nowadays the usefulness of other biomarkers has been considered such as: circulating immunocomplexes (CIC), Toll-like receptors TLRs), and acute phase proteins (APP). All of them serve as valuable biomarkers for disease progression, treatment efficacy, and relapse detection in CanL. We will evaluate all of them at the oral presentation.

New approaches for treating canine leishmaniasis in Brazil

Leucio Camara Alves (UFRPE, Brazil), Fabio dos Santos Nogueira (Fundação Educacional de Andradina, Brazil), Renata Pimentel Bandeira de Melo (Department of Veterinary Medicine, Brazil)

Canine leishmaniasis, caused by *Leishmania infantum*, represents a major concern in small animal clinical practice in Brazil, with prevalence rates reaching up to 60% in certain regions. In this way infected dogs constitute the urban reservoir of the parasite and play a crucial role in transmission of the infection to humans. After the diagnosis, dogs must be treated and miltefosine is currently the only molecule authorized for canine treatment in Brazil. However, canine leishmaniasis treatment typically involves a combination of molecules such as miltefosine associated with allopurinol, which can help to prevent the relapse. Despite available treatment in Brazil, achieving a sustained therapeutic response remains challenging. Clinicians often struggle to identify clinically effective drug combinations while monitoring the clinical status, immunological parameters, and parasite load of the affected animals. A couple of years ago, marbofloxacin, a fluoroquinolone antibiotic, started to be used in combination with other treatments. Right now, a new formulation called Marbox-Leish has been launched in Brazil for the treatment of canine leishmaniasis. Containing marbofloxacin as the active compound, this product aims to promote safe and effective remission of clinical signs in infected dogs. This presentation summarizes the therapeutic possibilities for Canine leishmaniasis in Brazil.

Current situation of Canine Leishmaniosis in Argentina, Paraguay, and Uruguay

Jose Octavio Estevez (Veterinaria del Oeste, Argentina and *Brasileish)*

In last 30 years spreading of human and canine Visceral Leishmaniosis to Paraguay, Argentina and Uruguay was observed. Reliable data on the number of canine cases in each country is very low. Hence the real number of affected animals is estimated from limited and specific studies from differents regions. During the nineties, cases of Canine leishmaniosis were communicated in Paraguay's Central Region followed by the first human cases in 2000. After this initial reporting, canine and human cases appeared in other areas, mainly in the southeastern and northern regions of the country. In 2006 on the Northeast of Argentina the first human and canine VL autochthonous cases were reported. Afterwards a progressive spreading towards the south and northwest areas of the country began. In Uruguay dogs with leishmaniosis were reported near the Brazilian and Argentinian borders in 2015. Today this area is under a constant increase of reported cases. In 2018 the

first human case was verified by the authorities. Regarding Canine leishmaniosis, different management criteria were developed in each countries: in Paraguay, euthanasia of infected animals is still mandatory though in practice many dogs are under treatment. Argentinian and Uruguayan authorities suggest the euthanasia albeit treatment is not forbidden and an increasing number of dogs are under treatment today. Different programs of preventive measures are being developed everywhere in these countries including, educating the public, monitoring of vector presence, focal spraying of affected areas and widespread usage of deltamethrin impregnated collars in certain cities in Uruguay for example. Nowadays, concentration of foci of Leishmania in many country borders (like northeast or northwest of Argentina or in the northwest of Uruguay), highlights the need for an integrated scheme among neighboring countries in order to share control measures, diagnosis criteria, modality of animal treatment and healthcare policies.

Hematological alterations in canine leishmaniosis

Paulo Cesar Rodrigues Tabanez (Clínica Veterinária Tabanez, Brazil and *Brasileish)*

Canine leishmaniosis is a systemic infectious disease with frequent and significant hematological involvement. Anemia, leukopenia, and thrombocytopenia are the most common findings and can influence both disease progression and prognosis. Anemia in affected dogs is typically non-regenerative and normocytic-normochromic, resulting from chronic inflammation, bone marrow suppression, and immune-mediated mechanisms. Leukopenia, especially lymphopenia, is associated with immunosuppression and correlates with worse outcomes. Thrombocytopenia may arise from splenic sequestration, immune-mediated platelet destruction or secondary coagulopathies. The pathogenesis of these disorders involves inflammatory cytokines, immune complex deposition and direct parasitic effects on the bone marrow. Bone marrow alterations such as dysplasia and hypocellularity are observed in advanced cases. Early recognition of hematological abnormalities through complete blood counts, bone marrow examination and coagulation profiles is critical for clinical management. Hematological changes not only serve as diagnostic markers but also help guide therapeutic strategies and assess prognosis. Understand and address the hematological disorders is essential to improve clinical outcome and quality of life in dogs affected by leishmaniasis.

Room 2
Oral Communication

Sustainable Strategies for Gastrointestinal Nematode Control in Ruminants

Session Chair: Felipe Torres-Acosta (Autonomous University of Yucatan, Mexico)

Implementation of sustainable gastrointestinal nematode control strategies on extensively managed sheep farms

Eilidh Geddes (Moredun Research Institute, United Kingdom), Claire Morgan-Davies (Scotland's Rural College, United Kingdom), Ann McLaren (Scotland's Rural College, United Kingdom), Philip J. Skuce (Moredun Research Institute, United Kingdom), Jade M. Duncan (Moredun Research Institute, United Kingdom), Neil Sargison (University of Edinburgh,

United Kingdom), Fiona Kenyon (Moredun Research Institute, United Kingdom)

Extensive farming systems play an important role in global sheep production yet face many innate management and production challenges compounded by narrow economic margins which threaten their future sustainability. Gastrointestinal nematodes (GINs) pose a significant challenge to the productivity, health and welfare of grazing ruminants. While traditionally extensive sheep farms were not regarded as having significant GIN challenge, previous work following nine extensive sheep farms has demonstrated a production limiting challenge and anthelmintic resistance on all of them, indicating the need for sustainable control strategies. Develop and implement sustainable GIN control solutions suitable for extensive production systems. Improved GIN control solutions were subsequently developed and trialled for two years on those nine farms, accounting for prior parasitological challenge and management. To measure the impact and assess the practicality of these solutions, production data and longitudinal parasitological data including faecal egg counts, anthelmintic efficacy testing and molecular GIN species identification were collected. Furthermore, semi-structured interviews were conducted to capture management and socioeconomic-related impacts. Solutions applied on farm were unique due to varying management systems, however had similar core themes including increased evidence-based decision making, strategic use of anthelmintic and/or improved grazing management. Impacts observed included reduced anthelmintic use and improved lamb performance. Based on the evidence collected, farmers were also motivated to continue implementing these solutions beyond the project end. This demonstrates that the implementation of sustainable GIN management strategies on extensive farms is achievable, however flexibility is required to adapt solutions to fit individual management systems.

Weight gain based-targeted selective treatments for gastrointestinal nematode control: a field trial in a large-scale cattle farm

Candela Canton (CIVETAN, CONICET, UNCPBA, Argentina), Laura Ceballos (CIVETAN, CONICET, UNCPBA, Argentina), Estanislao Quiroga (Private agricultural engineer, Argentina), Eduardo Peres (Private agricultural engineer, Argentina), Paula Domínguez (CIVETAN, CONICET, UNCPBA, Argentina), Lucila Moriones (CIVETAN, CONICET, UNCPBA, Argentina), Juan Manuel Torres (CIVETAN, CONICET, UNCPBA, Argentina), Luis Alvarez (CIVETAN, CONICET, UNCPBA, Argentina), Carlos Lanusse (CIVETAN, CONICET, UNCPBA, Argentina)

Parasite control in grazing ruminants through targeted selective anthelmintic treatments (TST) has been proposed as a novel and sustainable strategy to delay the development of anthelmintic resistance by preserving nematode populations in refugia. TST involves treating only those individuals likely to benefit most from anthelmintic intervention. In the present study, we evaluated a weight gain-based TST approach for the control of gastrointestinal nematodes (GIN) in grazing cattle predominantly exposed to *Haemonchus* spp. and *Cooperia* spp. A total of 189 recently weaned beef calves were enrolled in the trial. All animals received a levamisole treatment at the beginning of the study (baseline treatment). From March to July, calves were monitored monthly and treated only if their weight gain fell below the prede-

termined minimum threshold for that period. Treatments consisted of drug combinations (abamectin + oxfendazole or ivermectin + levamisole) with >99% efficacy, as established by a prior fecal egg count reduction test (FECRT). Most calves required only a single treatment during the study; five animals were never treated, as their growth rates consistently exceeded the threshold. Significant differences in average daily weight gain were observed between treated and untreated animals. Notably, treated calves with initially lower growth rates exhibited a marked improvement in weight gain following treatment. Their post-treatment gains were significantly higher than both their own pre-treatment values. In contrast, daily weight gain in untreated calves remained relatively stable, suggesting limited impact of GIN parasitism in this subgroup. These findings indicate that the growth response to anthelmintic treatment is most pronounced in animals with suboptimal pre-treatment performance, supporting the effectiveness of weight gain-based TST in selectively targeting individuals most in need of intervention.

FAMACHA© and BCS help identify sheep and goats with low worm burden during fall and spring in Mississippi commercial farms

Lindsey Dearborn (ALDI Inc.), Juan Felipe Torres-Acosta (Universidad Autónoma de Yucatán, México), Javier Ventura Cordero (Universidad Autónoma de Yucatán, Mexico), Leyla Rios (Mississippi State University, USA)

The use of eyelid mucosal color (FAMACHA©) and body condition scores (BCS) are used as indicators to select animals requiring deworming against gastrointestinal nematodes (GIN) on farms using Targeted Selective Treatment (TST) schemes. These indicators need evaluation at different times of the year in each region of the world. To identify the specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV) of FAMACHA© 4 to 5 and BCS <2.5 to identify sheep or goats with GIN egg counts > the 9th percentile, in autumn and spring on farms in Mississippi, USA. Five sheep and five goat farms were sampled in two seasons (fall 2021 and spring 2022). During each visit, all the adult animals were sampled for FAMACHA© score, BCS, and individual fecal samples. The GIN egg counts (eggs per gram of feces, EPG) were estimated with the McMaster method. The specificity, sensitivity, positive predictive value (PPV), and negative predicted value (NPV) of BCS<2.5 and FAMACHA© 4 to 5 to identify animals with EPG higher than 90% percentile (cut-off point). The FAMACHA© 4 to 5 and the BCS < 2.5 had poor sensitivity to identify goats or sheep with EPG > 9th percentile in autumn and spring. These indicators had > 70% specificity in both species and in both seasons. Consequently, FAMACHA© 4 to 5 and BCS < 2.5 had poor PPV (< 22.2%) in both seasons. The low PPV is due to the high percentage of thin or anemic animals with low EPG counts (< the 9th percentile). Those animals may have other health problems or a diet failing to meet their production requirements. On the other hand, a high NPV (> 90%) for both phenotypic indicators in goats and sheep in both seasons suggests that goats and sheep with FAMACHA© 1 to 3 and BCS > 2 are likely to have low GIN burdens. FAMACHA© values of 1 to 3 and BCS >2 can be used to identify animals with low GIN loads in the sheep and goat farms of Mississippi during fall and spring, and those animals can be left without AH treatment in a TST scheme.

The impact of regenerative grazing on nematode infections in sheep

Phoebe Beal (Moredun Research Institute, UK), Jade Duncan (Moredun Research Institute, UK), Gillian Mitchell (Moredun Research Institute, UK), Leigh Andrews (Moredun Research Institute, UK), Yolanda Corripio-Miyar (Moredun Research Institute, UK), Fiona Kenyon (Moredun Research Institute, UK), Adam Hayward (Moredun Research Institute, UK)

Productivity loss caused by gastrointestinal nematodes (GIN) is a major problem in the livestock industry. Management of GIN has relied on anthelmintic drugs, but the evolution of anthelmintic resistance means that this is unsustainable. Consequently, there has been a shift towards control strategies that rely on boosting the natural defences of the animals, including regenerative grazing strategies that may reduce parasite exposure and improve nutritional status. In July–October 2023 and June–October 2024, 120 lambs were maintained under 4 grazing treatments in a 2x2 design. Lambs were kept on one of two 'pasture' treatments (ryegrass or 'improved' pasture) and 'grazing' treatments (set-stocked or rotationally grazed). Every two weeks, we monitored weight gain, GIN faecal egg count (FEC) and several immunological parameters including GIN-specific antibody responses and cytokine secretion. Faecal microbial diversity was assessed using 16S rRNA sequencing. Sward composition was conducted annually in August, which showed limited establishment of the improved pasture. Analysis from both grazing seasons shows a limited impact of grazing treatments on weight gain and FEC, but differences between groups emerged towards the end of the seasons, through differences in immunological variables. Animals which were set stocked had lower total protein but higher serum IgG levels, compared to their rotationally grazed counterparts. Animals rotationally grazed on the 'improved' pasture, had higher gut microbiota diversity than the other three groups. Resistance to GIN infection was characterised by a negative relationship between strongyle FEC and serum IgA, IgG, and Interleukin-4. These results provide the first quantitative insight into how regenerative grazing strategies can impact defence against infection in ruminant livestock and its potential role in mitigating the impact of GIN on sheep.

Which clinical markers can predict goats with higher faecal egg counts for targeted selective treatment in Austrian farms?

Miguel Peña-Espinoza (University of Veterinary Medicine Vienna, Austria)

Targeted selective treatments (TST) against gastrointestinal nematodes in small ruminants relies on identifying animals with high faecal egg counts (FEC). However, individual FEC is not always a realistic option for farmers and other practical markers may help to identify goats for TST. Here, we evaluated associations between individual FEC and clinical markers in a large sample of goats in Austria. We performed individual FEC (eggs per gram [EPG]) and on-farm clinical evaluations of 582 goats (not dewormed in the last 8 weeks) in 21 farms. Individual animals sampled for FEC were clinically evaluated by: a) FAMACHA©; b) body condition score (BCS); c) presence of submandibular oedema; d) dag score, and e) hair coat condition. Spearman rank correlations were performed between EPG and all clinical markers. Generalised linear models (GLM) were implemented with EPG as

response variable and clinical markers as fixed factors. From all examined goats with FEC ≥ 1000 EPG (n=145/582), 72.4% had BCS scores of 1–2 (thin/emaciated) and 59.3% FAMACHA scores of 1–2 (no anaemia). Negative (weak) correlations were found only between EPG vs BCS (R = –0.15; P<0.001). GLM analyses revealed that specific FAMACHA and BCS scores had significant associations with FEC. FAMACHA score of 4 (pink-white/anaemic) was the only score associated with higher FEC (FAMACHA-4 goats = 1820 mean EPG vs FAMACHA-1–3 animals = 646–840 mean EPG; P<0.01). For BCS, animals with score 1 (extremely thin) had higher FEC than goats with BCS ≥ 2 (BCS-1 animals = 1356 mean EPG vs BCS-2–4 goats = 643–755 mean EPG; P<0.001). FAMACHA=4 and BCS=1 significantly predicted goats with high FEC, with BCS (scores 1–2) showing promise as a practical marker to inform TST in goats under Austrian conditions. We are currently studying how age group, production system and dominant nematode species in the different farms influence associations between FEC and FAMACHA/BCS that may help to further validate and implement TST markers.

Targeted selective treatment in growing lambs for sustainable anthelmintic use

Cristina Santos Sotomaior (Pontifícia Universidade Católica do Paraná, Brazil), Saulo Henrique Weber (Pontifícia Universidade Católica do Paraná, Brazil), Maria Christine Rizzon Cintra (Pontifícia Universidade Católica do Paraná, Brazil), Fernanda Rosalinski-Moraes (Universidade Federal de Uberlândia, Brazil), Rüdiger Daniel Ollhoff (Pontifícia Universidade Católica do Paraná, Brazil)

Anthelmintic (AH) resistance increasingly threatens sustainable sheep production. Targeted selective treatment (TST) offers an alternative for nematode control by selectively treating only individuals exhibiting clinical parasitism, thereby preserving a refugia population. Identifying lambs needing timely treatment remains a challenge. This abstract details research findings from applying and refining TST protocols for growing lambs. Earlier studies compared monthly AH treatment with FAMACHA (F)-based and daily weight gain (DWG)-based treatments in lambs. Monthly treatment yielded the lowest FEC (1044.5 EPG) and highest DWG (198.4 g; p<0.05), while F-based treatment resulted in the highest FEC (4845.5 EPG) and lowest DWG (90.5 g; p<0.05). Regarding the total mean number of AH treatments, the F-based treatment strategy resulted in the lowest frequency of treatment application (p<0.05); however, this approach likely lacked the sensitivity to adequately identify all lambs requiring treatment. Data indicated a low sensitivity of the F score in growing lambs, ranging from 13.9% to 30.8% for hematocrit (Ht) cutoff values of ≤22% and ≤18%, respectively, suggesting its inadequacy as a sole criterion for the control of haemonchosis in young animals. A critical aspect, however, of using DWG as a criterium is the determination of an appropriate DWG threshold. To address this, a mathematical growth curve was fitted, and lambs with weight gain below the curve's estimate were identified as needing treatment. In a trial with a commercial flock, growth curve-guided treatment significantly reduced AH use (42 vs 182 treatments) compared to monthly treatments, with no significant difference in DWG (204 vs 254 g/day). This demonstrates that integrating growth curve-based DWG assessment is a promising strategy for refining

TST in growing lambs, enabling reduced AH usage without impacting growth performance.

Towards sustainable worm control: field validation of a decision support tool for targeted treatment of gastrointestinal nematodes in first-season grazing dairy cattle

Janne Goes (Ghent University, Belgium), Johannes Charlier (Kreavet, Belgium), Evi Canniere (Inagro, Belgium), Luna De Veerman (Ghent University, Belgium), Ophélie Degryse (Inagro, Belgium), Edwin Claerebout (Ghent University, Belgium)

Gastrointestinal nematode infections in cattle are often controlled by routine anthelmintic treatments without diagnostic support, contributing to anthelmintic resistance and threatening the long-term efficacy of available drugs. Targeted treatment (TT), where only at-risk herds receive treatment, offers a sustainable alternative. This study aims to validate a non-invasive decision support tool to implement TT in first-season grazing dairy heifers, in order to reduce anthelmintic use while maintaining growth and controlling infection levels. A field study was conducted on 78 dairy farms in Belgium, monitoring 1234 heifers in 153 groups. Risk scores for gastrointestinal nematode exposure were calculated per group at the beginning of the pasture season, based on pasture management and previous anthelmintic use. Treatment advice for TT groups (N = 77) was tailored to these risk scores, whereas no treatment advice was provided to the control groups (N = 76). Outcomes (anthelmintic use, serum pepsinogen levels, average daily weight gain) were compared between TT and conventionally treated heifers. TT reduced anthelmintic use by 21%, with no significant difference in weight gain or serum pepsinogen between groups. 'Cumulative egg-free days' was used as a quantitative measure of anthelmintic use, i.e. the total number of days animals remained free of egg-shedding following treatment. Despite the shorter grazing period in the control group, cumulative egg-free days were significantly lower in the TT group. Moreover, TT heifers experienced 16.7% more grazing days without anthelmintic pressure, supporting improved sustainability. Both groups' mean serum pepsinogen values (1.4 U Tyr) fell within the desired reference range (1.2 - 3.5 U Tyr). This study demonstrates the potential of a non-invasive decision support tool to reduce anthelmintic use without compromising performance.

16:00 – 17:30

Room 1
Round Table

Prevention of zoonotic vector-borne pathogens affecting pet animals

Prevention of vector-borne diseases of pets

Domenico Otranto (University of Bari "Aldo Moro", Italy)

Dogs represent a common and available blood source for arthropods (e.g., ticks, phlebotomine sand flies, mosquitoes, and fleas), which may act as vectors of pathogens. The circulation of canine vector-borne pathogens (CVBPs) within a population of animals is influenced by a plethora of individual (e.g., genetics, immunocompetence, and use of preventative measures), and environmental factors. Therefore, control strategies should be tailored considering ecological and behavioural peculiarities of arthro-

pod vectors, availability of susceptible hosts, co-circulation of multiple pathogens, individual risk factors (e.g., hunting, shepherd dogs as opposed to dogs kept as domestic indoor companions), as well as socio-economic conditions. Importantly, several aspects of the vector-pathogen-host interaction (e.g., pathogen transmission routes and times, feeding behavior, blood feeding duration) influence the efficacy of parasiticides, being some pathogens inoculated soon after the blood feeding begins (e.g., *Leishmania* spp.), and others later, after some hours to days (e.g., *Rickettsia* spp. and *Babesia* spp., *Anaplasma* spp., *Borrelia* spp.). Knowledge about all the above factors is fundamental to plan control strategies to reduce their feeding activities on the hosts. At present, the prevention of CVBP transmission in companion animals is achieved through the administration of products that can repel or rapidly kill arthropods, thus preventing or interrupting feeding before transmission occurs. These products may act by killing (i.e., insecticidal and/or acaricidal products, formulated for topical or oral applications) and/or repelling (i.e., repellent products, formulated for topical application only) arthropods. Therefore, the above formulations can reduce arthropod feeding (anti-feeding effect) and/or block the feeding process in its early stages, ultimately reducing the risk of VBP transmission.

Isoxazolines and vector-borne diseases

Frederic Beugnet (Boehringer Ingelheim Animal Health, France)

Parasiticides represent the first largest segment of the global animal health market, accounting for more than 25% of the veterinary medicine market (i.e., €7 billion/year, in a €25 billion market). Few novel parasiticides have been introduced during recent decades. One exception is the success story of isoxazolines (afoxolaner, fluralaner, lotilaner, and sarolaner), which first entered the market in 2014. Isoxazolines are active against insects and acarians, they bind to a unique and specific receptor on GABA-gated chloride channels in nerve cells of arthropods, blocking transfer of chloride ions across cell membranes. Following oral or topical administration, they are quickly absorbed and bind to plasma proteins. They provide a systemic insecticidal and acaricidal effect through ingestion by arthropods, and do not provide any repellent effect. One key question is their ability to prevent the transmission of pathogens by arthropods. Isoxazoline formulations provide a sustained ectoparasiticide activity with a short speed of kill (e.g. 6 to 12h against fleas, 8 to 48h against ticks, depending on the molecule, formulation, dosage and if it concerns curative or preventive efficacy). It is also probable that before dying, arthropods like ticks stop their feeding behavior. It has been confirmed under field and experimental conditions that isoxazoline products are able to prevent the transmission of certain canine vector borne pathogens (e.g., *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Babesia canis*, and *B. rossi*. In addition, it has been demonstrated that they present a high insecticidal efficacy against several mosquito species including *Aedes aegypti*, and *Culex pipiens*, and several sandfly species like *Phlebotomus perniciosus*, 48 to 72h after feeding on treated dogs or after artificial membrane feeding. Although if the use of isoxazolines cannot prevent the transmission of pathogens to dogs by mosquitoes or sandflies, it may reduce the population of insect vectors and, therefore, the dynamic of pathogen transmission. This has been observed

in sheltered dogs for the incidence of infection with *Leishmania infantum* and *Diroflaria immitis* in highly endemic area. Moreover, due to their sustained killing effect within 72h against mosquitoes, their use on already infected dogs could reduce the risk of transmission to uninfected ones because female mosquitoes rest for a few days to digest and lay eggs before taking new blood meal. Mosquitoes and sandflies ingesting *Leishmania* amastigotes or *Dirofilaria* microfilariae would also die before the maturation of the pathogens.

Growing diversity of vector-borne diseases

Marcos Rogério André (UNESP, Brazil)

Over the past few decades, the number of reports on vector-borne pathogens (VBP) and associated diseases has increased exponentially. Although multiple factors may contribute to this rise, three main drivers can be identified: (i) globalization, which facilitates the movement of people, animals, and vectors across regions; (ii) the encroachment into natural habitats, promoting closer interactions among humans, vectors, and both wild and domestic animals; and (iii) significant advances in molecular biology, which have enhanced the detection and characterization of VBP. In this presentation, we will highlight recent discoveries in the field of VBP, focusing on two groups studied by our research team: Piroplasmids (tick-borne apicomplexan protozoa) and *Bartonella* spp. (arthropod-borne bacteria). Based on a multi-locus sequence approach, our research group have expanded Piroplasmida phylogenetic reconstruction, adding five novel clades: “Phyllostomidae bat group”; “South American Marsupialia Group”; “South American Rodentia Group”; “*Tapirus terrestris* group”; and “Capybara group”. The same approach allowed the description of novel piroplasmids in Brazilian wildlife, namely *Theileria terrestris* in tapirs, *Babesia goianiaensis* in capybaras, *Babesia pantanalensis* in crab-eating foxes, and *Cytauxzoon brasiliensis* in little-spotted pumas, ocelots, and domestic cats. Digital PCR assay has shown the occurrence of piroplasmids and *Bartonella* spp. in wild animals from Brazil in which these agents has been scarcely detected using conventional/real-time PCR assays. Multiplex real-time PCR assays based on the ITS-1 revealed single or coinfection by *Babesia divergens, Babesia microti*, and *Babesia odocoilei* in humans from the USA. Hybrid sequencing platforms (Illumina NovaSeq and Nanopore) has allowed the description of *Bartonella machadoae* and *Bartonella harrusi*, two novel *Bartonella* species isolated from rodents and marsupials, respectively, from Brazil. Phylogenomics and ANI (Average Nucleotide Identities) were used to differentiated both species that could not be distinguished from each other using multi-locus sequencing. These findings underscore the importance of taking advantage of novel molecular approaches to unravel the cryptic diversity of vector-borne agents.

Room 2
Round Table

Sustainable Parasite Control. How to build communities of practice?

Session Chair: Laura Rinaldi (University of Naples Federico II, Italy)

Building a European Community of Practice in Sustainable Worm Control for ruminants

Johannes Charlier (Kreavet, Belgium), Laura Rinaldi (University of Napoli Federico II, Italy),

Eric R. Morgan (University Belfast, Northen Ireland), Edwin Claerebout (Ghent University, Belgium), Dave J. Bartley (Moredun Research Institute, UK), Smaragda Sotiraki (Veterinary Research Institute, Greece), Marcin Mickiewicz (Toinen Pro Art Fundacja, Poland; Warsaw University of Life Sciences-SGGW, Poland), Maria Martinez-Valladares (Universidad de León, Spain), Natascha Meunier (Animal Health Ireland, Ireland), Tong Wang (Kreavet, Belgium), Alistair Antonopoulos (Kreavet, Belgium), Fanny Baudoin (Flanders Research Institute for Agriculture, Fisheries and Food, Belgium), Leen Lietaer (Flanders Research Institute for Agriculture, Fisheries and Food, Belgium)

Anthelmintic resistance is an escalating problem in Europe and the environmental consequences (soil and aquatic health) related to anthelmintic use are an increasing matter of concern. Several sustainable worm control practices are available now. These include the increased use of diagnostics and decision support enabling a targeted use of anthelmintics. Complementary control measures, referred to as the 'Basket of Options', include plant-based control, grazing management, nematode destroying fungi and selective breeding and can also reduce the need for anthelmintic use. Their use is more complex than the simple use of anthelmintics and their uptake has remained relatively low. Equipped by recent studies on the barriers and drivers for the uptake of sustainable worm control by farmers, we are now building a Community of Practice across Europe, termed SPARC – Sustainable Parasite Control in grazing ruminants, involving all relevant stakeholders at local, national and European level to achieve sustainable worm control together. We will present recent activities, learnings and results from this effort.

Equine Parasite Control: Working Towards Global Consensus

Martin K. Nielsen (Aarhus University, Denmark)

Recommendations and guidelines for equine parasite control have often been found conflicting and contradictory depending on the source and context. Until 2012, there were no sanctioned guideline documents available for this purpose anywhere in the world. That year the American Association of Equine Practitioners (AAEP) published their first guideline document, and since then several other countries have followed suit. Guidelines are now available in Sweden, Denmark, the Netherlands, United Kingdom, and Australia. In addition, there is a general European equine parasite guideline document published by the European Scientific Counsel Companion Animal Parasites (ESCCAP). While this development is very positive, it also comes with new challenges. Since these different guidelines are written by different authors in different parts of the world, the texts are far from identical, and, hence, they can leave an impression of lack of consensus. However, at the 2024 International Equine Infectious Disease Conference authors of the aforementioned guideline documents got together in a special session to compare and discuss differences and similarities. Overall, all guidelines shared the same goals: 1) to decrease the risk of equine parasitic disease, and 2) to delay anthelmintic resistance development as much as possible. The main target parasites species/categories were generally the same with cyathostomins and ascarids receiving the most attention. Some differences

were observed with regards to the perceived importance of equine tapeworms, and only the two Scandinavian countries had a main focus on the equine bloodworm *Strongylus vulgaris*. The same anthelmintic classes were available in all countries with only Australia differing substantially due to a large portfolio of combination products not available in the other countries. Similarly, differences in diagnostic products available impacted the guidance provided in the different countries. Most importantly, the guidelines for anthelmintic resistance testing were generally aligned with the Fecal Egg Count Reduction Test guideline document published by the WAAVP in 2022. Several research needs were identified, including the need for better methods for effective communication of these guidelines to the end-users.

Parasites of domestic dogs in traditional societies of sub-Saharan Africa: need for interventions vs. coexistence and conservation of evolutionary processes

Andrei Daniel Mihalca (University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania)

Sub-Saharan Africa is home to a treat variety of culture, traditions, landscapes, wildlife and beauty. Animals, play an integral, vital role in many traditional societies from this fascinating part of our planet. A lot of effort and resources had been invested in parasite surveillance and control of livestock, mainly due to their economic value and resources provided for the welfare or even survival of people. However, dogs are also an important part of most pastoral or hunter-gatherer communities in sub-Saharan Africa, but, compared to livestock, rather neglected regarding their parasitic diseases. Part of these diseases pose a real threat to their survival or welfare, but the knowledge on the true diversity and impact of these parasites is at best limited to isolated studies. On one hand, parasites play an important evolutionary role and are key components of natural selection, with strong evolutionary implications, even in domestic animals. On the other hand, many diseases, including parasitic ones can significantly influence the life expectancy of dogs, challenging rabies control programs for instance. Control of parasites in such dog populations poses various challenges related on one hand to financial or logistic reasons, or lack of efficacy studies against most tropical parasites. On another note, the use of parasiticides poses ethical concerns because of their environmental impact or disruption of the evolutionary processes. Nevertheless, such approaches should consider the balance between the benefits and undesired impacts, including cultural perceptions.

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10:30 – 12:00

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Room 1
Oral Communication

Equine Helminths: Epidemiology, Immunity, Microbiota and Control Strategies

Cyathostomin species identifications in 89 German horse farms based on cytochrome c oxidase I sequence analysis

Jennifer Sandra Schmidt (Freie Universität Berlin, Germany), Jürgen Krücken Freie Universität Berlin, Germany), Sandro Andreotti (Freie Universität Berlin, Germany), Sarah Sparmann (Berlin Center for Genomics in Biodiversity Research, Germany; Leibniz Institute for Research on Evolution and Biodiversity, Germany), Susan Mbedi (Berlin Center for Genomics in Biodiversity Research, Germany; Leibniz Institute for Research on Evolution and Biodiversity, Germany), Thore Schenk (Freie Universität Berlin, Germany), Jürgen Bartz (Virbac Tierarzneimittel GmbH, Germany), Eric Bousquet (Virbac, France), Georg von Samson Himmelstjerna (Freie Universität Berlin, Germany)

Grazing horses are usually affected by multispecies infections with parasitic nematodes. Differentiation of the 50 cyathostomin and 14 Strongylin species represents a major challenge, which makes surveillance and diagnosis of pathogenic species such as large strongyles challenging. In order to obtain data on the nemabiome of German horses, a cross-sectional study was carried out with 970 horses from 96 farms in 6 federal states. Fecal samples were examined for the presence of GINs using Mini-FLOTAC. DNA was isolated from strongyle eggs at farm level and used for PCR to amplify a 653±3 bp partial cytochrome c oxidase I (COI) fragment. PCR products were sequenced on an Illumina MiSeq (2×300 bp paired-end reads) and processed through a bioinformatics pipeline, allowing differentiation of cyathostomin and strongylin species. Strongyle were detected in 47.4% (95%CI 44.3-49.8%) of the horses and 95.8% of the farms. In 90 samples, 6210 amplicon sequence variants (ASVs) with 1,189,311 reads could be assigned to a species. Further 412 ASVs (63,026 or 5.3% of the reads) could not be classified. Strongyle communities consisted of 4 to 21 species, assuming that the unclassified ASVs represented at least one other species. No significant difference in the number of species was found between farms that dewormed two or more times a year compared to farms with no or only one deworming per year (Man-Whitney test p=0.328). The 5 most prevalent species were *Cylicostephanus longibursatus*, *Cyathostomum catinatum*, *Cylicostephanus minutus* OTUII, *Cylicostephanus goldi* and *Cylicocyclus nassatus*. *Strongylus vulgaris* was detected on five farms, but only one among the five farms treating ≤1-time/year. The described method for characterizing the nemabiome allows a reliable identification of an increasing number of species as well as their prevalence and abundance, which will enable future investigations on the ecology and pathogenicity.

Epidemiological patterns of cyathostominae burden in warmblood horses in Mexico.

Cintli Martinez-Ortiz-de-Montellano (Universidad Nacional Autónoma de México, México), Laura González-Reyes (Universidad Nacional Autónoma de México, México), Hugo Oswaldo

Toledo-Alvarado (Universidad Nacional Autónoma de México, México)

A significant challenge in equine parasitology is the comprehension of the epidemiology of cyathostomosis, particularly in large herds. It is imperative to recognize that when egg excretion counts (FEC) are conducted, the results necessitate interpretation. A retrospective study spanning five years was conducted at a Warmblood farm in Avándaro, State of Mexico, to address this knowledge gap. The data obtained over the years corresponds to sex, age, month, year, management type, as well as monthly FEC of an estimated growing population of 100 to more than 400 horses, a very robust database. A generalized linear model with a negative binomial distribution was employed, utilizing the function glm.nb from the MASS library in R version 4.3.0, with the following formulation: $ln(EPG)=Intercept+Sex+Age+Month+Year+Handling+e$ where EPG represent the eggs per gram of faeces of the horses resulting from the FEC; Sex with male and female levels: Age is grouped into categories of 0, 1-3, 4-6, 7-9, 10-12, 13-15, and 16 or more. Month is grouped into categories from January to December. Year is grouped into categories from 2020 to 2024. Management is grouped into categories of stable, paddock, and breeding stock, e is the associated error. The exponent for the estimates of the levels of each factor was estimated, as well as the estimated marginal means with their 95% confidence intervals. The results of the database demonstrate that there are no statistically significant differences in EPG excretion between sexes. However, it is evident that age groups 1-3, 4-6, and 7-9 deviate from the remainder of the groups. Group 10-12 also differs from both group 0 and >16. The annual elimination ranges are found to be statistically significant, indicating a progressive decline in EPGs. The months with the highest elimination significance are November and December (p=0.01). Finally, the characterization of management indicates that those in the flock are high dispersers of EPGs (p=0). These findings underscore the impracticality of employing deworming protocols based on rigid, closed treatment schedules. This finding underscores the need for a paradigm shift in established practices, compelling veterinarians to adopt a more discerning approach when implementing preventative medicine strategies. This approach is predicated on the imperative to circumvent anthelmintic resistance within the context of sustainable equine parasite management.

Prevalence and risk factors of *Parascaris* spp. infection in equids under two years old in france

Bourrier Kenza (Anses, Animal Health Laboratory, France), Karadjian Gregory (Anses, Animal Health Laboratory, France), Merlin Aurélie (Anses, Animal Health Laboratory, France)

Severe infections of *Parascaris* spp. (nematode) in young equids can cause respiratory signs, intestinal obstruction, and sometimes death. The prevalence of this parasite has been assessed in various countries but not in France. The aim of this study was to estimate the prevalence of *Parascaris* spp. infection in foals under two years old in the Normandy region and to identify the associated risk factors. Faecal samples were collected from 720 equids kept on 66 different farms between February and December 2024, and a questionnaire study was conducted. Faecal egg counts were performed using the Mini-FLOTAC method. A generalized linear mixed model was implemented to identi-

fy risk factors for this infection at animal and farm levels. The prevalence of *Parascaris* spp. infection was 12.9%. Eggs of strongyles, *Anoplocephala* spp., *Strongyloides westeri* and *Oxyuris equi* were also identified in 95%, 26.5%, 8.1% and 0.8% of animals. Three factors influenced significantly the presence of *Parascaris* spp. eggs: age, time between the last treatment and sampling and number of equids per farm. Foals aged [2-4] months were less likely to be infected than animals aged]4-8] months and more likely than animals aged]16-20] and]22-24] months. Equids that had received an anthelminthic treatment in the last 2.5 months were less likely to be infected than those that had not been treated since birth or had been dewormed for more than 2.5 months. However, 10.5 % of these animals continued to shed *Parascaris* spp. eggs, raising the possibility of dewormer-resistant parasites. Equids kept in large farms were less infected than those kept in medium-sized farms were. No difference was observed between small and large farms. This study provided for the first time the *Parascaris* spp. prevalence in french equids. To go further, it would be interesting to investigate the effectiveness of deworming and the underlying characteristics of the medium-sized farms leading to a higher prevalence of infection.

A Stable Relationship? Local and Systemic Immune Responses to Equine Cyathostomin Infection

Orla Byrne (University College Dublin, Ireland), Callum Donnolly (University of California, USA), Carrie Finno (University of California, USA), Hanne Jahns (University College Dublin, Ireland), Kirsty L Lightbody (Austin Davis Biologics Ltd, UK), Corrine Austin (Austin Davis Biologics Ltd, UK) Jacqueline B Matthews (Austin Davis Biologics Ltd, UK), Nicola Walshe (University College Dublin, Ireland), Grace Mulcahy (University College Dublin, Ireland)

The equine hind gut has a major influence on the health and welfare of horses, and typically contains a population of nematodes, chiefly cyathostomins. Major knowledge gaps exist in how these resident nematodes influence the immune system. We aimed to establish baseline data for immune cell infiltration of the hindgut mucosa in horses with cyathostomin infection, and to characterise equine peripheral blood mononuclear (PBMC) responsiveness to mitogen and cyathostomin antigens. We obtained necropsy samples of the mucosal lining of 11 horses that had cyathostomin burdens (range: 0 - 1,670 epg) but no related clinical signs, and characterised immune cell infiltration and cyathostomin burdens in these animals. In a separate study, we measured PBMC responsiveness to adult cyathostomin somatic antigens (n= 450 worms, 9 cyathostomin species) in 97 horses with known strongyle egg shedding status (range:0-180 epg), and serum scores indicating low exposure status. The main immune cell types found in the mucosa were T-cells (41%), macrophages (30%) eosinophils (17%), and B-cells (10%), whereas the sub-mucosa had relatively fewer T-cells. Where present, cyathostomin larvae were mainly seen in the lamina propria mucosa surrounded by moderate numbers of macrophages, less commonly in the submucosa. Incubation of PBMC from horses of known strongyle egg shedding status, and low exposure as indicated by serum scores with the mitogen PHA or with cyathostomin antigens revealed an inverse relationship between mitogen responsiveness and a positive association between antigen responsiveness and strongyle egg shedding. We have produced

a multi-dimensional dataset on equine immune cell infiltration in the hindgut, PBMC proliferative responsiveness, and cyathostomin infection, including evidence of bystander immunoregulation via reduced PBMC proliferative mitogen responses in horses with even a low level of infection. These results contribute to understanding of the pathophysiology of equine cyathostominosis.

Microbiota dynamics in cyathostomin-infected horses under two parasite control strategies.

Cintli MARTINEZ-ORTIZ-DE-MONTELLANO (Universidad Nacional Autónoma de México, Mexico), Claudia C. Marquez-Mota (Universidad Nacional Autónoma de México, Mexico), Edgar Dantán-González (Universidad Autónoma del Estado de Morelos, Mexico), Armando Hernández-Mendoza (Universidad Autónoma del Estado de Morelos, Mexico), Hugo Oswaldo Toledo-Alvarado (Universidad Nacional Autónoma de México, Mexico), Rosa Estela Quiroz-Castañeda (Centro Nacional de Investigación Disciplinaria en Salud Animal e Inocuidad, México.), Noemí Estefanía González-Serrano (Universidad Nacional Autónoma de México, Mexico), Mariana Pérez-Olvera (Universidad Nacional Autónoma de México, Mexico), Fernanda Calixto (Universidad Nacional Autónoma de México, Mexico)

Cyathostomosis with anthelmintic resistance (AHR) is a growing global concern in equine farms, affecting both health and parasite management. The frequent use of anthelmintics, often without selective criteria, raises environmental and public health issues. This study explores sustainable strategies for parasite control, emphasizing responsible drug use and microbiota care via digestive support. We characterized the fecal microbiota of naturally infected horses under two control approaches: conventional Ivermectin (IVM) 1.87% treatment and a digestive support supplement. Over 30 days, fecal samples were collected from 19 horses on days 0, 15, and 30. DNA was extracted, and the V4 region of the 16S rRNA gene was sequenced to assess microbial profiles. Blood samples were also taken on the same days to measure inflammatory markers, while daily coproparasitoscopic analyses evaluated IVM efficacy. Results showed microbiota shifts linked to stability and resilience in supplemented horses, while IVM-treated animals showed changes reflecting redundancy and possible disruption. Inflammatory markers such as fibrinogen were higher in the IVM group. Notably, IVM showed rapid efficacy by day 2 post-treatment. However, its misuse is a risk factor for AHR, dysbiosis, ecotoxicity, and broader public health consequences. It is imperative to implement targeted selective treatment or strategic treatment grounded in sustainable equine parasite management to ensure long-term efficacy and microbiota health without ecosystem fragmentation.

Exploration of equine keepers' attitudes, beliefs and behaviours surrounding helminth control and anthelmintic resistance in the United Kingdom

Faye Ellen McTigue (Harper Adams University, United Kingdom), Dr Emma Bleach (Harper Adams University, United Kingdom), Professor Philip Robinson (Harper & Keele Veterinary School, United Kingdom), Jodie Perrett (Food and Rural Affairs (Government), United Kingdom), Dr Alison Pyatt (Food and Rural Affairs (Government), United Kingdom), Dr Ste-

phen Mansbridge (Harper Adams University, United Kingdom)

Historic blanket approaches to anthelmintic use have contributed to widespread resistance. Although positive changes to helminth controls have been reported in the UK equine sector, many still use traditional approaches. This study used an online questionnaire to identify current helminth controls used by UK equine keepers. There was a total of 383 respondents and 576 responses across all equine categories. Results revealed 57% of keepers remove dung twice weekly and most return the animal as per their normal routine to the same pasture post anthelmintic administration. Moxidectin was the most used anthelmintic; 80% of equines received a dose within the last 12 months. Five themes were identified as factors influencing pharmaceutical controls: diagnostic testing; targeting helminths; time of year; routine anthelmintic administration and irregular anthelmintic administration. Results revealed 88% uptake of faecal egg counts and 79% uptake of faecal egg count reduction tests (FECRTs). However, further analysis found that 55% of total respondents did not know the anthelmintic resistance (AR) status of their premises and 57% stated that the AR status was identified by a FECRT, suggesting confusion surrounding AR status and FECRTs. Increased helminth-associated disease affecting UK equines was considered the main consequence should anthelmintics become ineffective. Nearly all (98%) respondents were aware of AR, 84% were concerned about it and 72% would like to increase their AR knowledge. Most (70%) keepers implemented quarantine measures specifically for helminth control, however there was considerable variation in the measures used. Although positive practices were identified, a significant number of equines could be at increased risk of helminth infection due to suboptimal practices, particularly surrounding pasture management. These findings will inform future strategies aimed at improving equine anthelmintic stewardship, thereby slowing the rate of development of AR.

Room 2
Oral Communication

Lungworms in Companion Animals: Epidemiology, Risk Assessment, and Control Strategies

Impact of environmental conditions on the spread of canine neural angiostrongyliasis in Australia, 2020-2024

Phoebe Rivory (University of Sydney, Australia), Rogan Lee (Westmead Hospital, Australia), Michael P. Ward (University of Sydney, Australia), Jan Slapeta (University of Sydney, Australia)

Neural angiostrongyliasis (NA), caused by the rat lungworm (*Angiostrongylus cantonensis*), is an emerging zoonotic disease on Australia's east coast, with an increasing number of cases since 2010. This study aimed to understand spatial and temporal dynamics of canine NA (CNA), diagnosis and the parasite genetic diversity. We analysed cerebrospinal fluid samples from 180 clinically suspected cases (2020-2024) using AcanR3990 qPCR to confirm infection. We examined the genetic diversity of *A. cantonensis* through partial *cox*1 sequencing and assessed environmental factors influencing CNA using generalised linear modelling. Infection was confirmed in 93 cases, primarily around Brisbane and Sydney, with a peak in 2022 (32 cases). CNA occurrence was significantly asso-

ciated with immediate and long-term rainfall (1 and 10-12 month lags) and medium-term temperature changes (5-7 month lags). Genetic analysis identified Ac13 as the dominant haplotype (9/15). Comparison with an established ELISA using 50 randomly selected samples showed substantial agreement (κ = 0.66). The study highlights the significant role of environmental factors in CNA occurrence and the genetic diversity of *A. cantonensis*. With many cases likely undiagnosed, NA remains a critical One Health issue in Australia, necessitating improved diagnostic and preventive measures.

Epizootiological update on the occurrence of *Angiostrongylus vasorum* and respiratory *Capillaria* spp. in dogs from Italy

Donato Traversa (University of Teramo, Italy), Simone Morelli (University of Teramo, Italy), Angela Di Cesare (University of Teramo, Italy), Chiara Astuti (University of Teramo, Italy), Alessandra Barlaam (University of Foggia, Italy), Mariasole Colombo (University of Teramo, Italy), Fabrizia Veronesi (University of Perugia, Italy), Barbara Paoletti (University of Teramo, Italy), Raffaella Iorio (University of Teramo, Italy), Raffaella Maggi (Freelance Veterinary Practitioner, Italy), Alessandra Passarelli (Clinica Veterinaria Città di Bari, Italy), Alessia Pede (Boehringer Ingelheim Animal Health, Italy), Linda Rossi (University of Teramo, Italy), Manuela Diaferia (University of Perugia, Italy)

Angiostrongylus vasorum, *Capillaria aerophila* and *Capillaria boehmi* are major nematodes affecting the lungs of dogs. Adult stages of *A. vasorum* reside in the pulmonary arteries, while those of *C. aerophila* and *C. boehmi* live in the trachea/bronchi and nasal cavities/paranasal sinuses, respectively. Clinical consequences of *A. vasorum* infections are unpredictable and may be potentially fatal, while pulmonary and nasal capillariosis are characterized by respiratory signs of varying severity. This study has investigated the occurrence of *A. vasorum*, *C. aerophila* and *C. boehmi* in dogs living in Italy, i.e. in Umbria (Site A – n. 400), Marche (Site B – n. 400), Abruzzo (Site C – n. 400), Molise and northern Apulia (Site D – n. 366) and Latium (Site E – n. 434). A binomial logistic regression was performed to investigate statistically significant associations between positivity and possible risk factors. Larvae of *A. vasorum* were found in 62 (3.1%) dogs, i.e. 39 (10.2%), 12 (3%), 6 (1.5%), 2 (0.5%), 3 (0.7%) in sites A-E, respectively. Eggs of *C. aerophila* were found in 217 (10.8%) samples, i.e. 62 (15.5%), 63 (15.7%), 20 (5%), 7 (1.9%), 65 (15%) in sites A-E, while those of *C. boehmi* were present in 44 (2.2%) samples, i.e. 21 (5.2%), 4 (1%), 6 (1.5%), 13 (3.2%) in sites A-C and E, respectively. Infection by *A. vasorum* was significantly associated with the occurrence of cardiorespiratory signs ($p < 0.001$), mollusk ingestion ($p < 0.001$), and permanent outdoor housing ($p = 0.029$), while *C. aerophila* infection was significantly associated with hunting dogs ($p = 0.020$). A stable enzootic of *A. vasorum* in the examined areas is confirmed, while respiratory *Capillaria* spp. were found with higher rates than in the past. These results underline the need for increased awareness and implemented control strategies against dog angiostrongylosis and respiratory capillarioses.

Lungworms and other endoparasites in domestic cats living in the States of Rio de Janeiro and Rio Grande do Sul, Brazil

Angela Di Cesare (University of Teramo, Italy), Simone Morelli (University of Teramo, Italy),

Donato Traversa (University of Teramo, Italy), Sandra Márcia Tiez Marques (UFRGS, Brazil), Lebana Fernandes Knopp (Fluminense Federal University, Brazil), Caio dos Santos Gomes (UFF, Brazil), Eduarda Nóbrega Fialho Tavares (UFF, Brazil), Júlia Pereira da Silva (UFF, Brazil), Aline Silva de Mattos Queiroz (UFF, Brazil), Frederic Beugnet (Boehringer-Ingelheim Animal Health, France), Karin Botteon (Boehringer-Ingelheim Animal Health, France), Ilaria Lallone (University of Teramo, Italy), Luciano Antunes Barros (UFF, Brazil)

Aelurostrongylus abstrusus, *Troglostrongylus brevior* and *Capillaria aerophila* are the main respiratory nematodes infecting cats. *Aelurostrongylus abstrusus* has a worldwide distribution and it occurs in different countries, including Brazil, while *T. brevior* and *C. aerophila* are enzootic in Europe but never unequivocally reported in cats in Brazil. The present study provides updated epizootiological data on lungworm infections and other feline endoparasites in two States of Brazil. Overall, 537 cats were sampled, i.e. 521 in metropolitan area of Rio de Janeiro and 16 in Porto Alegre from Rio Grande do Sul. Fecal samples were examined via Baermann test (n. 521) and Sheater's flotation (n. 537). DNA was extracted from each Baermann sediment and subjected to two separate nested PCRs to identify *A. abstrusus* and *T. brevior* according to a previously validated protocol. To date, PCRs were run on 214 samples. Larvae of *A. abstrusus* were identified at Baermann in 3 (0.6%) samples from Rio de Janeiro. The most frequent parasites found at flotation were Ancylostomatidae (n. 30; 5.8%), *Toxocara cati* (n. 14; 2.7%), *Cystoisospora felis* (n. 8; 1.5%). At PCR, *A. abstrusus* DNA was found in 4 samples (1.87%), i.e. in the 3 positives at Baermann and in one sample from Porto Alegre positive for larvae which were unidentifiable microscopically. Even though the results herein presented are still incomplete, (i) the enzooticity of *A. abstrusus* in the investigated areas of Brazil and (ii) the usefulness of PCR when larvae are not detected, or a single stool sample is analyzed, are shown. Further molecular examinations will be performed to investigate the genetic make-up of *A. abstrusus* isolates herein found. Given the clinical impact of lungworms and the zoonotic potential of other nematodes here detected, a high level of vigilance towards feline parasites in Brazil is warranted.

Aelurostrongylus abstrusus infections, a health threat for cats entering animal shelters in the Netherlands?

Rolf Nijsse (Utrecht University, Netherlands), Ruth van der Leij (Utrecht University, Netherlands), Shevaun den Bakker (Utrecht University, Netherlands), Carolien van Beuningen (Utrecht University, Netherlands)

Around 45,000 cats are taken in by animal shelters (AS) in the Netherlands every year. Standard procedures after admission are deworming and neutering. For both procedures it is important to know what parasitic infections are present. First, for the choice of anthelmintic, but even more urgently, with regard to lungworms, to assess the risk of anesthesia. The aim of this pilot study is to assess the level of parasitic infections in cats entering AS. Participating AS were instructed on how and when to collect fecal samples from newly entering cats. Coproscopic examination, using the centrifugation flotation (CSF) method and the Baermann technique, was performed on fecal samples of these cats before deworming while still in quarantine. Shelters supplied information about the anthel-

mintics they rountinely use for new admitted cats? A mobile veterinary clinic working on a trap, neuter and return project also provided fecal samples from stray cats. Fourteen animal shelters were included in the study. They provided fecal samples from 80 cats. The mobile clinic provided 10 fecal samples. The CSF resulted in 32% of the samples testing positive for at least one parasite. *Toxocara cati* was most frequently found (25.8%). *Capillaria* sp. eggs were identified in 3.3% of the samples. Furthermore, 2.2% of the samples contained strongyle-type eggs, possibly from *Ancylostoma tubaeforme.* Oocysts of *Cystoisospora* spp. were determined in 4.3% of the samples. The Baermann resulted in 10% positive samples for the first-stage larvae of *Aelurostrongylus abstrusus*. None of these cats showed respiratory symptoms. Anthelmintic treatment in the AS was never aimed at treating lungworm infections and six of the shelters used a product that was not licensed for lungworm infection. Lungworm infections in cats are an underrecognized risk for cats entering AS. Cats should be tested before surgery, as infection can pose a health risk for cats during anesthesia.

Latest update on the use of eprinomectin against cat lungworms

Donato Traversa (University of Teramo, Italy), Angela Di Cesare (University of Teramo, Italy), Simone Morelli (University of Teramo, Italy), Fabrizia Veronesi (Department of Veterinary Medicine, University of Perugia, Perugia, Italy), Giulia Morganti (Department of Veterinary Medicine, University of Perugia, Perugia, Italy), Eric Tielemans (Boehringer Ingelheim Animal Health, France), Camille Gamblin (Boehringer Ingelheim Animal Health, France), Giulia Rigamonti (University of Perugia, Italy), Paolo Emidio Crisi (University of Teramo, Italy), Frederic Beugnet (Boehringer Ingelheim Animal Health, France)

Aelurostrongylosis, troglostrongylosis and capillariosis caused by the nematodes *Aelurostrongylus abstrusus*, *Troglostrongylus brevior* and *Capillaria aerophila* respectively, are major respiratory diseases of domestic cats. Macrocyclic lactones are among anthelmintics that are efficacious for treatment and/or prevention of these diseases. A spot-on formulation containing eprinomectin (0.4%), esafoxolaner (1.2%) and praziquantel (8.3%) has been recently evaluated for its parasitological and clinical efficacy against *A. abstrusus*, *T. brevior* and *C. aerophila*. A clinical study has demonstrated that this formulation assures the parasitological and clinical recovery of 36 cats infected either by *A. abstrusus* and/or *T. brevior*. Two administrations 4 weeks apart reduced 100% the larval shedding but, even more importantly under a practical standpoint, they guaranteed significant clinical and radiographic improvement in infected cats. A complete clinical recovery and remission of radiographic signs occurs in almost all cats within 8 weeks after the first administration. In severely infected cats the clinical recovery was obtained in 12-16 weeks. Another field trial conducted on 40 cats naturally infected by *C. aerophila* showed that this formulation assures an egg shedding reduction of 99.5-100% after 2 administrations 2 weeks apart. A clear clinical efficacy was also assessed, with a significant post-treatment improvement of clinical and radiographic findings. Most treated cats showed a marked recovery 2 weeks after the second administration of eprinomectin. In a few cats clinical and radiographic signs may persist after the treatment, likely due to chronic lesions. These findings

confirm that eprinomectin is safe and effective against cat lungworms. A monthly clinical and radiographic follow-up is recommended after treatment until complete healing. Also, it is underlined the need for an early diagnosis and an efficacious therapy due to the risk of persistent lung damages.

The efficacy of Simparica Trio® (sarolaner, moxidectin, pyrantel) for the treatment of adult *Angiostrongylus vasorum* infections in dogs

Jessica Rodriguez (Zoetis, USA), Anne Lloyd (Zoetis, Belgium), Lore Van Mechelen (Zoetis, Belgium), Carleen Van Overloop (Zoetis, USA), Padraig Doherty (Forbairt Táirgí Iorras Teoranta, Ireland), Jakob L. Willesen (University of Copenhagen, Denmark), Fabrizio Solari Basano (Arcoblu srl, Italy), Thomas Geurden (Zoetis, Belgium)

Angiostrongylus vasorum is a parasite of dogs, foxes, and related species. It is primarily transmitted by the ingestion of snails and slugs. Adult worms live in the pulmonary arteries, and infections can result in pneumonia, heart failure, and coagulopathies. Two studies were performed to demonstrate the safety and efficacy of Simparica Trio® (ST), at minimum dose (1.2 mg/kg sarolaner, 24 µg/kg moxidectin, 5 mg/kg pyrantel) for the treatment of adult *A. vasorum* infection in dogs. These studies were conducted under the principles of Good Clinical Practice. Study 1 was performed in laboratory-induced infections in beagle dogs with 3 treatment groups (T01, T02, T03: 7 or 8 dogs each). T01 received placebo on Days 0 and 28, T02 received ST on Day 0 and placebo on Day 28, and T03 received ST on Days 0 and 28. In groups T02 and T03, there was a 98.3% and 99.6%, respectively, reduction in adult worms compared to group T01 (placebo) ($P < 0.0001$). Study 2 was a field study in Italy and Denmark on naturally infected dogs, diagnosed by Baermann examination. Informed consent was signed by dog owners before inclusion. There were two treatment groups: T01 (66 dogs) received ST at label dose, and T02 (37 dogs) received Advocate™ (10 mg/kg imidacloprid, 2.5 mg/kg moxidectin; Elanco) spot-on (positive control), at least once monthly. If larvae were detected on visit 2 (Day 30), dogs received a second treatment. For T01, reduction of geometric mean larval counts compared to pre-treatment was 96.7% after one treatment (66 dogs) and 99.1% after two treatments (11 dogs). Simparica Trio was non-inferior to Advocate for the reduction at both larval count timepoints. The results of these two studies demonstrate that Simparica Trio is safe and effective for the treatment of angiostrongylosis in dogs by killing adult *A. vasorum*.

Plenary Oral Communication

Tick-Borne Diseases and Control Strategies in ruminants: From Pathogen Detection to Field Interventions

***Babesia bovis* enolase is expressed in intracellular merozoites and contains predicted B-cell epitopes that induce neutralizing antibodies**

Juan Mosqueda (Universidad Autonoma de Queretaro, Mexico), Alma Cardenas-Flores (Universidad Autonoma de Queretaro, Mexico), Minerva Camacho-Nuez (Universidad Autonoma de la Ciudad de Mexico, Mexico), Massaro Ueti (Animal Diseases Research Unit, USDA-ARS), Diego Josimar Hernández-

-Silva (Universidad Autonoma de Queretaro, Mexico), Masahito Asada (Obihiro University of Agriculture and Veterinary Medicine, Japan), Shin-ichiro Kawazu (Obihiro University of Agriculture and Veterinary Medicine, Japan)

Bovine babesiosis is one of the most important tick-borne diseases in cattle worldwide; *Babesia bovis* is the species that causes the most serious clinical disease. The development of vaccines involving technologies like immunobioinformatics and reverse vaccinomics includes the discovery of novel antigens. Enolase is a "Moonlight" enzyme of glucose metabolism, that has been shown to have potential as a vaccine against various pathogens. However, this protein has not been studied in *B. bovis*. In this study, the enolase gene of two *B. bovis* isolates was sequenced. The gene consisting of 1,366 bps was identified and its transcription in intra-erythrocytic parasites was confirmed by RT-PCR. The predicted sequence of 438 amino acids was obtained, and two peptides containing predicted B-cell epitopes were identified. Synthetic peptides were were used to obtain hyperimmune sera in rabbits, generating antibody titers up to 1:256,000. The specific antibodies recognized intraerythrocytic merozoites by confocal microscopy and bound to a protein of approximately 47 Kda from *B. bovis* culture lysates by Western Blot. A neutralization assay was performed with the specific anti-sera in a *B. bovis in vitro* culture. The antibodies generated against peptide 1 had no effect, while the antibodies against peptide 2 reduced the parasitemia 71.99%. We conclude that *B. bovis* enolase contains conserved B-cell epitopes that induce neutralizing antibodies and can be considered as a vaccine candidate.

Molecular identification of hemopathogens and morphological analysis of ectoparasites in water buffaloes (*Bubalus bubalis*) in Minas Gerais, southeastern Brazil.

João Paulo Soares Alves (Federal University of Minas Gerais, Brazil), Pedro Henrique Cotrin Rodrigues (Federal University of Minas Gerais, Brazil), Nícolas Colácio (Federal University of Minas Gerais, Brazil), Markus Vinicius Vieira de Araújo (Federal University of Minas Gerais, Brazil), Christopher Gerald de Almeida Vargas Crawford (Federal University of Minas Gerais, Brazil), Stella Assunção de Almeida Costa (Federal University of Minas Gerais, Brazil), Bruna Kathleen Cunha Soares (Federal University of Minas Gerais, Brazil), Olívia Diniz Lacerda Silva (Federal University of Minas Gerais, Brazil), Daniel Sobreira Rodrigues (Agricultural Research Company of Minas Gerais, Brazil), Júlia Angélica Gonçalves da Silveira (Federal University of Minas Gerais, Brazil)

Vector-borne diseases pose a major threat to humans and animals, especially in tropical climates like Brazil. Buffaloes, valued for their resilience, face health risks in multi-species farming systems, requiring deeper clinical and epidemiological understanding. This study investigated hemopathogens and ectoparasites in water buffaloes in Minas Gerais, Brazil. Blood samples were collected from 0- to 12-month-old calves in extensive or semi-extensive systems on 17 dairy farms in the Bom Despacho microregion. Samples were taken via jugular vein puncture, followed by ectoparasite inspection, and transported to the PROTOVET Laboratory at UFMG's Veterinary School. After DNA extraction, PCR or nested PCR assays were performed to detect *Anaplasma marginale, Babesia*

bovis, B. bigemina, Theileria sp., hemotropic *Mycoplasma* spp. (*Candidatus* Mycoplasma haemobos and *M. wenyonii*), and monocytic *Ehrlichia* sp. Among bacteria, *A. marginale* was the most prevalent, detected in 65.34% of samples (164/251). Hemotropic *Mycoplasma* spp. were found in 33.06% (83/251), with *C.* M. haemobos in 74.70% (62/83) and *M. wenyonii* in 6.03% (5/83). Monocytic Anaplasmataceae were found in 1.19% (3/251). Among protozoa, *B. bovis* was detected in 8.36% (21/251) and *B. bigemina* in 3.58% (9/251), while *Theileria* sp. was not detected. Coinfections were common, with *A. marginale* and *C.* M. haemobos in 15.93% (40/251). More complex coinfections, including *B. bovis, A. marginale*, and *C.* M. haemobos, were found in 1.20% (3/251). At least one hemopathogen was detected by PCR on each farm. Ectoparasite analysis identified *Rhipicephalus microplus* ticks on 15 farms. On three farms, *Haematopinus tuberculatus* lice were found, both serving as key vectors. The study highlights a high prevalence of hemopathogens and their vectors in buffaloes, emphasizing their role as infection sources, facilitating pathogen transmission. The detection of monocytic *Ehrlichia* sp. is a novel finding, awaiting sequencing confirmation.

Enzootic status of tick-borne pathogens in cattle from two climatically distinct regions of Paraíba, Northeast Region of Brazil

Felipe Boniedj Ventura Álvares (Federal University of Campina Grande, Brazil), Jordania Oliveira Silva (Federal Institute of Paraíba, Brazil), Basílio Felizardo Lima Neto (Federal University of Campina Grande, Brazil), Geraldo Moreira Silva Filho (Federal University of Campina Grande, Brazil), Samira Pereira Batista (Federal University of Campina Grande, Brazil), João Victor Inácio Santos (Federal University of Campina Grande, Brazil), Arthur Willian de Lima Brasil (Federal University of Paraíba, Brazil), Marcelo Bahia Labruna (University of São Paulo, Brazil), Thais Ferreira Feitosa (Federal Institute of Paraíba, Brazil), Vinícius Longo Ribeiro Vilela (Federal Institute of Paraíba, Brazil)

Bovine tick-borne diseases (BTBD), primarily caused by *Babesia bovis*, *Babesia bigemina*, and *Anaplasma marginale*, and mainly transmitted by *Rhipicephalus microplus*, remain a major health and economic challenge for cattle farming in Brazil. The aim was to determine the prevalence by molecular diagnostic methods of tick-borne disease agents in cattle from the semiarid and humid tropical climates in the state of Paraíba. Blood samples were collected from cattle from 228 animals (up to 6 animals/ farm) across 21 farms in the Sertão region (semiarid) and 21 farms in the Brejo region (humid tropical). DNA was extracted from the blood samples and tested via multiplex-nested PCR to detect infections by *B. bovis*, *B. bigemina*, and *A. marginale*. Farms were classified as BTBD-positive (all PCR-positive animals), BTBD-negative (all PCR-negative animals), or BTBD-mixed (both positive and negative animals). The overall pathogen prevalence was 73.2% (167/228), with *A. marginale* at 70.2%, *B. bigemina* at 49.1%, and *B. bovis* at 33.8%. Co-infections occurred in 36.8% (84/228), mainly by *A. marginale*/*B. bigemina* (23.7%, 54/228) co-infections, followed by triple infections (10.1%, 23/228) and *A. marginale*/*B. bovis* (3.1%, 7/228) co-infections. A total of 5 (11.9%) farms were BTBD-positive, showing only *A. marginale* infections. BTBD-mixed classification was observed in 18 farms (42.9%), with 11 farms exhibiting *Anaplasma marginale* dominance. BTBD-negative was detected in only 2

farms (4.8%), both located in the Sertão region. There was an elevated prevalence of BTBD pathogens, predominantly *A. marginale*, with heterogeneous pathogen distribution across both regions. This pattern suggests enzootic instability in the Sertão (semiarid), due to inconsistent pathogen exposure, and positive enzootic stability in the Brejo (humid tropical), as all farms were BTBD-positive.

Efficacy of a novel pour-on fluralaner ectoparasiticide, NexLaner (Ourofino Saúde Animal Ltda.), against the cattle tick, *Rhipicephalus microplus*, *Haematobia irritans*, *Dermatobia hominis* larvae, and for controlling and preventing myiasis caused by *Cochliomyia hominivorax* larvae

Alvimar José da Costa (Instituto de Pesquisas em Saúde Animal Ltda., Brazil), Breno Cayeiro Cruz (Ourofino Saúde Animal Ltda., Brazil), Carolina Buzzulini (Instituto de Pesquisas em Saúde Animal Ltda., Brazil), Lucas Vinicius Costa Gomes (Instituto de Pesquisas em Saúde Animal Ltda., Brazil), Daniel Pacheco de Melo (Instituto de Pesquisas em Saúde Animal Ltda., Brazil), Milenni Garcia Michels (Ourofino Saúde Animal Ltda., Brazil), Patricia Chiba Tagava (Ourofino Saúde Animal Ltda., Brazil), Marcus Antônio Martins Buso (Ourofino Saúde Animal Ltda., Brazil), Ferdinando Nielsen de Almeida (Ourofino Saúde Animal Ltda., Brazil), Igor Renan Honorato Gatto (Ourofino Saúde Animal Ltda., Brazil)

Fluralaner, the only ectoparasiticide available for cattle not yet affected by resistance, is an isoxazoline highly efficient against insects and acari. NexLaner (NXL) is an alternative fluralaner product for cattle. Studies here presented, all GCP-compliant, adhering to international standards and applicable laws, evaluated its: efficacy against natural and experimental infestations by *Rhipicephalus microplus*; efficacies against natural infestations by *Haematobia irritans*, *Dermatobia hominis* larvae and *Cochliomyia hominivorax* larvae; and preventive efficacy against myiasis by *C. hominivorax*. All efficacy trials were conducted following EMA and WAAVP applicable guidances, using adequate numbers of animals and experimental groups, as well as all specified and recommended procedures. In the stall test against *R. microplus*, efficacy indexes >90% were observed right after 3 days post-treatment (DPT), and maximum efficacy (100%) was registered as soon as 8 DPT, up to 47 DPT. NXL had high efficacy over ticks' reproductive parameters. On the field trial against ticks, efficacy overcame 98% on 1 DPT, and 100% efficacy persisted up to 35 DPT. In tests against ticks, Exzolt® (MSD Animal Health) was added as a positive control, and formulations were always statistically equivalent. On remaining trials, efficacy against *H. irritans* remained over 95% for 7 DPT. Infestations of treated animals only reached 50% of initial burdens after 42 DPT. Efficacy against *D. hominis* larvae remained at 100% up to 35 DPT, exceeding 95% in 42 DPT. For control of myiasis by *C. hominivorax* larvae, 100% efficacy was reached on 01 DPT, and maintained up to 03 DPT. 100% efficacy was also observed for prevention of *C. hominivorax* infestation for up to 05 DPT. Conclusion: NXL, administered on its recommended dose, 1 mL/10 kg (2.5% fluralaner; 2.5 mg/kg), is highly efficient against *R. microplus*, *H. irritans*, *D. hominis* larvae, and for controlling and preventing myiasis by *C. hominivorax* larvae.

Challenges in maintaining a cattle tick-free zone in Southern Brazil: Insights from the

investigation of *Rhipicephalus microplus* outbreaks

Jose Reck (Inst. Pesq. Vet. Desiderio Finamor, Brazil), Greice Gonchoroski (UFRGS, Brazil), Marco Rocha Pereira (Municipal Veterinary Office, Brazil), Rovaina Doyle (Inst. Pesq. Vet. Desiderio Finamor, Brazil), Guilherme Klafke (Inst. Pesq. Vet. Desiderio Finamor, Brazil), Crsitina Trein (State Veterinary Office)

The cattle tick, *Rhipicephalus microplus*, is a highly invasive parasite infesting bovines across vast regions of the Americas, Asia, and Africa. In Brazil, it threatens cattle production, causing estimated losses of billions of dollars annually. The parasite is widespread in Brazil, except for a tick-free zone covering approximately 5,500 km^2 in two southernmost municipalities of Rio Grande do Sul State. This tick-free area, south of latitude 32°S, is ecologically unfavorable for R. microplus. It borders Uruguay to the south, is separated from the rest of Brazil by a wildlife refuge to the north, and is bounded by water bodies to the east and west. Since 1951, the state veterinary service has regulated cattle entry into this zone. Despite these measures, sporadic cattle tick outbreaks occur within the tick-free zone. This study investigated border and buffer zones, identifying contributing factors. Monthly farm visits over two years recorded seven outbreaks. Three outbreaks were linked to farmers illegally grazing cattle in the northern wildlife refuge, where roaming cattle from both tick-infested (north) and tick-free (south) areas were observed. Two outbreaks followed the legal transport of cattle treated with acaricides and verified by state officers, but resistance to fluazuron and organophosphate-pyrethroid mixtures likely allowed immature ticks to evade detection. Another outbreak was tied to tick-infested horses returning from a rodeo, and one to potential illegal cattle movement. The large population of invasive chital deer is under investigation for its role in tick dispersion. These findings highlight the challenges of maintaining tick-free areas amid acaricide resistance and non-compliant practices. Results will inform efforts to enhance surveillance and strengthen management of Brazil's tick-free zone.

Emerging patterns of acaricide resistance in *Rhipicephalus microplus* ticks across diverse agroclimatic zones of Bihar, India

Shyma, K.P. (Department of Veterinary Parasitology), Kumar, A. (Department of Veterinary Parasitology), Sharma, R.K. (Department of Veterinary Parasitology), Gupta, J.P. (Bihar Animal Sciences University, India)

A study was conducted to assess the acaricide resistance patterns of *Rhipicephalus microplus* ticks collected from four districts representing distinct agroclimatic zones of Bihar, India. The efficacy of commonly used acaricides, deltamethrin, cypermethrin, fenvalerate, ivermectin, and malathion was evaluated using standard larval bioassays. Ticks collected from Banka district (Agroclimatic Zone IIIb) exhibited Level I resistance to deltamethrin and ivermectin, indicating the early phase of resistance development. However, these tick populations remained susceptible to cypermethrin, fenvalerate, and malathion. In Gopalganj (Agroclimatic Zone I), tick isolates demonstrated Level II resistance to deltamethrin, while remaining susceptible to cypermethrin, fenvalerate, ivermectin, and malathion. Ticks from Patna (Agroclimatic Zone IIIa) showed Level II resistance to deltamethrin and Level I resistance to ivermectin, but retained susceptibility to cypermethrin, fenvalerate,

and malathion. Notably, tick isolates from Khagaria (Agroclimatic Zone III) exhibited Level I resistance to deltamethrin, cypermethrin, and ivermectin, suggesting early signs of resistance in this zone as well. These differential resistance patterns indicate that *R. microplus* populations are responding variably to acaricide pressure based on geographical and agroecological factors. The findings emphasize the necessity of region-specific resistance management strategies and rotational use of acaricides to delay resistance progression. Continuous monitoring and integration of agroclimatic data into tick control programs are essential to ensure sustainable management. Further molecular investigations into the mechanisms underlying resistance in these populations are recommended to develop targeted interventions for effective tick control in Bihar.

Proof-of-concept of the efficiency of MALDI-TOF mass spectrometry in the determination and discrimination of North American ixodid tick species

Ian Daniel (Texas A&M University, USA), Amber Holley (The University of Texas Medical Branch, USA), Samantha R. Hays (Texas A&M University, USA), Pete D. Teel (Texas A&M University, USA), Tanguy Tchifteyan (The University of Texas Medical Branch, USA), Guilherme G. Verocai (Texas A&M University, USA), Maureen Laroche (The University of Texas Medical Branch, USA)

Matrix-assisted laser desorption/ionization coupled with time-of-flight mass spectrometry (MALDI-TOF MS) is a fast-emerging and robust method for rapid and accurate characterization of arthropod vectors. It has been extensively used for tick identification based on leg proteins. Correct identification of ticks is key in monitoring medically relevant ticks and associated pathogens; however, this depends on proper diagnostics. In North America, MALDI-TOF MS has not been explored for comprehensive identification of ticks, except for a few studies focusing on a limited number of species. In this study, we aimed to generate proteomic spectral profiles of nine North American tick species obtained from laboratory-maintained colonies. A total of 407 high-quality MS spectra were generated in this study; 44 were deposited in our newly made database and used as reference spectra. All remaining MS spectra were used to query and validate the database. All specimens were identified correctly to the species level using MALDI-TOF MS, with reliable Log Score Values (LSVs) ranging from 1.72 to 2.86 and median and mean values of 2.41 and 2.40, respectively. Only in two cases were tick species misidentified. As a cost-effective, user-friendly, and high-throughput method, MALDI-TOF MS can serve as an alternative for accurately identifying tick species of veterinary and public health importance in North America, thereby supporting tick-borne disease surveillance efforts.

14:00 – 15:30

Room 2
Oral Communication

Gastrointestinal nematodes Infections in Companion Animals: Epidemiology, Resistance, and Therapeutic Advances

Occurrence of Endoparasites in Dogs and Cats in Various Regions of Brazil

Karin Denise Botteon (Boehringer Ingelheim), Ana Letícia Ferreira Bicalho (Hospital Veterinário São Francisco de Assis, Brazil), Marcy

Lancia Pereira (Universidade Federal de Santa Catarina, Brazil), Debora Azevedo (Clínica Veterinária Cães e Gatos angra dos Reis, Brazil), George Rêgo Albuquerque (Universidade Estadual de Santa Cruz, Brazil), Luciano Antunes Barros (Universidade Federal fluminense, Brazil), Leucio Camara Alves (Universidade Federal de Pernambuco, Brazil), Livio Martins Costa Junior (Universidade Federal do Maranhão, Brazil), Estevam Guilherme Lux Hoppe (Universidade Estadual Paulista, Brazil), Patricia Mendes Pereira (Univesidade Estadual de Londrina, Brazil), Daniel Guimarães Gerardi (Universidade Federal do Rio Grande do Sul, Brazil), Marielle Servonnet (Boehringer Ingelheim Animal Health), Frederic Beugnet (Boehringer Ingelheim Animal Health)

Brazil is the largest and most populous country in South America. It is also the world's third largest country in terms of owned dog and cat population, according to the Brazilian Institute of Geography and Statistics. Despite the benefits of human-animal interaction, the transmission of zoonoses still represents an often-neglected risk, as pets can serve as reservoirs for various pathogens. Considering that helminths, especially those transmitted through soil contamination, represent an epidemiological risk to public health, the objective of this study was to survey the occurrence of these parasites in the population of owned dogs and cats in various locations in Brazil. Between September2023 and November2024, a total of 1,837 healthy owned dogs and cats (995 dogs and 842 cats) living in urban and sub-urban environment were recruited during a veterinary consultation from 10 veterinary centers in Brazil. They did not receive any anthelmintic treatment for at least 2 months before being included in the study. Of the 1,837 animals, the faeces of 843 dogs and 761 cats were assessed using the Mini-FLOTAC floatation technique, a qualitative and quantitative method for the identification of eggs, larvae, oocysts, and cysts of endoparasites. Endoparasites were identified in 17.2% of dogs and 14.8% of cats. Among nematodes, the *Ancylostomatidae* family (14% of dogs and 5.9% of cats) and the *Toxocaridae* family (4% of dogs and 1.8% of cats) were the most frequently detected gastrointestinal parasites. *Trichuris vulpis*was found in 2% of the dog samples. Other intestinal parasites detected included nematodes: *Strongyloides* spp., trematodes (*Platynosomum* spp.), cestodes (*Dipylidium caninum, Spirometra* spp., and*Taenia* spp.). Protozoans were also observed, i.e. *Cystoisospora* spp. *Neospora* spp., and *Giardia*spp.The findings underscore the importance of monitoring and controlling parasitic infections to protect public health.

Morphological and molecular data on canine hookworm species from Italy

Renata Fagundes Moreira (University of Bari "Aldo Moro", Italy; University of Pavia, Italy), Marcos Antonio Bezerra-Santos (University of Bari "Aldo Moro", Italy), Riccardo Paolo Lia (University of Bari "Aldo Moro", Italy), Jairo Alfonso Mendoza-Roldan (University of Bari "Aldo Moro", Italy), Jan Slapeta (University of Sydney, Australia), Domenico Otranto (University of Bari "Aldo Moro", Italy; City University of Hong Kong, Hong Kong, China)

The value of morphological studies in helminthology, enhanced by molecular characterization, should be recognized as crucial for the advancement of the discipline. We conducted studies to establish procedures for the morphological identification of *Ancylostoma caninum*

and *Uncinaria stenocephala* in naturally infected dogs in Italy. Studies generically refer to "hookworms" as a group of parasites, though *U. stenocephala* is the predominant species infecting dogs in Europe and less susceptible to anthelminthics than *A. caninum*. Fecal samples were collected in Basilicata (*n*=15) and Puglia (*n*=95) regions of Italy and processed using the Mini-FLOTAC®, followed by a modified filter paper method. Larvae were retrieved on days 5 and 8 using the Baermann technique. Eggs and larvae were identified based on morphometry and the number of intestinal cells in third-stage larvae (L3). Species were confirmed via cPCR targeting the ITS-1 region and sequencing. L3 of *U. stenocephala* ranged from 557.2 µm to 622.0 µm in body length (mean: 583.6 µm ± SD 22.3 µm) and from 23.4 µm to 30.4 µm in body width (26.2 µm ± 2.4 µm), with 32 well-defined intestinal cells. Dense lipid granules in *A. caninum* larvae hindered their visualization. Egg morphometry supported the occurrence of both species, with *U. stenocephala* eggs measuring 82.0 µm to 85.0 µm length (83.5 µm ± 2.9 µm) and 48.5 µm to 49.0 µm width (48.7 µm ± 1.0 µm), while *A. caninum* measured 51.5 µm to 55.0 µm (53.2 µm ± 2.6 µm) × 41.7 µm to 60.0 µm (50.9 µm ±12.2 µm). Molecular data confirmed the morphological identification, with nucleotide ITS-1 difference of 8% between species. Overall, *U. stenocephala* was detected in 35.5% (*n*=39/110) and *A. caninum* in 3.6% (*n*=4/110). Both species were detected in Puglia (i.e., *U. stenocephala* in 35.8% [*n*=34/95]; *A. caninum* in 4.2% [*n*=4/95]), whereas only *U. stenocephala* was detected in Basilicata [*n*=5/15]. The modified coproculture method showed to be effective for morphological differentiation of hookworm species in dogs, supporting accurate field diagnosis, epidemiological studies, and treatment strategies.

Global Molecular Epidemiology Study of Benzimidazole Resistance in the Canine Hookworm *Ancylostoma caninum*

Mahya Dini (University of Calgary, Canada), John S. Gilleard (University of Calgary, Canada), Libby Redman (University of Calgary, Canada), Rebecca Chen (University of Calgary, Canada), Pablo D. Jimenez Castro (Antech Diagnostics), Christian M. Leutenegger (Antech Diagnostics), Christian Savard (Biovet Inc.)

Anthelmintic resistance in *Ancylostoma caninum*, a zoonotic canine hookworm, is an emerging concern. While multi-drug resistant populations have been widely documented in racing greyhounds and pet dogs in the USA, the extent of benzimidazole resistance mutations worldwide remains unclear. Understanding the distribution and evolution of resistance alleles is critical to informing diagnostic and control strategies in both veterinary medicine and public health contexts. We are investigating the molecular epidemiology of benzimidazole resistance in *A. caninum* from multiple geographic regions worldwide. The project will identify and characterize canonical resistance-associated single nucleotide polymorphisms (SNPs) in the β-tubulin isotype-1 gene, particularly at codons 134, 167, 198, and 200, and scan for additional novel resistance mutations. Fecal DNA samples from over 2,000 hookworm-positive dogs across North and South America, Asia, Oceania, and the Caribbean will be analyzed. Illumina amplicon sequencing will quantify the frequency of known SNPs, and Oxford Nanopore long-read sequencing will enable near full-length β-tubulin gene scanning to detect novel mutations. Phylogenetic and haplotype network analyses will elucidate the genetic relationships

and potential transmission pathways of resistance alleles across regions. This research will generate a large-scale global map of benzimidazole resistance mutations in *A. caninum*, uncovering both the prevalence and genetic diversity of resistance alleles. The findings will inform the development of molecular diagnostics and guide resistance management strategies to protect both animal and public health.

Widespread occurrence of key SNPs in benzimidazole-resistant *Ancylostoma caninum* in Australia

Swaid Abdullah (The University of Queensland, Australia), Thomas Stocker University of Sydney, Australia), Hyungsuk Kang (The University of Queensland, Australia), Ian Scott (Massey University, New Zealand), Douglas Hayward (Vetnostics NSW - North Ryde Laboratory, Australia), Susan Jaensch (Vetnostics NSW - North Ryde Laboratory, Australia), Michael P. Ward (University of Sydney, Australia), Malcolm K. Jones (The University of Queensland, Australia), Andrew C. Kotze (The University of Queensland, Australia), Jan Šlapeta (University of Sydney, Australia)

Canine hookworm (*Ancylostoma caninum*) is a gastrointestinal nematode that primarily infects the small intestine of domestic dogs and poses a zoonotic risk. In the USA, *A. caninum* infecting greyhounds and pet dogs has shown resistance to multiple anthelmintics. This study aims to investigate benzimidazole resistance in *A. caninum* from dogs in Australia and New Zealand. To conduct a molecular survey of benzimidazole resistance in *A. caninum* samples collected from dogs in Australia and New Zealand, and to assess the prevalence of specific β-tubulin mutations associated with benzimidazole resistance. We employed an ITS-2 rDNA deep amplicon metabarcoding sequencing approach to identify hookworm species in diagnostic samples. The frequency of F167Y and Q134H isoform-1 β-tubulin mutations, which confer benzimidazole resistance, was evaluated using the same sequencing method. Egg hatch assays were performed on a subset of *A. caninum* samples to correlate IC_{50} to thiabendazole with the presence of the F167Y mutation. *Ancylostoma caninum* was the predominant hookworm species detected in 90% (83/92) of diagnostic samples, with *Uncinaria stenocephala* identified in 11% (10/92). Coinfection with both species was found in one sample. The F167Y and Q134H β-tubulin mutations were present in 67% and 49% of *A. caninum* samples, respectively. The F167Y mutation was also recorded in *U. stenocephala* for the first time. No mutations were found at codons 198 and 200. A significant correlation was observed between IC_{50} to thiabendazole and the F167Y mutation, with higher IC_{50} values in samples with >75% F167Y mutation. 14% of dogs had *A. caninum* with >75% F167Y mutation. The study indicates widespread benzimidazole resistance in *A. caninum* across various regions of Australia. To mitigate resistance selection and spread, a risk assessment-based approach to limit unnecessary anthelmintic use is recommended for future parasite control strategies

Transcriptional responses to in vitro macrocyclic lactone exposure in *Toxocara canis* third-stage larvae

Theresa A. Quintana (Auburn University, USA), Matthew T. Brewer (Iowa State University, USA), Jeba R.J. Jesudoss Chelladurai (Auburn University, USA)

Toxocara canis is a parasitic ascarid of canids with a complex lifecycle. While macrocyclic lactones (MLs) are effective against adult worms, they fail to eliminate somatic third-stage larvae (L3s), contributing to persistent infections and zoonotic risk. In this study, we characterized transcriptomic changes in hatched *T. canis* L3s following *in vitro* exposure to 10 µM ivermectin, moxidectin, or control media (RPMI 1640) for 12 hours in triplicate. We extracted total RNA with Trizol, depleted rRNA, and sequenced using Illumina Stranded mRNA libraries on a NextSeq. Reads were mapped using the RNA STAR pipeline, and differential expression was analyzed via DESeq2. Ivermectin-treated larvae exhibited 608 differentially expressed genes (DEGs), including 453 upregulated and 155 downregulated genes. Moxidectin-treated larvae had 1,413 DEGs, with 902 upregulated and 511 downregulated. Genes affected were involved in transcriptional regulation, energy metabolism, neuronal structure and function, physiological processes, excretory/secretory molecule production, host-parasite response mechanisms, and parasite elimination. In particular, we analyzed the expression of known ML targets and transporters, including glutamate-gated chloride channels (GluCls) and ATP-binding cassette (ABC) transporters, subfamily B. Our study revealed that the expression of *Tca-glc-3* and six ABCB genes, particularly four P-glycoproteins, were significantly altered in response to ML treatment. Compared to controls, *Tca-glc-3*, *Tca-Pgp-11.2*, and *Tca-Pgp-13.2* were downregulated in ivermectin-treated larvae, while *Tca-abcb1*, *Tca-abcb7*, *Tca-Pgp-11.2*, and *Tca-Pgp-13.2* were downregulated in moxidectin-treated larvae. Conversely, *Tca-abcb9.1* and *Tca-Pgp-11.3* were upregulated in moxidectin-treated larvae. These findings suggest that MLs broadly impact transcriptional regulation in *T. canis* larvae.

Cry-ing for a pipeline of deworming drugs: the IBaCC platform for anthelmintics

Kelly Flanagan (University of Massachusetts Chan Medical School, USA), Hanchen Li (University of Massachusetts Chan Medical School, USA), Nicholas Cazeault (University of Massachusetts Chan Medical School, USA), Florentina Rus (University of Massachusetts Chan Medical School, USA), Qian Ding (University of Massachusetts Chan Medical School, USA), Ernesto Soto (University of Massachusetts Chan Medical School, USA), Stefani Diaz Valerio (Georg-August University Gottingen, Germany), Yolanda Bel (University of Valencia, Spain), Jacqueline Hellinga (Freie University Berlin, Germany), Georg von Samson-Himmelstjerna (Freie University Berlin, Germany), Baltasar Escriche (University of Valencia, Spain), Heiko Liesegang (Georg-August University Gottingen, Germany), Gary Ostroff (University of Massachusetts Chan Medical School, USA), Raffi V Aroian (University of Massachusetts Chan Medical School, USA)

Gastrointestinal nematodes (GINs) pose significant health threats to livestock, companion animals, and (as soil-transmitted helminths) approximately 1.5 billion humans worldwide, contributing to substantial morbidity. The widespread use of anthelmintics has led to the emergence of drug-resistant GINs. Cry5B, an anthelmintic crystal (Cry) protein derived from *Bacillus thuringiensis* (Bt), has been developed into IBaCC for *I*nactivated *Ba*cteria with *C*ytosolic *C*rystals. IBaCC is a promising and safe paraprobiotic therapy for treating GIN infec-

tions across various host species. To date we have focused on Cry5Ba but have looked to expand the number of anthelmintic Cry proteins given the evolution of Cry proteins from Bt in nature. Here, we will talk about our approaches to turn IBaCC into a platform for production of a pipeline of anthelmintics. Recently, the use of Cry14Ab for control of plant-parasitic nematodes has been published, and we have set out to study whether this protein as IBaCC has anthelmintic functions as well. Further, based on bioinformatic and novel screening approaches, we have identified many new putative anthelmintic Cry proteins and turned them into IBaCC. Here, we will update our studies to discover new anthelmint Cry's using IBaCC as a platform. Our ultimate objective is to optimize a Cry-stacked *Bacillus* strain to deliver synergistic and resistance-busting Cry proteins at maximal potency against a wide spectrum of GIN targets. These findings represent significant strides toward the development of a Cry-based anthelmintic therapy as a practical solution in veterinary and human medicine.

Successful treatment of experimental canine hookworm infections with a single administration of a fluralaner, moxidectin and pyrantel chewable tablet in a non-terminal study design

Heinau, L. (MSD Animal Health Innovation GmbH, Germany), Petersen, I. (MSD Animal Health Innovation GmbH, Germany), Rohdich, N. (MSD Animal Health Innovation GmbH, Germany) , Chiummo, R. (MSD Saúde Animal, Brazil), van der Mescht, L. (ClinVet International (pty) Ltd, Republic of South Africa; University of the Free State, South Africa), Liebenberg, J. (ClinVet International (pty) Ltd, Republic of South Africa), Barrett, A. (Merck Animal Health, USA)

In the development program for a chewable tablet containing fluralaner (FLU, 10 mg/kg), moxidectin (MOX, 0.025 mg/kg) and pyrantel (PYR, 5 mg/kg), a non-terminal study design was successfully implemented to determine anthelmintic efficacy. Three non-terminal studies were conducted to investigate efficacy of a FLU, MOX and PYR chewable tablet against hookworms. In these single-site, GCP-compliant studies, dogs were inoculated with third stage larvae of *Uncinaria stenocephala* (Studies 1 and 2) or *Ancylostoma caninum* (Study 3). Using fecal egg counts (FECs), dogs were blocked and randomized to two groups per study (10 dogs per group in Studies 1 and 3; 8 per group in Study 2) and either treated once orally on day 0 with FLU, MOX and PYR chewable tablet, or left untreated. Twelve days after treatment, both groups in all studies were treated orally with Profender® tablets for dogs as diagnostic dewormer (DDW). Primary efficacy was determined by counts of expelled worms recovered after DDW treatment. Copro Antigen-ELISA (PetCheck™ IP, IDEXX), PCR and FECs were completed at key time points. Adequacy of infection was demonstrated in control groups. Relative to the control groups, statistically significant reductions of mean worm counts were found in the FLU, MOX and PYR chewable tablet groups in Studies 1, 2 and 3 (99.8%, $P < 0.0001$, 100%, $P = 0.0002$ and 100%, $P < 0.0001$, respectively). All control dogs had positive FECs until the DDW treatment, while no eggs were detected after day 0 in 26 of the 28 FLU, MOX and PYR chewable tablet treated dogs. Results of molecular analyses were confirmatory of the conclusions from worm counts and FECs. A single dose of FLU, MOX and PYR chewable tablet is

effective in the treatment of canine infections with adult hookworms (*U. stenocephala*, *A. caninum*). Non-terminal studies present a valid means of determining the anthelminthic efficacy against hookworms in dogs.

Plenary Oral Communication

Education, Technology and Extension: Enhancing Parasite Control through Knowledge Translation

Session Chair: Fernando de Almeida Borges (Federal University of Mato Grosso do Sul, Brazil)

NCVP grant program, impacting parasitology research since 2013

Scimeca RC (Oklahoma State University, USA), Little S (Oklahoma State University, USA), Starkey L (Oklahoma State University, USA), Duncan K (Oklahoma State University, USA), Sundstrom K (Oklahoma State University, USA), Reichard MV (Oklahoma State University, USA)

The National Center for Veterinary Parasitology (NCVP) provides training, research, and service in veterinary parasitology. As part of the parasitology research support, the NCVP established in 2013 an annual grant program opened to all members of the American Association of Veterinary Parasitologists (AAVP). The purpose of the NCVP grant program is to advance veterinary parasitology by supporting innovative research, promote the participation of undergraduate, graduate, and veterinary students in veterinary parasitology research, and advocate interest and involvement of AAVP membership. During the twelve funding cycles, a total of $1,096,766.3 have been granted to twenty-eight different institutions in the United States, Canada and the Caribbean, supporting 73 research projects. The purpose of this presentation is to provide an overview of the NCVP grant funding initiative, its goals and current allocation among AAVP members to continue expanding basic and applied parasitology research advances.

University extension in favor of animal, human, and environmental health

Larissa Reifur (Federal University of Paraná, Brazil), Yasmin Souza Garcia Redondo (Federal University of Paraná, Brazil), Isabela Akemi Nenoki (Federal University of Paraná, Brazil), Camille De Souza Moraes (Federal University of Paraná, Brazil), Edinara Cristina Mariano Da Silva (Federal University of Paraná, Brazil)

The One Health approach, which integrates the pillars of human, animal, and environmental health, proves essential in educational contexts due to its potential in disease prevention and the promotion of collective well-being. Aiming to address educational gaps related to this theme, our university extension project, composed of a multidisciplinary team, carries out activities in communities from Curitiba city and its Metropolitan Region, southern Brazil. The activities are planned based on local needs and employ active methodologies such as games, book reading, discussion circles, theatrical plays, and other interactive strategies. Topics addressed include responsible pet ownership, parasites in companion animals, zoonoses, dengue, oral and personal care, pediculosis, sexual education, and hygiene of food and utensils. Visits occur periodically with the aim of identifying needs, promoting educational interventions, and strengthening health promotion. Following the Brazilian guidelines of university extension

— dialogical interaction, interdisciplinarity, the inseparability of teaching, research and extension, and social and educational transformation — our team develops customized educational and scientific materials to work with communities and schools in disadvantage areas. In addition, environmental and animal fecal samples are analysed for parasitological diagnosis and prevention. Visits to non-governmental organizations, technical and research centers are also carried out to encourage knowledge exchange and expand academic experiences. Since 2019, around 120 university students from 11 courses worked in the project and more than 4 thousand people, ranging from 4 to 80 years old, have been directly impacted. The exchange of experiences between students and the community not only fosters social transformation but also contributes to the holistic education of students, who come to understand diverse realities and improve their communication and educational skills.

Scientific knowledge applied at Brazilian buffalo milk herds for parasite control

Eduardo Bastianetto (Universidade Federal de Minas Gerais, Brazil), Cristiano Barros de Melo (Universidade de Brasília, Brazil)

Water buffalo (*Bubalus bubalis*) is relevant to food security in tropical countries. Buffalo breeding in Brazil is not one ancient activity as at Asiatic countries but currently specialized buffalo herds are present to produce and sell pure buffalo milk to cheese industries. Regarding parasitic diseases, is important highline that parasitic infections are responsible for serious health injuries on buffalos, particularly specific parasites like *Paracooperia nodulosa, Eimeria bareillyi* and *Haematopinus tuberculatus*, demanding greater attention on its control. Calf mortality, animal's performance reduction caused by endoparasites and stress derivate of *H. tuberculatus* skin injuries motivate Brazilian research to give attention to all epidemiological aspects related to parasitic infection on buffalo herds at different brazilian regions.
Supported by diagnose and looking to the challenges at all type of buffalo farm, strategic parasitic control practices were suggested. To evaluate the impact of Universities extension program and veterinary services to disseminate correct practices to reduce parasitic impact at buffalo health, and the adoption of buffalo parasitic control strategies by farmers, authors performed data analyze the form with data related to management practices on milk buffalo farms located at Minas Gerais State (MG) in Brazil (n=120) and also face-to-face visit (n=48). As result is possible indicate *H. tuberculatus* were eradicated at 93,75% of herds, anthelmintic drugs on calves under 15 days and specific medication to control *Eimeria sp*. infection are in use respectively at 70,83% and 47,91% of herds. These preventive practices reduced hardly buffaloes mortality, in special young animals. Is now necessary improve a better control of subclinical helminthic infection using abundant scientific information available, promote *refugia* parasite population and consider drugs toxicity for buffalos.

Are we too late to get producers to use anthelmintics rationally?

Juan Felipe de Jesús Torres-Acosta (Universidad Autónoma de Yucatán, México), María Gabriela Mancilla-Montelongo (Universidad Autónoma de Yucatán, México), Andrew Greer (Lincoln University, New Zealand), Fiona Kenyon (Moredun Research Institute,

Scotland), Cristina Sotomaior (Pontifícia Universidade Católica do Paraná, Brazil)

For over 40 years, conventional anthelmintics (AHs) have been employed to control gastrointestinal nematodes (GIN) in sheep and goat herds. These AHs have frequently been used by individuals who have received minimal training or guidance on the use of the various products, contributing to the development of anthelmintic resistance. Is there still an opportunity to train these individuals on the best practice use of AHs? To identify and analyze elements needing improvement among various groups involved in sheep and goat production regarding the rational use of AHs, within the context of sheep and goat herds. Opportunities for improving the use of dewormers are proposed based on four key blocks: (a) the workers who administer dewormers, (b) vendors and veterinarians, (c) the pharmaceutical industry and industry bodies, and (d) education providers that train future veterinarians or consultants. Solutions are suggested for each element at every block. The approach must encompass all four blocks involved. Language and concepts should be adapted and aligned to effectively intervene in each region of the planet. It is essential to consider the different ecological zones of each region and country, taking into account their climates, parasites, the zootechnical goals of production systems, grazing conditions, and animal type. Additionally, we must consider the human factor, acknowledging the unique attitudes and behaviors of workers, veterinarians or advisors to foster teamwork toward the shared objective. This comprehensive strategy should be implemented in courses offered by teams of experts from various educational providers, who will collaborate in this effort. We propose key elements for training producers, workers, veterinarians and advisors on the best practice use of AHs to ensure their future viability.

Assessing the future uptake of precision livestock farming tools by sheep and goat farmers in the meat and dairy industries

F. Kenyon (Moredun Research Institute, UK), A. McLaren (SRUC, Hill & Mountain Research Centre, UK), L. Grøva (NIBIO, Norway), A. de Boer (NIBIO, Norway), T.W.J. Keady (Teagasc, Ireland), B. McClearn (Teagasc, Ireland), V. Giovanetti (AGRIS Sardegna, Italy), M. Acciaro (AGRIS Sardegna, Italy), L. Depuille (IDELE, Campus INRAe, France), R. Klein (UNIDEB, Hungary), A. Godo (ARO, Israel), P. Piirsalu (EULS, Estonia), I. Halachmi (ARO, Israel), I. Llach (EULS, Estonia), C. Morgan-Davies (SRUC, Hill & Mountain Research Centre, UK)

Precision livestock farming (PLF) tools could be used to optimise anthelmintic use for roundworm control. Examples include the use of automated egg counting tools, or using electronic identification (EID) tags and weigh crates to record individual animal weight and use this as indicator for treatment. However, the uptake of PLF and digital tools has been poor among small ruminant farmers. The Sm@RT project aimed to improve the uptake of PLF tools by small ruminant farmers. To understand factors which influence the rate and peak level of uptake, stakeholder groups in the 8 Sm@RT countries (Estonia, France, Hungary, Ireland, Israel, Italy, Norway, UK) used the Adoption and Diffusions Outcome Prediction Tool (ADOPT) (https://adopt.csiro.au/home.aspx). Questions were in 4 categories; characteristics of the tool/technology; characteristics of the farming population; advantage of using the tool/technology; and learnability. Sessions were completed on 30

different tools/technologies presented by the project as possible solutions to needs previously identified. The predicted rate and peak level of uptake varied between tools and countries. For example, predicted rate and peak level of uptake for an EID stick reader (across 4 different countries) ranged from 9-24 years and 72-97% of the population. In the UK, peak adoption of the automated egg counting system, FECPAK G2, was 58% in 8 years. Overall results indicate that answers relating to what proportion of the population will need new skills/knowledge, and the proportion of farms that could benefit from the tool/technology, are influential in terms of rate and peak level of uptake respectively. The results will allow better targeted knowledge exchange to address small ruminant farmers concerns for uptake of new tools.

Parassess, a digital OWNER-FACING tool based on algorithm to assess parasite risk for dogs and cats.

Antoine L. (Boehringer Ingelheim, France), Guillot J. (Nantes Veterinary College (Oniris VetAgroBio), France), Papadopoulos E. (Aristotle University of Thessaloniki, Greece), Otranto D. (University of Hong Kong, SAR, China), Wright I. (The Mount Veterinary Practice, UK), Morgan E. (Queen's University, Northern Ireland), Beugnet F. (Boehringer Ingelheim, France)

Parassess is an interactive digital risk checker based on the most up-to-date understanding of parasite risk factors and control recommendations from scientific associations such as ESCCAP, TROCCAP, CAPC and WAAVP. The major parasites of dogs and cats are included in this assessment, fleas, ticks, mites, roundworms, hookworms, whipworm, tapeworms, lungworms, heartworm and *Leishmania infantum*. The risk factors are related to each parasite biology and epidemiology, and to each dog or cat, such as their sex and age, and other external factors, like location, behavior, feeding, and antiparasitic treatments. One algorithm has been built for dogs, another for cats. It is based on 13 to 20 short and comprehensive questions to be answered by the pet owners. Some questions are qualitative, others are quantitative including single or multiple answers. A few questions refer to the possible presence of clinical signs like pruritus, fatigue, coughing, and diarrhea. The algorithm calculates scores and provides a final score from 0 to 100 for each parasite. The final scores correspond to a risk assessment for the most common parasites. The digital questionnaire is easy to fill out in a few minutes. Parassess is based on the biology and epidemiology of parasitic diseases and not product-related. It can be adapted to specific geographies by adding or removing certain parasites. It offers veterinarians and pet owners the opportunity to better discuss and understand the risks, and to tailor the parasite prevention strategies.

Comprehensive epidemiology and management of trypanosomosis with special emphasis on multi-faceted research from Punjab, India

Lachhman Das Singla (Department of Veterinary Parasitology Guru Angad Dev Veterinary and Animal Sciences University, India)

Trypanosomosis is a significant hemoprotozoan parasitic disease affecting livestock across various regions of India, with extensive research contributions emerging from Punjab State. This presentation will summarize key findings from a series of studies on bovine and equine trypa-

nosomosis conducted in our region. *Trypanosoma evansi* remains the predominant causative agent in India, impacting cattle, buffaloes, horses, and camel. Our surveillance studies have revealed notable epidemiological trends in Punjab, where the disease is primarily transmitted by tabanid flies. Clinically infected animals exhibit intermittent fever, progressive anaemia, emaciation, and a marked decline in productivity. Sero-epidemiological investigations using CATT, along with molecular diagnostic tools, have demonstrated prevalence rates ranging from 5.8% to 17.2% across different agro-climatic zones, with considerable seasonal and management-based variation. Acute outbreaks have been documented, particularly in crossbred cattle, and risk factors associated with *T. evansi* infection have been identified.Our research has led to the development and validation of advanced diagnostic methods, including real-time PCR, multiplex PCR, and duplex PCR, facilitating the concurrent detection of *T. evansi* alongside other hemoparasites such as *Babesia bigemina*, *Anaplasma marginale*, and *Theileria equi*. Additional investigations have explored the immunomodulatory role of levamisole in infected cow-calves, the pharmacokinetics of diminazene combinations in buffalo calves, immune responses to hemorrhagic septicemia vaccination in *T. evansi*-infected animals, and the activity of human serum against *T. evansi* in experimental models. Challenges to effective control include the limited availability of field-level diagnostics, emerging drug resistance, and insufficient vector management strategies. This presentation will comprehensively discuss our findings related to disease detection, management of latent infections, therapeutic interventions, and key research priorities required to reduce the burden of trypanosomosis in Punjab and other affected regions of India. A One Health approach integrating veterinary services, research institutions, and policy frameworks is essential to address this growing threat to livestock health and productivity.

August 21 - 2025
9:00 – 10:00

August 21 - 2025
9:00 – 10:00

Plenary
Plenary Session

Parasites and their endosymbionts: evolution, coevolution and applications

Claudio Bandi (Università degli Studi di Milano, Italy), Davide Sassera (Università degli Studi di Pavia, Italy), Francesco Comandatore (Dipartimento di Scienze Biomediche e Cliniche), Greta Bellinzona (Università degli Studi di Pavia, Italy), Sara Epis (Università degli Studi di Milano, Italy), Michele Castelli (Università degli Studi di Pavia, Italy)

The eukaryotic cell likely originated around two billion years ago, following the symbiotic joining of prokaryotic organisms— life forms that had already inhabited Earth for over a billion years. Since then, prokaryotes and eukaryotes have coexisted, with prokaryotic life displaying a level of abundance and ubiquity that we have only begun to appreciate in recent decades. In this light, we can say that eukaryotic life emerged from prokaryotes and continued to evolve "surrounded" by them. Unsurprisingly, this has led to the evolution of numerous symbiotic relationships—not only between prokaryotes and eukaryotes, but also among different eukaryotic species. A key factor in determining the evolutionary trajectory of a symbiosis is the mode of transmission of the symbiont. When symbionts are strictly vertically transmitted—generally from mother to offspring—the alignment of host and symbiont fitness favors the evolution of mutualistic relationships. This is seen in tsetse flies and lice, where their endosymbionts *Wigglesworthia* and *Riesia* have become essential for host development and reproduction. However, some vertically transmitted prokaryotic symbionts adopted a different strategy: reducing the fitness of host individuals who do not participate in their transmission, such as males or uninfected females. The most striking example is *Wolbachia*, a bacterium capable of sterilizing uninfected females (through cytoplasmic incompatibility, or CI) or biasing sex ratios toward females via mechanisms like feminization, male killing, or parthenogenesis. CI enables *Wolbachia* to spread through insect populations, where it can also confer resistance to viral infections—an application currently exploited in the control of mosquito-borne viral diseases. Conversely, in filarial nematodes, *Wolbachia* is required for filarial reproduction and long-term survival, which has led to the development of anti-filarial therapies targeting this bacterium with antibiotics.

10:30 – 12:00

Room 1

Tungiasis Symposium: A Neglected Disease Demanding Integrated Research and Sustainable Control

Session Chairs: Georg von Samson-Himmelstjerna (Freie Universität Berlin, Germany), and Jürgen Krücken (Freie Universität Berlin, Germany)

The ramifications of zoonotic tungiasis in endemic communities: a call to action

Francis Mutebi (Makerere University, Kampala, Uganda), Berrick Otieno (The Aga Khan University, Nairobi, Kenya), Lynne Elson (KEMRI-Wellcome Trust Research Program-

me, Kenya; University of Oxford, United Kingdom), Ulrike Fillinger (International Centre of Insect Physiology and Ecology, Kenya), Hermann Feldmeier (Charité University Medicine, Germany), Amina Abubakar (The Aga Khan University, Nairobi, Kenya), Georg von Samson-Himmelstjerna (Freie Universität Berlin, Germany), Jürgen Krücken (Freie Universität Berlin, Germany)

Tungiasis is a zoonosis which is caused by adult female sand fleas (*Tunga penetrans*) when they penetrate and develop within the skin of people and animals. A wide range of mammalian hosts including pigs and dogs and to a small extent ruminants and chicken get infected. The embedded sand fleas frequently induce inflammation, which is amplified by high infection intensities and bacterial superinfections. Tungiasis is an extremely neglected tropical disease which is endemic in Latin America and sub-Saharan Africa. This presentation aims to highlight the significance of tungiasis among animals and humans and the peril of endemic communities. This is a narrative review, which is based on the available literature on how tungiasis affects animals and humans, and its implications on affected persons, animals and communities. Tungiasis is a self-limiting disease that is frequently complicated by secondary bacterial infections, which together with persistent and high infection intensities contribute to severe morbidities. Severe morbidities may contribute to deformations of affected body parts especially the feet and fingers, contributing to variable disabilities and hence low economic productivity. Affected children are unable to walk to school or concentrate in class which results into poor academic performance. Superinfections may present with septicaemia and wound infections with *Clostridium tetani* might be fatal. Moreover, tungiasis is associated with stigma and was recently shown to contribute to poor neurocognitive and scholastic outcomes among children. Animal infections may affect their production, merchantability and pose significant animal welfare concerns. Tungiasis is an impoverishing zoonosis contributing to chronic poverty in endemic communities. One health interventions are indicated towards tungiasis elimination and improved quality of life in endemic communities.

The microbiome of human and pig *Tunga penetrans* biopsies is dominated by vertically transmitted bacteria while *Staphylococcus* spp. were the most abundant opportunistic bacteria causing superinfection

Francis Mutebi (Makerere University, Uganda), Sandro Andreotti, Maike Boje (Freie Universität Berlin, Berlin, Germany), Georg von Samson-Himmelstjerna (Freie Universität Berlin, Berlin, Germany), Lynne Elson (KEMRI-Wellcome Trust Research Programme, Kenya; University of Oxford, United Kingdom) Ulrike Fillinger (Human Health Theme, Kenya), Charles Waiswa (Makerere University, Uganda), Jürgen Krücken (Freie Universität Berlin, Berlin, Germany)

Tungiasis is a neglected skin disease occurring in Latin America and sub-Saharan Africa which is caused by adult female sand fleas when they penetrate the skin of humans and animals. Pigs are considered the major animal hosts for *Tunga penetrans* in Uganda. Tungiasis is associated with bacterial superinfections which exacerbate inflammation. *Tunga penetrans* have also been shown to harbour endosymbionts such as *Wolbachia*. The study aimed to identify the repertoire of vertically transmitted endosymbion-

ts and characterise bacteria associated with the secondary infections of flea lesions in pigs and humans from pig- and non-pig-keeping households. Skin biopsies (n = 89) were collected from pigs and humans. The microbiome was characterised using a near full-length 16S rRNA PCR product and PACBIO sequencing. The majority of the sequence reads represented vertically transmitted bacteria. Three *Wolbachia* supergroups were found with prevalences of 15.7, 88.8 and 95.5%. *Rickettsia belli* and *Spiroplasma chrysopicola* were present in 92.1 and 15.7% of the fleas. Superinfection-associated bacteria were dominated by *Staphylococcus* spp., *Pseudomonas* spp. and Enterobacteriaceae. No pathogenic *Clostridium* spp. were detected. Analysis of β-diversity of bacteria associated with superinfections based on relative abundance or presence/absence data of amplicon sequence variants revealed overlapping but clear differences between fleas collected from pigs and from humans from non-pig-keeping households while fleas from humans from pig-keeping household were between these two groups. On the genus level, these differences were less pronounced. The diversity of vertically transmitted bacteria was larger than expected from previous studies. Although the β-diversity analysis revealed differences between pigs and humans, the overlapping spectra of bacteria suggest that pigs can be used as a good model for parasite/host interaction studies.

Efficacy of natural and synthetic insect growth regulators for the control of sand flea (*Tunga penetrans*) off-host stages: From laboratory bioassays to a randomised control field trial.

Abneel Kaur Matharu (Freie Universität Berlin, Germany), Paul Ouma (International Centre for Insect Physiology and Ecology, Kenya), Tullu Bukhari (International Centre for Insect Physiology and Ecology, Kenya), Lynne Elson (University of Oxford, UK), Juergen Kruecken (Freie Universität Berlin, Berlin, Germany), Ulrike Fillinger (International Centre for Insect Physiology and Ecology, Kenya)

Tunga penetrans causes a neglected, severe skin disease (tungiasis) with infective off-host stages in the environment. Integrated control strategies including a prevention arm are needed. Pyriproxyfen (PPF) is a widely used insect growth regulator. Neem oil (NO) has similar properties on many insects. To implement concentration-response bioassays under controlled laboratory conditions to assess the efficacy of PPF and NO against *flea* larvae and to establish their field applicability for interventions. Effects of PPF and NO on larval development and adult emergence were quantified. The optimum concentration of pyriproxyfen was tested in a randomised control trial with three arms including household floor spraying with PPF/water emulsion, water only and without any treatment. After four weekly applications, the impact on infection prevalence and intensity was evaluated. While all unexposed larvae developed into adults, PPF prevented pupation of 75% of the larvae at 0.0007 ppm. The remaining larvae died after a mean of 18 days. No cocoons developed at ≥0.007 ppm. NO was only effective at concentrations ≥ 41.4 ppm at which 17% of the larvae died before pupation and 27% pupated but did not emerge. Successfully pupated larvae did so by day 10, similar to unexposed controls. At 110.4 ppm, 50% of the larvae died before pupation and 46% did not emerge from cocoons. For the field trial only 1 ml of PPF per

32 m2 was used. Prevalence of embedded live fleas was reduced by 50% by PPF (OR=0.50, 95% CI 0.33-0.76, *p<0.001*) and 21% by water (OR=0.79, 95% CI 0.54-1.15, *p=0.221*). Infection intensity was reduced by 87% by PPF (RR=0.13, 95% CI 0.09-0.18, *p<0.001*) and by 61% by water (RR=0.39, 95% CI 0.29-0.52, *p<0.001*). Spraying PPF significantly reduced infection prevalence and intensity, though water treatment also showed some effects. NO required extremely high application rates and hence would not be a feasible product to take forward.

A Community-driven One Health based approach for effective and sustainable control of tungiasis in rural Uganda

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Tungiasis is a neglected tropical disease affecting primarily low-income communities in Latin America and Sub-Saharan Africa, particularly children below 15 years. The condition is caused by female sand fleas *Tunga penetrans* which burrow into the skin of humans and animals, resulting in severe local inflammation, itching and pain. Most cases are treated by mechanical removal of fleas, a method deemed inappropriate and unhygienic. This Rotary-funded project aimed at establishing a One Health approach for effective and sustainable control of tungiasis in an endemic area in Bugiri district, Uganda. Medical, educational, veterinary and environmental approaches for tungiasis control were combined and implemented in 521 affected households from 40 villages in Bugiri district, Uganda for two years starting February 2022. Affected humans were treated with dimeticone oil and animals with an organophospate. Furthermore, the floors of affected households were sprayed with a pyrethroid and sensitization meetings on tungiasis control were held in communities and schools. Four household surveys were conducted to assess the prevalence and infection intensity in humans and their animals. During four visits of the same 521 households, ca. 2400 people per visit were examined. While initially 800 (33.3%) were infected, prevalence decreased to 1% and 0% at the second and fourth visits, respectively. Similarly, the prevalence in household animals dropped from 24.5% to 0%. Community and school-based trainings were conducted on diagnosis and treatment of tungiasis through established Village Tungiasis Response Teams. At least 4500 pupils and 75 focal teachers from 12 primary were sensitized on tungiasis treatment and control. The community-based One Health tungiasis control approach reduced the tungiasis prevalence and median intensity to zero.

Room 2
Oral Communication

Anthelmintic Resistance in Small Ruminants: From Field Efficacy to Molecular Insights

Anthelmintic Resistance in Sheep Farms: Is the Risk of Resistance Increasing Worldwide?

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Research to estimate the frequency of sheep flocks with resistant gastrointestinal nematodes (GIN) to the anthelmintic (AHs) classes benzimidazoles (Bz), imidazothiazoles (Lev), and macrocyclic lactones (ML) began decades ago. These AHs are still used in most countries, hence, the risk of finding AH-resistant GIN populations could be higher at present. To compare the risk of recording sheep farms with GIN populations resistant to Bz, Lev, and ML in published articles using the Faecal Egg Count Reduction Test (FECRT) during two periods: 1996–2010 and 2011–2023. A scoping review was conducted (PRISMA guidelines) for the years 1996-2023. Five databases were included, resulting in 148 articles reporting FECRT results. The number of resistant, suspect, or susceptible sheep farms in each article was counted for each AH class (Bz, Lev, and ML). Respective 2 x 2 contingency tables were used to calculate the odds ratio (OR) and its 95% confidence interval (95% CI) to establish the risk of declaring resistance on farms for each AH class. The period 1996–2010 was used as baseline. For 1996–2010 vs 2011–2023 periods, the Bz resistance frequency increased from 56.9% to 77.9%, representing a 2.61 (95% CI = 2.14–3.19)-fold increased risk of resistance ($P < 0.05$). For the Lev class, frequence of resistance remained stable with 56.9% and 55.1% (OR = 0.97; 95% CI = 0.79–1.18). For the ML class, risk of resistance increased from 26.9% to 50.8%, representing a 4.62 (95% CI = 3.96–5.39)-fold increase ($P < 0.05$). Compared to the 1996-2010 period, the risk of diagnosing farms with GIN resistant to Bz and ML increased during 2011–2023, possibly due to its prolonged use and the lack of policies to promote their rational use. The situation for Lev remains unchanged possibly because it is less used due to toxicity concerns, personal preference, or its unavailability in some countries.

Reduced field efficacy of eprinomectin against gastrointestinal nematodes in austrian goat farms

Barbara Hinney (University of Veterinary Medicine Vienna, Austria), Miguel Peña-Espinoza (University of Veterinary Medicine Vienna, Austria), Nina Schlifelner (University of Veterinary Medicine Vienna, Austria), Claudia Pitterle (University of Veterinary Medicine Vienna, Austria)

Eprinomectin (EPM) is the only anthelmintic registered for goats in Austria and widely used due to its zero-day milk withdrawal and the high

proportion of adult dairy goats (~40%). However, its current efficacy against gastrointestinal nematodes (GIN) in Austrian goat-farms is unknown. We evaluated EPM field efficacy on 19 goat farms (6 federal states) from Sep 2023–Jan 2024 using WAAVP Faecal egg count reduction (FECR) test guidelines. Farms were selected from a previous prevalence study (Aug 2023) based on pooled faecal egg counts (FEC ≥200 eggs per gram (EPG)). A total of 583 goats were screened, and 285 were included in FECR tests (n=7–23/treatment group/farm). Individual liveweights were recorded for accurate dosing. EPM was administered as topical (Eprinex®, 1 mg/kg) or injectable (Eprecis®, 0.2 mg/kg). Injectable EPM was tested on 11 farms, topical on 3, and both on 5 farms. In 6 farms, moxidectin (MOX; Cydectin®, 0.4 mg/kg, oral) was also assessed. Post-treatment faecal samples were collected on day 14. Pooled pre- and post-treatment larval cultures were used for morphological and molecular identification of L3 larvae. FECR% and 90% CI were calculated, with efficacy targets set at 99% (lower threshold 95%). Reduced efficacy was observed across nearly all farms: topical EPM (FECR = 41–95%; 90% CI = 4–98), injectable EPM (FECR = 23–99%; 90% CI = 0–99). Only one farm retained high efficacy with injectable EPM (FECR = 99%; CI = 98–99). Where both formulations were tested, injectable EPM consistently outperformed topical (63–99% vs 45–95%). MOX was highly effective on 5 farms (FECR ≥ 99%), with one exception (FECR = 91%). These results demonstrated anthelmintic resistance to EPM in GIN infecting goats in Austria, as defined by the WAAVP guidelines. Resistance to MOX also emerges where EPM resistance is advanced. Continued monitoring and sustainable anthelmintic use are essential to delay further resistance development.

Detection of anthelmintic drug resistance in sheep flocks infected by gastrointestinal nematodes in the northwest of spain using *in vivo*, *in vitro* and molecular assessment

Laura González del Palacio (University of León, Spain), Elora Valderas García (The Spanish National Research Council, Spain), Marta Ruíz Somacarrera (University of León, Spain), Rafael Balaña Fouce (University of León, Spain), María Martínez-Valladares (The Spanish National Research Council, Spain)

The aim of the study was to evaluate the level of anthelmintic resistance (AR) present in sheep flocks from northwest Spain infected by gastrointestinal nematodes (GIN) applying *in vitro* techniques such as Egg Hatch Test (EHT) to detect resistance to drugs of the benzimidazole (BZ) family, and *in vivo* Faecal Egg Count Reduction Test (FECRT) against macrocyclic lactones (ML). Molecular deep amplicon sequencing approaches were also applied for targeting the nemabiome and detection of Single Nucleotide Polymorfisms (SNPs) to determine the resistance status on farms and the strongyle species involved. The evaluation of AR against BZ was carried out in 47 flocks. Pooled faecal samples were collected from all farms for the EHT at a discriminating dose of 0.1 µg/ml thiabendazole. For detection of AR to LM, 18 flocks were tested after selecting those with the highest egg shedding for the FECRT. For this purpose, animals were treated with ivermectin orally at a therapeutic dose (0.2 mg/kg bw), and the mean number of eggs per gram in faeces was compared between the day of treatment and 2 weeks later. Results for EHT revealed hatching percentages indicative of BZ resistance in 5/47 flocks (10.6%), with hatching ratios ≥ 50%. For AR against LM, treatment with oral ivermectin was

considered effective in 8/18 flocks (44.4%), with a FECRT greater than 95% (CI90%); 10 of the remaining flocks (55.5%) were declared resistant with a FECRT less than 95% (CI90%). When comparing AR levels in the study area compared to previous years, a significant increase in AR to LM was observed. The results of the species composition of GIN in the flocks and studies of anthelmintic resistance (detection of SNPs) in GIN larvae by molecular biology techniques (*deep amplicon sequencing*) are currently being conducted and will be presented at the congress. This study has been funded by project PID2020-119035RB-I00. LGP and MRS are funded by JCyL (LE096-20 and CV: BOCYL--D-17102024-8, respectively), EVG is funded by European Union Next Generation EU/PRTR (Margarita Salas Grant for the training of young doctors).

From FECRT to PCR: Unveiling anthelmintic resistance in Czech sheep

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The latest WAAVP guidelines recommend the faecal egg count reduction test (FECRT) as the gold standard for detecting anthelmintic resistance in ruminants. However, its interpretation is limited when multiple strongylid species with varying drug sensitivities coexist. In the Czech Republic, data on anthelmintic resistance in small ruminants remain scarce. Due to the increasing impact of *Haemonchus contortus* on production in Czech small ruminant farms, we assessed the efficacy of nine different anthelmintics in a Suffolk sheep flock in the Czech Republic. A total of 120 three-month-old lambs from a single flock were sampled, weighed, and treated with one of nine anthelmintics (12 animals per group). Faecal samples were collected pre- and 14 days post-treatment. FECRT was performed alongside multiplex real-time PCR targeting *Haemonchus* spp., *Teladorsagia* spp., *Trichostrongylus* spp., *Chabertia* spp., and *Nematodirus battus* to provide species--specific insights. FECRT revealed resistance to all tested anthelmintics except monepantel. Combining FECRT with real-time PCR provided a more detailed assessment of resistance at the strongylid species level. Notably, this is the first report of triple-resistant *H. contortus* (benzimidazoles, levamisole, and macrocyclic lactones) in sheep in the Czech Republic. Additionally, an avermectin-resistant (ivermectin, eprinomectin, doramectin) *N. battus* population was detected. Our study highlights the importance of integrating FECRT with real-time PCR for precise anthelmintic resistance diagnostics. The emergence of multi-drug-resistant *H. contortus* and avermectin resistant *N. battus* underscores the urgent need for sustainable parasite control strategies in Czech small ruminant farming.

Application of nemabiome targeted sequencing and an automated larval migration assay to characterize anthelmintic resistance in small ruminant flocks in Greece

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Tours, France), Alexandre Vernudachi (Invenesis France Sàrl, France), Elizabeth Redman (University of Calgary, Canada), Rebecca Chen (University of Calgary, Calgary, Canada), Cédric Neveu (Université de Tours, France), Lucien Rufener (Invenesis, Switzerland), John Stuart Gilleard (University of Calgary, Canada), Smaragda Sotiraki (Hellenic Agricultural Organization (ELGO-DIMITRA), Greece)

Preventive anthelmintic use remains important for ovine gastrointestinal nematode (GIN) control, but intensive use has led to widespread anthelmintic resistance (AR). In Greece, GINs are a challenge, since husbandry systems depend on grazing, but AR has only been reported anecdotally. This study aimed to determine GIN infection intensity, species diversity and anthelmintic resistance status in small ruminants in 4 main sheep production regions in Greece. Faecal samples were collected from 20 ewes from 20 farms per region. Faecal Egg counts (FECs) were determined by modified McMaster and species composition by ITS2 nemabiome metabarcoding of harvested larvae. Benzimidazole (BZ) and levamisole (LV) resistance SNP frequencies were determined by Illumina deep amplicon sequencing of iso-1 B-tubulin and acr-8, respectively. Larvae were analyzed for macrocyclic lactone resistance using a motility trap assay (MTA). GIN prevalence was 99.8% of farms (mean FEC range 3.15 to 2,643 EPG). There were striking differences between regions. ITS2 nemabiome metabarcoding revealed that *Haemonchus contortus* predominated in mainland Greece, while *Teladorsagia circumcincta* dominated in Crete. Deep sequencing revealed high BZ resistance SNP levels for *H. contortus* on the mainland (97.22% prevalence, 63.71% mean frequency). On the contrary, for *T. circumcincta* higher reistance SNP levels detected on Crete (94.44% prevalence, 93.22% mean frequency) compared to mainland (11.11% prevalence, 61.68% mean frequency). LV resistance was not detected on any farm. The MTA detected ivermectin and moxidectin resistance on 37.5% and 14.3% of the farms respectively. AR was confirmed *in vivo* by Faecal Egg Count Reduction Test in selected farms. Based on an original combination of molecular, phenotypic and parasitological approaches our results facilitate the implementation of sustainable worm control practices tailored to region-specific management systems.

Characterisation of eprinomectin resistant *Haemonchus contortus* collected in dairy ewe farms: from phenotype to genome

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Anthelmintic resistance significantly impacts both animal welfare and productivity. At the heart of this issue is *Haemonchus contortus*, a highly pathogenic gastrointestinal nematode of small ruminants. In this study, we examined eprinomectin (EPR) resistance in *H. contortus* using a unique set of samples collected from French dairy sheep farms in a small geographic area of southwest France, where EPR is routinely used as a deworming treatment, with se-

veral cases showing alarming clinical failures. Six worm isolates characterized for their susceptibility or resistance to macrocyclic lactones (ML) using a motility assay on 3 stage larvae were used in this study (see abstract A. Lespine). DNA was extracted from L3 for advanced genome-wide approaches based on sequence comparisons. In addition, adult males and females were recovered from infested animals in order to conduct a transcriptomic study. The dataset provides a unique opportunity to link significant genomic and gene expression variations with worm phenotypic data and farm treatment histories. In EPR-resistant worms, a discrete genomic region on chromosome V exhibited significant genetic differentiation, which had previously been identified as a signature of ivermectin resistance in *H. contortus*. Moreover, we observed variations in the expression of genes located on chromosome V that could be associated with the resistance phenotype.
This paves the way for identifying novel molecular targets, forming the basis for improved diagnostics and treatments for helminth infections.

European COST Action ENVIRANT: Environmental impact of anthelmintics in livestock and alternatives to minimize their use

María Martínez Valladares (CSIC-University of León, Spain), Laura Rinaldi (University of Naples "Federico II", Italy), Martin Danaher (Teagasc Food Research Centre, Ireland), Dimitrios Karpoyzas (University of Thessaly, Greece), Stefan Geisen (Wageningen University & Research, Netherlands), Johannes Charlier (Kreavet, Belgium), Mahmut Sinan Erez (Afyon Kocatepe University, Türkiye), Orla Keane (Teagasc, Ireland), Edwin Claerebout (Ghent University, Belgium), Eric Morgan (Queen's University Belfast, United Kingdom), Nikolaos Mittas (Democritus University of Thrace, Greece), Marcin Mickiewicz (Warsaw University of Life Sciences, Poland), Zorana Kovačević (University of Novi Sad, Serbia), Smaragda Sotiraki (Veterinary Research Institute, HAO DIMITRA, Greece)

Pasture-based animal production systems play a vital role in promoting sustainable farming practices in Europe. These systems rely on grass grazing as an affordable feed source on the farm. However, these systems have certain disadvantages, particularly concerning animal health since grazing livestock are very susceptible to infections caused by helminth parasites. These infections can impose a substantial economic burden on the food production system and the most commonly used method for their control is the administration of anthelmintic drugs. However, these drugs have been considered recently emerging contaminants because their presence in both terrestrial and aquatic ecosystems poses significant environmental risks. Under this context, the objective of ENVIRNAT is to advance, consolidate and disseminate research and knowledge on the environmental occurrence and ecological impact of anthelmintics administered to livestock and to propose more sustainable practices and methods to minimize their use in the control of helminth infections. This Action aims to (i) to monitor the sale, use and efficacy of anthelmintics in European livestock farming; (ii) to investigate factors related to farming practices, the environment and climatic conditions that favour the persistence of anthelmintics in the environment; (iii) to assess the impact of anthelmintic residues on ecosystems; (iv) to develop sustainable methods to reduce the use of anthelmintics in a variety of European settings; (v) to conduct socio-psychological research on

barriers that may arise in the implementation of sustainable methods; (vi) to model the impact of anthelmintic use, considering both animal health benefits and ecosystem risks through benefit-risk assessment. A COST Action is an interdisciplinary research network that brings researchers and innovators together to investigate a topic of their choice for 4 years. COST Actions offer an inclusive, pan-European environment for individuals of all levels of seniority to grow their professional research networks and boost their careers. Action funded by COST, CA23154.

Plenary Oral Communication

Advances in Heartworm Research: Epidemiology, Resistance, and Control in Companion Animals

Living fossil: What does population genomics reveal about the origin of *Dirofilaria immitis*?

Rosemonde I. Power (University of Sydney, Australia), Swaid Abdullah (University of Queensland, Australia), Heather S. Walden (University of Florida, USA), Guilherme G. Verocai (Texas A&M University, USA), Tiana L. Sanders (Texas A&M University, USA), Joe L. Luksovsky (Texas A&M University, USA), Andy R. Moorhead (North Carolina State University, USA), Michael T. Dzimianski (University of Georgia, USA), Jeremy M. Foster (New England Biolabs, USA), Michelle L. Michalski (University of Wisconsin Oshkosh, USA), Alicia Rojas (University of Costa Rica, Costa Rica), Samuel C. Chacón (Healthy Pet Veterinary Hospital SC, Panamá), Georgiana Deak (University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania), Andrei Mihalca (University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania), Patrizia Danesi (Istituto Zooprofilattico Sperimentale delle Venezie, Italy), Elias Papadopoulos (Aristotle University of Thessaloniki, Greece), Piyanan Taweethavonsawat (Chulalongkorn University, Thailand), Dung Thi Bui (Vietnam Academy of Science and Technology, Vietnam), Anh Do Ngoc (Vietnam Military Medical University, Vietnam), Reuben Sharma (Universiti Putra Malaysia, Malaysia), Simon Y. W. Ho (University of Sydney, Australia), Steve Doyle (Wellcome Sanger Institute, UK), Jan Slapeta (University of Sydney, Australia)

Heartworms (*Dirofilaria immitis*) are parasitic nematodes causing significant cardiopulmonary morbidity and mortality in canids worldwide. This study aimed to conduct the largest population genetics analysis of heartworms to date, utilizing whole-genome sequencing to uncover their evolutionary history and global dispersal patterns. We performed whole-genome sequencing on 127 adult heartworms from mammalian carnivores across four continents. Our transcontinental genome-wide analysis included admixture analyses to trace the origins and dispersal of heartworms. Our findings revealed a deeper origin and dispersal history of heartworms than previously understood. Ancestral canid hosts played a crucial role in the evolution and dissemination of heartworms globally, rather than solely human-mediated dispersal of dogs. Admixture analyses indicated an Asian origin for Australian heartworms, consistent with the arrival of dingoes thousands of years ago. Genetic relatedness between European and Central American heartworms suggests modern dispersal linked to human colonization of the Americas. Anthropogenic

influences, such as drug treatment, climate change, and global connectivity, are likely to rapidly shape heartworm diversity. This study enhances our understanding of the global population dynamics and evolutionary history of heartworms. The insights gained are critical for developing effective surveillance and control strategies, which should consider geographical variation and transmission dynamics to ensure sustainable parasite control.

Resistance to macrocyclic lactone heartworm preventives: Current prevalence, diagnostic tests and concerns

Roger Prichard (McGill University, Canada)

Heartworm disease, caused by *Dirofilaria immitis*, can result in severe morbidity and death in dogs. It also causes disease in cats and is zoonotic. The infective stage L3 larvae are transmitted by many species of mosquitoes. Prevention of adult infection is key to reducing disease. Currently, all preventives are macrocyclic lactones (MLs) which kill the L3/L4 stages prior to the pathogenic adults establishing. However, resistance to MLs has developed in the USA. The incidence of heartworm infection in the USA has increased and appears to be increasing in other parts of the world. The first confirmed case of ML-resistance was in 2011 in a dog imported into Canada from Louisiana, USA. Genetic markers have been developed to monitor for genotypic evidence of ML-resistance in *D. immitis* and been used in surveys for resistance to ML heartworm preventives. The microfilariae suppression test (MFST), as well as the use of experimental infections with animals under heartworm preventive regimes, have been used to prove phenotypic resistance. Recent studies show that ML-resistance is now widespread in USA. There is potential for resistance to spread to other countries with the transport of infected dogs as has occurred in Canada and Italy. It is time to consider how to reduce the risk of spreading ML-resistance and what can be done to maintain heartworm prevention in the face of ML-resistance. This should include monitoring for resistance, paying attention to animals that are transported to new locations, and the use of the most effective preventive regimes. The latter involves the choice of ML used, dose rate and full compliance when mosquitoes may transmit the parasite. Further steps are needed to reduce mosquito transmission of *D. immitis*. This involves using combinations with ectoparasiticides which prevent mosquito reproduction and/or repel mosquitoes. Finally, novel heartworm preventives which are not affected by ML-resistance are needed.

Surveillance of single nucleotide polymorphisms correlated to macrocyclic lactone resistance in *Dirofilaria immitis* from client-owned dogs across the United States

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Dirofilaria immitis is a filarial nematode and the causative agent of heartworm disease. Heartworm disease is managed via macrocyclic lactone (ML)-based chemoprophylactics. Through opportunistic sampling, genotypically and phenotypically confirmed ML-resistant *D. immitis* isolates have been isolated in the Lower Mississippi River Valley region (LMRV);

however, the pervasiveness of resistant isolates in the USA has not been evaluated. This study aimed to evaluate the geographic distribution of genotypically ML-resistant heartworms in client-owned dogs across the USA over a 3-year period. Owner consent was obtained to collect microfilaremic blood samples from heartworm-positive dogs from participating clinics. Veterinarians completed a questionnaire on the known history of each dog, including treatment and travel history. Microfilariae were filtered from blood, DNA extracted utilizing the QIAGEN QIAamp DNA Micro Kit and samples sequenced by the Génome Québec Innovation Centre to determine allele frequencies at nine SNP sites previously correlated with ML resistance. The highly predictive 2-SNP model was used to identify genotypically susceptible, mixed, and resistant populations. A total of 310 microfilaremic blood samples were collected from 45 geographically diverse veterinary clinics located in 22 states. Computational analysis indicated 111 (35.8%) were genotypically susceptible, 96 (31.0%) were genotypically resistant, and 103 (33.2%) were genotypically mixed. The genotypically mixed and ML-resistant infections were located within and outside of the endemic LMRV, as far north as Michigan. These findings indicate canine populations outside of the LMRV are at increased risk for transmission of potentially ML-resistant heartworm infections than was previously hypothesized. Veterinary practitioners across the USA need to be aware of the potential risks of ML-resistant heartworm infections in their regions and ensure compliance with recommended prevention protocols.

Heartworm Alert: Development of a Spatial Decision Support System for the Management of Canine Heartworm in Australia

Kennedy K Mwacalimba (Zoetis), Sahil Arora (The University of Queensland, Australia), Nicholas J Clark (The University of Queensland, Australia), Richard L'Estrange (Zoetis), Andrea Wright (Zoetis), Ricardo Soares Magalhaes (The University of Queensland, Australia), Jessica Rodriguez (Zoetis)

Canine heartworm disease, caused by *Dirofilaria immitis*, remains a major veterinary concern in Australia, necessitating enhanced surveillance and management to better understand and mitigate its impact. Traditional epidemiological studies have been constrained by small-scale datasets and heterogeneous clinical records, limiting the ability to comprehensively map demographic risks and treatment outcomes on a national level. To address these limitations, we developed Heartworm Alert—a spatial decision support system that integrates advanced natural language processing (NLP) techniques with big data analytics. Data were extracted from over one million electronic patient records from VetCompass Australia, and a tailored NLP algorithm was implemented to mine unstructured consultation texts. This algorithm achieved an accuracy of 98.7% and a sensitivity of 99.87% by effectively resolving clinical abbreviations, synonyms, and misspellings. Our analysis showed a steady increase in heartworm testing and positive diagnoses from 2014 onward, with significant geographic clustering along Queensland's coastline. Regions such as Townsville and Cairns exhibited the highest incidence rates, highlighting localized disease hotspots. The HeartwormAlert platform, developed using R-Shiny, provides an interactive dashboard with real-time geographic and temporal visualizations, detailed maps, demographic breakdowns, treatment outcomes, and an alert functionality

to notify veterinarians and dog owners of location-specific risks. Integrating big data with state-of-the-art NLP dramatically improves the surveillance and clinical management of canine heartworm disease. The HeartwormAlert platform supports data-driven decision-making for veterinary professionals, promotes proactive owner education, and encourages preventive healthcare. Future research will incorporate additional environmental and socioeconomic variables to refine risk assessments and guide targeted intervention strategies.

Comparative study of oral treatments for canine heartworm disease using milbemycin oxime and doxycycline

Ana Clara Moreira Pessoa Monteiro (State University of Santa Cruz, Brazil), Cassia Matos Ribeiro (State University of Santa Cruz, Brazil), Laura Padilha Figueira Louro (State University of Santa Cruz, Brazil), Hllytchaikra Ferraz Fehlberg (State University of Santa Cruz, Brazil), Anaiá Paixão Sevá (State University of Santa Cruz, Brazil), Fábio Barbour Scott (Federal Rural University of Rio de Janeiro, Brazil), Maureen Angela Kelly (Texas A&M University, USA), Guilherme G Verocai (Texas A&M University, USA), George Rego Albuquerque (State University of Santa Cruz, Brazil), Pabasara Weerarathne (Texas A&M University, USA), Renada Joana Costa Cruz (State University of Santa Cruz, Brazil)

Dirofilariosis is a vector-borne disease that primarily affects dogs, potentially leading to severe illness and death. Despite the efficacy of the current adulticidal treatment available, the drug is not available in several countries, highlighting the need for accessible alternatives. This study aimed to compare two oral treatment protocols in dogs naturally infected with *Dirofilaria immitis* in Southern Bahia state, Brazil. Twenty-six client-owned dogs confirmedly heartworm-positive via modified Knott's and antigen detection tests (SNAP® 4Dx®, IDEXX Laboratories, Inc.) were enrolled. Dogs were randomly assigned to two groups (n=13): ML (milbemycin oxime – 0.5–2.5mg/kg, monthly) and Doxy+ML (same milbemycin + doxycycline – 10mg/kg, BID for 30 days, repeated after 6 months). Microfilariae Counts (mfC) were performed monthly until one year, as well as qPCR assays targeting *D. immitis* and *Wolbachia* DNA. On day 180 post-treatment (dpt), dogs in the ML group were administered doxycycline due to the absence of a significant reduction in mfC. Thereafter, the ML group was reassigned to the Doxy+ML group and monitored from the baseline. Significant differences were observed between treatment groups ($p=0.003$), and between treatment and time ($p<0.001$). To assess the variation in microfilaremia within each protocol, repeated measures ANOVA was applied to dogs with complete records across time points. Within the Doxy+ML group, mfC progressively decreased from 120 until 180 and in a stationary pattern to 330 dpt compared to baseline ($p<0.005$). Within the ML group, a significant increase in mfC was observed from 30 to 150 dpt ($p<0.005$). The mfC between groups differed significantly at every time point from 60 to 180 dpt ($p<0.005$). Currently, some dogs are still under evaluation and have not yet completed the full treatment period. Continued monitoring is essential to assess the efficacy of both protocols and to support more effective management of heartworm disease in the region.

Epidemiology, diagnosis and clinical features of feline dirofilariosis in the Mediterranean Basin

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Dirofilaria immitis and *Dirofilaria repens,* causing heartworm disease and subcutaneous dirofilariosis, respectively, are filarioids of global zoonotic concern. Information on their distribution in the Mediterranean basin in cats is mainly limited to case reports. We assessed the occurrence of feline dirofilariosis, its risk factors, clinical picture and clinicopathological abnormalities in cats from six countries of the Mediterranean Basin. In addition, *Wolbachia* spp. endosymbionts infection were assessed in *Dirofilaria* spp.-positive cats.
Blood and sera samples were collected from cats living in Spain (*n*=354), Portugal (*n*=287), Italy (*n*=125), Greece (*n*=116), Israel (*n*=101) and France (*n*=100). Sera were tested by both direct antigen detection (SNAP test) and indirect antibodies (in-house ELISA) serological tools, and blood samples by real time and conventional PCR. A statistical analysis was run to assess the link between *Dirofilaria* spp. infection and clinical records, as well as among feline immunodeficiency virus (FIV) and/or feline leukaemia virus (FeLV) co-infections, .
Overall, 3.8% (41/1,083) cats scored positive for *Dirofilaria* spp. with prevalence ranging from 2% in Israel to 7.8% in Greece. Of the 41 positive cats, 16 were infected by *D. immitis* (by SNAP test and/or PCR) and two by *D. repens* (by PCR); the remaining animals were exposed to *Dirofilaria* spp.. *Wolbachia* DNA was detected in one *D. immitis*-infected cat. Dirofilariosis positivity was significantly associated with age, breed, decreased appetite, dandruff, and dyspnoea. This study provides data on the prevalence of *Dirofilaria* spp. infection in cats from the Mediterranean Basin, suggesting that *D. immitis* mainly circulates in cats from islands. Overall data shown the importance of performing strategic chemoprophylactic treatments for cats living in areas where canine dirofilariosis is endemic.

DIROGEN: *Dirofilaria immitis* and *D. repens* in Austria – A global change example and why deciphering the genetic diversity on a global perspective is of need

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Dirofilaria repens and *D. immitis* are filarioid helminths with domestic and wild canids as main hosts and mosquitoes as vectors. They are

causative agents of zoonotic diseases. Both *D. immitis* and *D. repens* are known as potential invasive species, and their distribution seems associated with climate change. Until recently, both species were nonendemic in Austria. The first autochthonous case of *D. immitis* was documented in a cat in the easternmost province of Austria, Burgenland, in 2020, and more recently in a red fox (*Vulpes vulpes*) in January 2025. Since then, no further autochthonous cases were reported which can be associated with the way of dog keeping in Austria (dogs are not staying outside overnight). Cases of the canine heartworm *D. immitis* in Austria are still limited to imported cases in dogs, but our findings in a red fox and cat indicate its spread from Hungary to Austria. Furthermore, the establishment in Austria is discussed as realistic with the establishment of new competent day-biting mosquito vectors – tiger mosquitoes (*Aedes albopictus*) which establishment process is ongoing in Austria. Despite the global distribution of *D. repens* and *D. immitis*, knowledge of their genetic diversity and transmission dynamics remains limited. To address this, our recently started project, DIROGEN, aims to assess the genetic diversity of *D. repens* and *D. immitis* across continents using metabarcoding, and mitochondrial and whole genome sequencing, to identify species and haplotypes infecting humans to elucidate zoonotic transmission routes and investigate genetic variations among adult worms infecting individual canine hosts. Samples from dogs, cats, wildlife, and humans will undergo DNA extraction, PCR, and advanced sequencing (Sanger, NGS). Data will be analyzed using bioinformatics tools for population genetics and phylogenetics. This research addresses critical gaps in *Dirofilaria* epidemiology, aiding global control efforts and understanding zoonotic risks.

14:00 – 15:30

Room 1
Oral Communication

Coccidiosis and Other Protozoa: Epidemiology, Diagnosis, and Advances in Control

First epidemiological report of *Neospora caninum* in sheep from four municipalities in Colombia

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Neosporosis is a disease of little interest to sheep producers in Colombia because it is only associated with abortions in cattle. In the country, numerous epidemiological studies of Neospora caninum have been carried out in cattle, but to date no serological studies have been carried out in ewes or verification of abortions due to *N. caninum* in sheep by histopathology or molecular techniques. The objective of the study was to analyse the prevalence of infection by *N. caninum* in breeding ewes from four municipalities (Cumaral, Pacho, Alvarado and El Cocuy). For this purpose, 187 blood samples were collected from sheep in the reproductive stage, which were analysed by indirect ELISA and the association between seroprevalence, and some management factors were estimated by means of a logistic regression analysis. For

N. caninum, a seroprevalence of 9,09% (17/187) was identified, positive animals were found in 47,62% of the farms (10/22). At the municipality level the estimated prevalence was Cumaral 10,8% (5/46), Pacho 4,3 % (2/46), Alvarado 2,78% (1/36) and El Cocuy 15,2% (9/59). The variables Ground level feeders (OR 2,4) and presence of cattle (OR 4,2) were identified as risk factors for *N. caninum* infection. Although the prevalence was low in this first study, is necessary to continue investigating other municipalities and verify if it is the cause of abortions.

Molecular Analysis of *Eimeria* Species Causing Bovine Eimeriosis in Sulawesi Districts, Indonesia

Fitrine Ekawasti (National Research and Innovation Agency, Indonesia), Humaira Putri (Universitas Gadjah Mada, Indonesia), Dyah Ayu Kurniawati (Agricultural Instrument Standardization Agency, Indonesia), Vika Ichsania Ninditya (Universitas Gadjah Mada, , Indonesia), Raden Wisnu Nurcahyo (Universitas Gadjah Mada, Indonesia)

Eimeria spp. is a protozoan parasite that inhabits the digestive tract of cattle and poses a significant challenge in the livestock industry due to its impact on animal health and productivity. This parasite is a primary cause of coccidiosis, which manifests as acute diarrhea, particularly in young cattle, and accounts for a mortality rate of approximately 75% in severe cases. Early and accurate identification of *Eimeria* species is critical for implementing effective control and prevention strategies. Molecular methods including nested PCR with ITS-1 markers have emerged as reliable tools for detecting and differentiating *Eimeria* species. This study aims to analyze and identify *Eimeria* species in beef cattle feces using the nested PCR method with the ITS-1 marker to improve diagnostic accuracy and support control efforts. Seventeen fecal samples from quarantine farms in Gorontalo and Pare-Pare in Sulawesi district were analyzed. DNA was isolated, and nested PCR with ITS-1 primers was performed to detect *Eimeria*. The PCR products were visualized using electrophoresis, followed by sequencing. BioEdit and MEGA V.11.0.13 were used for sequence alignment and phylogenetic analysis. PCR analysis revealed that 57% of the fecal samples tested positive for the *Eimeria* genus. Electrophoresis demonstrated distinct band sizes corresponding to specific species: *Eimeria alabamensis* (184 bp), *Eimeria bovis* (238 bp), *Eimeria zuernii* (344 bp), and *Eimeria ellipsoidalis* (148 bp). Sequencing further confirmed the presence of *E. alabamensis*, *E. ellipsoidalis*, and *E. zuernii*. Phylogenetic analysis using the Neighbor Joining method and the p-distance model showed high similarity between the identified species and sequences in the NCBI database. The findings highlight the prevalence of *Eimeria* species in beef cattle and underscore the effectiveness of Nested PCR with ITS-1 markers in species identification. This approach provides a valuable diagnostic tool for managing coccidiosis in cattle and can inform targeted prevention strategies.

Emerging bovine besnoitiosis in Italy: preliminary insights from serological and clinical investigations

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derico II, Italy), Nicola Lattero (University of Naples Federico II, Italy), Michele Garaguso (Regional Breeders Association of Basilicata, Italy), Alessia Gazzonis (Università degli Studi di Milano, Italy), Vincenzo Veneziano (University of Naples Federico II, Italy)

Bovine besnoitiosis (BB) is an emerging disease caused by *Besnoitia besnoiti*, affecting the skin and mucous membranes. Though rarely fatal, it causes symptoms like weight loss, edema, skin thickening, abortions, and reproductive issues, leading to economic losses. BB is endemic in parts of southern and eastern Europe, but data from Italy are limited, and it is not officially considered endemic in the country. The "BESNOBIT" project aims to improve understanding of *B. besnoiti* in Italy and develop effective control strategies. A comprehensive serological survey was conducted on dairy and beef cattle across northern, central, and southern Italy. Based on an estimated farm-level seroprevalence of 5%, farms were randomly selected and serum samples collected from 10 native cattle (>12 months) per farm, then stored at –20°C. During sampling, information on breed, age, sex, and clinical status was recorded, and a questionnaire on farm management and biosecurity was administered. In total, 147 farms (44 in Lombardia, 52 in Umbria and Marche, and 51 in Campania, Basilicata and Molise) and 1,470 animals (440, 520, and 510, respectively) were tested using the ID Screen® *Besnoitia* Indirect 2.0 ELISA (IDVET, France). Western Blot analysis is underway for confirmation. Overall, 246 animals (16.7%) from 47 farms (32%) tested seropositive: 27 animals (6.13%) from six farms (11.3%) in Lombardia; 18 animals (3.46%) from eight farms (15.38%) in the central regions; and 201 animals (39.4%) from 33 farms (64.7%) in the southern regions. Clinical examinations revealed both acute and chronic signs in some herds, and PCR of skin biopsies confirmed infection. A clinical case series documented at the time of sampling will be discussed. These findings indicate that BB foci are present across Italy, with a potential hotspot in Basilicata, emphasizing the need for heightened awareness among practitioners and veterinary authorities for early detection and effective control measures.

Development and evaluation of a Gamma-irradiated vaccine strain for *Toxoplasma gondii* infection

Mohamed A. Helal (Veterinary Research Institute, National Research Centre, Egypt), Hany M. Elsheikha (University of Nottingham, UK)

Toxoplasma gondii is an obligate intracellular parasite that infects birds and mammals, including humans, causing toxoplasmosis, a latent infectious disease affecting approximately one-third of the global population. The establishment of *T. gondii* infection requires attachment, invasion, tachyzoite replication, and differentiation into cysts, all of which are crucial for disease progression. This study aims to evaluate the *in vitro* effects of gamma-irradiated tachyzoites from *T. gondii* RH and ME49 strains at absorbed doses of 0.2, 0.4, and 0.8 kGy on their infectivity, replication capacity, and functional antigenicity. The viability of irradiated tachyzoites was assessed using a 0.4% trypan blue exclusion test in PBS (pH 7.3). Phenotypic characterization of irradiated parasites, including their ability to invade, replicate, and form plaques, was evaluated in microglial cell models using Giemsa staining, CFSE parasite staining, Acridine Orange staining, and crystal violet staining in infected monolayers. The impact of *T. gondii* infection on microglial

cell viability was analysed using AlamarBlue® viability, cell proliferation, SRB, and MTT viability assays, comparing irradiated and non-irradiated strains with uninfected control cells. The results indicated that tachyzoites remained viable and withstood higher doses of gamma irradiation, as confirmed by the trypan blue exclusion test. Irradiation allowed tachyzoites from both strains to invade host cells even at higher exposure doses but inhibited their replication and plaque formation at 0.4 and 0.8 kGy for the ME49 and RH strains, respectively. Cell viability significantly decreased ($p < 0.05$) in a dose- and time-dependent manner, with the lowest viability observed in cells infected with wild-type strains, 0.2 kGy-irradiated ME49 tachyzoites, and 0.2–0.4 kGy-irradiated RH tachyzoites at all time points (3-, 24-, 48-, and 72-hours post-infection). Gamma irradiation at absorbed doses of 0.4 kGy (ME49 strain) and 0.8 kGy (RH strain) attenuates *T. gondii* tachyzoites, allowing them to invade host cells but preventing intracellular replication while preserving their immunogenicity. This attenuation strategy suggests that irradiated *T. gondii* tachyzoites could serve as a potential vaccine candidate, capable of inducing a humoral immune response and providing partial protective immunity against natural infection.

Characterisation of *Giardia duodenalis* assemblages from veterinary clinical cases in Ireland

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Giardia duodenalis (syn. *G. intestinalis* or *G. lamblia*) is an intestinal flagellated protozoan that is a leading cause of diarrhoea in humans and animals globally. Infection typically occurs through waterborne transmission or the faecal-oral route. Genetic analysis has identified eight assemblages (A-H), with varying degrees of host specificity and zoonotic potential. The objective of this study was to identify the assemblages present in clinical cases in animals in Ireland using a multilocus genotyping (MLG) approach. The study included 140 faecal samples from dogs, cats, cattle, lemurs, seals and alpacas, submitted to the diagnostic laboratory of UCD Veterinary Hospital, external veterinary practices, wildlife parks and animal sanctuaries. Following detection of *G. duodenalis* cysts by coprologic examination using faecal flotation or identified as positive by a commercial immunochromatographic kit, *G. duodenalis* assemblages were characterised based on Sanger sequences of fragments of the beta-giardin (*bg*) and glutamate dehydrogenase (*gdh*) genes. Preliminary analysis of the *bg* gene fragment indicated that canine samples contained the expected assemblages C and D, typically associated with dogs. Unexpectedly, assemblage E, commonly found in ruminants, was also prevalent across all the other animal groups tested, except lemurs. When these samples were tested using the *gdh* gene, there was strong agreement with the previous gene for assemblages B (κ =.656), D (κ =.407) and F (κ =.885). However, canine samples that were identified as assemblage E (κ =.038) using the *bg* locus were largely identified as assemblages C and D. These findings highlight the importance of using a MLG approach when assigning *Giardia* assemblages. Further characterisation using a third gene (triosephosphate isomerase) may

help reinforce these results and potentially identify mixed assemblage infections.

Longitudinal follow-up of *Giardia* infection in dogs shows impaired immune development

Bregt Decorte (Ghent University, Belgium), Peter Geldhof (Ghent University, Belgium), Edwin Claerebout (Ghent University, Belgium)

Giardia infection in dogs is well-documented, yet its role in clinical disease and whether dogs develop immunity against infection remains unclear. While some dogs seem to eliminate the parasite, others exhibit chronic cyst excretion, raising questions about immune response efficacy and infection persistence. This study investigated *Giardia* infection dynamics in young and adult dogs, assessed immune development indicators (e.g., cyst excretion reduction) and evaluated the association between infection and fecal consistency. A longitudinal follow-up study was conducted on puppies over a five-month period and on adult dogs over a two-month period. Cyst excretion per gram of feces (CPG) was measured regularly to assess infection persistence. Fecal consistency was scored to analyze correlations between clinical signs and CPG levels. To evaluate the impact of puppy origin on infection rates, prevalence was compared between dams and their litters from large- and small-scale breeder facilities. A significant proportion of puppies remained chronically infected with consistently high CPG levels, suggesting lack of protective immune development. Initial infection status strongly predicted chronicity, with puppies from professional breeders at higher risk than those from non-professional breeders. The prevalence study confirmed significantly higher *Giardia* rates in both dams and litters at large-scale breeding facilities. Similarly, adult dogs exhibited high infection rates, with most consistently excreting high cyst levels. A significant correlation was observed between *Giardia* prevalence, cyst excretion levels and stool consistency. These findings highlight the chronic nature of *Giardia* infection in both young and adult dogs and the ineffective immune development in infected animals. The strong correlation between CPG levels and loose stool in adult dogs suggests that CPG quantification could guide treatment decisions, though further research is needed to establish clinical cutoff values.

High prevalence of gastrointestinal parasites of dogs from Saipan, Northern Mariana Islands, including the zoonotic *Ancylostoma ceylanicum*

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Gastrointestinal (GI) parasites of dogs, including helminths and protozoans, are of substantial relevance to veterinary medicine and public health. Nevertheless, epidemiological data is scarce from many areas worldwide, especially in remote locations. The emergence of novel technologies and diagnostic platforms facilitates comprehensive screening of multiple GI parasites. Our study aims to establish a baseline prevalence for GI parasites in dogs from Saipan,

Northern Mariana Islands. Fecal samples were collected from dogs (n=420) from May to June 2023 during a spay-neuter campaign. Frozen samples had total nucleic acids extracted and were molecularly screened with a rapid turnaround test for endoparasites called KeyScreen® GI Parasite PCR (Antech Diagnostics). Overall, parasites were detected in 267 (63.5%) of canine samples. The most detected parasite genus was *Ancylostoma* sp. (n=224; 53.3%), followed by *Giardia* (n=67; 15.9%), *Trichuris* (n=39; 9.2%), *Dipylidium* (n=25; 5.9%), *Toxocara* (n=15; 3.5%), *Cystoisospora* (n=10; 2.3%), and *Cryptosporidium* (n=6; 1.4%). Zoonotic assemblages of *Giardia* and the benzimidazole-resistance marker (codons F167Y and Q134H) in *Ancylostoma caninum* were not detected. Considering risk factors significantly associated with infection were: age, sex, district and ownership for *Trichuris;* age and ownership for *Ancylostoma*, *Giardia*, and *Dipylidium*; and ownership for *Toxocara* and *Cystoisospora*. Hookworm positive samples were further characterized to species level using qPCR. Overall, *A. caninum* and *A. ceylanicum/A. duodenale* were confirmed in 196 (46.7%) and 57 (13.5%) dogs, respectively. Further NGS sequencing confirmed the presence of the zoonotic *A. ceylanicum* in at least 21 samples; approximately 5% of dogs geographically distributed across all districts. The high prevalence found for multiple parasites of One Health importance reinforces the need for surveillance and implementation of control strategies, in special targeting *A. ceylanicum*.

Room 2
Oral Communication

Next-Generation Sequencing Applications in Anthelmintic Resistance and Gastrointestinal Nematode Diversity

Screening for anthelmintic drug resistance mutations in parasitic nematodes using Oxford Nanopore long-read amplicon sequencing

Rebecca Chen (University of Calgary, Canada), Mahya Dini (Faculty of Veterinary Medicine,University of Calgary), Elizabeth Redman (Faculty of Veterinary Medicine ,University of Calgary), Lynsey Melville (Moredun Research Institute , Scotland), Dave Bartley (Moredun Research Institute , Scotland), John Gilleard (University of Calgary, Canada)

Illumina short-read next generation amplicon sequencing is increasingly applied to study the molecular epidemiology and screen for anthelmintic resistance SNPs. However, a major limitation is the short length of fragment sequenced (<600 bp) which restricts our ability to detect resistance SNPs across the whole open reading frame of target genes. Oxford nanopore (ONT) long-read sequencing offers a solution. However, its lower accuracy still makes individual SNP calling and Amplified Sequence Variant (ASV) generation more challenging. Use benzimidazole (BZ) resistance as a system to develop ONT amplicon sequencing for reliable low
frequency anthelmintic resistance SNP detection in parasite populations. A bioinformatic pipeline for non-synonymous SNP detection from ONT amplicon sequence was developed. *Nematodirus battus* from UK sheep farms was chosen for validation due to previous Illumina data showing many populations with a low frequency of the isotype-1 β-tubulin 167Y and 200Y isotype-1 β-tubulin BZ resistance SNPs. Isotype-1 β-tubulin from six PCR replicates from 17 farms *N. battus* gene was ONT amplicon sequenced

and results were compared with more accurate PacBio SMRT and Illumina amplicon sequencing data. The validated assay was then applied to a large geographically diverse set of *Haemonchus contortus* and *Ancylostoma caninum* populations. F167Y and F200Y SNP frequencies were very consistent across replicates for the *N. battus* validation and comparable to the Illumina and PacBio sequencing results. Application of the validated ONT amplicon sequencing to the full-length isotype-1 β-tubulin gene from *Haemonchus contortus* and *A. caninum* populations detected additional non-synonymous SNPs which will be presented. ONT long-read amplicon sequencing is reliable for low frequency SNP detection in parasite populations and uncovers additional non-synonymous isotype-1 β-tubulin SNPs.

Development and field-evaluation of a deep amplicon sequencing assay to detect the levamisole-resistance associated SNP (S168T) in the *acr-8* gene

Elizabeth Redman (University of Calgary, Canada), Osama Zahid (University of Calgary, Canada), Ray M. Kaplan (St. George's University, Grenada), Sue B. Howell (University of Georgia, USA), John S. Gilleard (University of Calgary, Canada)

A non-synonymous Single Nucleotide Polymorphism (SNP) in (S168T) in the *H. contortus acr-8* gene has been shown to be associated with levamisole resistance. The aim of this work was to develop a deep amplicon sequencing assay to detect this SNP in ovine GIN field populations, and to determine its frequency in archived samples whose levamisole resistance phenotype had previously been established using a Larval Development Assay (LDA). Primers designed to amplify the relevant *acr-8* fragment from three main ovine GIN species, *H. contortus*, *T. circumcincta* and *T. colubriformis*, were tested against single species isolates to determine specificity. Illumina Miseq paired-end sequencing was performed on amplicons from various field populations and a bespoke DADA2 based denoising pipeline developed to generate Amplicon Sequence Variants (ASVs). ASVs were taxonomically assigned to GIN species reference sequences, and the S168T SNP frequency determined for each species. The sequencing assay was used to determine the S168T SNP frequency in US field populations of known levamisole resistance phenotype. A total of 68 USA farm samples submitted to the University of Georgia (2015 and 2019) were classified as either susceptible (n=18), suspected-resistance (n=7), low-resistance (n=27) and high-resistance (n=16) by LDA. The mean frequencies of the S168T SNP, combined for each GIN species, were 10.9% and 32.9% in the susceptible and high-resistance groups, respectively. We suggest that the detection of the S168T SNP in "susceptible" populations and the moderate overall correlation between the LDA resistance phenotype and S168T SNP frequency ($R^2 = 0.4288$) likely results from limitations in LDA sensitivity. Finally, field populations from UK, US, Canada and Greece were used for further validation and the presence and frequencies of the S168T SNP broadly reflected the levamisole usage histories in the different geographical regions.

Unraveling benzimidazole resistance in cattle gastrointestinal nematodes through Next-Generation Sequencing

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Gastrointestinal nematodes (GIN) infection represents one of the most significant health challenges in ruminants and are the primary cause of economic losses in livestock production systems worldwide. Their control relies almost exclusively on the use of synthetic antiparasitic compounds. Inappropriate use has led to therapeutic failures associated with the development of resistant nematode parasites. The advancement in high-throughput sequencing technologies has enabled the development of molecular-based techniques for resistance diagnosis. This study describes the first molecular identification of GIN parasitizing cattle across 6 commercial farms located in Argentina, using the ITS-2 gene metabarcoding. Additionally, the fecal egg count reduction test and sequencing of the β-tubulin isotype-1 gene were used to assess benzimidazole (BZD) resistance under different anthelmintic treatment regimens (BZD alone or BZD+macrocyclic lactones). Seven GIN species were identified: *H. placei (64.1%), C. punctata (26.6%), O. radiatum (3.6%) O. ostertagi (3.5%)*, *H. contortus (1.1%), C. oncophora (0.9%) and T. axei (0.2%).* Among the 21 anthelmintic treatments applied across six farms, two farms exhibited overall efficacies above 95% for all treatments, while four farms displayed efficacies below 95% for either BZD alone or combined treatments. While *Cooperia punctata* and *Ostertagia ostertagi* were the main species resistant to BZD, *Haemonchus placei* was found to be BZD-susceptible on all the farms. BZD resistance associated SNPs in codons 167, 198 and 200 of the isotype-1 β-tubulin gene were present in both *C. puntacta* and *O. ostertagi*, being the F200Y allele recovered with the highest frecuency. Finally, BZD resistance associated SNPs were found at low frequencies even when the *in vivo* FECR was >95% on the field, demonstrating the potential of β-tubulin amplicon sequencing to screen for the early emergence of resistance mutations. Monitoring the prevalence and distribution of tubulin gene polymorphisms is crucial for tracking the emergence and spread of BZD resistance, which is now being used for the first time (as a model) in the large extension cattle ranches of the *Argentina´s Pampa Húmeda*.

Nemabiome metabarcoding shows variable resistance levels and species dynamics in anthelmintic resistant gastrointestinal nematode populations

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Gastrointestinal nematodes (GINs) pose a major threat to livestock production by reducing yields in milk, meat, and wool. Although faecal egg count reduction tests (FECRTs) measure phenotypic resistance to anthelmintics, they do not reveal the underlying dynamics and diversity of the resistant GIN populations. This study aimed to elucidate the species composition and diversity of anthelmintic-resistant GIN populations in sheep farms in southeast England by integrating molecular techniques with standard FECRTs. Eighteen farms were sampled, with faecal collections from three groups of 10 lam-

bs at treatment (administered with recommended doses of ivermectin, levamisole, or their combination) and 14 days post-treatment. After performing faecal egg counts (FECs), eggs were hatched, and DNA was extracted from first-stage larvae. The extracted DNA underwent nemabiome metabarcoding using next-generation Illumina sequencing. Sequence data were processed to identify and quantify GIN species and their corresponding amplicon sequence variants (ASVs). Alpha and beta diversity analyses were employed to differentiate the sampling groups. Post-treatment FEC reductions ranged from 30% to 99%, with combination treatments generally achieving the highest efficacy, though very few samples exceeded a 95% reduction, indicating widespread resistance. Pre-treatment analyses identified nine GIN species, with *Haemonchus contortus*, *Teladorsagia circumcincta*, and *Trichostrongylus vitrinus* being predominant. After treatment, profiles were dominated by *T. circumcincta* and, on certain farms, *Trichostrongylus colubriformis*, while *H. contortus* and *T. vitrinus* were largely diminished. Diversity analyses revealed statistically significant differences between pre- and post-treatment samples but not among treatment groups. Combining FECRTs with next-generation molecular analyses provides critical insights into the diversity and dynamics of anthelmintic-resistant GIN species. The observed differential resistance among species supports the need for tailoring anthelmintic regimens to individual farm conditions, thus offering a valuable resource for future research and improved parasite management strategies in sheep farming.

Metabarcoding of equine strongyle communities reveals unexpectedly frequent *Strongylus* spp. occurrence in treated horses

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Grazing equines usually harbor complex communities of Strongylidae (Nematoda) comprising multi-species infections. Currently, the cyathostomins are most prevalent, while the *Strongylus* species are only rarely detected. Strongyle eggs and, in most cases, infective larvae cannot be differentiated to species level, with the exception of *Strongylus* spp. To employ deep amplicon sequencing using the cytochrome *c* oxidase subunit I (COI) region for better equine intestinal strongyle species complex resolution compared to sequencing of internal

transcribed spacer 2 (ITS-2) regions. Nemabiome analyses were conducted using DNA extracted from third stage larvae, representing strongyle communities from regularly treated (RT) and never treated (NT) equine populations from Brazil, France (only RT), Germany, Ukraine, the UK, and the USA. Samples were predominantly from horses, but some were obtained from Przewalski's horses (Ukraine), donkeys (Germany, Ukraine) and kulans (Ukraine). Most sequence reads (87.7%) were identified to the species level, but unclassified reads occurred more frequently in donkeys and kulans than horses. No obvious difference in species diversity and richness was observed between RT and NT equines. However, there were significant differences in species composition between the RT and NT groups. While *Strongylus* spp. were significantly more abundant in the NT groups, in some farms their reads were high in RT groups. *Cylicocyclus nassatus*, *Cylicostephanus longibursatus,* and *Cyathostomum catinatum* were more abundant in the RT group. Our findings suggest that strongyle communities in domestic equines may have been shaped by anthelmintic treatments in the last decades. The decreased classification success for reads from non-caballine equines suggests that there are more strongyle species specific for this rarely-investigated group and that additional efforts are needed to improve the sequence database.

Analysis of the gastrointestinal nemabiome of wild and domestic canids from Costa Rica

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A wide variety of nematode species can infect domestic and wild canids such as coyotes, *Canis latrans*, due to their close evolutionary relationship, some of which are considered zoonotic. To molecularly characterize the gastrointestinal nematode communities in coyotes and dogs from Costa Rica by using microscopical and amplicon sequencing of the *cox*1. Scats from coyotes and fecal samples from free-roaming and in-house dogs were collected. Coprological analysis was conducted to all samples. Then, DNA was extracted, and coyote species identification was confirmed by RFLP and NGS of vertebrate 12S gene and genotyped by sequencing to avoid sampling bias. To determine nematode species, amplicon sequencing of *cox*1 was performed and obtained sequences were classified with GenBank and BOLD databases. Finally, the genetic diversity of the identified nematodes was phylogenetically analyzed. 149 dog and 135 coyote samples were collected from which 51 samples were successfully genotyped, identifying 31 coyotes. Thirty-nine percent (12/31) of coyotes and 30% (44/149) of dogs had at least one parasite in their feces. *Ancylostoma caninum* was the most frequent parasite found in both coyotes and dogs. In coyotes, other species such as *Toxascaris* sp., *Strongyloides* sp., *Taenia* sp., *Spirometra* sp.,

and *Alaria* sp. were detected. In dogs, *Toxocara canis* and *Spirocerca lupi* were also found at lower frequencies. Phylogenetic analyses of *A. caninum* indicated that the same haplotypes circulate in both coyotes and dogs in Costa Rica, suggesting that the parasite infects both canid species indiscriminately. This represents the first nemabiome analysis in carnivores, finding a high frequency of nematodes in coyotes and dogs from Costa Rica. Importantly, *A. caninum* was demonstrated be transmitted between dogs and coyotes, highlighting the role of wild canids as parasite reservoirs.

Deep amplicon sequencing and faecal egg count reduction tests reveal poor efficacy of anthelmintic treatments against gastrointestinal strongyle nematodes in bactrian and dromedarian camels in Germany due to presence of resistant *Haemonchus contortus* and *Trichostrongylus colubriformis*

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Strongyle parasites cause a considerable health risk and economic burden to livestock, including camelids. This study examined the strongyle communities of Old-World Camels (OWCs) in Germany and evaluated anthelmintic efficacy. Nine German OWC farms were visited in spring 2023. Treatments were planned by the farms/veterinarians. FLOTAC (multiplication factor 1) was used to determine the strongyle eggs per gram faeces (epg) in 100 OWCs (*Camelus bactrianus*: 86.0%; *Camelus dromedarius*: 6.5%; hybrids: 7.5%) before and after treatment. The faecal egg count reduction (FECR) was calculated by eggCounts and bayescount. Nemabiome analysis based on ITS-2 sequencing was used to determine prevalence and relative abundance of strongyles. The combination of nemabiome and FECRT data aimed to determine community structures of parasites in OWC and determine treatment efficacy. No scales were available on the majority of farms and weight was estimated. Egg shedding intensity/prevalence differed widely between farms. A significant decrease in FECR was found on 6/8 farms post anthelmintic treatment ($p < 0.05$, paired Wilcoxon test). The FECR was calculated for seven farms (range 26.6-90.8%) after treatment with albendazole, fenbendazole, ivermectin, moxidectin or doramectin indicating anthelmintic resistance. Only monepantel showed full efficacy (>99% FECR). The most abundant species were *Trichostrongylus colubriformis*, *Haemonchus contortus, Camelostrongylus mentulatus*, *Cooperia oncophora* and *Trichstrongylus axei*. Only *C. mentulatus* has not been detected in German domestic ruminants before. After deworming, *T. colubriformis* and *H. contortus* strongly dominated strongyle communities while *C. mentulatus* and *C. oncophora* were consistently eliminated. Anthelmintic treatment of German OWCs showed poor efficacy. Since treatment efficacy

was species dependent, not underdosing but resistant strongyle species probably lead to treatment failures.

Plenary Oral Communication

Vector-Borne Pathogens in Companion Animals: Global Perspectives and Transmission Dynamics

High seroprevalence of selected vector-borne pathogens in dogs from Saipan, Northern Mariana Islands

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Canine vector-borne diseases (CVBDs) are illnesses caused by pathogens transmitted by blood-feeding arthropods such as ticks and mosquitoes. Several CVBD, including dirofilariosis, anaplasmosis, and ehrlichiosis, are zoonotic, making epidemiological surveillance a joint veterinary and public health effort. In this study, we determined the seropositivity of four pathogens from dogs on Saipan, Northern Mariana Islands, a US Commonwealth located in the Pacific Ocean. Blood samples (n=443) were collected from client-owned, owner-surrendered, and shelter dogs that participated in an island-wide spay-and-neuter event in 2023. All samples were assessed using a point-of-care enzyme-linked immunosorbent assay, IDEXX SNAP® 4Dx® Plus test, to detect *Dirofilaria immitis* antigen and antibodies against *Ehrlichia* spp., *Anaplasma* spp., and *Borrelia burgdorferi* sensu lato. Risk factors were assessed for each pathogen through a univariate analysis, followed by a multivariable logistic regression. Overall, 66.1% (n=300/443) of the dogs tested positive for at least one pathogen, with the highest prevalence observed for *Ehrlichia* spp. (58.0%), followed by *Anaplasma* spp. (43.1%) and *D. immitis* (14.8%). Among the dogs with a single pathogen detected (30.9%) *Ehrlichia* spp. was most prevalent (64.9%), followed by *Anaplasma* spp. (23.3%) and *D. immitis* (11.6%; n=16/137). For co-detection of two or more pathogens (36.7%; n=163/443), *Ehrlichia* spp. + *Anaplasma* spp. presented the highest frequency (70.5%; n = 115/163), followed by *Ehrlichia* spp. + *D. immitis* (6.7%; n=11/163), *Anaplasma* spp. + *D. immitis* (3.6%; n=6/163), and *Ehrlichia* spp. + *Anaplasma* spp. + *D. immitis* (19.0%; n=31/163). We analyzed risk factors that could be associated with exposure to these CVBDs. This study shows high seropositivity for CVBDs in a dog population living in a poorly studied area. The results of this study suggest that control strategies for these CVBDs should be reinforced on the Island of Saipan.

Diversity of pathogens from ticks of dogs from two Caribbean islands

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Ticks and the pathogens they transmit are found globally. There is, however, limited information on the diversity of ticks and tick-borne pathogens of dogs in some Caribbean islands. This study aims to identify ticks and tick-borne pathogens from Trinidad and Tobago using a high-throughput real-time microfluidic PCR system. A total of 293 ticks were removed from 126 dogs and identified using morphological keys and PCR. Nucleic acids were extracted from individual ticks, and a high-throughput microfluidic real-time PCR system was used to screen for 49 bacterial species (10 genera), 18 protozoan species (six genera) and four tick species (two genera). Representative PCR products were selected to validate results using conventional PCR and sequencing. Two species of ticks were identified, including *Rhipicephalus sanguineus* s.l. (n=272) and *Amblyomma ovale* (n=21). Overall, the DNA of 12 pathogens belonging to eight genera (*Anaplasma, Babesia, Borrelia, Ehrlichia, Hepatozoon, Mycoplasma, Rickettsia* and *Theileria*) were detected in (46 of 293; 15.7%) ticks removed from dogs. The most prevalent pathogen was *Hepatozoon canis* (18 of 293, 6.1%), detected in *R. sanguineus* s.l. only. Co-infections with two (3 of 293; 1%) and three pathogens (2 of 293, 0.7%) were also detected, in both tick species. The high diversity of pathogens detected in this study, with some of veterinary and public health importance, highlights the strength of the high-throughput microfluidic real-time PCR system as a surveillance tool for the efficient and rapid detection of tick-borne pathogens in ticks in the Caribbean.

Feline vector-borne pathogens in Iran

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Feline vector-borne pathogens (FeVBPs) are common in tropical and subtropical countries, mainly due to favorable climate conditions for arthropod perpetuation coupled with limited preventive measures. However, data regarding the actual burden of these infections among cats is still scarce compared to dogs. The present study aimed to provide an overview of the prevalence of FeVBPs infections in Iran. From December 2018 to February 2023, a total of 848 cats of both sexes, different ages, and with ou-

tdoor lifestyle living in seven provinces of Iran were blood sampled and molecularly screened for *Hepatozoon* spp., *Babesia* spp., *Cytauxzoon* spp., *Dirofilaria* spp., and *Leishmania* spp. Overall, 5.4% of cats scored positive for at least one VBP, with *Hepatozoon* spp. being the most common (3.8%), followed by *Leishmania* spp. (2.5%) and *Dirofilaria immitis* (0.7%). The *Hepatozoon*positive cats lived in localities from the eastern, western, and central-northern regions; most of them (*n*=25) were infected by *Hepatozoon felis,* and the remaining (*n*=3) by *Hepatozoon canis*. *Leishmania* spp.-infected cats were detected from the east, center, and west of the country, while *D. immitis*-positive animals lived in central-north areas. To our knowledge, this is the first large-scale molecular epidemiology study of vector-borne pathogens in cats in Iran. The circulation of several VBPs, including those with zoonotic potential (*i.e.*, *D. immitis* and *Leishmania* spp.) highlights the importance of endo- and ectoparasite control measures in owned cats and suggests that controlling the population of feral animals (*e.g.*, through spaying and neutering campaigns) would contribute to reducing the risk of transmission of VBPs.

Vectors and vector-borne pathogens of companion animals in Greece.

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Vector-borne pathogens (VBPs) pose a growing global threat, presenting unprecedented challenges for veterinarians, acutely in endemic regions. Greece, located in the Mediterranean basin, offers favorable environmental conditions for pathogen transmission, compounded by the poor management of stray dog and cat populations. Prior studies reported Greece as endemic for various VBPs, primarily based on serological analyses. This study aimed to estimate the presence of VBPs in dog and cat populations across diverse environmental conditions, utilizing molecular analyses to identify circulating species. Blood samples collected from 1,224 owned dogs and 378 household cats habiting across the country. Ectoparasites present on the animals were also collected. All animal samples were molecularly analysed for the presence of *Ehrlichia* spp. (16S rRNA), *Anaplasma platys* (16S rRNA), *A. phagocytophilum* (16S rRNA), *Bartonella henselae* (gltA), *Babesia* spp. (18S rRNA) and *Hepatozoon* spp. (18S rRNA). Cat samples were also analysed with qPCR for the presence of *Leishmania infantum* (kDNA). Sequencing analyses revealed the presence of *E. canis* in 2.3%, *A. platys* in 1.4%, *H. canis* in 6.1%, *B. canis vogeli* in 0.4% and *B. gibsoni* in 0.2% of dog samples. In cats, *A. platys* was identified in 0.5%, *H. felis* in 25.6%, *Bartonella* spp. in 0.3% and *Babesia* spp. in 0.3%. *L. infantum* was detected in 2.9% of cat blood samples, with parasitic load up to 5,888 genome copies/ml. Most ticks collected from both animals belonged to the *Rhipicephalus sanguineus* s.l. group, while fleas predominantly collected from cats identified as *Ctenocephalides felis*. Molecular analysis of ticks/fleas is ongoing. The results indicated significant regional differences in VBP prevalence, confirming the presence of specific pathogens across all areas. Overall, our findings provide a comprehensive epidemiological framework tailored to each region, serving as a valuable tool for designing preventive treatment schemes for pets.

Occurrence of Ectoparasites and Vector--Borne diseases in Dogs and Cats in Various Regions of Brazil.

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Brazil, the largest and most populous country in South America, holds the third placein the world interms of owned dog and cat population,according to the Brazilian Institute of Geography and Statistics. Despite the benefits of human--animal interaction, the transmission of zoonoses still represents an often-neglected risk, as pets can serve as reservoirs for pathogens. This study surveyed the occurrence of ectoparasites in the pet dog and catpopulations,in various locations in Brazil. Between September2023 and November2024, a total of 1,837 healthy owned dogs and cats living in urban and sub-urban environment were recruited during a veterinary consultation from 10 veterinary centers in Brazil. All animals were examined for ectoparasites by physical examination of body surface, skin scrapings, scotch test, and ear wax examination. Dogs were also screened for *Dirofilaria immitis,Anaplasma phagocytophilum/A. platys*, *Borrelia burgdorferi*, *Ehrlichia canis/E. ewingii*and *Leishmania*spp., through SNAP tests. In cats, the SNAPFeline Triple was used to detect *D. immitis*, Feline leukemia virus and Feline immunodeficiency virus. 12% of dogs and 8.6% of cats were infested by fleas, while 5.7% of dogs and only one cat showed tick infestations. Other ectoparasites identified in dogs were: *Demodex canis*, *Sarcoptes scabiei*, *Otodectes cynotis*, and *Trichodectes canis*. In cats, *Lynxacarus* spp., *Felicola subrostratus*, *O. cynotis*, *Demodex cati*, and *S. scabiei*were found. 25.3% and 7.2% of dogs were found *Ehrlichia* spp. or *Anaplasma* spp. positive, respectively. 3.6% were positive for *D. immitis*and 3.1% for *Leishmania* spp.. One dog was positive for *Borrelia burgdorferi*. In cats, 0.23% tested positive for *D. immitis*. Although FIV and FeLV infections are not vector-borne diseases, it is worth mentioning that 6% and 6.7% of cats were positive, respectively. The findings highlight the importance of ectoparasite controlto protect the health and welfare of pets,and public health.

Assessment of the speed of transmission of *Ehrlichia canis*, *Anaplasma phagocytophilum*, and *Borrelia burgdorferi* sensu stricto by infected ticks through an in vitro experimental system

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Canine vector-borne diseases (CVBDs) have significant clinical and public health implications. This experimental study used an adapted version of the USDA-developed continuous flow in vitro feeding system (CFIFS) for ticks to investigate the speed of transmission (SOT) of three tick-borne pathogens (TBPs): *Ehrlichia canis* by laboratory reared infected *Rhipicephalus sanguineus* (18.3% tick infection rate), *Anaplasma phagocytophilum* by laboratory reared infected *Ixodes ricinus* (56%), and *Borrelia burgdorferi* sensu stricto by laboratory reared infected *I. ricinus* (76%). All ticks began to attach and feed three hours after being introduced in the feeding system. PCR tests were used to detect the presence of pathogens in the blood flow collected every three hours. Swab samples from the inner face of the feeding membrane were also collected and tested every six hours during the *B. burgdorferi* study. In this experimental in vitro design, *Ehrlichia canis* had a SOT of 6-9h, *A. phagocytophilum* of 15-18h, and *B. burgdorferi* of 45-48h in blood but only 3-6h on inner membrane swabs. The early detection of *Borrelia* spirochetes on the membrane indicates a delay in their bloodstream entry. This in vitro system allows to test and compare many tick pathogen transmission pathways. The findings of this study highlight the transmission time of tick-borne pathogens, emphasizing the the possible difficulty to prevent pathogen with high speed of transmission like *Ehrlichia* and *Anaplasma* using acaricides.

Poster Presentation
Day 18

Poster Presentation - Day 18

Drug resistance and drug resistance mechanisms

Dynamics of benzimidazole resistance in a field-selected isolate of *Haemonchus contortus*

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Single nucleotide polymorphisms in beta-tubulin isotype-1 at codons 134,167,198, & 200 are associated with benzimidazole (BZ)resistance in strongylid nematodes. However, multiple lines of evidence suggest additional mechanisms exist. Examine the impact of field selection with a BZ drug on drug susceptibility, beta-tubulin allele frequency, and haplotype diversity in *Haemonchus contortus.* 20 goats infected with a drug-susceptible isolate of *H. contortus* were assigned to treatment (T; n=14) or control (C; n=6), and grazed separately. Group T received 10 subtherapeutic fenbendazole doses at 4–8-week intervals, which were chosen to yield a reduction in fecal egg count (FEC) of approximately 50% (0.25–0.75 mg/kg). Pre- and post-treatment samples were collected for FEC, egg hatch (EHA) and larval development assays (LDA), and genetic analysis. Group C was sampled accordingly. Mean efficacy of the 10 subtherapeutic treatments was 53.7%. Resistance was confirmed by a final full-dose treatment (5 mg/kg), yielding an efficacy of 58.8% (90% CI; 24.8-83.7). IC_{50} shifted >18-fold and >5-fold in the EHA and LDA, respectively. The F200Y beta-tubulin mutation was selected, increasing from 1.9% to fixation (100%). A strong quadratic relationship was observed between EHA/LDA IC_{50} and F200Y frequency (R^2=64%/89%). High beta-tubulin haplotype diversity (n=14) was maintained throughout the study, but only 3 resistant haplotypes survived full-dose treatment. Our drug-selection strategy using subtherapeutic dosages with natural reinfection succeeded in maintaining high haplotype diversity, and progressively selected for resistance. Notably, F200Y fixation occurred at relatively low IC_{50}, suggesting additional mechanisms contribute to high-level resistance. Whole-genome sequencing of archived samples is being performed to identify additional loci under selection, providing further insights into the genetic basis of BZ resistance.

Genomic and transcriptomic analysis of long-term evolutionary ivermectin resistance in the parasitic nematode model *Caenorhabditis elegans*

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Caenorhabditis elegans serves as a key model organism for studying parasitic nematodes, particularly in the context of antiparasitic drugs. It is widely used in understanding molecular mechanisms underlying current and new treatments and identifying novel drug targets. As a free-living nematode, it is especially well-suited for experimental evolution due to its

easy maintenance. To investigate the effects of population size on ivermectin (IVM) resistance evolution and identify genes involved in resistance. Twelve populations each of 2000, 1000, and 200 worms were exposed to stepwise increasing IVM concentrations over 40 generations. Both ancestral and evolved resistant populations were analyzed using genomic and transcriptomic approaches. Preliminary genomic analysis revealed difference in SNP and INDEL profiles between ancestral and evolved resistant populations. Transcriptomic analysis showed distinct patterns of gene expression between these groups. First, analysis of the control populations to the ancestor found that there was the largest difference in gene expression for the smallest population. Second, there was dissimilarity of gene expression between the resistant and control populations, which decreases as the population size decreases. Thirdly, the gene expression overall is similar for the different population sizes when exposed to the same IVM concentration but there were small discriminatory differences. Lastly, the concentration of IVM in the environment dictated how strongly some genes were expressed. C-type lectins, detoxification and pathogen stress response, and general stress response pathways were significantly up- and down-regulated. Population size influences the rate of ivermectin resistance during the evolution experiment, shaping transcriptomic responses to varying IVM concentrations and extending beyond resistance mechanisms to include natural defenses, metabolism and structural pathways.

Efficacy of ivermectin against gastrointestinal strongylid nematodes in cattle from a farm in the Cajamarca valley, Peru

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Ivermectin (IVM) is a widely used endectocide for cattle globally and has been employed for decades. However, its extensive and repeated use has resulted in the emergence of anthelmintic resistance in various regions. This study aimed to evaluate the efficacy of IVM in treating gastrointestinal strongylid nematodes (GISN) in cattle from a farm located in the Cajamarca district, Cajamarca region, Peru. Twenty Fleckvieh cattle (aged three to 12 months, both sexes, ≥100 EPG [eggs per gram]), raised under a grazing system, were selected (Huacaríz Farm). These animals had not received any anthelmintic treatment for at least three months before the study and had a natural infection with GISN. Ten animals were assigned to the untreated control group, while the remaining ten received IVM. Fecal samples were collected directly from the rectum. On day 0, parasitological analyses were performed to determine baseline parasite load and to assign animals to homogeneous groups based on EPG levels, followed by IVM administration (0.2 mg/kg BW, SC). Efficacy was assessed 14-, 28-, and 42-days post-treatment via the Fecal Egg Count Reduction Test (WAAVP guidelines), using the modified McMaster technique (INTA chamber) and larval cultures (Roberts and O'Sullivan) for nematode recovered (Baermann method) and genus identification (Keith's keys). The efficacy of IVM (95% CI) at the three post-treatment time points was 66.89% (64.49–69.29%), 65.82% (63.48–68.16%), and 64.64% (62.44–66.84%),

respectively. At the genus level, IVM demonstrated 100% efficacy against *Trichostrongylus*/*Oesophagostomum* while showing 0% efficacy against *Ostertagia*/*Haemonchus*. Although IVM was effective against two genera, its ineffectiveness against the others highlights the urgent need for pre-treatment larval cultures to inform antiparasitic selection and the necessity for alternative parasite control strategies to improve herd health management.

Genome-wide analysis of *Teladorsagia circumcincta* from different treatment regimes reveals stronger selection for ivermectin resistance in frequently treated lambs

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Teladorsagia circumcincta is the predominant and the most commonly resistant gastrointestinal nematode species infecting sheep in temperate regions including the UK. *T. circumcincta* infection in sheep results in significant production losses for farmers and highly compromises the health of infected animals. Controlling these infections relies on anthelmintics such as ivermectin (a macrocyclic lactone). As with other anthelmintics, intensive use of ivermectin has led to widespread resistance with over 50% of sheep farms reporting resistance in the UK. Many studies have been carried out to try to understand ivermectin resistance, with a recent study identifying a region under selection on Chromosome 5 in different populations. However, limited information is currently available on the causal variants linked to ivermectin resistance in this region. This study investigated evidence of selection by ivermectin treatment using a genomewide approach. The samples used were from a six-year field trial following implementation of two different ivermectin drenching regimes: neo-suppressive treatment (NST), where the entire flock was drenched every four weeks and targeted selective treatment (TST) where drenching was done on individual lambs based on their ability to attain required weight as per the Happy Factor™ tool. In the trial, lambs were grazed on replicate paddocks assigned to different treatment regimes. Strongyle eggs collected bi-weekly from individual lambs were pooled by treatment regime and hatched to the first larval stage (L1). DNA was extracted from the L1 pools followed by whole-genome sequencing. Genetic analysis of nucleotide diversity of *T. circumcincta* populations in both treatment regimes showed they were similar, suggesting that ivermectin resistance has evolved over time with resistance alleles being on diverse haplotypes. However, FST comparisons between the two treatment regimes consistently highlighted the highest degree of differentiation on Chromosome 5 between ~25 - 50Mb. Genome-wide analysis of *T. circumcincta* showed evidence of stronger selection in the NST treatment group, reflecting its rapid reduction in ivermectin efficacy as compared to TST group that showed weaker selection for anthelmintic resistance.

Detection of three independent benzimidazole resistance mutations in a single *Ancylostoma caninum* population from a Brazilian breeding kennel

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Anthelmintic resistance is a growing concern in the canine hookworm *Ancylostoma caninum*. Two SNPs conferring benzimidazole resistance (Q134H and F167Y) are prevalent in North America. However, the F200Y SNP has rarely been observed. This study has genetically characterized a single Brazilian *A. caninum* isolates that contained three different canonical benzimidazole resistance SNPs: Q134H, F167Y, and F200Y. A fecal sample from a Golden Retriever breeding kennel in São Paulo, Brazil, was tested using the KeyScreen® GI Parasite PCR Test. Allele-specific qPCR assays were followed by both Illumina and Oxford Nanopore amplicon sequencing to confirm and quantify resistance SNPs in the isotype-1 β-tubulin gene. qPCR detected Q134H, F167Y, and F200YSNPs Illumina and Nanopore sequencing confirmed their presence and showed the three SNPs were present on separate haplotypes, indicating genetic independence. Nanopore sequencing of the full-length gene identified two additional non-synonymous mutations (S37N and V368A), but their functional relevance remains unclear. This is the first report of three independent benzimidazole resistance mutations in a single *A. caninum* population and shows the value of Nanopore amplicon sequencing to confirm qPCR results. They also highlight the high drug selection pressure in breeding kennel environments and the need for molecular surveillance to monitor anthelmintic-resistant hookworm emergence.

A selected lipid-metabolite profiling secreted by *Haemonchus contortus* may depend on nematode ABC transporters activity for its extrusion

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The secretion of lipids by parasitic nematodes as part of the parasite-derived biomolecules (PBDM), is an incipient subject in “multi-omics” research in parasitology. Several lipid-based signalling molecules have been described to play roles in host-parasite interactions and in nematode physiology. In multicellular organisms such as parasitic nematodes, lipids may be exported through several mechanisms, including the energy-dependent function displayed by the ABC transporters. These efflux pumps have been localized in the plasma-membrane of mammalian cells, and they have been linked to anthelmintic resistance (AR) in veterinary nematodes, including *Haemonchus contortus*, particularly to the lipophilic macrocyclic lactone (MLs) anthelmintics. Our aim was to investigate the role of nematode ABC transporters in the lipids profile secreted by *H. contortus*. We conducted an ex-vivo culture of adult worms from *H. contortus* with different ABC transporter inhibitors. Furthermore, we collected the media at different time-points and carried an untargeted-global lipidomics analysis. Among the lipids with significant fold change (FC) affected by ABC transporter inhibitors in the media were found Sterols, Lysophosphatidic acids, Lysophosphatidylinositols, Prenols, Saccharolipids, N-acyl amides, Sulfatides and Polyketide secondary metabolites. In addition, the expression profile of *H. contortus* ABC transporter genes tended to increase depending on the inhibitor applied, suggesting a transcriptional plasticity to adapt their efflux capacity. Our results indicate that several lipids with a signifi-

cant FC reduction have structural features that make them suitable substrates for nematode transporters translocation. Overall, our findings provide evidence about the involvement of ABC transporters to extrude a selected panel of lipids out of *H. contortus*, participating on the PBDM releasing mechanism in nematodes, with potential implications in AR resistance.

Detection of benzimidazole resistance on horse farms using *in vitro* methods

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Cases of anthelmintic resistance (AR) in equine nematodes are currently increasing and becoming significant worldwide. The aims of the study were to determine the occurrence of benzimidazole (BZ) resistance in strongylid nematodes on horse farms in Slovakia using *in vitro* tests with analysis of associated risk factors and to optimise *in vitro* methods by identifying the indicators of early resistance detection. Faecal samples from 77 horses from 8 farms were selected to perform the *in vitro* egg hatch test (EHT) and the larval development test (LDT). The hatching of strongyle eggs in threshold concentration 0.1 µg/ml thiabendazole (TBZ) in the EHT has been monitored. The number of infectious third-stage larvae (L3) was assessed for each TBZ concentration in the LDT. The infectious L3 isolated from the highest TBZ concentrations were differentiated according morphological features. The hatching of strongyle eggs in the EHT at 0.1 µg/ml TBZ indicated the presence of resistant nematodes in all farms. The percentage of hatched eggs ranged from 8.5% to 91.0%. Random forest algorithm of the EHT results showed that egg hatching at 0.1 µg/ml TBZ was the most important predictor of BZ resistance detection. In the LDT, only cyathostomin L3 were found at highest TBZ concentrations (0.08–1.28 µg/ml TBZ) in 81.8% of horses. A comparison of LDT results did not show a statistically significant agreement with EHT. The monitoring of hatching at selected concentrations in the *in vitro* EHT could estimate proportion of resistant strongyle population on horse farms and could serve as an indicator for early detection of BZ resistance. The results of LDT showed that test was not suitable for BZ resistance detection.

Prevalence and Drug Resistance Profiles of Canine African Trypanosomosis in Nigeria

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Canine African trypanosomosis (CAT) is an endemic protozoan disease of significant economic and public health importance in sub-Saharan Africa. Chemotherapy remains the widely used method of CAT control, although emerging reports of drug resistance are concerning. In Nigeria, the prevalence of CAT and drug-resistant canine trypanosomes (CT) is underreported. This study was conducted to determine the prevalence of CT in Nigeria and assess their sensitivity to trypanocides. A total of 300 dogs were randomly sampled in Udenu and Igbo-Eze Local Government Areas (LGA) and screened for trypanosomes, using standard parasitolo-

gical and molecular (ITS-PCR, TgsGP-PCR, and DNA sequencing) methods. Haematological parameters, including packed cell volume (PCV), total leucocyte count (TLC), and red blood cell (RBC) count, were also determined. Trypanosome-positive samples were subjected to single- and multi-dose tests to evaluate their sensitivity to diminazene aceturate (DA) and isometamidium chloride (IC). Clones were derived from some of the multidrug-resistant CT isolates and further assessed. The CAT prevalence in Udenu and Igbo-Eze LGAs were 12.7% and 6%, respectively, with an overall prevalence of 9.3% (28/300 dogs). The CT species identified were *Trypanosoma brucei, T. evansi,* and T. *congolense*, with *T. brucei* predominating. Infected dogs had significantly lower PCV, TLC, and RBC counts ($p < 0.0001$) than uninfected dogs. Drug-sensitivity studies revealed a 4.7% and 3.3% prevalence of drug-resistant CT in Udenu and Igbo-Eze LGAs, respectively, with an overall 4% prevalence. Among the drug-resistant isolates, 58.3%, 16.7%, and 25% were resistant to DA, ISM, and both trypanocides, respectively, with CD_{50} ranging between 15.43 and 34.19 mg/kg. Clonal isolates exhibited high drugs (CD_{50} = 35.19 and 37.19 mg/kg). In conclusion, CAT and drug-resistant CT are endemic in Udenu and Igbo-Eze LGAs, Nigeria, underscoring the need for an integrated trypanosomosis control strategy.

Unravelling resistance: Transcriptomic insights into *Cooperia oncophora* resistance to macrocyclic lactones

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Macrocyclic lactone (ML) resistance in gastrointestinal nematodes (GIN)poses a significant challenge to livestock health and productivity. *C. oncophora* is among the most prevalent ML-resistant GIN in cattle; however, studies have primarily focused on a limited set of candidate genes. This study aimed to elucidate ML resistance mechanisms in *C. oncophora* by integrating gene expression profiling and SNP discovery. We compared two *C. oncophora* isolates: a laboratory-maintained susceptible strain (ColvSus) and a resistant field isolate (ColVR08) under four conditions: ColvSus non-exposed, ColVR08 non-exposed, ColVR08 exposed to ivermectin, and ColVR08 exposed to moxidectin. Following de novo assembly, differentially expressed genes (DEGs) were identified using edgeR (FDR< 0.05, fold-change ≤ 0.5 and fold-change ≥ 2) and automatically annotated. We further conducted a manual analysis of two gene families implicated in ML response: ligand-gated anion channels and ATP-binding cassette (ABC) transporters. Additionally, SNPs were identified using pooled transcriptomic reads. The de novo assembled transcriptome consisted of 44,509 transcripts with 89.4% completeness (BUSCO). Among the top ten DEGs, secretory-excretory protein and cuticle collagens genes were the most frequently downregulated in ColvR08 compared to ColvSus. The glc-6 anion channel was constitutively downregulated in resistant isolates, whereas ABC transporter genes abt-2b, wht-5b1, and abt-8 were significantly upregulated. SNP analysis revealed five common SNPs, including polymorphisms at madd-2, cdkl-1, and nas-5, genes associated with axon guidance, neural development, and pharyngeal function. Our findings suggest that ML resistance in *C. oncophora* involves multiple mechanisms, including altered neurotransmission, enhanced

efflux, and structural adaptations. These insights advance our understanding of resistance mechanisms in this parasite.

Proven effectiveness of doramectin in a horse breeding farm with history of fenbendazole resistance

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Currently, Anthelmintic Resistance (AHR) in parasitic populations of horses represents a major threat due to the frequent and indiscriminate use of anthelmintics (AH), which compromises treatment effectiveness and equine health. Benzimidazoles (BDZ) and Macrocyclic Lactones (LM) were the first drugs utilized due to their broad spectrum of action and high therapeutic index. However, in recent years, cases of RAH to these molecules have been reported globally, including Mexico, therefore, new molecules have emerged. The objective of this study was to evaluate Doramectin (DOM) to demonstrate its AH efficacy using the Fecal Egg Count Reduction Test (FECRT).The research was conducted in a Warmblood horse breeding farm located in Avandaro, State of Mexico. From an initial sampling of 300 horses, 40 horses were selected. These equines had a parasite burden of ≥150 EPG and had not been treated with AH in the last 60 days. The animals were randomly distributed in 20 each per group: Control Group (CG) and Treated Group (TG) with DOM at a dose of 200 mcg/kg. The Microsoft Excel program RESO.exe© was used for data evaluation. Parasite burden was determined using the modified McMaster technique. Prior to the administration of the treatment, a larval culture was performed for the identification of the parasite species found, where the presence of larvae corresponding to cyathostomine species was identified. An average parasite burden of 1,020 EPG pre-treatment and 0 EPG post-treatment was obtained. The result of the percentage of egg reduction was 100%. LM are effective in infections caused by strongylids, however, they must be used with specific criteria taking into account the location and stage of the parasites, including hypobiosis in the case of cyathostomins. This study promotes good Integrated Parasite Management practices by evaluating a newly introduced AH molecule through the *FECRT* test for preventing the development of RAH and maintain its effectiveness.

Evaluation of anthelmintic effectiveness in a English Thoroughbred horses in the State of Mexico

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A wide range of gastrointestinal helminths have been observed in horses, with strongyle nematodes being particularly prevalent. The indiscriminate and prolonged use of anthelmintics (AH) has favored the development of resistance, posing a challenge to parasite control. Therefore, it is essential to evaluate the efficacy of AH by the Fecal Egg Count Reduction Test (FECRT). The objective of the present study was to evaluate the efficacy of three AH in a Thoroughbred horse farm naturally infected

with strongylids, using the FECRT. The study was conducted in the State of Mexico. Parasite burden was assessed in a population of 150 equids using the McMaster technique. Eighty horses were selected that met the inclusion criteria: age > 3 months and parasite burden > 150 HPG. Four groups of 20 animals each were then formed. Group A received oxibendazole (OXB) 10 mg/kg, group B ivermectin (IV) 0.2 mg/kg, group C moxidectin (MX) 400 µg/kg and the control group. For the post-treatment evaluation, samples were collected at two time points: seven and 15 days after treatment. The decision on the time of sample collection was made by the AH. In addition, the larval cultures of each group were processed for larval identification before and after treatment. The percentage of effectiveness was determined by employing the Microsoft Excel©RESO.exe© program. The effectiveness of IV and MX was demonstrated to be 100%, while OXB exhibited a value of 58%. Pre- and post-treatment larval identifications revealed a 100% prevalence of Cyathostominae species. The study promotes sustainable equine parasite management by detecting AH resistance and reducing unnecessary drug use through responsible management.

Therapeutic failure of ivermectin and high efficacy of albendazole, against *Lamanema chavezi* in naturally infected llamas (*Llama glama*) in The Peruvian Central highlands

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The raising of llamas (*Llama glama*) has constituted a major economic activity for the Peruvian highlands communities for many centuries. The intestinal nematode *Lamanema chavezi* shows high prevalences of infection in llama herds all across the Andean region, causing liver condemnation at slaughterhouses and subclinical disease. The frequent use of anthelmintic drugs in domestic camelids has led to the rise of anthelmintic resistance. There is a scarcity of reports regarding the efficacy of different drug classes against *L. chavezi* in Peruvian llama farms. In order to evaluate the efficacy of albendazole; and ivermectin, against *L. chavezi* in llama herds from Pasco, at the Peruvian Central highlands, naturally infected animals were treated, orally with albendazole (n=20), or, subcutaneously, with ivermectin (n=18). Fecal samples were obtained at the treatment day (day 0) and 10 days post treatment. The Mini FLOTAC test was used to determine the fecal egg count (FEC). The therapeutic efficacy was determined following the WAAVP guidelines by measuring reduction in FEC, using the R package egg Counts (Wang *et al.*, 2018). A bootstrapping method was used to obtain the 95% confidence intervals (95%CI). Our results show therapeutic failure for ivermectin (69.80 and 95%CI of 30 to 86%) and a high efficacy for albendazole (97.9, with 95% of 95 to 99). These results indicate the need for constant monitoring of drug efficacy against *L. chavezi* to ensure the success of the control programs in llama herds at the Peruvian highlands.

The status of synthetic pyrethroid, organophosphate and macrocyclic lactone resistance in buffalo flies carried by Australian beef cattle.

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Buffalo flies cause considerable economic losses in the Australian cattle industry through decreased productivity, weight loss, and an increased susceptibility to other diseases. On properties where buffalo flies are present in high numbers, chemical treatments are essential in their control. Resistance to synthetic pyrethroids has already been reported and it is emerging for organophosphates. Consequently, there is a need for further research into insecticide resistance status within this species. In this study, the degree and extent of insecticide resistance within sampled populations of buffalo flies was conducted over summer seasons of 2020 to 2023. A graded bioassay was used to assess fly mortality and derive LC50 values for the insecticides abamectin (macrocyclic lactone), diazinon (organophosphate), and zeta-cypermethrin (synthetic pyrethroid). The results indicated high levels of resistance to synthetic pyrethroids in populations sampled in NSW and QLD. Organophosphate resistance has reached a critical point for reduced efficacy in most grazing areas. This study also suggests that resistance to macrocyclic lactones is emerging. The findings provide insight into the current resistance status of populations across different regions of Australia, and this is crucial for developing effective strategies to manage the growing threat of resistance. There are methodological limitations in data collection due to the variability introduced by field conditions and the absence of a true susceptible reference population. The results of this study are best suited to generate hypotheses and guide the design of future, prospective studies on buffalo fly resistance.

Genetics and genomics

Molecular detection of Rdl mutation in field strains of Ctenocephalides felis felis from the metropolitan area of Rio de Janeiro

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The cat flea (*Ctenocephalides felis felis*) is the most important flea in veterinary medicine due to the diseases it can transmit. Various methods are used to control these pests, with chemicals being the main one. Fipronil is an insecticide commonly used on cats and dogs, acting on GABA-gated chloride channels. Nevertheless, the preventive use of insecticides has been associated with the emergence of tolerance to previously lethal doses. The aim of this study was identify possible mutations in the gene *Rdl* (*Resistance to Dieldrin*), which may be associated to cross-resistance to Fipronil. The research was performed at the Federal Rural University of Rio de Janeiro, in the Laboratory of Experimental Chemotherapy in Veterinary Parasitology (LQEPV), located in the municipally of Seropédica, state of Rio de Janeiro, Brazil. Over the

course of one year, adult *C. felis felis* specimens were obtained from the Parasitology diagnostic sector at LQEPV. All fleas were collected from cats or dogs in the metropolitan area of Rio de Janeiro. A total of 120 fleas from 60 animals (2 fleas per animal) were analyzed. Genomic DNA was extracted using phenol-chloroform protocol. Detection of mutation in the *Rdl* gene was performed by PCR-RFLP. Exon 7 region was amplified using the forward primer Ex7F and the reverse primer Exon7R. After amplification, the restriction enzyme *BsmAI* was used to digest the amplicons, allowing identification the alleles based on fragment sizes. Fleas were classified as susceptible (SS), resistant (RR) or heterozygous (RS). From the 120 fleas analyzed, 110 were collected from dogs and 10 from cats. Among the dog-derived fleas, 90,90% (100/110) contained RR genotype, 4,54% (5/110) were SS and 4,54 (5/110) were RS. For cat-derived fleas, 90% (9/10) were RR and 10% (1/10) were RS. The predominance of the RR genotype suggests that flea populations may by under constant selective pressure from Fipronil exposure.

Maximizing reads utilization for transcriptome analysis in *Haemonchus contortus*

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Haemonchus contortus is an important parasite for small ruminants and its genetic diversity can directly influence its anthelmintic resistance status. In this study we performed a *de novo* assembly of an *H. contortus* transcriptome, enabling future analyses of changes in the gene expression profile between different isolates. Three studies involving experimental infections were conducted to obtain adult parasites. In the first, three groups of sheep were infected with isolates sensitive (1 group) and resistant to ivermectin (2 groups, one exposed to ivermectin). The second followed the same design but using benzimidazole sensitive and resistant isolates, goats as hosts and albendazole. In the third, 25 goats were infected with mixed infection larvae and phenotyped for resilience to parasitism. Total RNA was extracted from three pools of male *H. contortus* from each euthanized animal in each experimental group resulting in 80 samples. Samples were sequenced using both long (Iso-Seq) and short read (RNA-Seq) strategies. *De novo* transcriptome assembly was performed using the Trinity tool (v2.15.1) and evaluated with rnaQUAST tool (v2.3). The assembly generated a total of 1.000.382 transcripts, with a GC content of 43.4%. Alignment of the assembled transcripts to the *H. contortus* genome showed that 904.914 (90%) transcripts were aligned and of these, 95% were uniquely aligned to the genome. The average alignment length was 605 bp. RNA-Seq reads were mapped to both the reference genome and the assembly. The overall alignment to the genome showed a rate of 58.79%, with 43.15% of the reads concordantly and uniquely aligned. The alignment to the *de novo* assembled transcriptome showed an overall rate of 88.32%, with 74.35% of the reads aligned in a multiple concordance manner, indicating high representation of the reads in the assembly. These assemblies will be combined in order to capture as much gene expression information as possible at the differential expression analysis stage.

Genomic insights into *Leishmania infantum* isolates from naturally infected dogs and cats

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To investigate the genomic diversity of *Leishmania infantum* infecting dogs and cats in endemic areas of São Paulo, Brazil, isolates from both hosts were expanded, and whole genome sequencing (WGS) was performed using the Illumina® platform. Trimmed reads were mapped to the *L. infantum* reference genome, followed by single nucleotide polymorphism (SNP) identification. High-quality genomic data were obtained from five isolates (two from dogs and three from cats). The reads aligned well (>93%) with the reference genome, providing sufficient depth for SNP calling. The SNP analysis identified 821 unique variants, with 58.2% exclusive to feline isolates and 8.2% exclusive to canine isolates. Canine isolates from Ilha Solteira (C2 and C28) exhibited high genetic similarity, suggesting low intra-population variability. In contrast, feline isolates (G19, G35, G36) displayed greater genetic divergence, particularly G35, from a recently endemic area (Votorantim), possibly reflecting adaptive responses to local environmental pressures. Principal component analysis (PCA) confirmed host-specific clustering, with feline isolates clearly separated from canine ones. Notably, G35 exhibited a higher proportion of heterozygous SNPs, which may indicate a parasite population still adapting to a newly established transmission cycle. Variant Effect Prediction (VEP) identified high-impact mutations in cat-derived isolates, including variants predicted to introduce premature stop codons. Functional analysis of genes carrying these mutations revealed associations with biological processes such as pathogenicity and drug resistance. The observed genetic diversity among isolates appears to be influenced by host species, geographic location, and the year of isolation. This study provides genomic data on feline *L. infantum* isolates in Brazil, highlighting host-specific genomic diversity and reinforcing the importance of including cats in molecular epidemiology and surveillance strategies.

Metagenomics of the tropical bont tick *Amblyomma variegatum* and co-evolution implications of its endosymbiont *Rickettsia africae*

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The tropical bont tick *Amblyomma variegatum* which is widespread in Africa and the Caribean Islands is of both medical and veterinary importance as the principal vector of intracellular bacterial pathogens *Ehrlichia ruminantium* and *Rickettsia africae*. Tick endosymbionts such as *Rickettsia* species are involved in the synthesis of components of certain essential pathways in the host tick such as vitamin B synthesis, tick metabolism, and fitness. However, the *A. variegatum* complete mitochondrial genome (mitogenome) has not been reported. The lack of a mitochondrial genome hampers studies on its evolution, phylogeography, systematics, and phylogenetic analysis. Hypothesizing that *A. variegatum* and the endosymbiont *R. africae* co-evolve is a significant and scientifically intriguing proposition. To assess our hypothesis, we conducted complete mitogenome sequencing of 41 *A. variegatum* ticks that were sourced from cattle in Malawi and Uganda. Multi-locus sequence typing (MLST), of six housekeeping genes of *R. africae* was also carried out. Further, the co-evolutionary relationship between *A. variegatum* and the endosymbiont *R. africae* was also investigated. The complete mitogenome of *A. variegatum* was 14630 to 14651 bp long. There was geographical population sub-structing in the mitogenomes of *A. variegatum*. The prevalence of *R. africae* in the examined ticks was 100%. The tanglegram showed non-strict co-cladogenesis between *A. variegatum* and *R. africae*. Furthermore, the Procrustes Application to Cophylogenetic (PACo) analysis and residuals of host-parasite associations showed no statistically significant association between *A. variegatum* and *R. africae* samples. This observation supports both the vertical and horizontal transmission of the endosymbiont *R. africae* in *A. variegatum*. This study was designed to understand how pathogenic/endosymbiotic bacterium *R. africae* has evolved with its host tick *A. variegatum* using the mitogenomic approach.

Adapt or Die: Conditional Screens Reveal Essentiality, Plasticity, and Parasite Persistence

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Apicomplexan parasites, including *Toxoplasma gondii* and *Plasmodium falciparum*, rely on a suite of specialized adaptations to invade, replicate within, and exit host cells. These processes are orchestrated by parasite-specific molecular machinery, much of which remains functionally undefined due to the deep evolutionary distance from model organisms. Comparative genomics offers limited insight, and although CRISPR/Cas9-based approaches have enabled some functional annotation, assigning precise roles to many genes remains challenging. To overcome these limitations, we developed a conditional splitCas9 system in *T. gondii*, enabling stage-specific and temporally controlled gene disruption. This system allows scalable pheno-

typic screens while distinguishing between early essential genes, late essential genes, and those whose deletion parasites can adapt to over time. Using a reporter strain tracking F-actin dynamics and apicoplast segregation, we screened 320 candidate genes for functions in the asexual life cycle. We identified and characterized two critical regulators: signalling linking factor (SLF), required for early induction of egress, and conoid gliding protein (CGP), essential for motility and parasite exit. Conditional knockouts generated using the DiCre system confirmed their distinct roles in temporal control of egress. The power of splitCas9 to conditionally regulate gene deletion across defined developmental windows unlocks the potential for genome-wide dissection of gene essentiality in Apicomplexans—differentiating stage-specific functions and uncovering adaptive mechanisms. This platform not only accelerates functional annotation in deeply divergent parasites but also provides a framework for identifying novel therapeutic targets and understanding the robustness of the parasite's cellular systems.

Immunity and antigens

Protective, immunomodulatory and anti-inflammatory effect of *Echinococcus granulosus*' laminated layer in a mouse model of ovalbumin-induced asthma

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Asthma is an inflammatory disease of the airways characterized by an excessive type 2 immune response. Our team have also highlighted the anti-inflammatory effect of the helminth *Echinococcus granulosus* and its laminated layer (LL, outer layer of the cyst) during chronic inflammatory pathologies. In this sense, the aim of our study was to investigate the effect of LL during an experimental model of asthma. We developed an experimental model of asthma induced by ovalbumin in female Balb/c mice and subsequently treated simultaneously with LL. We measured the level of specific anti-OVA IgE and nitrites. The lungs were used for histological study with H&E and PAS staining. In addition, the study of cytokines and transcription factor expression by quantitative RT-PCR was carried out in the lung and spleen. Our results showed that treatment with LL decrease the levels of allergen-specific IgE and nitrites, as well as improved the histological appearance, inflammatory cells infiltration and hyperplasia of the goblet cells in the lungs. In addition, the expression of effector cytokines was significantly increased in the lung (IL-1β, IL-17A, IL-4) and spleen (IFN-γ, IL-1β, IL-17A). However, this expression was significantly reduced by LL, in the lung (IL-1β) and in the spleen (IFN-γ, IL-1β, IL-17A). Interestingly, mRNA levels of Foxp3, IL-10 and TGF-β were significantly up-regulated in the lung and spleen after treatment with LL. Our data confirm the immunomodulatory and therapeutic effect of *Echinococcus granulosus*' LL and show the value of using LL as adjuvant therapy in inflammatory and allergic pathologies.

Immunomodulatory and anti-tumoral effects of *Echinococcus granulosus* hydatid fluid in a cell-derived breast cancer xenograft model

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Breast cancer remains the most frequently diagnosed cancer in women worldwide, with conventional treatments often limited by serious side effects and therapeutic resistance. In recent years, the ability of various helminths and derived products to suppress cancer growth has been well documented, both in humans and in experimental models. We aimed to investigate the potential antitumor effects of *Echinococcus granulosus* hydatid fluid (HF), known for its immunomodulatory properties, in a cell-derived breast cancer xenograft (CDX) model. For this purpose, immunosuppression was first induced in Wistar rats using cyclosporine A (CsA). Subsequently, HF treatment was followed by xenograft of human HEP2 tumor cells. Key parameters monitored throughout the experimental protocol included the rats' overall health statuts, body weight, circulating immune cell populations, nitric oxide (NO) and interleukin-6 (IL-6) production. HF treatment resulted in notable changes in the overall health and immune responses of the rats. Treated rats showed improved weight regain, increased monocytes and neutrophils counts, and increased NO/IL-6 production, indicating enhanced immune response activation. Remarkably, only 20% of HF-treated rats developed tumors, compared to 80% in untreated rats, with a significant reduction in tumor size. Additionally, HF-treated rats exhibited lower levels of hepatic transaminase, indicating reduced toxicity. These finding suggest that hydatid fluid not only enhances the immune response but also effectively inhibits tumor growth with minimal toxicity. This study highlights the potential of hydatid fluid as a promising therapeutic approach for breast cancer. Further research is recommended to understand the molecular mechanisms underlaying the effects of HF. This new antitumor strategy could open new perspectives in the development of highly immunogenic anticancer vaccines.

Comparison of manual and QuPath methods for counting CD3+ T lymphocytes in *Haemonchus contortus*-infected sheep: enhancing reproducibility and accuracy in immune response analysis

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lmmunohistochemical analysis has proven effective for evaluating the cellular immune response in sheep, facilitating comparisons among different animal genotypes, environmental conditions, and dietary supplementation. Nonetheless, the conventional cellular count relies on a manual count of ten random regions within a defined-area graticule, potentially compromising reproducibility and accuracy. The utilization of digital images associated with semi-automated cell counting software such as QuPath may reduce subjectivity and increase accuracy. This study compared the manual counting to the QuPath method for CD3+ cells (T lymphocytes) labeled by
immunohistochemistry in the abomasum of *Haemonchus contortus*-infected animals supplemented with different particle sizes of zinc oxide. The manual was conducted by examining ten distinct regions of the abomasum utilizing a 1 mm2 graticule at a magnification of 400x. Ten random images were captured at 400x magnification for the QuPath count, with counting performed in a similar graticule area. A two-tailed paired t-test (Wilcoxon test), Spearman correlation, and Linear regression were assessed to evaluate the similarity, correlation, and relationship between the methods, respectively, using Statistical Analysis System (SAS). The count of CD3+ cells was similar between manual and QuPath methods, showing no significant difference ($p = 0.5071$), with means of 1544±109.4 and 1656±145.9, respectively. A strong positive correlation ($r = 0.76$, $p < 0.0001$) was observed between the methods, suggesting a close relationship between the two approaches ($R^2=0.65$, $p<0.0001$). The immune response to nematode infection is characterized by elevated T lymphocyte counts (CD3+ cells), which may lead to an undercount when assessed through a manual method. QuPath, as a semi-automated tool and available as free software, arises as an alternative to minimise variability and improve reproducibility and accuracy.

Characterization of a *Rhipicephalus microplus* histamine binding protein

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The cattle tick *Rhipicephalus microplus* is an ectoparasite that causes high economic impact to livestock, mainly in tropical and subtropical areas. The lack of effective vaccines against tick infestation has hindered its control and the identification of new molecular targets for recombinant vaccine development arises as an alternative strategy. Tick saliva is known as a reservoir of potential vaccine targets, including several proteins involved in attachment, immune response modulation and feeding that targeting might affect tick infestations. Among these proteins, histamine binding proteins (HBPs) sequester host histamine molecules reducing itching and pain response, allowing that ticks successfully attach to the host and get a complete blood meal. The aim of this study is to functionally and immunologically characterize a *R. microplus* HBP (Rm-HBP). Recombinant Rm-HBP expression was performed using pET24a(+) vector in *Escherichia coli* BL21 pLysS. Histamine binding activity of Rm-HBP was assessed using a histamine quantification test. Recombinant Rm-HBP was produced as His-tag fusion protein and recovered as a 24 kDa polypeptide. The histamine binding assay

showed that free histamine concentration reduced after the incubation with recombinant Rm-HBP, suggesting that this protein binds to histamine. These results suggest that recombinant Rm-HBP might act as a bona fide HBP, potentially modulating host response upon tick infestation. However, it is necessary to perform further assays to confirm histamine binding activity and immunomodulatory potential of Rm-HBP. The vaccine potential of recombinant Rm-HBP will be performed through immunological characterization assays, including rabbit vaccination and assessment of the protective immune response.

Identification of neutralizing epitopes in a serpin from the cattle tick *Rhipicephalus microplus*

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Previous studies from our research group showed that three *Rhipicephalus microplus* serpins (RmS-3, RmS-6 and RmS-17) are secreted into saliva during feeding and interfere with the host immune responses. Additionally, antibodies raised against these serpins neutralize their function. Recently, neutralizing epitopes from RmS-17 were identified using an *in vitro* approach. Despite the identification of epitopes remaining a major challenge, the adjustment of various algorithms has allowed for the detection of epitopes that are similar to those identified through *in vitro* approaches. This method offers a cost-effective way to pinpoint epitopes crucial for the development of anti-tick vaccines. To evaluate the algorithms performance in predicting B-cell epitopes and identifying neutralizing epitopes for RmS-6. In this study, the performance of *in silico* algorithms that predicted B-cell epitopes was evaluated, testing different parameters to identify a strategy that would replicate results obtained using an *in vitro* PEPSCAN analysis for RmS-17. Comparatively best algorithm was employed to predict epitopes for RmS-6. Them, the tertiary structure of RmS-6 was predict to screen for candidate epitopes that are close to their reactive center loop (RCL). Peptides were synthesized based on predictions and used to generate rabbit anti-sera against the synthetic peptide. Finally, purified lgGs were used to test the capacity of antibodies to neutralize the RmS-6 inhibitory activity. Linear B-cell epitopes were best predicted by ABCpred with a sensitivity of 100%, PPV of 32.66% and a MCC value of 0.2299 and employed to predict epitopes for RmS-6. The antibodies raised against p1RmS-6 neutralize the rRmS-6 activity by 48%, anti-p3RmS-6 by 22% and a combination of both by 25%. Antibodies against the peptides identified in this work neutralized the RmS-6 inhibitory activity. Additionally, our approach might be used to identify neutralizing epitopes in other tick salivary proteins related to tick feeding.

Therapeutic vaccine applied to the control of canine visceral leishmaniasis

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Canine Visceral Leishmaniasis (CVL) is a severe zoonosis caused by the protozoan *Leishmania infantum*, primarily transmitted through the feeding of female vectors *Lutzomyia longipalpis*. Dogs are considered the main urban reservoir of the parasite, and due to their proximity to humans, CVL cases often precede Human Visceral Leishmaniasis (HVL). Clinical signs of CVL include weight loss, lymphadenopathy, anemia, and enlargement of the liver and spleen. In Brazil, the only approved drug for treatment is Milteforan™ by the Ministry of Health (MS) and the Ministério da Agricultura e Pecuária (MAPA). However, Milteforan™ can lead to resistant strains over time and does not provide a parasitological cure, resulting in recurrent disease relapses in dogs. In this context, immunotherapy emerges as a promising alternative to combat CVL. The aim of this study is to evaluate the effectiveness of the immunobiological L22 in treating CVL, and its combination with Milteforan™ to explore potential solutions for reducing CVL transmission and mortality. The animals were divided into three groups: one treated with Milteforan™ only (N=4), another with Milteforan™ plus L22 (N=5), and a third treated exclusively with L22 (N=5). The study included xenodiagnosis and biological sample collection before and 30 days after the immunotherapy. The analyses included hemograms, biochemical parameters, leukocyte immunophenotyping by flow cytometry, serum antibody levels by ELISA, and parasitic load quantification by qPCR. Preliminary results showed a significant reduction in parasitic load in bone marrow across all treatment groups. However, the group treated with the combination of L22 and Milteforan™ exhibited the greatest reduction, with a 99.99% decrease. This suggests that combining these two therapies may be more effective than using Milteforan™ alone. These results highlight the importance of investigating treatment combinations to achieve better outcomes in controlling the parasitic load in bone marrow.

One Health and welfare

MiRNAs: new perspectives in the control of cystic echinococcosis

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Cystic echinococcosis (CE), caused by the larval stages of *Echinococcus granulosus*, is a global public health priority disease due to its impact on human and animal health, and is recognised

as needing effective control efforts. Therefore, new diagnostic and therapeutic targets are urgently needed to improve control programs against this zoonosis. MicroRNAs (miRNAs) have been suggested as potential therapeutic targets for treatment and control for metacestode infections. According to that, this study aimed to identify the miRNAs in different parasitic stages of *E. granulosus*, in order to discover potential new targets for diagnosis and development of a new therapeutic approach against CE. Germinative membranes, protoscoleces were collected from ruminants (sheep, cattle and water buffaloes) naturally infected by CE. Specifically, small RNA libraries were constructed from germinative membrane and protoscoleces using the Illumina sequencing technology. Finally, the expression levels of selected miRNAs were evaluated using real-time quantitative PCR (qPCR). The results of the bioinformatic analysis revealed the presence of 145 mature miRNAs in germinative membrane and protoscoleces samples. Among the mature miRNAs, egr-miR-10a-5p resulted the most abundant, followed by egr-let-7-5p and egr-miR-71-5p. The qPCR results showed that no significant differences of miRNAs level expression were found between the animal species ($p>0.05$), whereas the difference of miRNA-71 level expression between protoscoleces and membranes was statistically significant ($p<0.0001$). The results of this study could form the basis for the development of innovative strategies based on RNA technology to diagnose and control CE.

From research to implementation: tackling cystic echinococcosis in southern Italy

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Cystic echinococcosis (CE), caused by the larval stage of *Echinococcus granulosus*, is recognized as a One Health priority due to its global distribution and significant impact on both human and animal health. Control programs targeting CE are regarded as long-term public health initiatives that necessitate an integrated approach with robust surveillance systems. Over the years, several Mediterranean countries—including southern Italy—have implemented measures to control CE, aiming to reduce infection rates in both definitive and intermediate hosts. In recent decades, the Campania region of southern Italy has implemented targeted intervention strategies against CE, aiming to translate scientific research and innovation into concrete veterinary policy measures. These efforts have included public education, diagnostic approaches in ruminants and dogs—such as ultrasound, the Mini-FLOTAC technique, organ inspection at slaughterhouses, and microsatellite analysis. Surveillance has been enhanced using geographic information systems, GPS data loggers, and camera traps. Control measures have included the use of the EG95 vaccine, praziquantel-laced baits, and drones for the treatment of stray canids. Since 2022, the control of *E. granulosus* in animals has been officially recognized as a mandatory objective within the regional planning document of the veterinary services in Campania. Over the past two years, this initiative has led to the implementation of control measures on more than 350 farms, where sheep, cattle, and buffaloes had tested positive for CE during slaughterhou-

se inspections. On these farms, over 700 dogs were treated with praziquantel, and 3,300 lambs were vaccinated. The effectiveness of these strategies will be assessed a few years after the initial interventions. Furthermore, the successful implementation of these strategies in southern Italy has facilitated their transfer to other endemic regions in the Mediterranean area.

Prevalence of parasites in feces of maned-wolves and dogs in the Serra da Canastra National Park

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The Serra da Canastra National Park is a Conservation Unit located in the state of Minas Gerais and represents a significant remnant of the Brazilian Cerrado. In this region, humans, domestic and wild animals live nearby. Domestic dogs may have contact with populations of maned wolves (*Chrysocyon brachyurus*), which are officially considered endangered. Recognizing the importance of parasitic diseases for the conservation programs of endangered species, the objective of this research was to identify parasitic infections and calculate the prevalence of parasites present in fecal masses of maned wolves and domestic dogs that inhabit the PNSC region. The fecal samples of both species of canids were stored in plastic jars, duly identified, containing liquid preservative (ethyl alcohol 70º GL) and taken to the laboratory where they were processed by the Sheather (centrifugal flotation in saturated sucrose solution) and HPJ (simple sedimentation) techniques. Among the dog samples, 22.5% (9/40) tested positive for at least one parasite species. Of these, 44.4% (4/9) were positive to hookworm eggs, 22.2% (2/9) to *Toxocara canis*, 11.1% (1/9) to *Cystoisospora canis* and trematode eggs. Among maned wolves, the prevalence was 75% (30/40) of feces positive for at least one parasite species. Among these, 86.6% (26/30) corresponded to capillariid eggs, 16.6% (5/30) to Toxocara canis, 10% (3/30) to hookworm eggs and *Dioctophyma renale*, 6.6% (2/30) to *Cystoisospora canis*, 3.3% (1/30) to *Physaloptera tupinambae* and trematode eggs. The presence of *P. tupinambae* in wolf feces indicates cases of pseudoparasitism. The presence of *D. renale* eggs in the feces indicates that the lobes urinate in the feces, probably as a territorial marking habit. Given the clinical impact of parasites diseases, a high level of vigilance towards domestic canine parasites and wild is warranted.

Microbiological and parasitological parameters of water consumed by humans and animals in a rural settlement Paraná, Brazil

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The lack of basic sanitation infrastructure in rural environments contributes to the contamination of water sources intended for human and animal consumption, favoring the spread of waterborne diseases. The study aimed to evaluate the microbiological quality of water consumed by humans and animals, detect *Giardia* spp. in cattle feces and water, and associate the findings with the observed sanitation conditions. Samples were collected in a rural settlement in Paraná, Brazil. Centrifugal flotation in a saturated sucrose solution was performed to determine the concentration of *Giardia* spp cysts and *Cryptosporidium* spp oocysts in feces. Microbiological analysis of water was carried out using Colilert®IDEXX kit, and parasitological analysis of both water and feces was performed by nested and seminested PCR, aiming at the amplification of the 18S-rRNA genes of *Giardia* and for the *TPI*, β-*Giardina* genes, seminested PCR for the *GDH* gene. The positive amplicons were characterized by Sanger sequencing. *Giardia* spp. was detected in 8 out of 24 water samples by the *GDH* gene, and four also amplified the TPI gene, confirming the presence of *Giardia duodenalis* in the water. In feces, 4/60 (6.6%) were positive, three amplified all target genes, characterizing *G. duodenalis*, assemblage E. One sample did not show amplification in the additional genes tested. Among the water sources evaluated, 14/24 (58.3%) corresponded to springs, of which only 7/14 (50%) were adequately protected; the others had improvised covers, such as tarps and asbestos sheets. Microbiological analysis showed that 79.1% of the samples were contaminated by total coliforms and 66.6% by *Escherichia coli*. Protected springs presented lower microbial contamination compared to unprotected ones. The results highlight the presence of *Giardia* spp. and *E. coli*, the sanitary vulnerability of water sources and the potential zoonotic risk, exacerbated by the lack of sanitary infrastructure and inadequate management.

Cystic echinococcosis in cattle and buffalo caused by *Echinococcus granulosus* genotype G5 in Chennai, India

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Cystic echinococcosis is a zoonotic disease of great public health and economic significance, caused by the larval stage of the dog tapeworm *Echinococcus granulosus*. The incidence of this disease has been reported affecting cattle and buffaloes in India. However, there is a paucity of molecular data describing the genotypes of this important cestode in domestic ruminant intermediate hosts. Thirteen hydatid cysts from the lungs and/or livers of slaughtered beef cattle (9 samples) and buffaloes (4 samples) were collected from abattoir in Chennai, India. The samples were genotyped at the mitochondrial loci, *cox1.* Sequences were analyzed for their identity to determine species genotypes using NCBI – BLAST and the most similar genotypes were identified. Out of the 13 hydatid cysts collected, 12 were found to be fertile. Based on *cox1* sequences obtained from 13 hydatid cysts, the NCBI – BLAST analysis of the fertile hydatid cysts revealed that all the fertile hydatid cysts obtained from cattle and buffaloes were of *E. granulosus* G5 genotype. The sequence results

exhibited 95% to 98% homology with cox1 gene sequences of the G5 genotype in the NCBI database. All genotyped samples belonged to *E. granulosus* and the present study confirmed the presence of genotypes G5 in domestic cattle and buffalo intermediate hosts in Chennai, India and provide data for future diagnostic and epidemiological studies.

Livestock and environmental risk patterns of taeniasis in Thailand: a one health spatial analysis

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Taeniasis is a zoonotic parasitic disease endemic to Thailand, where close interactions between humans, livestock, and the environment create conditions for sustained transmission. A One Health approach is critical for understanding the interconnected drivers of taeniasis and guiding effective control strategies. To identify spatial risk patterns of taeniasis in relation to livestock density, environmental conditions, and socioeconomic factors using a One Health framework. Taeniasis case data from 2009 to 2014 were analyzed alongside livestock density maps (for cattle and pigs), environmental variables (elevation, precipitation, normalized difference vegetation index [NDVI], and travel time to cities and healthcare facilities), and socioeconomic indicators. All spatial layers were processed and integrated using geographic information system (GIS) tools. Spatial risk modeling was performed using the Integrated Nested Laplace Approximation (INLA) to identify high-risk areas and assess associations between taeniasis prevalence and One Health-related factors. The prevalence of taeniasis in 2014 showed a decline compared to 2008. Spatial analysis indicated that high-risk clusters had shifted over time—from the North, Northeast, and parts of the West—toward the western border regions of Thailand, particularly near the Thai Myanmar border. One Health-related factors, including human population density, livestock density, and environmental variables (altitude, precipitation, NDVI, and access to cities or healthcare), were significantly associated with taeniasis prevalence, highlighting the multifactorial and spatially dynamic nature of transmission. This study demonstrates the value of a One Health approach in understanding taeniasis risk across Thailand. Integrating human, animal, and environmental data through spatial modeling allows for the identification of shifting risk patterns and supports the development of targeted, cross-sectoral interventions for zoonotic disease control in endemic regions.

A novel genotype of *Taenia solium* in Japan: public health implications and evolutionary insights

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Taenia solium is a tapeworm that uses pigs and wild boars as intermediate hosts, and humans

as the only definitive hosts. Humans can also serve as the intermediate host, in which cysticerci develop in the muscles and central nervous system. *Taenia solium* is generally considered non-endemic in Japan, where sewage disposal systems limit intermediate hosts' access to human feces. Here, we report three cases of cysticercosis unexpectedly identified in wild boars in Nagano Prefecture, Japan, in 2014 and 2023. We performed genetic analyses on six cysticerci obtained from two wild boars independently captured in 2023. Based on three nuclear DNA markers (*pepck*, *pold*, and *rpb2*), the cysticerci were identified as *T. solium*. Using an amplicon-based sequencing approach, we constructed six complete mitochondrial genomes (13,712 bp), which were identical among all cysticerci, yet exhibited multiple nucleotide substitutions compared to the previously reported sequences from China and Peru. Haplotype network analysis using mitochondrial *cox1* and *cytb* sequences revealed that the cysticerci in Japan represent a novel haplotype distinct from haplogroups reported in other endemic regions, i.e., Asia, Africa/South America, and Bhutan. Subsequent phylogenetic analysis using mitochondrial 12 protein-coding sequences showed that the Japanese haplotype diverged first from other haplogroups, likely in the early to middle Pleistocene. Our findings indicate that the *T. solium* lifecycle can be maintained in regions generally considered non-endemic, highlighting a previously unrecognized risk of local transmission of cysticercosis. Furthermore, the phylogenetic analysis of *T. solium* suggests that the genetic divergence of the Japanese haplotype began long before the "Out of Africa" dispersal of modern humans—the current sole definitive host. Comparative genomics based on additional specimens will be essential to elucidate the full evolutionary history of *T. solium*.

Surveillance of hemopathogens and sars--cov-2 in domestic and wild mammals in the southeastern region of Brazil from a One Health perspective.

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Between January 2021 and March 2023, we investigated the presence of SARS-CoV-2 and hemopathogens in three distinct mammal populations in Belo Horizonte, Minas Gerais, Brazil. Different diagnostic methods were used, including RT-qPCR for SARS-CoV-2, PCR for hemopathogens, RFLP-PCR for *Leishmania* spp., and serological assays for Toxoplasma gondii. In the PetCOVID-19 Project, which included 48 dogs and 38 pet cats whose owners had been diagnosed with COVID-19, the infection rate for SARS-CoV-2 was 9.3%. Coinfections with hemopathogens were common, with prevalences of 47.7% for *Anaplasma/Ehrlichia* spp., 17.4% for *Mycoplasma* spp., 15.8% for *Bartonella* spp., and 37.5% for Piroplasmida. No animal tested positive for *Leishmania* spp., and the

seroprevalence of *T. gondii* was 15.8% among the cats. The Omicron variant (BA.1.) was identified in these animals. Among the 78 stray cats from Américo Renné Giannetti Municipal Park, 7.7% were positive for SARS-CoV-2. The prevalence of hemopathogens included 38.5% for *Anaplasma/Ehrlichia* spp., 7.7% for *Mycoplasma* spp., 38.5% for *Bartonella* spp., and 2.6% for Piroplasmida. *Leishmania* spp. was detected in 15.4% of the cats, with 14.1% testing positive for L. infantum. The seroprevalence of *T. gondii* was 23.1%. The Delta variant (B.1.617.2) was identified in the cats from the park. Among the 52 captive mammals at the Belo Horizonte Zoo, 18% tested positive for SARS-CoV-2. Coinfections with hemopathogens were observed, with prevalences of 14.6% for *Anaplasma/Ehrlichia* spp., 17.5% for *Mycoplasma* spp., 42.5% for *Bartonella* spp., and 22.5% for Piroplasmida. *Leishmania* spp. was detected in 33.3% of the Canidae family. The Alpha (B.1.1.7) and Omicron (BA.1.) variants were identified in the zoo animals. Significant associations were found between SARS-CoV-2 infection and the presence of hemopathogens in the park cats ($p = 0.032$) and between SARS-CoV-2 and *Bartonella* spp. in the zoo mammals ($p = 0.000$). These findings highlight the importance of epidemiological surveillance in animals, aiming not only at animal health but also at public health, particularly in pandemic contexts like COVID-19.

Parasites of fish, aquaculture, and wildlife

Diagnosis of foodborne zoonotic nematodes (Nematoda: Anisakidae) in fish species used in row fish-based meals in Uruguay

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In Uruguay, particularly in Montevideo, there is a growing interest in raw fish-based foods such as sushi, sashimi, and ceviche. This consumption habit poses a public health risk, as these foods can serve as a source of infection of anisakid larvae (Nematoda: Anisakidae), which causes a zoonotic disease known as anisakidosis. To diagnose and quantify anisakid larvae in fish species used for raw consumption. Methods: A total of 349 specimens from four fish species were analyzed: *Urophycis brasiliensis* (n=79), *Micropogonias furnieri* (n=66), *Paralichthys orbignyanus* (n=60), and *Merluccius hubbsi* (n=50). Diagnosis was performed on the coelomic cavity and muscle tissue (edible portion) using simple visual inspection, candling, and UV transillumination. Collected larvae were quantified and identified up to genus level to establish prevalence, mean abundance, and mean intensity. All evaluated species were found to be parasitized, with *M. hubbsi* and *P. orbignyanus* being the most affected, showing prevalences of 74% and 65%, respectively. Regarding the edible portion, only *M. hubbsi* and *U. brasiliensis* were affected, with prevalences of 14% and 2.5%, respectively. Morphological identification revealed three genera commonly associated with anisakidosis cases—*Anisakis*, *Pseudoterranova*, and *Contracaecum*—as well as the genus *Hysterothylacium*. Conclusion: The detection of anisakid larvae in *U. brasilien-*

sis, *M. furnieri*, and *P. orbignyanus* represents a new host record for Uruguay. Additionally, the identification of the genus *Hysterothylacium* in fish is reported for the first time in the country. Furthermore, the presence of these zoonotic parasites in the edible portions of *U. brasiliensis* and *M. hubbsi* is an unprecedented finding in Uruguay, which should be considered when evaluating the risk of anisakidosis from raw consumption of these fish species.

Morphometric and Molecular insights about *Hepatozoon* spp. in Wild and Synanthropic Rodents from Southern and Southeastern Brazil

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Small rodents constitute an important biomass and serve as hosts for various pathogens, including *Hepatozoon*. In Brazil, *Hepatozoon* infections in wild rodents have been reported since the last century, but to date, only *Hepatozoon milleri* has been described in *Akodon montensis*. This study aimed to advance knowledge on *Hepatozoon* spp. in wild and synanthropic rodents from southern and southeastern Brazil. Liver samples and blood smears were obtained from 289 rodents captured in municipalities of Paraná State and Rio de Janeiro State, Brazil. These rodents belonged to 14 species of the family Cricetidae and two species of the family Muridae. Smears were stained using the Giemsa method, and gametocytes were detected via microscopy in 10.72% (n = 31/289) of the rodents, with *A. montensis* being the most frequently parasitized species (n = 28/31). Significant morphometric differences were observed in gametocyte measurements from *Akodon* rodents. Using conventional PCR, *Hepatozoon* spp. 18S rDNA fragments were amplified in 24.91% (n = 72/289) of the rodents, including *Akodon cursor, A. montensis, Mus musculus, Oligoryzomys nigripes, Oxymycterus nasutus, Oxymycterus quaestor,* and *Sooretamys angouya*. Phylogenetic analyses revealed that the sequences obtained in this study (n = 41) clustered into a subclade with other sequences from small mammals in Brazil and belonged to four different haplotypes. Subsequently, three long sequences (>1600 bp) representing haplotypes 1, 3, and 4 were obtained and analyzed. Based on phylogenetic analysis, this study reinforces the trophic relationship between rodents and reptiles as a possible *Hepatozoon* transmission cycle in South America. Furthermore, our findings expand knowledge on *Hepatozoon* spp. hosts, describing *O. nasutus* and *O. quaestor* as new host species and identifying two novel circulating haplotypes in rodents from Paraná State, southern Brazil.

Report and description of the invasive cestode *S. acheilognathi* in Colombia

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The Asian tapeworm *Schyzocotyle acheilognathi* is considered the most successful invasive parasite in freshwater environments. Since its first description in Japan, it has exhibited rapid global spread due to its notable environmental tolerance and low host specificity. This parasite represents a potential threat to fish health, being responsible for high mortality rates, particularly in fish of the family Cyprinidae. Stablished the presence *S. acheilognathi* in Colombia. A total of 103 specimens of the native fish species *Parodon magdalenensis* were collected using cast nets and electrofishing. The fish were euthanized by immersion in a concentrated eugenol solution (300 mg/L), and subsequently examined for parasites. The cestodes found were identified morphologically using taxonomic identification keys and molecularly through phylogenetic analyses of the 28S, 18S, and 16S rRNA gene regions. Cestodes were found in the intestines of *P. magdalenensis*, with a parasitic frequency of 32 out of 103 individuals (31.07%). The parasites measured an average of 70.14 mm ± 31.32 in length and 1.4 mm ± 0.41 in width. They exhibited a whitish color and a long, flat body composed of multiple, distinctly separated proglottids. The observed morphological characteristics were consistent with the genus *Schyzocotyle*. Furthermore, this morphological identification was supported by molecular analyses of the ssrDNA (18S rRNA), larDNA (28S rRNA), and rrnL (16S rRNA) gene sequences identified at species level *S. acheilognathi*. This finding represents a significant advancement by providing the description of the invasive cestode *S. acheilognathi* in Colombia, as well as its first record in the native species *Parodon magdalenensis*. This highlights the need for further studies on Colombian parasitofauna, the potential spread of this parasite to other river basins, and the implications for the health of native fish species.

Molecular detection of *Hepatozoon* spp. in wild felids from Rio de Janeiro, southeastern Brazil

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The genus *Hepatozoon* spp. encompasses apicomplexan protozoa transmitted through ingestion of infected ixodid ticks by various species of vertebrates. In Brazil, few studies have reported molecular identity of these agents in wild felids. This study aimed to investigate the occurrence and phylogenetic positioning of *Hepatozoon* spp. in wild felids from Rio de Janeiro state. For this purpose, animal's carcasses roadkill between 2023 and 2024 on Rio de Janeiro highways were collected by concessionaires and then necropsied. Fragments of

organs (lungs, liver, heart, spleen, mesenteric lymph node, kidneys, and skeletal muscle) were collected. When possible, cavity effusions were collected and stored in tubes containing EDTA, which were frozen. Wild felid tissue fragments were subjected to DNA and PCR targeting the mammalian *gapdh* endogenous gene. Positive samples in the abovementioned PCR were subjected to a nested PCR targeting a 1120bp fragment of the 18S rRNA gene of *Hepatozoon* spp.. PCR products were purified and submitted to Sanger sequencing. Up to now, 11 carcasses have been analysed, including three of *Puma concolor*, three of *Leopardus pardalis*, three of *Leopardus guttulus*, one of *Leopardus wiedii*, and one of *Herpailurus yagouaroundi*, with a total of 85 samples collected. Out of these, *Hepatozoon* spp. DNA was detected in 39 (45.9%) samples, from three *P. concolor*, three *L. pardalis*, two *L. guttulus*, and one *L. wiedii*. The samples with the highest positivity were the kidneys (23.1%), followed by the lungs (17.9%). BLASTn analyses of 33 sequences showed 98.8 to 99.2% identity with *Hepatozoon felis* sequences deposited in GenBank (query cover=100%; E-value=0). Maximum Likelihood phylogenetic inference positioned the obtained sequences in a unique clade, separated from *Hepatozoon luiperdjie* and *Hepatozoon* spp. detected in *Felis silvestris* by a 75% bootstrap. This is the first report of *Hepatozoon* spp. in wild felids from the Rio de Janeiro state, Brazil.

Occurrence of *Trichostrongylus axei* and *Trichuris* spp. in giraffes (*Giraffa camelopardalis*) kept in a safari park

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Giraffes (*Giraffa camelopardalis*) in captivity are susceptible to various gastrointestinal parasites. These infections pose health risks and require specialized management strategies in zoological institutions. The nematode *Trichostrongylus axei* can infect giraffes, particularly those kept in captivity. This parasite is not specific to giraffes and can also be found in other ruminants. In zoo environments, giraffes may acquire *T. axei* infections from other animals sharing their enclosures. Additionally, *Trichuris* spp. infect various species, including giraffes and other wild ruminants in zoological and wildlife parks worldwide. These parasites can cause significant morbidity in giraffes under human care. This study aims to describe the occurrence of gastrointestinal parasites affecting the health and welfare of giraffes in captivity. Fresh fecal samples were obtained from six giraffes housed in a safari park in Portugal. The samples were examined using the modified McMaster method. The flotation solution used was a saturated sugar solution. Larval cultures were performed to identify the species of strongylid. Out of a total of six samples, three were found positive for strongylid eggs, and three positive for *Trichuris* spp. The strongylid egg counts ranged from 150–200, and from 50–200 for *Trichuris* spp. The positive samples for strongylid eggs were separately pooled and subjected to larval culture. The overall prevalence of parasites detected in the giraffes from the safari park in Portugal was 66.7% (4/6). The presence of strongylid eggs was 50% (3/6), and *Trichuris* spp. eggs 50% (3/6). The larval culture revealed 100% *Trichostrongylus axei* larvae. Zoo managers and veterinarians should be aware of the potential for *T. axei* and *Trichuris* spp. infections in captive giraffes, especially when housing them with other ruminant spe-

cies. Regular parasite monitoring and appropriate management practices can help control these infections in zoo environments.

Spirocerca lupi in a bush dog (*Spetohos venaticus*) from the Brazilian Cerrado

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Spirocerca lupi is a nematode parasite of canids, with dung beetles as intermediate hosts and several paratenic vertebrate hosts. They cause a variety of lesions that can evolve into sarcomas. Despite the pathogenic importance, little is known about its epidemiology. The bush dog (*Speothos venaticus)* is a small, near-threatened, Neotropical canid widely distributed but not very abundant.The carcass of an adult male bush dog was found in Mineiros, Goiás State. At necropsy, reddish parasites were recovered from gastric nodules, 06 males and 05 females, and stored in ethanol until morphological identification. Measurements are expressed as mean, in millimeters. Males (n=2) total body length (TBL) is 36.5, 0.70 in width. The esophagus (OES) is 5.88 long, and the glandular portion is 0.60. Nerve ring (NR) and excretory pore (EP) are 0.51 and 0.55 from anterior ending. The male tail has 04 pairs of pre-cloacal pedunculated papillae, 01 single sessile papilla anterior to the cloaca, 02 pairs of post-cloacal pedunculated papillae, and a set of small sessile papillae at the tail tip. Spicules are unequal, the left is 2.58 long and the short 0.68, with complex gubernaculum. The female (n=1) TBL is 53, 0.82 wide. The OES is 6.43 long and glandular portion 0.50, NR and EP are at 0.48 and 0.45 from the anterior ending. The vulva opening and anus are at 3.96 and 0.38 from the posterior ending. Eggs (n= 10) are 0.036 mm long and 0.014 mm wide. These features are consistent with *S. lupi*, which was first detected in a Brazilian bush dog. It is difficult to determine the impact of *S. lupi* on wild canids without continued surveillance. The region of this finding has an expressive livestock production, which could benefit some dung beetles species, facilitating the spread of *S. lupi* in anthropized environments. Therefore, it is necessary to investigate the possibility of pathogen spillover between wild and domestic animals in these areas.

Characterisation of Ixodid Ticks and Molecular Screening for *Borrelia* and *Anaplasma/Ehrlichia* spp. in Wild Mammals in Northern Portugal

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Ixodid ticks are ubiquitous hematophagous arthropods and vectors of pathogens relevant to animal and public health. Wild mammals may act as reservoirs for *Ehrlichia* spp., *Anaplasma* spp., and *Borrelia* spp., contributing to their persistence in natural ecosystems. This study aimed to characterise ixodid tick infestation in wild mammals in northern Portugal and to screen those specimens for *Borrelia* spp., *Anaplasma* spp., and *Ehrlichia* spp. Ticks were collected from wild mammals admitted to a wildlife rehabilitation centre in the north of Portugal and morphologically identified to species level by stereomicroscopy using taxonomic keys. A representative subset was screened for the presence of *Anaplasma/Ehrlichia* spp., and *Borrelia* spp. by PCR, using genus-specific primers targeting the *16S rRNA* and the *flaB* genes, respectively. PCR amplicons were sequenced, followed by phylogenetic analysis for species-level identification. In a total of 51 wild mammals, 372 ticks were collected, predominantly *Rhipicephalus sanguineus* (74,7%), followed by *Ixodes Ricinus* (13,7%), *I. hexagonus* (6,2 %), and *R. pusillus* (0,2%). Eighteen specimens could not be identified at either the genus or species level. Most ticks were adults [75,8%, mostly males (53,2%)]. PCR products of the expected size were obtained for *Borrelia* spp. in 24 ticks (4 *I. ricinus*, 19 *R. sanguineus*, and 1 *Rhipicephalus* spp.) and for *Anaplasma /Ehrlichia* spp. in 7 ticks (6 *R. sanguineus* and 1 *I. ricinus*). Phylogenetic analysis confirmed *Borrelia garinii* in a *R. sanguineus* tick from a genet. These results suggest the circulation of *Borrelia* spp. and *Anaplasma/Ehrlichia* spp. in ticks from wild mammals in northern Portugal. The detection of *B. garinii* in a *R. sanguineus* from a genet suggests the role of wildlife in maintaining zoonotic tick-borne pathogens and highlights the need for a One Health-based wildlife and vector surveillance.

Gastrointestinal Parasites in Wild Mammals, Birds, and Reptiles in Captivity: An Epidemiological Study in Northern Paraná

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The objectives of this study were to analyze the occurrence of gastrointestinal parasites in wild animals in Paraná, assess risk factors associated with infection, and evaluate the diagnostic sensitivity and specificity of flotation and sedimentation techniques. A total of 81 fecal samples were analyzed (18 mammals, 36 birds, 27 reptiles), obtained from IPEVS (56 samples) and private owners (25 samples). Coproparasitological techniques included flotation in sucrose solution and sedimentation. Risk factors were assessed using an epidemiological questionnaire. The occurrence of gastrointestinal parasites was 59.3% (48/81), with the highest rates in mammals (72.2%), followed by reptiles (70.4%) and birds (44.4%). Helminths identified included *Ascaris*, *Capillaria*, *Enterobius*, *Kalicephalus*, *Strongyloides*, *Synhimantus*, *Trichuris*, *Ancylostoma*, and other nematodes similar to oxyurids and strongyloids. Eggs from Cestoda, Trematoda, and coccidian oocysts (including *Choleoeimeria*) were also detected. *Ascaris* was the most frequent, with *Crotalus durissus* being the most infected species. *Enterobius* eggs were found in *Sapajus apella*. Risk factors

showed that animals in collective environments were significantly more susceptible to infection (85.7%) than those housed individually (50%). Regarding diagnostic methods, flotation showed a sensitivity of 66.7% and specificity of 93.9%, while sedimentation had a sensitivity of 83.3% and specificity of 93.9%. The agreement between techniques was slight (Kappa = 0.27). In conclusion, the study highlights a high occurrence of gastrointestinal parasites in wild animals, with collective housing as a risk factor. The sedimentation technique was more sensitive, underscoring the importance of multiple coproparasitological methods. This study also reports the first case of *Enterobius* eggs in a *Sapajus apella* in Brazil and the first global report of *Choleoeimeria* oocysts in *Phylodryas olfersii*.

Clinical leishmaniosis due to *Leishmania infantum* in captive wild animals from Spain zoos

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Leishmaniosis is an endemic zoonotic disease in the Iberian Peninsula, primarily affecting humans and dogs. It is caused by a protozoan, *Leishmania infantum*, and transmitted through the bite of phlebotomine sand flies. Since clinical leishmaniosis was detected in wallabies and orangutans in Madrid, more zoo veterinarians have included this disease in their differential diagnoses and have detected new cases of *Leishmania* infection such as in a European otter (*Lutra lutra*) in Murcia and two meerkats (*Suricata suricatta*) in Madrid. In this study, we investigated a total of 8 clinical cases of *Leishmania infantum* infection in wild captive animals from the zoological parks of Madrid. The animal species of the 8 positive cases included were as follows: chimpanzee (*Pan troglodytes*), rock wallaby (*Petrogale xantophus*), Bengal tiger (*Panthera tigris*), meerkat, South American sea lion (*Otaria flavescens*), two maras (*Dolichotis patagonum*) from Madrid Zoo Aquarium and Bennet's wallaby (*Macropus rufogriseus*) from Faunia. Samples were obtained during routine intervention by veterinarians of both zoos. Samples obtained in alive animals were blood, serum, oral, conjunctival and genital swabs. In deceased animals, spleen, liver, kidney, skin and lymph nodes were also collected. All samples were stored at -20oC. Antibodies against *L. infantum* were determined using a commercial immunochromatography technique based on the detection of the recombinant rK39 antigen. Nested PCR was carried out to detect *Leishmania* DNA in all biological samples. All animals were positive against *L. infantum* antibodies and nested PCR. Of the two animals (a chimpanzee and a mara) no showed signs compatible with leishmaniosis. The rest of the animals presented clinical signs such as alopecia, hyperpigmentation, epistaxis and chronic lameness. This study demonstrates the presence of clinical leishmaniosis in animals housed in zoos, including endangered species. Further studies are necessary to determine the

role of these species as susceptible species to leishmaniosis.

Molecular identification of *Anisakis* spp. larvae from fishery products in Portugal: preliminary results

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Parasitic third-stage larvae of the genus *Anisakis* (Nematoda: Anisakidae) are frequently detected in the viscera and muscles of commercially important fishes. These nematodes are a relevant zoonotic foodborne hazard due to their infectious and allergenic potential to human consumers and inflict an economic impact to the fishing industry. Fish intake in Portugal is one of the highest in the world and the recent introduction of raw fish consumption habits has risen the risk of anisakiasis. This study aimed to determine the molecular identification (PCR-RFLP) of larvae of *Anisakis* spp. infecting the viscera and muscle of some popular fishery products collected from establishments of first sale in Portugal. Larval anisakids were recovered from fishery products collected from commercial fisheries' landing facilities in Portugal during the winter of 2024 after individual enzymatic digestion of viscera and muscles of the hosts. Then, DNA was extracted from the recovered larvae of the genus *Anisakis* and amplification of the ITS region was performed by PCR followed by Restriction Fragment Length Polymorphism (RFLP) using *Hinf* I and *Hha* I enzymes. *Anisakis pegreffii* was the most prevalent species from the viscera and muscle of Atlantic horse mackerel (*Trachurus trachurus*) and Atlantic chub mackerel (*Scomber colias*), followed by *A. simplex* sensu stricto (s.s.) and hybrid genotypes between the two sibling species. Additionally, Atlantic mackerel (*S. scombrus*) harboured mostly larvae of *A. simplex* (s.s), particularly in the viscera. These preliminary results seem to indicate a predominance of *A. pegreffii*, a species that has been previously associated with warmer water temperatures. This study will ultimately contribute to an updated understanding of the epidemiology of *Anisakis* spp. in Portugal and allow a more robust risk analysis from a food safety perspective.

Infections by *Capillaria* sp. in maned wolves (*Chrysocyon brachyurus*): parasitism or pseudoparasitism?

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The maned wolf (*Chrysocyon brachyurus*) is endemic to South America and classified as vulnerable by the Brazilian Ministry of the Environment. Parasitological studies on freeranging animals provide crucial data for wildlife conservation and their interactions with domestic animals and humans. Considering the importance of parasitological diagnosis for maned wolf conservation programs, this study aimed to diagnose parasitic infections in maned wolves from Serra da Canastra National Park (PNSC), MG, calculate the prevalence coefficient of gastrointestinal parasites, and determine parasitism frequency in dry and wet seasons. From March 2017 to October 2024, fresh fecal samples were collected in PNSC, preserved in 70° GL ethanol, and analyzed at Fluminense Federal University using Sheather and HPJ coproparasitological techniques. Of the 151 samples, 53% (80/151) were from the wet season and 47% (71/151) from the dry season. Parasitic infection was detected in 53% (80/151) of samples, with 60% (48/80) positive for *Capillaria* sp. The dry season had a higher frequency (66.7%, 32/48) than the wet season (33.3%, 16/48) (p=0.0022). This may be due to lower fruit availability, leading wolves to prey on rodents, birds, and reptiles, which can host various parasites, including *Capillaria*. Reports indicate *Capillaria* sp. in maned wolves, but *C. hepatica* has only been confirmed post-mortem. Molecular tests on fecal eggs did not confirm *C. hepatica* or other canid capillariids, suggesting pseudoparasitism from ingested small vertebrates. Further research is needed to identify prey species and *Capillaria* taxa in these hosts. Distinguishing parasitism from pseudoparasitism is crucial for managing maned wolves in natural and captive environments, preventing misdiagnosis and unnecessary treatments. We believe that such questions contribute to a better understanding of parasitic ecology and trophic interactions among the involved host species.

Prevalence of parasites in coproparasitological exams of snakes at the herpetology and research center of the Vital Brazil Institute

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Reptiles, including snakes, harbor parasites that, for the most part, do not determine significant clinical changes, but occasionally can become harmful to the host compromising its health. Parasitological diagnosis is essential for the implementation of preventive management protocols, improvement of the quality of life of animals. The present study, conducted by the Vital Brazil Institute in partnership with the Laboratory of Diagnostic Support in Parasitic Diseases/UFF, aimed to identify the parasites present in the feces of snakes. Samples were collected during the regular management, using fecal masses naturally emitted by the animals.

The samples were stored in plastic vials containing 70oGL ethyl alcohol and sent to the Support Laboratory. From May to December 2024, 261 tests were carried out, using fecal samples from snakes kept in the quarantine sector. Among the tests performed, 33% (86/261) presented positive results for some parasitosis. Of these, 38.4% (33/86) were positive for eggs of *Ophidascaris* sp., 19.8% (17/86) for eggs or larvae of strongylids (Sf Strongyloidea) and 40.7% (35/86) for eggs and/or adults of *Ophionyssus* sp. in the feces. The parasites found can cause lesions and changes in the functioning of the gastrointestinal tract, anemia, weight loss and dermatological irritations. The parasitized animals were kept in quarantine and treated with antiparasitics (Ivermectin I.M 0.2mg/kg, Fipronil 0.25% and Albendazole V.O 50mg/kg). The release of the snakes to the herd was only performed after presenting at least two negative post-treatment results. For animals kept in captivity, coproparasitological diagnosis should always be a mandatory parameter, which allows the correct diagnosis, with the early identification of possible parasitosis and serves as a basis for the implementation of an appropriate treatment and assertive management. This process is essential to ensure the health and well-being of snakes kept in captivity.

Ancylostomatidae in wild canids and felids from Romania

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Hookworms (Ancylostomatidae) significantly affect the health of domestic animals and humans worldwide, with some having zoonotic potential. Despite the relatively common reports of hookworms in pets from Europe, mainly in coproscopic studies, data on their occurrence in wild carnivores is limited. To address this, the primary objectives of this study were to investigate the diversity, prevalence, and distribution of hookworms in wild canids and felids from Romania, using both morphological and molecular analyses. Between 2013 and 2025, 319 carcasses belonging to 6 species of wild canids and felids from Romania (23 gray wolves (*Canis lupus*), 137 golden jackals (*Canis aureus*), 79 red foxes (*Vulpes vulpes*), 2 raccoon dogs (*Nyctereutes procyonoides*), 70 European wildcats (*Felis silvestris*), and 8 Eurasian lynxes (*Lynx lynx*)) were collected as roadkills or legally hunted. Necropsies were performed to

collect hookworms from the intestinal tract. Hookworms were collected in formalin for morphological identification and in absolute ethanol for genetic analysis. The genomic DNA was extracted and analyzed using a PCR targeting a barcode region of the second nuclear ribosomal internal transcribed spacer (ITS-2), followed by sequencing. The sequences were compared with other entries from GenBank™. Overall hookworm infection prevalence was 19.7%, with the infection detected in 4 wolves (17.4%), 23 jackals (16.8%), 11 wildcats (18.6%), 4 foxes (5%), 2 raccoon dogs (100%), and 1 lynx (12.5%). Three hookworm species were identified: *Uncinaria stenocephala* (in wolf, jackal, fox, raccoon dog, and wildcat), *Ancylostoma caninum* (in wolf, jackal, wildcat, and lynx), and *A. tubaeforme* (in wildcat). This is the first study assessing the diversity of hookworms in wild carnivores in Romania, showing new host-parasite associations and highlighting the importance of these hosts as reservoirs for domestic pets and humans.

Mortality associated with severe pneumonia caused by *Dictyocaulus* spp. in reintroduced European bison and farmed red deer

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Dictyocaulosis is a parasitic disease caused by pulmonary nematodes of the genus *Dictyocaulus*, affecting various ungulate hosts. In heavily infected individuals, fatal outcomes can occur. This study reports cases of severe pneumonia in free-ranging and farmed wild ruminants in Romania. A male European bison (*Bison bonasus*) from a reintroduction area in Nucşoara village, Făgăraş Mountains, and four farmed red deer (*Cervus elaphus*) from BT Wild Ranch, Cluj County, Romania were found dead following severe respiratory failure. Postmortem examination confirmed pulmonary nematode infection in all individuals. Histopathological analysis of the lungs from both species revealed severe verminous pneumonia, characterized by ectatic bronchi and bronchioles, filled with numerous adult nematodes in the mucus. Additionally, foamy fluid and an inflammatory infiltrate consisting of lymphocytes, macrophages, and eosinophils were observed. Larvae were found within the alveoli, resulting in pulmonary edema and congestion. Morphological and molecular analyses confirmed *Dictyocaulus viviparus*

in the European bison and *Dictyocaulus cervi* in the red deer. These findings underscore the importance of rigorous health monitoring in wildlife management and reintroduction programs. For threatened species such as the European bison, continuous health monitoring is essential to ensure population stability and conservation success. Furthermore, the detection of *Dictyocaulus cervi* represents the first report of this species in Romania, highlighting the need for further epidemiological studies and its impact on local wildlife.

Babesia in wildlife: genetic diversity in northern Italy

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Babesiosis, caused by intraerythrocytic protozoan parasites of the genus *Babesia*, is a globally relevant tick-borne disease. Over 100 species infect wild and domestic animals and six are recognized as human pathogens. In Europe, human babesiosis is primarily associated with *Babesia divergens*, *Babesia venatorum* and *Babesia microti*, all of which are transmitted by *Ixodes ricinus* ticks. A regional-scale survey was carried out in Emilia-Romagna (Northern Italy) in 2023–2024 to assess the presence of zoonotic *Babesia* species in wildlife. Tissue samples (ear, spleen) and ticks were collected and analyzed using molecular methods. DNA was extracted with a commercial kit (INDICAL) and screened by Real-Time PCR (Stanczak et al., 2015). Positive samples were confirmed with conventional PCR (Casati et al., 2006), targeting an approximately 460 bp fragment of the 18S rRNA gene. In total, 1510 wild animals were sampled, yielding 2550 specimens (ear, spleen, lymph node). Wild animals belonged to various species including ungulates (roe deer, red deer, fallow deer), carnivores (fox, wolf, marten, badger), rodents (mouse, rat, squirrel), and bats. Out of all samples, 568 (22.2%) tested positive for *Babesia/Theileria* DNA. These were further analysed by conventional PCR targeting the 18S rRNA gene and sequenced through Sanger method. A total of 215 high-quality sequences were used for phylogenetic analysis, identifying nine distinct *Babesia* species, including the zoonotic *B. venatorum* and *B. divergens*. While *Babesia* species are typically host-specific, some can infect humans, especially immunocompromised individuals. This study confirms a notable diversity of *Babesia* circulating in Northern Italy and highlights a concrete risk of infection in this environment.

Serological detection of *Toxoplasma gondii* in bats from Bahia, Brazil: comparison of whole blood and filter paper samples

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Bats are known reservoirs of zoonotic agents, but their role in the transmission of *Toxoplasma gondii* remains understudied. Environmental degradation in Bahia, Brazil, may influence host-parasite interactions. To determine the seroprevalence of *T. gondii* in bats and to compare diagnostic performance of whole blood versus filter paper samples. Forty-five bats were captured in urban and rural areas using mist nets or hand nets. Blood was collected by intracardiac or venipuncture and applied to filter paper. Liver samples were obtained from euthanized animals. Direct agglutination tests were used for antibody detection. Diagnostic agreement was evaluated using sensitivity, specificity, McNemar's test, and Kappa coefficient. Nine bats (20%) tested seropositive. Seven were positive in both whole blood and filter paper samples; one tested positive only in liver. Filter paper showed 92.31% specificity and 60% sensitivity, indicating a high false-negative rate. The Kappa coefficient (0.5231) indicated moderate agreement (p=0.0036). Although filter paper demonstrated good specificity and negative predictive value, its reduced sensitivity limits diagnostic reliability. Whole blood remains the most consistent sample type for *T. gondii* serodiagnosis in bats.

Detection of *Sarcocystis* spp. in opossums and coatis killed by roadkill in Paraná, Brazil

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Increasing urbanization has intensified the proximity of wildlife to urban environments, and the coatis (*Nasua nasua*) and opossums (*Didelphis albiventris*) are frequently found in these areas. Infection by *Sarcocystis* spp. has been reported in several animals and may facilitate its dissemination. In this context, this study aimed to detect the presence of *Sarcocystis* spp. in coatis and opossums killed by roadkill on highways in Paraná, Brazil. Between 2020 and 2022, carcasses of run-over animals were collected and subjected to autopsy. Organ fragments (brain, tongue, heart, lung, liver, and spleen) were collected for histological and molecular evaluation. The fragments were fixed and processed according to the routine for histology, and the sections were stained with hematoxylin-eosin. For molecular analysis, tissue extraction was performed from each organ, and a nested PCR targeting the 18S rRNA region. The n-PCR products were subjected to digestion with the restriction enzymes. In total, samples from 16 opossums and 7 coatis were collected. Microscopic examination revealed circulatory alterations such as hemorrhage, congestion, and edema, mainly in the lungs, liver, and brain. Inflammatory processes associated with parasites were also observed, predominantly in the digestive system. Mild to moderate interstitial pneumonia was detected in both species. In the n-PCR, 43.7% (10/23) were positive, with four samples showing cleavage patterns com-

patible with *Sarcocysts*. After sequencing the samples, three showed high similarities to Sarcocystis spp. The results identified the presence of the DNA of the studied agent in the animals run over in the study region. Understanding the relationship between urbanization, environmental changes, and the impacts on the ecology of wildlife is increasingly important for monitoring infectious diseases in animals and humans. Further studies should be conducted to evaluate the role of these animals in sarcocystosis epidemiology.

Monitoring of *Leishmania infantum* infection in captive Maned Wolves (*Chrysocyon brachyurus*) from Belo Horizonte Zoo, Minas Gerais, Brazil

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Visceral leishmaniasis is a globally distributed infectious zoonosis. Caused by biphasic and obligatorily intracellular protozoa of the genus *Leishmania*, its transmission occurs through the hematophagous activity of infected female sandflies, which act as vectors of the disease. In Brazil, *Lutzomyia longipalpis* is the primary vector, *Leishmania infantum* is the predominant etiological agent, and domestic dogs are the main reservoirs. This study aimed to evaluate the dynamics of *Leishmania* spp. infection in captive *Chrysocyon brachyurus* individuals from the Foundation of Municipal Parks and Zoobotany, located in Belo Horizonte, Minas Gerais, Brazil, endemic area of *L. infantum*. Whole blood, bone marrow, and skin samples were collected from four individuals before and after treatment with leishmaniostatic and leishmanicidal drugs. The detection of *Leishmania* spp. was performed by conventional PCR, targeting the internal transcribed spacer (ITS-1) of the ribosomal DNA (rDNA) gene, followed by enzymatic digestion with *HaeIII* for species identification through restriction fragment length polymorphism (RFLP) analysis. Six months after treatment, all individuals tested negative for *Leishmania* spp.; however, 3/4 reverted to positivity one year later, while the remaining individual became positive two years post-treatment. In all cases, *L. infantum* was confirmed as the circulating species. Bone marrow and skin samples were the most sensitive for parasite detection, whereas whole blood showed the lowest positivity rate.These results

highlight the exposure of these animals to the parasite, reinforcing the need for continuous health monitoring, especially in endemic areas. Furthermore, they provide valuable insights for guiding health managers in developing management strategies and public policies aimed at species conservation.

Special topic – Fasciola

Investigating *in vitro* competition between *Fasciola hepatica* and *Calicophoron daubneyi* in the intermediate snail host, *Galba truncatula*.

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In the UK and Ireland, as in many parts of Europe, *Galba truncatula* is the primary intermediate host for *Fasciola hepatica*. With the increase in *Calicophoron daubneyi* prevalnce, which is transmitted via the same snail species, it has been proposed that co-infection may occur, and that *C. daubneyi* may outcompete the more pathogenic *F. hepatica* within *G. truncatula* populations. However, there are few studies that demonstrate co-infection in the field and experimental competition experiments remain rare. This study aimed to test the susceptibility of wildtype (WT) *G. truncatula* populations to infection with *C. daubneyi* alone, *F. hepatica* alone and co-infection with both species simultaneously. A laboratory-maintained *G. truncatula* stock (SS) and three WT *G. truncatula* populations (BK, HR, AC) were initially assessed for susceptibility by exposure to miracidia from each trematode. Two WT snail populations, AC and HR, were most susceptible to infection from either *F. hepatica* or *C. daubneyi*. Three replicate batches of 50 snails were taken and each snail was exposed to either one *F. hepatica* miracidium, one *C. daubneyi* miracidium, or two miracidia (one of each parasite species). The number of snails infected was determined by cercarial shedding at 7-11 weeks post-exposure, dissection of the snails at 11 weeks post-exposure and multiplex PCR to detect *F. hepatica* and/or *C. daubneyi* DNA in the snail tissues. There was no significant difference in the number of snails shedding cercariae when snails were exposed to *F. hepatica* or *C. daubneyi* alone, compared to snails exposed to both parasites. However, snail dissections and multiplex PCR showed a significantly greater proportion of snails were infected when exposed to only one parasite ($p>0.001$). A significantly higher number of metacercariae were shed from co-exposed snails compared to those exposed to one parasite species ($p<0.05$). None of the *G. truncatula* exposed to both parasites subsequently shed cercariae of both species, with no evidence of co-infection present in dissected snails. However, multiplex PCR detected *F. hepatica* and *C. daubneyi* DNA in 29 individual snails (n=29/282, 10.28%). Only seven of these 29 snails survived to shed cercariae, with six of them shedding only liver fluke cercariae. In all experiments ~15% of snails remained uninfected. Based on the work presented here there is no evidence to that intramolluscan parasite competition directly impacts transmission of either *F. hepatica* or *C. daubneyi* and that a sufficiently large reservoir of snails persists that can drive transmission of both parasites on farm.

Waterborne and foodborne zoonotic helminth adaptation to extreme environmental con-

ditions: Fasciola hepatica ecopathology in tropical Andes

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Fasciola hepatica is a zoonotic trematode that affects farm animals in the Andean region of Peru. However, as a multihost parasite, it has also been reported in wild herbivores and the human population, creating an epidemiological bridge by transporting domestic artiodactyls to the Andean ecotones. The objective of this study was to determine its adaptation and distribution patterns in the Tropical Andes of Peru, in comparison with other helminthic zoonoses linked to aquatic environments, as well as the climatic factors that influence its biogeographic patterns. A search strategy based on bibliographic data and reports from our research team was used. It was found that *F. hepatica* is capable of infecting native domestic animals such as *Cavia porcellus*, *Lama glama*, and *Vicugna pacos*, as well as native wild animals such as *C. aperea*, *V. vicugna*, *L. guanicoe*, *Odocoileus virginianus*, and *Hippocamelus antisensis*. The presence of *F. hepatica* is reported in the snail *Galba viatrix* and in *V. pacos* in La Raya (Melgar, Puno), at an altitude between 4136 and 5470 masl, at a temperature between 14.75 °C and - 14.88 °C, and with an average rainfall of 625 mm and high average annual evaporation. In comparison, *Hysterothylacium* sp., *Heterophyes heterophyes*, *Centrocestus formosanus*, *Mesostephanus* sp., *Pygidopsis* sp. and *Ascocotyle longa* have been reported in fish from coastal wetlands in Peru; while in Amazonian wetlands: *Eustrongylides* sp. (L4), as well as *Contracaecum* spp. and *Clinostomum* sp., the latter two have adapted to diverse ecoregions of Peru along the entire climatic gradient. The observed distribution and adaptation patterns are related to environmental factors such as rainfall, humidity, droughts and floods, temperature, altitude, as well as the adaptation strategies of the free-living stages and intermediate hosts.

Combined albendazole-clorsulon treatment to control *Fasciola hepatica*: integrated pharmacokinetic and efficacy evaluations in sheep

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Fasciola hepatica causes fasciolosis, a growing zoonotic disease that affects both livestock and humans worldwide. The WHO has recognized it as a significant emerging neglected disease. Anthelmintic treatments are the main strategy to control fasciolosis in livestock animals, based on drugs such as triclabendazole (TCBZ), albendazole (ABZ), clorsulon (CLOR), nitroxynil, closantel, and rafoxanide. Only TCBZ is available for *F. hepatica* control in human medicine. However, the intensive use of TCBZ in veterinary medicine has exerted a significant selection pressure, leading to the development of resistant populations of *F. hepatica*. The use of drug combinations has been proposed as a strategy to preserve efficacy and delay the development of parasite resistance. The goal of the current work was to characterize the pharmacokinetic disposition and flukicidal efficacy for the ABZ+CLOR co-administration in *F. hepatica* infected sheep. The pharmacokinetic (PK) study included three (3) treated experimental groups: ABZ, CLOR and ABZ+CLOR. An untreated control group was included to perform the controlled efficacy test. Fourteen (14) days after treatment, animals were sacrificed, and adult *F. hepatica* specimens were removed from bile ducts and counted to evaluate the efficacy. The CLOR disposition kinetics was not affected by its co-administration with ABZ. Enhanced systemic exposure of the ABZ sulphoxide metabolite was observed after the co-administration ABZ+CLOR compared to the ABZ alone treatment. The flukicidal efficacy was 85% (ABZ), 92% (CLOR) and 100% (ABZ+-CLOR). The work described here contributes to the characterization of the disposition kinetics of both flukicidal molecules showing that the combined ABZ and CLOR therapy may enhance flukicidal efficacy in sheep.

Old drugs for new uses: pharmacokinetic assessment to support oxfendazole repurposing as a flukicidal compound

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Fasciolosis is a parasitic disease caused by liver flukes Fasciola hepatica that affects domestic ruminants and humans. The disease leads to significant economic losses in animal production due to decreased productivity. Human fasciolosis is considered a neglected tropical disease by the World Health Organization. Chemotherapy remains the most effective strategy for controlling fasciolosis in animals: However, its indiscriminate use resulted in the development of resistance to most effective flukicidal drugs. Oxfendazole (OFZ) is an old and well-established nematodicidal drug, with very limited activity against the trematode F. hepatica. In an attempt to repurpose its use, promissory flukicidal efficacies have been observed when OFZ was used at higher dose rates both in sheep and pigs. The pharmacokinetic profiles, accu-

mulation within adult F. hepatica, and clinical efficacy of orally administered OFZ were assessed in sheep using two different dose levels: the nematodicidal therapeutic dose of 5 mg/kg (OFZ5) and a higher dose of 30 mg/kg (OFZ30). OFZ was the main analyte detected in plasma of treated sheep, with its systemic exposure (AUC0–LOQ) increased from 17.9 ± 3.71 µg.h/mL (therapeutic dose) up to 85.4 ± 22.6 µg.h/mL (high dose). The plasma Cmax value was approximately 4 times higher in the OFZ30 group compared to the OFZ5 group. The differences in OFZ systemic exposure (bloodstream) were reflected in its accumulation pattern into F. hepatica, which was 332 % higher in the OFZ30 (4.28 µg/g) compared to the OFZ5 group (0.99 µg/g). The data shown here demonstrates that increasing OFZ dose is associated with an enhancement in drug exposure and greater accumulation within the target parasite, which may explain the promissory/advantageous efficacy of OFZ against adult liver flukes observed at the 30 mg/kg dose rate. The reported pharmacological data may contribute to stimulate OFZ development and use as a repurposed flukicidal benzimidazole drug.

Development of an evaluation system for *Fasciola hepatica* vaccine candidates using *in silico* and CRISPR-Cas approaches

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Fasciola hepatica is a trematode responsible for fasciolosis, an important veterinary disease and emerging zoonosis. While current control strategies predominantly rely on anthelmintics, the growing issue of drug resistance highlights the urgent need for effective vaccines. In this context, bioinformatic analyses of omics data have become essential for the rational selection of vaccine candidates, enabling the identification of proteins with key biological functions and exposure at the host-parasite interface. To identify vaccine candidates from proteomic studies of *F. hepatica* and assess their potential through laboratory experiments prior to conducting vaccination trials. A comprehensive bioinformatic analysis was performed using proteomes from different *F. hepatica* stages (newly excysted parasites and intestinal, peritoneal, and hepatic stages), previously obtained from our laboratory models that replicate the parasite's early migratory route within the host. Candidate proteins were selected and screened *in silico* for subcellular localization, antigenicity, and functional domains. Immunofluorescence and CRISPR-Cas9 editing were then used to further evaluate their biological relevance. Three candidate proteins—cathepsin B3, legumain 1, and serpin 1—were selected from all *F. hepatica* stages. Immunofluorescence assays confirmed their localization on the parasite's tegument, with distinct distribution patterns observed across specific developmental stages. Additionally, proof-of-concept CRISPR-Cas9-mediated gene editing of cathepsin B3 and legumain 1 resulted in slight disruptions of the targeted genes. Vaccine candidate proteins from *F. hepatica* were successfully identified and localized to the parasite's surface. Although CRISPR-Cas9 editing is still being optimized, it achieved partial gene disruption, demonstra-

ting the potential of this approach for validating and identifying future vaccine candidates.

In vitro evaluation of copper sulfate on miracidia stage of *Fasciola hepatica*

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Fasciolosis, caused by *Fasciola hepatica*, is a disease of major veterinary and public health significance. The free-swimming miracidia stage is essential for host infection and represents a potential target for environmental control strategies. Active biometals are innovative solutions and may be developed as new therapeutic products. This study aimed to evaluate the *in vitro* effect of copper sulfate ($CuSO_4$) solutions on the viability and motility of miracidia of *F. hepatica*. Newly hatched miracidia were exposed to $CuSO_4$ solutions from 62.5 to 8000µmol L^{-1}. A control group was maintained in distilled water, and a positive control was performed using nitroxinil 34% (100µmol L^{-1}). Observations were conducted from time zero (T0h) at regular intervals until complete loss of motility at each concentration. Mortality was defined as total absence of movement. A non-linear regression analysis was performed to determine the IC_{50} and goodness of fit (R^2). The solution showed a concentration-dependent motility effect. At 8000µmol L^{-1}, miracidia were immobile within 10 minutes. Complete mortality occurred at 1h20min for 4000µmol L^{-1}, 2h30min for 2000µmol L^{-1}, 3h10min for 1000µmol L^{-1}, and 3h40min for 500µmol L^{-1}. At 250µmol L^{-1}, miracidia were immobile at 3h50min, while 125µmol L^{-1} and 62.5µmol L^{-1} induced total mortality at 4h10min and 4h40min, respectively. In the control group, miracidia remained fully motile throughout 5h00min. The response yielded an IC_{50} of 1384µmol L^{-1} and an R^2 of 0.96, indicating a strong fit of the model. $CuSO_4$ demonstrated a toxic effect on *F. hepatica* miracidia, with ≥2000µmol L^{-1} inducing rapid mortality. Likewise, the high R^2 value supports the reliability of the *in vitro* test and the concentration-response relationship. The $CuSO_4$ solution had a clear larvicidal effect that may be used in the environment to reduce fasciolosis. Moreover, further studies are needed to assess tissue toxicity and its practical use in endemic areas.

Dynamics of *F. hepatica* infection in alpine pastured cattle in Austria

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Alpine regions are amongst those with the highest prevalence rate of *Fasciola hepatica* (F. hepatica) in Europe. In recent year, prevalence was observed to be increasing, likely due to climatic changes and inadequate control strategies. This study observed the infection dynamics of liver flukes in dairy cattle in Tyrol, Austria across different age groups over one grazing season on 14 dairy farms and two seasons on

four of these farms (focus farms) to understand epidemiology and inform sustainable parasite management. Faecal samples were collected before and after the alpine season on all farms. Focus farms underwent additional monthly sampling during two seasons, and ten other farms were sampled once mid-season. Bulk tank milk (BTM), individual milk, and blood samples were also collected on focus farms. Faecal samples were analyzed using quantitative sedimentation (Flukefinder®) and Fasciola copro-antigen ELISA. Blood and milk samples were tested for antibodies using ELISA. The study found the highest infection rate in dairy cows (40-100%), remaining mostly constant throughout the season, with some increases in late summer. First-season grazers were largely Fasciola-negative until October, while on some farms, infections appeared from August onwards at a 20% infection rate. Second-season grazers had infection rates ranging from 0-40%, with notable increases observed in June. These results help identify high-risk pastures, emphasizing dairy cows' significant role in parasite transmission. Recommendations include improved treatment strategies, pasture management, intermediate host-habitat exclusion, and slurry hygiene. The data will validate a hydro-epidemiological climate model adapted for predicting infection maxima in alpine conditions.

Neurotoxic Spider Venoms as Novel Flukicides Against *Fasciola hepatica*

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The parasite *Fasciola hepatica*, commonly known as the liver fluke, is responsible for enormous economic losses in the livestock industry globally. Triclabendazole (TCZ) is the most effective treatment for controlling *F. hepatica* infection in animals. The emergence of TCZ--resistant strains necessitates the development of novel flukicidal agents. Neurotoxic spider venom toxins (SVTs), which target invertebrate ion channels, have demonstrated potential as biopesticides and could provide a new method of parasite control. The purpose of this study is to assess the efficacy of certain neurotoxic SVTs against *F. hepatica* and to further understand their mechanisms of action. We employed an *in vitro* culture system to evaluate the effects of recombinant SVTs on *F. hepatica* newly excysted juveniles (NEJs). Toxins were selected based on their established ability to inhibit invertebrate ion channels. These ion channels were identified as present in the genome of *F. hepatica* through comprehensive bioinformatics analysis. A spider venom recombinant toxin, known as a positive allosteric modulator (PAM) of nicotinic acetylcholine receptors (nAChRs), was screened against newly excysted juvenile (NEJs) *F. hepatica* at varying treatment concentrations. Post-exposure parasite viability was assessed, and morphological alterations were observed. The recombinant toxin had a significant impact on the survival of *F. hepatica* juveniles when compared to the untreated control, with 50% survival rate after 96 hours. After 24 hours of toxin incubation, motility was significantly reduced, with no movement observed at all the treatment doses. Juvenile area measurements revealed a 45% reduction in growth of treated juveniles compared to untreated controls, suggesting the recombinant toxin's impact on parasite development. These results demonstrate the potent flukicidal activity of Neurotoxic SVTs against *F. hepatica*, likely mediated through disruption of the activities of their ion channels. These findings provide

support for further investigation into SVTs as viable candidates for the development of novel flukicides, providing a viable alternative for tackling populations of drug-resistant liver flukes.

Characterizing local and systemic cytokine responses in sheep infected with *Fasciola hepatica*

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*Fasciola hepatic*a is a globally distributed trematode that causes chronic liver disease in ruminants, leading to major economic losses. Control of fasciolosis is challenged by the lack of effective vaccines and the emergence of anthelmintic resistance. A deeper understanding of host immune responses is essential for guiding the development of new immunological tools and therapies. In this study, we aimed to characterize the cytokine response in sheep experimentally infected with *F. hepatica* using a broad protein-level approach. We performed ex vivo stimulation assays using whole blood and hepatic lymph node (hLN) cell cultures incubated with *F. hepatica* excretory/secretory antigens. Supernatants were analysed using a multiplex ELISA cytokine array, allowing the simultaneous quantification of 20 cytokines. This strategy enabled the assessment of immune responses in both systemic and local compartments during early and chronic infection phases. Compared to traditional RNA-based analyses, this approach offers a direct snapshot of the cytokine environment and includes mediators rarely addressed in ovine fasciolosis, such as LIF, LEKTI, and Cadherin-6. Preliminary results confirmed the expected shift toward a Th2/regulatory profile in infected animals, and revealed modulation of specific cytokines with potential relevance in tissue repair and immune regulation. Our findings support the use of multiplexed immunoassays in helminth research and contribute to broadening the current picture of the ovine immune response to *F. hepatica* infection. Further analyses are ongoing to explore individual variability and temporal dynamics.

Biochemical changes of the liver enzymes activity in naturally infected cattle after anti-Fasciola treatment

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The liver fluke *Fasciola hepatica* affinity to the hepatic parenchyma might be reflected in altered liver enzymes activity with expected changes after anthelmintic treatment. The study aimed to investigate the hepatic and cholestatic

enzymes activity after treatment with three anthelmintics: ivermectin+clorsulon (IVM+CLOR), levamisole+oxyclozanide (LEV+OXY), albendazole (ABZ) in cattle with fasciolosis. In total of 48 individuals were arranged into 4 groups (3 treatment, 1 untreated group) and three sampling days were established, as follows: Day 0 – day of treatment, Day 7 and Day 21 – after-treatment days, wherein blood and faecal samples were collected. The hepatic and cholestatic enzymes activity was determined spectroscopically according to standardized protocols of IFCC and after-treatment activity change was calculated. The faecal samples were analysed by quantitative sedimentation technique and anthelmintic efficacy was evaluated by *in vivo* faecal egg count reduction test (FECRT). The GLMM analysis confirmed significant decreased activity of the cholestatic enzyme gamma-glutamyl transpherase (GGT) within after-treatment days by 18.03%–27.87%, as well as for all treatment groups by 15.25%–25.42%. The RM ANOVA showed significant faecal egg count reduction (FECR) for LEV+OXY group on Day 7 and 21 by 97.83%–100% and for IVM+CLOR group on Day 21 by 92.67%. A comparison of %FECR and %GGT-change showed moderate negative correlation ($r_s(34) = -0.334$, $p=0.046$). The GGT activity decrease could be indicated as a biomarker of the liver regenerative processes after treatment of chronic fasciolosis, as well as useful diagnostic tool for the evaluation of the treatment efficacy in naturally infected cattle.

Pilot study on practical liver fluke ultrasound as a diagnostic tool for *Fasciola hepatica* in cattle

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Infections with *Fasciola hepatica* affect animal health, welfare and production, but do not Always cause disease. Distinguishing healthy from subclinically infected animals is instrumental to prevent unnecessary use of anthelmintics. Targeted selective treatment (TST) based on disease specific pathophysiological parameters of cattle allows a minimization of treatments while preventing (sub)clinical consequences of infection. The aim of this pilot study is to distinguish subclinically infected and healthy animals using practical liver fluke ultrasound (PLUS). This study aims to: 1. Determine whether PLUS can differentiate between infected and non-infected animals, and 2. Assess the correlation of PLUS findings with other pathophysiological parameters of *F. hepatica* disease. 42 cows from farm A with a 100% prevalence of infections and 19 cows from farm B with 0% prevalence of infection were assessed using PLUS, identifying 3 findings: calcification in the bile ducts (BD), and aberrations in the liver parenchyma (P) or gall bladder (GB). Findings on PLUS images were recorded independently by two observers and were correlated with infection at the animal level as determined by EPG. On a third farm (C), with animals affected by *F. hepatica* disease, PLUS was performed on Fasciola FEC positive and control cows. Cows found positive on PLUS findings were assessed for pathophysiological blood markers validated and commonly used to assess liver damage. BD showed perfect agreement (1.00) and specifici-

ty of 100% (CI: 82% – 100%) but low Se of 17% (CI: 7% - 31%). GB showed a substantial agreement (0.82) and a Specificity of 84% (CI: 60% – 97%) and Se 7% (CI: 1% – 19%). P demonstrated a moderate agreement (0.52) and a specificity of 89% (CI: 82% - 100%) with a sensitivity of 7% (CI: 7% - 31%). The inter-observer agreement was between 0.52 and 1.00 for different PLUS findings. The specificity of different findings was between 84% (CI: 60% – 97%) and 100% (CI: 82% - 100%), with a sensitivity between 7% (CI: 1% – 19%) and 17%. All animals that demonstrated positive findings on PLUS had elevated blood levels of markers. The high specificity and high correlation with pathophysiological blood parameters suggest that detecting liver fluke infections using PLUS under field conditions is possible. The sensitivity for detecting FEC positive animals is too low to be of practical use. The sensitivity and specificity of animals demonstrating elevated pathophysiological blood parameters needs to be studied further to conclusively validate PLUS for the purpose of TST.

A contemporary map of *Fasciola hepatica* distribution in sheep and cattle in New South Wales

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Fasciola hepatica is a global threat to livestock production, human health, and food security. In New South Wales (NSW), Australia, data on the distribution of *F. hepatica* are more than 50 years out of date and lack species-specific insights for cattle and sheep. Accurate, up-to-date distribution data are essential for livestock producers to implement targeted control programs, for veterinarians to provide timely and effective treatment recommendations, and for researchers to identify emerging trends, such as those influenced by climate change. This study addresses this knowledge gap by using diagnostic samples submitted to the Elizabeth MacArthur Agricultural Institute (EMAI) between 2019-2023 to update the distribution of *F. hepatica* in cattle and sheep in NSW. Diagnostic records were extracted, cleaned, analysed and geospatially mapped at the postcode level to reveal temporal and spatial trends by livestock species. Our findings suggest that *F. hepatica* hotspots in sheep are concentrated in the southeastern regions of NSW, whereas in cattle, hotspots extend along the coast. These results reinforce the existing dogma of *F. hepatica* distribution in NSW, providing evidence-based insights that are key to improved surveillance, refining precision parasite management, and mitigating the ongoing impacts of *F. hepatica* on animal health and production in NSW.

Daily egg excretion pattern of *Fasciola hepatica* in chronically infected cows

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Fasciola hepatica causes infections in cattle leading to (sub)clinical symptoms and production losses, especially at farms in peat meadow areas, the habitat of its intermediate host. Faecal egg counts (FEC) are the diagnostic method for in vivo diagnosis of active *F. hepatica* in-

fections, although eggs are irregularly excreted with the faeces. False negatives may occur if faecal sampling is performed during periods of low egg excretion. This study aimed to examine the daily pattern of *F. hepatica* egg excretion in faeces of infected cows to determine if and when cows have a common period of high egg excretion. Ten *F. hepatica* chronically infected dairy cows from the same farm, selected based on high FECs, were sampled on three consecutive days around five time points: 7:15h (morning milking), 8:30h (turnout to pasture), 13:30h (on the pasture), 18:15h (evening milking), 19:40h (turnout to pasture). Faecal samples were collected by catching spontaneous defecation. FECs were performed using the Botvanger®, a method comparable with the Flukefinder®, and were defined as eggs per gram faeces. The daily egg excretion pattern was visualized with a polynomial regression model. In total, 103 samples were analysed. Descriptive analysis showed variation in FECs between cows at all time points, with the greatest differences observed in the afternoon and the evening. Overall, the modelled daily excretion pattern showed that FECs were the lowest in the early morning (6:59h: μ = 44.1, 95% CI -12.1 - 101.3), after which it increased throughout the morning, as highest FECs were observed in the afternoon (13:31h: μ = 195.6, 95% CI 133.1 - 258.1) and the evening (19:20h: μ = 187.0, 95% CI 131.2 - 242.9). Highest *F. hepatica* egg counts were observed in the afternoon and the evening. Since egg excretion is often low in cows and the diagnostic performance of the Botvanger® has certain limitations, these periods with high FECs are optimal moments for faecal sampling when aiming to detect *F. hepatica* infected cows within a herd.

Fasciola hepatica on dairy farms in European alps - increasing prevalence, impact on milk yield and quality and risk factor for _S._ Dublin infections

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Fasciola hepatica (*F. hepatica*) infections impair animal health and productivity in livestock worldwide. Recently, the prevalence has increased in the UK, Denmark, and Switzerland, likely due to climate change. Infections were also associated with a higher risk for infections with *Salmonella* Dublin (*S.* Dublin), a zoonotic pathogen that causes diarrhoea, abortions, and pneumonia. Dairy farming in Tyrol, a federal state in the mountainous region of western Austria, is characterized by alpine summer pasturing with periods of high rainfall, small-holder farms and the predominant use of dual-purpose breeds. In 2005, a herd level prevalence of 73% for *F. hepatica* based on bulk tank milk (BTM) serology was reported in Tyrol. We aimed to re-assess the seroprevalence of *F. hepatica* antibodies in Tyrolean dairy farms and their impact on milk yield and quality. In 2023, BTM samples from 3645 farms were tested using ELISA (IDEXX Fasciolosis Verification Test, Montpellier, France), and a multivariate regression model was built to analyse

the association between *F. hepatica* infection and milk production parameters. In 2022, *S.* Dublin antibody analysis of BTM samples was conducted on 3670 farms. 86.1% of BTM samples tested positive for *F. hepatica*. The increase of positive samples by 13.1% over time was significantly associated with a decrease in milk yield, relative fat and protein ($p < 0.001$). Farms with *F. hepatica* infections were significantly more likely to be *S.* Dublin-positive compared to negative farms (OR = 11.6). The increasing *F. hepatica* prevalence suggests that current control strategies are insufficient, while favourable conditions for the parasite are associated with changes in climatic conditions favouring extended pasturing. Future research will examine climate impacts on liver fluke prevalence. The link between liver fluke and *S.* Dublin highlights the broader relevance of *F. hepatica* beyond direct health effects.

First report of multi-resistance against triclabendazole and albendazole in *Fasciola hepatica* in sheep from Northern Germany

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The trematode *Fasciola hepatica* is a global health problem, causing economic losses in the sheep sector. Several drugs are available for the treatment of these endoparasites. Only triclabendazole (TCBZ) is able to kill juvenile and adult *F. hepatica,* but TCBZ resistance was first described 30 years ago, although cases in Germany have been rare to date. The purpose of the study was to determine the efficacy of TCBZ and albendazole (ABZ) against *F. hepatica* infected sheep in Northern Germany. A faecal egg count reduction test (FECRT) was performed on 12 farms using FLUKEFINDER® on day 0 and 14 to 15 days after treatment. The FECs were compared with a commercially available copro-antigen ELISA. Furthermore, recovered *F. hepatica* eggs were examined for ABZ susceptibility using an in vitro egg hatch assay. Farms (n = 130) were screened for the presence of fluke eggs from December 2023 to January 2025 and 22.3% were positive for liver fluke, 22.3% for rumen fluke and 13.8% of the farms were positive for both parasites. Cohen's κ of agreement between coproscopic and copro-antigen results was 0.71. One farm had an intermediate FECR of 67.6% and one farm showed evidence of resistance against TCBZ, with a 17.4% FECR. Groups of animals from this farm, previously treated with TCBZ, were then treated with either twice the normal TCBZ dose (20 mg/kg) or a single dose of ABZ or closantel. No reduction in FECs occurred after ABZ or double-dose TCBZ treatments. In contrast, treatment with closantel completely suppressed egg shedding. Despite apparent in vivo ABZ resistance, the in vitro egg development test classified this population as susceptible to ABZ according to the results at a critical concentration of 0.5 µM ABZ. The present study represents the second report of TCBZ resistance and the first of TCBZ/ABZ multi-resistance of *F. hepatica* in Germany. Close monitoring, especially if resistance spreads to more farms, is highly warranted.

In vitro and _in vivo_ assessment of triclabendazole's efficacy against _Fasciola hepatica_

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Fasciola hepatica causes significant economic losses in livestock due to its parasitism in the liver. The increasing use of anthelmintic drugs has led to the development of parasitic resistance, making these disease control a challenge. This study evaluated the antiparasitic efficacy of triclabendazole, a fasciolicide, through *in vitro* and *in vivo* tests. Fecal samples were collected from naturally infected animals on a property in Alegre, Espírito Santo, an endemic region for fasciolosis in Brazil. The diagnosis was made by the coproparasitological technique Flukefinder® for the detection of *F. hepatica* eggs. After confirmation of infection, the animals were treated with Triclabendazole 10% (Saguaymic Plus®), administered orally at the recommended dose of 6 mL/50 kg of live weight. Twenty-one days after treatment, a new coproparasitological analysis revealed the absence of *F. hepatica* eggs, indicating 100% *in vivo* efficacy. In the *in vitro* evaluation, the Egg Hatching Test was performed, with eggs obtained directly from the gallbladder of positive slaughtered cattle in the southern region of the state and incubated in 24-well Elisa plates for 15 days with different concentrations of triclabendazole (20, 40, 60, 80 and 100 ppm). Then, the plates were exposed to a 100 W incandescent lamp for three hours, with hourly observations of miracidia hatching. No eggs hatched in the treated groups, while in the control group, incubated only with distilled water, 92% of the eggs hatched, with free miracidia and empty eggs observed. The efficacy calculation showed 100% inhibition of egg hatching, confirming the efficiency of triclabendazole *in vitro*. The results obtained indicate that triclabendazole is highly effective in controlling this population of *F. hepatica*, both in laboratory tests and under natural conditions of infection. The results confirm triclabendazole's efficacy against *Fasciola hepatica* in these area in Brazil, highlighting its potential for improved control.

Analysis of bovine fascioliasis prevalence in the State of Rio de Janeiro from 2021 to 2024

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Fascioliasis is a parasitic disease caused by the trematode *Fasciola hepatica*, which affects the liver of different mammals, especially ruminants, making it of significant economic importance in Brazil. Fascioliasis is still included in the list of neglected tropical diseases, and according to WHO, at least 2.4 million people have been infected in more than 75 countries. In Brazil, fascioliasis is highly prevalent in the southern regions and in some states of the southeastern region, and it is still geographically expanding. The objective of this study is to analyze the prevalence of bovine fascioliasis in slaughtered animals in the state of Rio de Janeiro, from 2021 to 2024. Data from the State Inspection Servi-

ce (SIE) were collected regarding the disposal of bovine livers affected by *Fasciola hepatica* in slaughterhouses in Rio de Janeiro. The data were calculated using descriptive statistics of the frequencies of positive animals in relation to the total number of animals slaughtered and by mapping the locations with the highest occurrence in the state. Between 2021 and 2024, the average prevalence of fascioliasis in the state was 9.41%, with the following rates distributed across the four analyzed years: 4.43%, 12.25%, 10.61%, and 10.35%. When analyzing the distribution in the mesoregions of Rio de Janeiro, the Northwest Fluminense region presented the highest prevalence rates of fascioliasis, 63.48% in 2021, 51.38% in 2022, 59.02% in 2023, and 59.83% in 2024, followed by the North Fluminense region with rates of 28.89%, 22.53%, 24.19%, and 25.61% in the same years analyzed. This demonstrates high rates of parasitism in these regions, allowing for the rapid spread of the disease since all regions of the state have reported cases of *Fasciola hepatica* in cattle every month of the years. Therefore, it is necessary to establish criteria for identification and control of the disease, minimizing economic losses for farmers and risks to animal and human health.

An assessment of liver fluke (F. hepatica) diagnostics in naturally infected cattle in New Zealand

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Farmers and their advisors require liver fluke diagnostic methods that not only indicate past or current infection but also quantify the number of flukes present. The aim of these studies was to investigate the characteristics of liver fluke diagnostics in naturally infected, grass fed cattle. In Study 1, 39 naturally infected cattle were faecal and serum sampled with livers recovered at slaughter. Diagnostics included faecal egg count (FEC) and coproantigen ELISA, IDEXX serum antibody ELISA and total liver fluke count. In Study 2, 120 dairy cows each from two herds were blood and faecal sampled. Diagnostics included FEC, coproantigen ELISA, and IDEXX serum antibody ELISA. A Bayesian latent class model (LCM) was used to test the characteristics of the three diagnostic tests. In Study 1 there was a significant relationship between coproantigen ELISA value (Val) and fluke count ($p<0.0001$), with Val of 20.1 predicting 10 flukes and Val of 44.8 predicting 30 flukes. The predictive model was *fluke count = 1.407 x Val.* There was a significant effect of total fluke count ($p=0.03$) on liveweight at slaughter, with liveweight falling 20.4kg for each unit increase in loge (total fluke count). In Study 2 Bayesian LCM found the coproantigen ELISA was the most accurate for diagnosing liver fluke infection (Se=0.98, Sp=0.95) compared to IDEXX ELISA (Se=0.39, Sp=0.86) and FEC (Se=0.23, Sp=0.92). Farmers and their advisors should be encouraged to use the coproantigen ELISA to diagnose liver fluke infection in individual cattle.

Liver Fluke (*Fasciola hepatica*), prevalence and impact of infection on milk production in dairy cows on the West Coast of the South Island of New Zealand

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Cows in dairy herds on the West Coast of the South Island of New Zealand, calve in spring and are fed a pasture-based diet without housing. This potentially exposes cows to metacercariae of liver fluke year round, especially in this temperate climate. However, neither the prevalence of infection nor the impact on milk production has not been previously investigated. The objectives of this study were to investigate the prevalence of liver fluke in dairy herds in the autumn and spring, and the impact of infection on milk production parameters. Milk samples from herds were analysed for antibody titre to *F. hepatica* in the autumn (n=395) and spring (n=377) of 2017. To investigate the impact of infection during lactation on milk production parameters, blood samples from cows in four herds collected in the spring and autumn were analysed for antibody titre to *F. hepatica* The IDEXX Fasciolosis Verification ELISA was used for both milk and serum analysis. Milk production parameters for each cow were monitored three to four times during the same lactation. Liver fluke infection in dairy herds was very common in the autumn and spring with 75% (298/395) and 67% (254/377) respectively having antibodies present. Of the 388 herds sampled on both occasions, 57% in the autumn (223/388) had an antibody titre indicating a within herd prevalence of >20%, while 72% (160/223) of these herds in the spring again had an antibody titre indicating a within herd prevalence of >20%. Cows that were categorised *negative* in the spring and autumn were analysed against cows that were *negative* and seroconverted to *strong positive*. Cows that seroconverted had a decrease in milk fat percentage of 0.24 percentage points, with a calculated financial cost of NZD 60.20 per cow over that lactation. Liver fluke is indeed a very common parasite of dairy cows in this region, with infection during the lactation period resulting in a decrease in milk fat percentage at a cost of NZD 60.20 per infected cow.

Morphological and molecular updating on *Fasciola* spp. isolated from sheep in a Mediterranean area

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Fasciolosis is a hepatobiliary helminthic disease caused by trematodes of *Fasciola* genus; the two species *Fasciola hepatica* and *Fasciola gigantica* leads to major economic losses in domestic ruminants. Despite its significant impact, few studies have highlighted the prevalence of fasciolosis in Tunisian sheep, while the occurrence and morphology of the flukes have never been investigated. Therefore, the present study aimed to characterize the Tunisian liver

flukes by morphometric and molecular analyses. A total of 300 flukes were collected from 66 sheep livers in Bizerte governorate, Northwest Tunisia between January and March 2021. Five morphometric parameters were determined for all the liver flukes, as follows: total body length (BL), distance between ventral sucker and the tail (VS-T), distance between oral sucker and ventral sucker (OS-VS), abdomen diameter (AD), tail diameter (TD) and the body length to width ratio (BL/BW). The molecular and phylogenetic analysis of the fluke specimen's identification was carried out by targeting a 680 bp sequence of ITS1 gene. The morphometric measurements showed that the mean of the total body length of the adult flukes was 21.1 ± 2.7 mm with minimum and maximum lengths of 13 and 31 mm, respectively. The PCR-RFLP analysis revealed a single profile consisting of three bands of approximately 370, 100, and 60 bp. *Fasciola* sequences described in the present study (GenBank numbers: OQ457027 and OQ457028) showed 99.58–100% identity to *F. hepatica* isolated from different hosts and different regions throughout the world. Molecular and phylogenetic analyses confirmed the presence of a single liver fluke species: *F. hepatica* in the Sejnane region of northwest of Tunisia. However, further studies are needed to determine the occurrence of *Fasciola* species in other Tunisian regions.

First report of *Pseudosuccinea columella* (Mollusca: Lymnaeidae) naturally infected with *Fasciola hepatica* (Trematoda: Fasciolidae) in the Espírito Santo State, Brazil

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Fasciolosis is a parasitic disease in expansion in Brazil, with high prevalences reported in cattle, mainly in the South and Southeast regions of the country. On the other hand, studies on the snails that transmit *Fasciola hepatica* are scarce and ancient, which revealed the lymnaeid *Pseudosuccinea columella* naturally infected in some Brazilian states (SP, MG, RJ, and RS). This study aims to report the infection of *P. columella* with *F. hepatica* in an endemic area for bovine fasciolosis in southern Espírito Santo State. A long-term malacological survey was carried out monthly from March 2024 to February 2025 in waterbodies located in three cattle farms with a history of occurrence of fascioliasis. New cattle fecal samples were subjected to coproparasitological tests. The snails were collected, sent to the laboratory and morphologically identified. To evaluate the infection with larval trematodes, they were subjected to the photostimulation test to induce the cercarial emergence. All snails negative after this test were crushed and examined for the presence of trematode intramolluscan stages (sporocysts and rediae). The morphology of the trematode larval stages was analyzed under a light microscope. As a result, eggs of *F. hepatica* were found in the feces of 51/150 (34%) bovines in the three evaluated farms. Moreover, 1021 specimens of *P. columella* were collected, of which 14 (1.37%) and 48 (4.7%) were found infected with trematodes after the photostimulation and crushing tests, respectively. Of these, two lymnaeids collected in the same farm in November 2024 were found harboring gymnocephalous cercariae morpholo-

gically compatible with *F. hepatica*. This is the first report of *P. columella* naturally infected with *F. hepatica* in the Espírito Santo State. The low infection rate verified in snails in an area of high infection of bovine points to the necessity of new integrated malacological studies aiming to characterize the disease transmission dynamics in the region.

Evaluation of different benzimidazole formulations against sheep naturally infected with *Fasciola hepatica* following the different WAAVP guidelines for the detection of anthelmintic resistance

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The aim of the present study was to measure the resistance or susceptibility of albendazole (ABZ) and triclabemdazole (TCBZ) in a naturally infected *F. hepatica* flock in Spain. The efficacy of fenbendazole (FENB) and oxfendazole(OXF) at different doses were also evaluated as alternative treatments since TCBZ is not commercialized in Spain. For this, a Faecal Egg Count Reduction Test (FECRT) was conducted and later evaluated using different statistical methods described for gastrointestinal nematodes in the two WAAVP guidelines published in 1992 and 2023. For the latter, two different methods are used, a bayesian approach, and a hybrid frequentist/bayesian analysis. For this purpose, in a first trial 4 groups of 10 animals each were established, which were administered ABZ at a dose of 7.5 mg/kg, OXF at 5 mg/kg, OXF at 30 mg/kg, and an untreated control group was also included (unpaired study design). This trial also included measures of FECR from the same animals (paired study design). In a second trial, FENB was evaluated at a dose of 10 mg/kg in a group of 19 sheep and compared with an untreated control group of 10 animals (unpaired study design). In a third trial the efficacy of TCBZ was measured in 28 animals at 10 mg/kg (paired study design). The mean number of eggs per gram (epg) in faeces was compared between the day of treatment and 2 weeks later, in the paired study design, or the comparison was done between the egp of the treated and control group at 2 weeks after treatment (unpaired study design). The ABZ resistance was confirmed to the adult stage of *F. hepatica* with FECR values between 63 and 73%, as paired and unpaired studies respectively, and with a confidence interval (CI) of 95%. The susceptibility to TCBZ was confirmed with a FECR of 98% ($CI_{95\%}$), according to the guideline published in 1992 and the bayesian approach, however, by the hybrid frequentist-bayesian method the result remained as “inconclusive”. FENB and OXF formulations improved treatment response, when compared to ABZ, but all analysis revealed reduced efficacies, between 68 and 80%. Further studies are needed to establish drug-specific targets for flukes and consequently which analytical methods should be preferred specifically for this trematode.

Special topic – Myiasis

Curative efficacy of three synthetic products against *Cochliomyia hominivorax* fly larvae in naturally infested Nellore cattle in the state of Maranhão, Amazon biome, Brazil

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Known as the screwworm of the New World, *Cochliomyia hominivorax* (Coquerel, 1858) causes primary myiasis in wild and domestic animals in the tropical and subtropical regions from Brazil. It is a holometabolous insect, going through the stages of egg, larva, pupa, and adult. Its parasitic phase begins when the adult female lays her eggs on the edges of wounds, mucous membranes, and other body orifices, causing myiasis and resulting in harm to both animal and human health. It is necessary to conduct tests in search of new formulations or drug combinations with curative potential against *C. hominivorax* larval infestations. The objective of this study was to evaluate the curative efficacy of 4% Ivermectin (IVM), 1% Doramectin (DRM), and a commercial product based on 1.1% Doramectin, 1.2% Abamectin, and 2% Ivermectin (DAI) against *C. hominivorax* larvae in Nellore cattle. The study was conducted in the municipality of Buriticupu, in the state of Maranhão, within the Amazon biome. A total of 24 Nelore cattle aged between 19 and 20 months were used. Three experimental groups (GT) were formed, with 8 animals in each group, treated via subcutaneous application with IVM 4% (800 mcg/kg) in GT1, DRM 1% (200 mcg/kg) in GT2, and DAI (220 mcg/kg; 240 mcg/kg; 400 mcg/kg) in GT3, respectively. The animals were naturally infested and evaluated from the 1st to the 10th day post-treatment (DPT). The efficacy value of the DAI combination was 100% on the 4th DPT. However, both IVM 4% and DRM 1% failed, each with 12.5% active myiasis on the 10th DPT. Based on the results of this study, both IVM 4% and DRM 1%, when used according to the demonstrated protocol, were considered ineffective in curing myiasis in naturally infested Nellore cattle.

Current situation and treatment of the screwworm in livestock in Mexico

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The reintroduction of the New World screwworm in Mexico poses a serious threat to animal health and human well-being. Favorable climatic conditions, the pest's high dispersal capacity, intensive livestock movement across the southern border, and various human-related activities (such as transporting infested live animals, hides, hunting trophies, and the use of contaminated transport vehicles) all increase the likelihood of reentry and spread, given the screwworm's ability to travel and reproduce rapidly. It has been over 30 years since Mexico was officially declared free of this pest. Today, the southeastern region remains the most vulnerable endemic zone, with a continuous risk

of the screwworm migrating northward. This report presents an overview of the current situation of the screwworm in Mexico, including its epidemiology and available clinical treatments. The recent discovery of the larval stage of *Cochliomyia hominivorax* in more than 700 confirmed cases marks the pest as a growing threat to livestock and other susceptible animal species. Given this, timely scientific updates on these cases are critical. The objective of this work is to document the treatment, three positive cases have been documented, two in cattle and one in horses in the state of Tabasco. These cases were successfully resolved using a standardized treatment protocol involving Negasunt®, Baymec®, and Asuntol® in bovines. Material and methods, healing time was recorded to document the effect of the treatment. A human infestation case was also recorded. In response, Mexico, through the National Service for Agro-Food Health, Safety and Quality (SENASICA), has strengthened its infrastructure at Federal Inspection and Verification Points in Catazajá, La Trinitaria, and Huixtla, Chiapas, along the border with Guatemala. At these checkpoints, trained dogs are used to detect infested wounds, and compliance with treatment protocols is verified by checking documentation of livestock bathing and ensuring transport vehicles are properly sealed and in good condition. Clinical treatment of this parasitic infestation typically involves the use of organophosphates, synthetic pyrethroids, carbamates, wound-healing agents, and low-concentration macrocyclic lactones. However, there have been reports indicating that avermectins may not be effective in controlling screwworm. For example, studies in Argentina have shown treatment failures with 3.15% Ivermectin and 1% Doramectin in preventing natural infestations of *C. hominivorax* larvae. These findings were based on both field efficacy tests and pharmacokinetic and pharmacodynamic analyses. This diagnosis remains the most critical tool in determining effective treatment In Mexico, once a case is reported, treatment is applied directly at the ones affected. The results indicate that the protocol with coumaphos, propoxur and endectocide has an outside-in and inside-out action to solve screwworm cases. Specialized people from CPA-OIRSA-SENASICA are responsible for monitoring and responding to these reports. The goal is to control and mitigate the spread of the screwworm, which is crucial not only for maintaining livestock trade but also for preventing significant economic losses to producers and the country. It is concluded that timely diagnosis and correctly applied treatment provide adequate results in cattle and horses.

Curative and preventive efficacy of Forbox® FT against *Cochliomyia hominivorax* larvae in experimentally infested cattle

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The *Cochliomyia hominivorax* fly is a parasite that requires living tissue as food, producing wounds on almost all domestic and wild animals and even humans. Historically, this fly's distribution extends from the south of the United States of America to the northeast of Chile, Argentina and Uruguay, causing damage to livestock. In Brazil, this fly is distributed throu-

ghout the country, causing economic losses in beef and dairy cattle. Treatment is mainly carried out with topical products, and failure on treatment in a timely manner can result in death of both young and adult animals. The aim of this study was to evaluate the larvicidal efficacy of a product based on cypermethrin 4%, imidacloprid 4% and fluazuron 3% (Forbox® FT) on treatment and prevention of cattle experimentally infested with *Cochliomyia hominivorax* larvae. The studies only began after approval by the UFRRJ's Ethics Committee for the use of animals. The animals in the curative study were experimentally infested on D-2 with 100 *Cochliomyia hominivorax* larvae and treated on D0. The animals in the preventive study were treated on D0 and then infested with larvae. The animals received Forbox® FT at a dose of 1 mL/10 Kg of body weight via pour on. The presence of live larvae was assessed on days D+1, D+2 and D+3 in both studies. On day D+3, live larvae were counted, and all larvae (live and dead) were removed from the treated and control animals. Forbox® FT showed 100% larvicidal efficacy in both curative and preventive tests, making it an excellent option for treatment and prevention of cattle affected by *Cochliomyia hominivorax* larvae.

Evaluation of the effect of anethole on the hatchability of Cochlyomia hominivorax (Coqueiral,1858)

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The species Cochliomyia hominivorax (Diptera: Calliphoridae) represents a category of insects of great sanitary and economic importance, which drives the search for effective alternatives for their control. Before the dissemination of synthetic compounds, essential oils from plants were already used to combat insects due to their insecticidal properties. In this context, the present study aimed to evaluate the effect of anethole, the main compound of anise (Pimpinella anisum), on the hatching of C. hominivorax eggs, investigating its ovicidal potential. For the experiment, 4,050 C. hominivorax eggs from a colony maintained were used. The eggs were collected within 6 hours after incubation and washed with a 1.2% NaOH solution for 5 minutes to separate the masses. Subsequently, the eggs were sieved, separated and distributed in Petri dishes (60 x 15 mm) containing filter paper impregnated with bovine blood and different concentrations of anethole (25, 50, 250, 500, 1000, 2500 and 5000 µg/mL) diluted in a 16% aqueous acetone solution. Control and placebo groups were also formed, with six replicates for each experimental group. The plates were incubated in a climate chamber at 35.5 ± 1 °C and relative humidity above 70 ± 10% for approximately 12 hours. Larval hatching was assessed using a stereoscope. The hatching percentage was corrected using the Abbott formula (1925), and the lethal concentration (LC50 and LC90) was determined by Probit analysis in the RStudio program, with a 95% confidence interval. The results showed that anethole concentrations caused mortalities of 8%, 10%, 28%, 74%, 99%, 99% and 99%, respectively, compared to the placebo group (0%). The LC50 was 352.14 µg/mL (107.20–499.45) and the LC90 was 864.20 µg/mL (789.24–1032.44). It was concluded that anethole showed a dose-dependent

effect on egg mortality and demonstrating ovicidal potential against C. hominivorax.

Evaluation of the in vitro effect of cinnamaldehyde on the hatchability of *Cochliomyia hominivorax* (Coqueiral,1858)

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The species *Cochliomyia hominivorax* (Diptera: Calliphoridae) represents a category of insects of great sanitary and economic importance, which drives the search for effective alternatives for their control. Before the dissemination of synthetic compounds, essential oils from plants were already used to combat insects due to their insecticidal properties. In this context, the present study aimed to evaluate the effect of cinnamaldehyde, the main compound of cinnamon (*Cinnamomum* sp.), on the hatching of *C. hominivorax* eggs, investigating its ovicidal potential. For the experiment, 4050 *C. hominivorax* eggs from a colony maintained were used. The eggs were collected within 6 hours after incubation and washed with a 1.2% NaOH solution for 5 minutes to separate the masses. Subsequently, the eggs were sieved, separated and distributed in Petri dishes (60 x 15 mm) containing filter paper impregnated with bovine blood and different concentrations of cinnamaldehyde (25, 50, 250, 500, 1000, 2500 and 5000 µg/mL) diluted in a 16% aqueous acetone solution. Control and placebo groups were also formed, with six replicates for each experimental group. The plates were incubated in a climate chamber at 35.5 ± 1 °C and relative humidity above 70 ± 10% for approximately 12 hours. Larval hatching was assessed using a stereoscope. The hatching percentage was corrected using the Abbott formula (1925), and the lethal concentration (LC50 and LC90) was determined by Probit analysis in the RStudio program, with a 95% confidence interval. The results showed that cinnamaldehyde concentrations caused mortalities of 18%, 24%, 39%, 47%, 86%, 100% and 100%, respectively, compared to the placebo group (0%). The LC50 was 602.10 µg/mL (578.40–877.20) and the LC90 was 1524 µg/mL (1152.70–2414.60). It was concluded that cinnamaldehyde had a dose-dependent effect on egg mortality, demonstrating ovicidal potential against *C. hominivorax*.

The chromosomal level assembly of the Australian sheep blowfly, *Lucilia cuprina dorsalis* genome using third-generation DNA sequencing and Hi-C analysis.

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The Australian sheep blowfly, *Lucilia cuprina dorsalis,* is a significant ectoparasite of sheep responsible for subcutaneous myiasis (flystrike) leading to production losses, injury or mortality. Current flystrike control management strategies predominantly rely on breech modification surgery (mulesing) and insecticide application; however, the widespread and accelerating development of insecticide resistance poses a major challenge. Despite this, the emergence and dissemination of resistance-associated alleles within Australian *L. c. dorsalis* populations remain poorly understood. To address this knowledge gap, we employed an integrated genomics approach, combining Oxford Nanopore Technologies (ONT) and Illumina sequencing with high-throughput chromosomal conformation capture sequencing (Hi-C), to achieve a chromosomal-level assembly of this important pest. Additionally, comprehensive long- and short-read RNA sequencing was utilized to construct a high-resolution *de novo* transcriptome. This multiomics approach provides valuable insights into the genetic architecture, evolutionary history, and molecular mechanisms underlying key biological processes in *L. c. dorsalis*. Furthermore, the chromosomal-level genome assembly enables the investigation of genetic variation within blowfly populations across Australia, facilitating the tracking and monitoring of resistance allele emergence and dissemination, which is essential for developing sustainable control strategies.

The Sheep Blowfly: Genetics and Behavior of Parasitism in *Lucilia cuprina*

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The Australian sheep blowfly, *Lucilia cuprina dorsalis*, is a facultative ectoparasite whose larvae feed on the living tissues of domestic sheep in agricultural regions. In contrast, the closely related subspecies, *Lucilia cuprina cuprina* – present in Australia and Brazil – primarily inhabits urban areas and exhibits limited parasitic behavior. This study investigates the genetic and behavioral factors underlying the trophic adaptations of these two subspecies, aiming to identify genes and genomic regions associated with their physiological diversification and feeding habits. Behavioral assays were conducted to assess larval feeding preferences by measuring responses to different diets (rotten and fresh meat) at two temperatures (33°C and 25°C). Survival assays were performed to evaluate if more larvae would survive in rancid meat at 25°C or fresh meat at 33°C. Additionally, comparative genomic analyses were conducted using Pool-seq data from *L.c. cuprina* (from Brazil and Australia) and *L.c. dorsalis* (Australia), encompassing both population and evolutionary analyses. Regarding the behavioral assays, we observed that *L.c. cuprina* larvae from Australia exhibited a stronger preference for fresh meat compared to *L.c. cuprina* larvae from Brazil. However, *L.c. dorsalis* larvae consistently displayed the highest preference for fresh meat. The genomic analyses revealed significant genetic divergence between the subspecies, particularly in genes related to insecticide resistance, olfactory and gustatory receptors, which may be associated with the

parasitic lifestyle of *L.c. dorsalis*. Furthermore, polymorphisms were identified in a chemoreceptor gene within a population of *L.c. dorsalis*, potentially contributing to its parasitic feeding behavior. Our findings contribute to the understanding of the genetic mechanisms driving trophic specialization and the evolutionary processes leading to the speciation of *Lucilia cuprina*.

Identifying potential insecticide protein targets in Australian sheep blowfly using RNA interference

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Blowfly strike, mainly caused by *Lucilia cuprina* larvae feeding on sheep, remains a significant welfare and economic issue in sheep farming countries like Australia. Current control methods include selective breeding, mulesing and the use of insecticides to prevent and reduce flystrike. However, chemical control is becoming increasingly challenging due to rising insecticide resistance in blowfly populations. The objectives of this study were to identify putative protein targets that are critical for the survival of blowfly larvae on the sheep. Protein targets were knocked down using RNA interference (RNAi) to identify those that produce lethal phenotypes. A sheep trial was conducted to analyse differential gene expression in larvae fed on sheep skin, meat and control media. Transcriptomic analysis identified 4,157 upregulated genes in larvae feeding on sheep-derived substrates. Proteomic analysis of larval excretory and secretory products revealed 347 proteins specific to larvae fed on sheep, leading to the identification of 79 putative protein targets. This list was further refined based on several criteria including lethality data inferred from the model organism *Drosophila melanogaster* resulting in the selection of five targets for initial screening using RNAi. dsRNA for each target gene was microinjected into blowfly embryos. No injection control and RNAi specific for an irrelevant gene (GFP) were also included in the experiment. Microinjection of dsRNA into *L. cuprina* embryos identified two lethal targets with >90% mortality. One of these (Target1) caused severe developmental defects, including posterior narrowing and significant growth reduction, preventing larvae from reaching second instar. Phylogenetic analysis showed Target 1 is conserved across insect species while sharing low diversity among flesh-eating insects. These findings highlight Target 1 as an essential, insect-specific protein, making it a promising candidate for novel insecticide development.

Vaccines and adjuvants

Multigenic DNA vaccine to reduce *Toxoplasma gondii* oocyst shedding in cats

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Felines play a crucial role in the transmission of *Toxoplasma gondii*, with domestic cats being particularly important due to their direct contact with humans. Following primary infection, these animals are capable of excreting millions of oocysts in their feces, contaminating the environment. After sporulation, these oocysts become infectious to both animals and humans. Over recent decades, the epidemiology of human toxoplasmosis outbreaks has changed, with the ingestion of oocysts now recognized as the main route of infection in such outbreaks. The present study aimed to evaluate a DNA vaccine formulated using nanotechnology with PLGA, associated with the genes *rop2*, *rop18*, *sag1*, *gra5*, and *gra7*, in reducing the shedding of oocysts by domestic cats. Cats were divided into three groups of three animals each (G1, G2, and G3). Animals in G1 received 20 ng of each plasmid containing *pcDNASAG1*, *pcDNAROP2*, *pcDNAROP18*, *pcDNAGRA5*, and *pcDNAGRA7*, totaling 100 ng, plus 4 ng of PLGA; animals in G2 received 100 ng of the pcDNA vector plus 4 ng of PLGA; and animals in G3 received PBS plus 4 ng of PLGA. Four doses were administered at 21-day intervals via the intranasal route. Thirty days after the final vaccine dose, all cats were challenged with 200 cysts of the VEG strain administered via a nasogastric tube. Sheather's technique was used for coproparasitological examination of feces, and oocyst counts were performed using a Neubauer chamber. The mean total number of oocysts eliminated per gram of feces during the experimental period showed that cats in G1 (OPG: 563 × 10^3) eliminated fewer oocysts than animals in G2 (OPG: 2,486 × 10^3) and G3 (OPG: 4,962 × 10^3). Vaccine efficacy, calculated by comparing groups G1 and G3, was 80%, and between G1 and G2, 63%. Cats that received the multigene DNA vaccine via the nasal route eliminated significantly fewer oocysts than felines in the control groups. Reducing oocyst shedding in cats is directly related to decreased environmental contamination and, consequently, a lower risk of toxoplasmosis outbreaks.

Efficacy of vaccine formulations based on salivary and intestinal antigens against *Amblyomma sculptum* using saponin as adjuvant

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Amblyomma sculptum is the tick of most significant medical importance in Brazil, as it is the main species associated with humans and is the primary vector of the bacterium *Rickettsia rickettsii*, which causes Brazilian spotted fever. Its control is mainly done through chemical acaricides, which can cause environmental contamination if not managed correctly. In the search for alternatives to optimize the control of *A. sculptum*, previous studies tested four proteins - rAs8.9kDa (8.9), rAsKunitz (K), rAsBasicTail (Bt) and rAsQuimera (Qui) - alone or

in combinations associated with Aluminum Hydroxide that showed efficacies higher than 80%, with egg fertility being the most affected parameter. To search for vaccines that induce higher female mortality and increased efficacies, novel formulations should be tested. Therefore, the objective of this study was to evaluate the efficacy of different vaccine formulations containing the 8.9, K, Bt, and Qui antigens using saponin as an adjuvant. Swiss mice were used in five experimental groups immunized as follows: C1-PBS; C2-PBS+adjuvant (adj); V1-8.9Qui+adj; V2-BtQui+adj; V3-KQui+adj; V4-8.9KQui+adj; V5-8.9KBtQui+adj. All antigens induced a humoral response with a significant increase in antigen-specific IgG ($p<0.05$). The percentage of total mortality of larvae fed on immunized mice ranged from 60.5 to 80%, but vaccine formulations did not induce higher mortality compared to controls. The total mortality of nymphs fed on immunized mice was 40% in groups C2 and V5, while group G6 had the lowest nymph mortality rate (13.3%). Low female mortality was seen only in groups V3 and V5. Vaccine efficacy was 43.4% in group V1, 49.2% in group V3, and 61.0% in group V5. The other groups did not show vaccine efficacy. Despite the production of antibodies (IgG) presented by the immunized mice, the vaccine formulations tested using saponin as an adjuvant were inferior to the formulations tested with aluminum hydroxide.

mRNA-LNP Vaccines Show Promise Against *Ornithodoros* Tick Vectors

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Ornithodoros erraticus and *Ornithodoros moubata* are the primary vectors of tick-borne human relapsing fever (TBRF) spirochetes and African swine fever (ASF) virus in the Mediterranean region and Sub-Saharan Africa, respectively. The development of effective anti-*Ornithodoros* vaccines could reduce *Ornithodoros* infestations and facilitate disease control. Recently, vaccinomics pipelines have enabled the prediction of several vaccine targets from the salivary glands and midguts of both *Ornithodoros* species. Some of these targets have conferred significant protection against *O. erraticus* and/or *O. moubata* when administered as recombinant proteins. Given that mRNA-LNP vaccines may preserve native conformational and glycan epitopes, they are expected to enhance the humoral immune response and improve protection. To produce four known protective antigens from *Ornithodoros* as mRNA-LNPs and compare their vaccine efficacy with that of their homologous recombinant counterparts in rabbits. We selected a salivary and an intestinal protective antigen from each species (*OeSOD, OeTSP1, OmPLA2, Om86*), along with luciferase as a control. IVT of mRNA and its encapsulation in LNPs were performed by GenScript, incorporating codon optimization, N1-methyl-pseudouridine modification, and C1 co-capping. Rabbits received three monthly doses, followed by a challenge with *O. erraticus* and *O. moubata* ticks 15 days after the final immunisation. The four mRNA constructs induced moderate to high humoral responses (IgG titres ranging from 1:6,000 to 1:12,000) and provided protec-

tion against both *Ornithodoros* species, comparable to or exceeding that conferred by their recombinant counterparts, particularly in the case of *Om86*. The mRNA-LNP platform is suitable for developing anti-argasid vaccines and can be employed for the screening and experimental validation of vaccine candidates predicted through vaccinomics pipelines.

A novel glycoengineered recombinant vaccine against the barber's pole worm *Haemonchus contortus*

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The H11 glycoproteins, located on the intestinal microvilli of the barber's pole worm *Haemonchus contortus*, comprise primarily a family of homologous aminopeptidases essential for the parasite's digestion of blood meals. Native H11s are highly effective vaccine antigens, capable of eliciting a robust protective immunity in sheep and goats against *H. contortus* infection. However, the recombinant production of H11 aminopeptidases using conventional expression systems and transgenic *Caenorhabditis elegans*, failed to replicate the protective efficacy of the native form. To address inappropriate glycosylation and suboptimal protein folding that significantly compromise the antigenicity, we developed a novel strategy to produce recombinant *Haemonchus* antigens in glycoengineered insect cells and evaluated their vaccine potential in a controlled animal trial. We modified the N-glycosylation pathways of *Trichoplusia ni*-derived Hi5 cells by introducing three *C. elegans* genes encoding essential glycoenzymes, enabling the production of H11 antigens with nematode-specific glycan epitopes. Glycoforms of the engineered antigens were characterised using HPLC and MALDI-TOF-MS/MS, and their biological activity was assessed through enzymatic assays and stimulation of ovine peripheral blood mononuclear cells (PBMCs) *in vitro*. A preliminary vaccine trial was carried out in sheep to validate the efficacy of the novel vaccine. The glycoengineered H11 antigens retained aminopeptidase activity and displayed nematode-specific glycan modifications, including the tri-fucosylated core and Galβ1,4Fuc epitope. *In vitro*, they stimulated cytokine secretion from ovine PBMCs, suggesting immunogenic property. The vaccine trial indicated their ability to induce protective immunity against *H. contortus* infection, as evidenced by a significant reduction in parasite egg shedding (81.1%) and worm burden (25.4%). These results highlight the feasibility of glycoengineering for producing bioactive *H. contortus* antigens and underscore their potential as novel vaccine candidates against this pathogenic nematode.

Precision glycoform engineering: combining plant and *in vitro* systems for helminth vaccine production

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Posttranslational modifications (PTMs), such as N-glycosylation, pose a significant bottleneck for the production of protein-based vaccines due to their impact on protein stability, folding and immunogenicity. In the development of a vaccine against the bovine abomasal parasite *Ostertagia ostertagi,* it was demonstrated that vaccine N-glycosylation plays a key role in eliciting a protective immune response. However, the native glycan of the *Ostertagia ostertagi* activation-associated 1 (OoASP-1) vaccine is not found upon production in conventional recombinant expression systems such as yeast or bacteria. Production of OoASP-1 with its' native glycans is feasible using a glyco-engineering approach in *Nicotiana benthamiana*, but requires optimization. Plant native (glyco-) enzymes hinder the generation of specific glycoforms, thereby affecting the homogeneity of the N-glycans on the plant-produced vaccine. In this study, we developed a precision glycoform engineering system to be able to homogeneously produce OoASP-1 with its' native N-glycans. An *in vitro* N-glycosylation system was set up with *Escherichia coli* produced glyco-enzymes, lacking their transmembrane region. By combining *in planta* glyco-engineering with *in vitro* glyco-engineering, we can produce a range of homogeneous glycoforms, covering high mannose, paucimannose, hybrid and complex N-glycan structures. The establishment of this platform enables the production of a variety of helminth vaccines with native N-glycans and opens the door to investigating the effect of specific N-glycans on immune responses and vaccine efficacy.

Modulation of immune response after LetiFend® vaccination: pathway enrichment analysis of upregulated proteins in peripheral blood mononuclear cells from dogs.

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Control of canine visceral leishmaniasis (CanL), caused by *Leishmania infantum*, is essential due to its zoonotic nature. Protective and effective vaccines are the best tool for the prevention of CanL. LetiFend®, (LETI Pharma), is a safe and effective veterinary vaccine able to reduce the risk of developing clinical symptoms. LetiFend® induces a strong humoral response insufficient to protect against infection. An in-depth knowledge of the mechanism of action of LetiFend® is required. To assess the immunological pathways elicited after LetiFend® administration to explain the mechanism underlying its efficacy. RNA from PBMCs restimulated with SLA from vaccinated (healthy, LetiFend® vaccinated) and unvaccinated (healthy, non-vaccinated) dogs were assessed by Illumina technology to obtain differentially expressed genes. Gene Set Enrichment Analysis was used to determine a set of genes with differences

statistically significant between vaccinated and unvaccinated. Gene Ontology (GO) database was used to define pathway maps. The pvalue cutoff for GO terms analysis was 0.05. R packages clusterProfiler and enrichplot were used to generate graphs. Immune pathways elicited by LetiFend® were defined, the top 15 enriched genes and related pathways were represented in a gene name concept map and the fold change for enriched genes were calculated using R. In vaccinated dogs, a significant increase in the expression of INF-γ IL-6, IL1-β, CCL1, CXCL10, INF-β1, RGS1, GBP6 y 5, STK3, TNFSF13β, SOCS3, CISH, CYSLTR1 and CD-40 was detected compared to unvaccinated. The genes were related to pathways involved in defense and immune responses, including T response, cytokine response, cellular and humoral response and response to external agents among others. A pathway enrichment analysis of PBMCs from vaccinated dogs indicates that the administration of LetiFend® could modulate the Th1 immune response via IFN-g, NO production and IL-4 reduction.

Differential gene expression pattern after LetiFend® vaccination: a whole-transcriptome analysis in peripheral blood mononuclear cells from dogs.

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Controlling canine visceral leishmaniasis (CanL) is crucial due to its zoonotic nature. Vaccines are the best tools for this. LetiFend® (LETIPharma) is a safe and effective vaccine that reduces the risk of clinical signs by inducing a strong humoral response and lowering levels of harmful immune complexes. However, its protective cellular response remains unclear. To set up a method based on NGS technology to identify differentially expressed genes between vaccinated and unvaccinated dogs. Groups (n=3): Vaccinated: healthy, vaccinated (LetiFend®); Unvaccinated: healthy, non-vaccinated. Blood samples (50ml) were collected for PBMCs isolation by Ficoll density gradient. Cells (5×106cells/ml) were stimulated with Soluble *Leishmania* Antigen (SLA) at 12µg/ml, 24h/37°C with 5% CO2. Then, RNA was isolated from PBMCs (RNeasy mini kit, Qiagen). Resources used: TruSeq Stranded Total RNA library Preparation Kit (Illumina) / NovaSeq 6000: library preparation; Real Time Analysis: base calling; Bcl2fastqV2.20: demultiplex and FASTQ format conversion; FastQC1V0.11.8: quality analyses; Hisat25V2.1.0 aligner: Alignment (*Canis familiaris* reference genome); Htseq-count8V0.11.2: count the reads mapping (genes); Deseq2R software package: differential expression analysis. NGS to assess gene expression in PBMCs from dogs was set up. This technology allowed to perform a comparison between vaccinated vs unvaccinated showing that data from each group can be clustered for further study. Heatmap showed differential expression of 74 genes in vaccinated, 59 of them had a positive log2 fold change. Using NGS technology, an in-depth study of the differential gene expression induced by LetiFend® vaccination could be realized. It could be concluded that vaccination induces strong transcriptional change in canine PBMCs restimulated with SLA, which translates into the upregulation of several genes, most of them related with activation of immune response.

Immunogenicity and efficacy of an Australian whole-cell killed *T. foetus* vaccine in

young bulls experimentally infected with *Tritrichomonas foetus*

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Tritrichomonas foetus is a protozoan parasite that causes bovine trichomonosis, a venereal disease-causing infertility and abortion in female cattle while bulls remain asymptomatic. Currently, the disease is managed by culling positive cattle as there is no vaccine available in Australia with the importation of a commercial vaccine deemed unfeasible due to quarantine implications. This study aimed to develop and evaluate the safety, efficacy and immunogenicity of a locally sourced *T. foetus* whole-cell killed vaccine in young bulls (1-2 years old). The TfOz5 Queensland *T. foetus* isolate was utilised as the vaccine strain, while the TfOz-N36 Northern Territory isolate was used as the challenge strain. The heat-inactivated vaccine, adjuvanted with Montanide ISA 61 VG, was administered subcutaneously to young *T. foetus*-naïve bulls (n=30) in two doses (5 x 10^7 cells/dose) one month apart. Control bulls (n=30) received a mock adjuvant Phosphate-buffered saline. Bulls were experimentally infected intraprepucially with live *T. foetus* cells at two- and four-weeks post vaccination. Monitoring of *T. foetus* infection was confirmed fortnightly through quantitative polymerase chain reaction and culture. The vaccine demonstrated safety, causing only mild local reactions, and no significant differences in weight and average daily gain between the two groups. Vaccinated bulls exhibited a significantly shorter average *T. foetus* infection duration compared to controls (7 vs. 16 days, $P = 0.003$, Mann-Whitney $U = 244.5$). The vaccine stimulated high serum anti-*T. foetus* IgG antibodies which were boosted after each *T. foetus* challenge. This study showed that the vaccine was well tolerated by bulls, effectively stimulated high serum anti-*T. foetus* IgG antibodies, and significantly reduced the average length of infection. These results highlight the vaccine's benefits for cattle and its potential uptake in the Australian beef industry.

Identification and selection of new vaccine targets against *Rhipicephalus microplus* (Canestrini, 1887), the cattle tick.

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Rhipicephalus microplus is the main tick species affecting livestock productivity, representing one of the greatest challenges for the production system in Brazil. This ectoparasite has major economic importance due to the significant reduction in meat and milk production resulting from intense blood feeding, and it also acts as a vector of the pathogens causing bovine babesiosis and anaplasmosis. Although chemical control using acaricides remains widely employed, the continuous and often improper use of these compounds has led to the emergence of resistant tick populations, significantly reducing treatment effectiveness and worsening field infestations. Furthermore, acaricides pose risks to animal, human, and environmental health, reinforcing the urgency to develop safer and more sustainable alternatives, such as vaccines. This study aimed to identify and evaluate new antigenic targets capable of disrupting the biological cycle of *R. microplus*. Initially, a proof-of-concept clinical trial was conducted in cattle using two vaccine formulations (VAC1 and VAC2), both associated with a saponin adjuvant. VAC1 achieved an efficacy rate of 56.75%, while VAC2 showed 33.38%. Subsequently, the antigen from VAC1 was selected to test in a murine model with two new formulations, varying only the adjuvant (Montanide or Aluminum Hydroxide - HA). The formulation with Montanide achieved an efficacy of 91.84%, while the HA formulation reached 56.01%. The adopted strategy successfully selected a potential vaccine formulation with high efficacy rate. Future work will focus on validating these results in cattle, aiming to provide effective and safe solutions for tick control. The development of national vaccines represents a strategic advancement, strengthening the competitiveness of Brazil's livestock industry by reducing economic losses associated with tick infestations and tick-borne diseases.

Evaluation of a DNA vector plasmid encoding a partial rop18 gene from *Toxoplasma gondii* in domestic cats as a vaccine candidate

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Toxoplasma gondii is a protozoan parasite that infects all warm-blooded animals, and cats are the definitive host, shedding millions of oocysts through feces, causing environmental contamination. Although cats play an important role in the epidemiology of toxoplasmosis, few studies have investigated vaccines against oocyst shedding in cats. Furthermore, studies with DNA vaccines have been improved in the last few years, and they have become an alternative in the control of toxoplasmosis. Based on this, the present study aimed to evaluate the effectiveness of a DNA vaccine containing rop18 against oocyst shedding in domestic cats. The partial rop18 gene was selected by reverse vaccinology. Four domestic cats (*Felis catus*) were used, of which two animals received 25 µg of pcDNA 3.1+rop18, and two received 25 µg of pcDNA 3.1. All animals received intramuscular immuniza-

tions with four doses every three weeks, along with 1.5% levamisole. Thirty days after the last immunization, the animals were infected with 300 tissue cysts from the ToxoDB #182 strain, a non-archetypal genotype isolated from a wild cat. Fecal examinations were performed for oocyst shedding. Enzyme-linked immunosorbent assay and western blotting analyses with recombinant ROP18 were performed to assess the humoral immune response. Animals that received plasmid containing the partial *T. gondii* rop18 gene produced specific lgG antibodies and shed 53.3% fewer oocysts than controls. The two groups of animals showed no statistically significant differences ($p > 0.05$) in oocyst shedding; however, they showed significant differences in the detection of anti-*Toxoplasma* antibodies ($p<0.05$). In conclusion, cats immunized with the rop18 DNA vaccine shed fewer *T. gondii* oocysts and showed better lgG antibody responses. Further studies using combinations of other genes and more animals should be conducted to improve the vaccine effectiveness.

Seroconversion in calves immunized with a recombinant nanovaccine against *Anaplasma marginale* under natural challenge in endemic area

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Cattle Tick Fever is a complex of diseases with a significant impact on livestock farming and worldwide distribution. In Brazil, it is caused by *Anaplasma marginale, Babesia bovis* and *B. bigemina*. *Anaplasma marginale* is responsible for Bovine Anaplasmosis. Several vaccines have been evaluated for disease control and prevention, but an effective and commercially viable option is still unavailable. The study aimed to test a recombinant nanovaccine to evaluate its protective capacity against bovine anaplasmosis in the field, impacting health, animal welfare, and productivity. The experiment was conducted on a commercial dairy farm in the metropolitan mesoregion of Belo Horizonte, Minas Gerais, Brazil. During the evaluations, the animals remained in a rearing system with low vector exposure. A total of 84 heifer calves, aged between 45 and 91 days, were randomly selected and divided into vaccinated (VG) and control (CG) groups. The protocol consisted

of three doses of the immunizing agent combined with an adjuvant, administered at 21-day intervals. Under the same protocol, the control group (CG) received a saline solution and adjuvant. Plasma samples were collected on day 0 (before immunization) and day 63 (21 days after the third dose) and subjected to Immunofluorescent Antibody Test (IFAT) to assess IgG against *A. marginale*. Of the 84 samples analyzed on D0, 14 were reactive in the CG (33.3%) and 19 in the VG (45.2%), a result that may be associated with passive immunity. By D63, seroconversion increased to 54.8% in the CG and 90.5% in the VG. *Anaplasma marginale* was detected in all experimental animals, demonstrating that all were exposed to the challenge. The results indicate that the nanovaccine induced an immune response against *A. marginale*, as evidenced by the significant increase in seroconversion in the VG. The clinical response and the duration of the convalescence period of immunized animals are currently under analysis.

Poster Presentation
Day 19

Poster Presentation - Day 19

Alternative control, bioactive, and natural products

In vitro nematocidal activity of hexane extract from spent substrate of *Pleurotus ostreatus* against *Panagrellus redivivus* (J_2)

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Parasitic nematodes such as *Haemonchus contortus*, *Nacobbus aberrans*, and *Meloidogyne* spp. pose significant threats to agriculture and livestock1. The edible mushroom *Pleurotus ostreatus* has demonstrated promising nematocidal against *H. contortus,* attributed to the production of bioactive secondary metabolites in both its fruiting bodies and spent substrate (SS)2. Despite these findings, the broader ecotoxicological profile of these compounds has not yet been widely explored. This study aimed to evaluate the toxicity of the hexane extract of spent substrate (HexSS) of *P. ostreatus* against the free-living nematode *Panagrellus redivivus* (J_2), employed here as an *in vitro* biological indicator. To evaluate the hexane extract derived from 30-day-old spent substrate (SS) of *P. ostreatus* against the free-living nematode *P. redivivus* (J_2 stage). An *in vitro* toxicity assay was conducted using serial concentrations of the HexSS were evaluated ranging 10 mg/mL to 1.25 mg/mL. Exposure times of 17 and 24 h were assessed with 50 larvae in each well. The assay included a positive control, ivermectin (IVM 1%) and a negative control (Tween 80 1%), with four replicates per treatment. Larval mortality was recorded, and data were analyzed using ANOVA followed by Tukey's test (p=0.05) in R program (version 4.4.1). The HexSS at 10 mg/mL induced 57% larval mortality at 17 h and 46% at 24 h, indicating a time-dependent decline in efficacy. At 5 mg/mL, mortality ranged between 33% and 36% at both time points, allowing over 50% survival of *P. redivivus* larvae. These findings suggest that while the HexSS exhibits toxic effects, concentrations below 5 mg/mL may help balance nematode control with reduced impact on non-target, beneficial organisms. This strategy will allow to optimize the use of the HexSS as a sustainable alternative in the management of nematodes. The HexSS of *P. ostreatus* at 10 mg/mL demonstrates significant *in vitro* toxicity against *P. redivivus* (J_2 stage) highlighting its potential as a sustainable nematode management strategy.

Nematocidal Activity of Hexane Extract from *Pleurotus ostreatus* Spent Substrate on Free-Living and Plant-Parasitic Nematodes

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The intensive and indiscriminate use of chemical nematicides has led to increased resistance in parasitic nematodes and collateral damage to non-target organisms, posing significant environmental risks. These concerns have intensified the search for sustainable control strategies, including natural products with nematocidal activity. *Pleurotus ostreatus* produce a variety of bioactive compounds, and their spent substrates (SS), often considered agricultural waste, may represent a valuable source of such metabolites1. This study investigates the nematocidal potential of a hexane extract from the SS of *Pleurotus ostreatus* against *Caenorhabditis elegans* and *Nacobbus aberrans*, two model organisms with relevance in parasitology. To assess the nematocidal activity of the hexane extract obtained from SS of *P. ostreatus* against *C. elegans* and *N. aberrans*. *In vitro* assays were conducted using juvenile stage of *N. aberrans* and *C. elegans* placed in 96-well microplates[2]. Treatments included ivermectin as a positive control and Tween 80 as a negative control with four replicates per treatment. The hexane extract was applied in serial concentrations of 10, 5.0, 2.5 and 1.2 mg/mL. Larval mortality was assessed after 72 hours for *N. aberrans* and after 24 hours for *C. elegans*. Statistical analysis was conducted using ANOVA followed by Tukey's test ($p < 0.05$) in R program (version 4.4.1). At a concentration of 10 mg/mL, mortality rates were 72.3% for *C. elegans* and 28.6% for *N. aberrans*. The differential susceptibility observed may be attributed to variations in cuticular structure and detoxification mechanisms. At 1.2 mg/mL, mortality in *N. aberrans* decreased to 1.9%, whereas in *C. elegans* it remained at 35.2%, indicating a dose-dependent effect 2. The hexane extract from SS of *Pleurotus ostreatus* showed nematocidal activity, with higher efficacy against *C. elegans* than *N. aberrans*. These results highlight its potential as a natural alternative for nematode control.

Comparison of the effect of soil and climatic period on the *in vitro* anthelmintic activity of *Gliricidia sepium*

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In the present work, the anthelmintic activity (AA) of dichloromethane extracts obtained from *Gliricidia sepium* was evaluated using the third-stage-larval *Haemonchus contortus* (L3) exsheathment inhibition test (LEIT). Extracts were tested at concentrations of 0.3, 0.6, 1.2, 2.5, 5.0, 10, 20, and 40 mg/mL. A comparison of anthelmintic activity was performed on the same plant grown in two different locations (different pH and mineral conditions) in the department of Tolima (Guamo and El Espinal) and collected during two different climatic periods (dry period and rainy period) using the same strain of the nematode *Haemonchus contortus* previously isolated. The average inhibitory concentrations 50-IC50- (three replicates per assay) were for dry period in Guamo (IC50 4,33 mg/mL) and El Espinal (IC50 3,93 mg/mL) not presenting statistically significant differences in one-way ANOVA ($p>0,05$). In the case of the

rainy period in Guamo (lC50 2,54 mg/mL) and El Espinal (lC50 2,24 mg/mL) also without significant statistical differences (p>0,05). The different environmental conditions in which the plant grows can influence the generation and concentration of metabolites previously described by our group (glycosylated flavonoids, methoxyphenols, fatty acids, phenylpropanoids, anthraquinone glycosides, amino acids, and glycosylated phenolic acids). Thus, harvesting and preparing extracts for concentrating metabolites from the Gliricidia plant is recommended during the rainy season, as this was the most important factor for anthelmintic activity under in vitro conditions. In the future, it is projected that nanoencapsulated forms of this plant extract will be generated under the most favorable conditions for the generation of metabolites with anthelmintic activity.

In vivo evaluation of the pour on combination of _Citrus aurantium_ var. amara essential oil with ivermectin against gastrointestinal nematodes

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This study developed a novel pour-on formulation combining essential oil from *Citrus aurantium* var. *amara* (OECa) with ivermectin (IVM), and evaluated its in vivo efficacy in cattle naturally infected with gastrointestinal nematodes. Efficacy was assessed using the fecal egg count reduction test (FECRT). On Day 0, animals were weighed and fecal samples collected for baseline egg counts. Cattle were randomly assigned to three treatment groups (n=10 per group): G1 received the combined pour-on formulation of OECa (50 mg/kg) + IVM (5 mg/kg); G2 received OECa alone (50 mg/kg); and G3 received IVM alone (5 mg/kg). On Day 15, fecal samples were recollected for post-treatment egg counts and larval culture. FECRT was calculated both overall and by nematode genus. The combination treatment resulted in a 40.6% overall reduction in egg counts, compared to 33.4% with IVM alone and only 4.9% with OECa alone. Notably, OECa alone was highly effective against *Haemonchus* spp. (93%, 95% CI: 90–96), and the combination achieved 88% efficacy (95% CI: 84–93). For *Cooperia* spp., the combination yielded 20% efficacy (95% CI: 14–27), while IVM alone reached 40% (95% CI: 29–51). Against *Oesophagostomum* spp., the combination showed 9% efficacy (95% CI: 6–11), and IVM alone achieved 51% (95% CI: 48–55). These results indicate that OECa exhibits targeted anthelmintic activity, particularly against *Haemonchus* spp., and may exert synergistic effects when combined with ivermectin. However, its efficacy against other genera was limited, highlighting the need for further investigation into its spectrum of activity and potential formulation optimizations.

Evaluation of linalool as an alternative acaricide for the control of *Rhipicephalus sanguineus*

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The brown dog tick, *Rhipicephalus sanguineus*, has a cosmopolitan distribution and holds significant importance in veterinary medicine and public health due to its ability to transmit pathogens responsible for hemoparasitic infections in dogs. The indiscriminate use of synthetic acaricides has contributed to the development of parasitic resistance, underscoring the need for effective and sustainable alternatives. Among these, the plant-derived monoterpene linalool has shown promise due to its neurotoxic properties and potentially synergistic interactions in tick control. This study aimed to evaluate the efficacy of linalool as an alternative acaricide for the control of *R. sanguineus*. Tick samples were collected from five locations, including shelters and independent animal rescuers, and classified as five distinct tick populations. Engorged females were incubated in a BOD chamber at 28°C until the end of oviposition. The Larval Packet Test (LPT) was used to determine the lethal concentrations (LC_{50} and LC_{90}) of linalool, which was tested at concentrations ranging from 0.0975% to 50%. Linalool exhibited acaricidal activity against all tested populations, with a mean LC_{50} of 0.4% (range: 0.272%–0.614%) and a mean LC_{90} of 1.0% (95% CI: 0.831–1.109%). Notably, LC_{90} values were consistent across populations, indicating efficacy regardless of geographical origin. Populations 3 and 4 demonstrated greater sensitivity (LC_{50} of 0.272% and 0.282%, respectively), possibly due to genetic or environmental factors. The low variability in LC_{90} reinforces linalool's potential as a robust alternative, even in the context of resistance to synthetic acaricides. Linalool demonstrated high potential as a natural acaricide for the control of *R. sanguineus*, supporting its viability as a promising alternative for future research in parasite management.

Synergistic effect of menthol in combination with anthelmintic drugs in *Haemonchus contortus*

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Control of resistant populations of *Haemonchus contortus* has become increasingly difficult globally. One alternative is attempting to enhance the effectiveness of drugs commercially available. In this study we evaluate whether menthol, an organic compound, can be useful as synergistic agent for different anthelmintic drugs against *H. contortus*. For this purpose, egg hatch assay (EHA) and larval migration (LMA) inhibition tests were performed using menthol in combination with ivermectin, tetramisole or thiabendazole in five concen-

trations. All tests were carried out with a field isolate (>93% *H. contortus*). In the EHA, when compared to menthol (EC50 154 µg/ml) and thiabendazole (EC50 0.5 µg/ml) alone; combination of both substances resulted in an EC50 130 µg/ml (-17%) and 0.41 µg/ml (-20%), respectively. In the LMA, combination of menthol and ivermectin did not show any improvement in their efficacy (EC50 1010 µg/ml and 2.52 µg/ml, respectively) compared to menthol (EC50 1051 µg/ml); and ivermectin (EC50 2.03 µg/ml) alone. However, combination of menthol with tetramisole in LMA improved efficacy in 50% (EC50 of 550 µg/ml and 0.2 µg/ml, respectively) compared to menthol (EC50 1051 µg/ml) or tetramisole (EC50 0.41 µg/ml) alone. These findings demonstrate the potential of some organic compounds to mitigate the problem of anthelmintic resistance.

In vitro effect of eugenol on larvae of multiresistant *Haemonchus contortus*

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Gastrointestinal nematode infections are one of the main causes of economic losses for sheep and goats. The control is mainly realized with synthetic anthelmintics. However, the intensive use of these drugs have selected resistant populations. Thus, new control strategies are being searched, such as the use of bioactive compounds. This study aimed to evaluate the anthelmintic activity of eugenol against third stage larvae (L3) of *Haemonchus contortus*. For this, the eugenol was obtained commercially and the larval migration inhibition test was performed using the Kokstad isolate multiresistant to benzimidazoles, levamisole and macrocyclic lactones. L3 were obtained from experimentally infected sheep and exsheathed. A solution with approximately 200 exsheathed larvae was incubated at 27°C for 18h with different concentrations of eugenol (4 to 0.125 mg/mL). Then, the solution was transferred to 24-well plates with a 25 µm mesh and incubated for 24h. Migrated larvae were counted under an inverted microscope. The negative and positive controls were tween 80 (3%) and ivermectin (0.008 mg/mL), respectively. For each treatment and control, five replicates and three repetitions were performed. The inhibition effects at each concentration of eugenol were analyzed using ANOVA and compared with the Tukey test ($P<0.05$). Effective concentration to inhibit 50% (EC50) of larval motility was calculated. GraphPad Prism® 6.0 software was used. At highest concentration (4 mg/mL), the eugenol efficacy was 71.36% ± 3.52 and differed statistically from positive control (98.02% ± 1.50). However, in all concentrations evaluated (4 to 0.125 mg/mL), the eugenol effect was statistically superior to negative control (1.34% ± 1.95). The eugenol effect was dose-dependent (EC50: 1.609 mg/mL). These findings indicate that eugenol reduces larval motility of *H. contortus*. Further studies should be performed to

evaluated the eugenol efficacy against *H. contortus* adults.

Identification of the metabolic pathway involved in the synthesis of fumagillin from edible mushroom *Pleurotus djamor* MPG-05 genome

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Nematode resistance to nematicides affects animal health and sustainability, prompting interest in natural alternatives. Edible mushrooms, such as *Pleurotus djamor*, show potential due to their ability to produce secondary metabolites with nematicidal activity. Bioinformatics tools help identify genes and biosynthetic pathways involved in the production of these compounds. In the genome of *P. djamor* MPG-05, the genes involved in fumagillin biosynthesis were identified, a compound previously reported to inhibit nematodes. Therefore, the objetive was to identify genes involved in fumagillin biosynthesis in *P. djamor* MPG-05. To this end, functional annotation of *P. djamor* MPG-05 genes was performed using KofamScan v1.0.1, while biosynthetic gene clusters were predicted with the antiSMASH fungal version. The functional annotation results were then compared between these software tools to accurately identify the genes. As a result, genomic analysis of *P. djamor* MPG-05 revealed genes related to fumagillin biosynthesis, confirmed by KEGG and antiSMASH annotations. Fumagillin is known for its inhibitory effects on protozoa and nematodes. Overall, genomic analysis of *P. djamor* MPG-05 reveals biosynthetic gene clusters for secondary metabolites production with potential nematicidal activity, notably the fumagillin pathway.

***In vitro* infection of *Ctenocephalides felis felis* pupae by *Heterorhabditis bacteriophora* (HP88) and *Heterorhabditis indica* (LPP30)**

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Fleas are significant ectoparasites in veterinary medicine, with *Ctenocephalides felis* being the most common species infesting dogs and cats. These infestations can lead to anemia, irritation, and pruritus, while also acting as vectors for pathogens that cause diseases in mammals. Flea control involves manual removal, environmental chemical treatments, and topical or oral formulations for affected animals. However, improper use of chemical control has resulted in financial burdens, environmental contamination, and the emergence of resistant populations. Researchers are investigating alternative methods to regulate flea populations, either independently or alongside chemical solutions. Entomopathogenic nematodes (EPNs), long used in agricultural pest control, show promise in managing invertebrates of veterinary importance. This study evaluated the susceptibility of naked pupae and pupae within pupal cases of *C. felis felis* to *Heterorhabditis bacteriophora* (HP88) and *Heterorhabditis indica* (LPP30) un-

der laboratory conditions. Bioassays included two experimental groups, each consisting of 10 Petri dishes containing 11 naked pupae and 11 pupae within pupal cases per plate. Each received 600µL of a solution containing 1200 infective juveniles of *H. bacteriophora* and *H. indica*. Five control groups were established, receiving only distilled water. Susceptibility was assessed through dissection, detecting EPNs inside pupae. No EPNs were found in the control group. Exposure to *H. bacteriophora* resulted in 100% susceptibility in naked pupae and 55.44% in pupae within pupal cases, while *H. indica* exposure led to 98.18% susceptibility in naked pupae and 64.99% in pupae within pupal cases. Results confirm that both naked and pupal-case-protected pupae are vulnerable to HP88 and LPP30 infection. While susceptibility was higher in naked pupae, infections occurred in both cases, supporting further studies on EPNs as potential tools for flea biological control.

Effect of Lippia grata essential oil on larvae *Haemonchus contortus*

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The essential oil of *Lippia grata* may be a promising alternative for the control of gastrointestinal nematodes (GIN) in small ruminants, as it may present major compounds such as thymol, eucalyptol and ocimene, some of these compounds already tested on GIN. In addition, other species of this genus have already been tested on *Haemonchus contortus* and presented promising results. Therefore, the objective of this study was to evaluate the effect of *L. grata* oil on migration larvae of *H. contortus*. For this, *L. grata* samples were collected in Iguatu, Ceará, Brazil. After, the oil essential was extracted by hydrodistillation Clevenger type. The oil chemical analysis was performed by Gas Chromatography coupled to Mass Spectrometry (GC-MS). To evaluate the anthelmintic efficacy of oil, the larval migration inhibition test (LMIT) was performed. For the recovery of infective larvae (L3), stool cultures were performed with feces from an experimental animal infected with *H. contortus*. A 400 µL of L3 solution and 400 µL of oil solution at different concentrations (2; 1; 0.5; 0.25; 0.12 and 0.06 mg/mL) were incubated. After 18 h, the larvae exposed to oil were transferred to the migration apparatus. Then, the oil inhibition capacity on larval migration was evaluated for 24 h. The LMIT was performed with two replicates with five replicates for the controls (positive - ivermectin 0.25 mg/mL and negative DMSO 1.5%) and treatments. GC-MS showed thymol (66.23%), eucalyptol (12.33%), o-cymene (06.01%) and thymol acetate (1.24%) as main compounds. The mean and standard deviation of the efficacies obtained at the abovementioned concentrations were 94.29 ± 5.43%; 90.92 ± 1.32%; 88.08 ± 1.32; 61.27 ± 6.65; 31.93 ± 6.27 and 24.33 ± 5.87. The mean of the controls was: positive 92.35 ± 0.11 and negative 2.66 ± 0.3, respectively. The in

vitro results obtained indicate that *L. grata* oil is promising on *H. contortus* control. Therefore the evaluation of the *L. grata* oil on adult nematodes of *H. contortus* is suggested.

Ovicidal effect of Lippia grata essential oil on Haemonchus contortus

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The use incorrect of synthetic anthelmintics has accelerated the development of anthelmintic resistance. Therefore, alternative control methods of nematodes gastrintestinais of small ruminnats have been evaluated, such as the use of essential oils. This study aimed to evaluate the effect of *Lippia grata* essential oil on *Haemonchus contortus* eggs. For this, *L. grata* samples were collected in Iguatu, municipaly of Ceará, Brazil. After, the oil essential was extracted by hydrodistillation Clevenger type The oil chemical analysis was performed by Gas Chromatography coupled to Mass Spectrometry (GC-MS). Then, the egg hatching test (EHT) was performed. Feces were collected from an experimental animal infected with a isolate of *H. contortus* resistant to benzimidazoles, imidatiazoles and macrocyclic lactones - Kokstad (KOK). Then, 250 µL of egg solution and 250 µL oil at different concentrations (0,5; 0.25; 0.12; 0.06 and 0.03 mg/mL) were incubated. The ability of oil to inhibit egg hatching was evaluated after 48h. The EHT was performed with three replicates and five replicates for controls (positive - thiabendazole 0.025 mg/mL and negative DMSO 1.5%) and treatment. The results were submitted to analysis of variance (ANOVA) and Tukey test ($P < 0.05$) by Graph Pad Prism®. GC-MS showed four main compounds: thymol (66.23%), eucalyptol (12.33%), o-cymene (06.01%) and thymol acetate (1.24%). The mean efficacy ± standard deviation of the oil at abovementioned concentrations were: 100 ± 0%; 99.82 ± 0.51%; 73.39 ± 11.13%; 19.39 ± 7.59%; 6.36 ± 3.10%, respectively. The efficacy of the positive and negative controls was 92.19 ± 4.28% and 3.34 ± 1.59%. The effective concentration to inhibit 50% 0.10 mg/mL Therefore, it is possible to verify that *L. grata* oil presents promising results on *H. contortus* eggs, requiring its evaluation in the other stages of the nematode's life.

Evaluation of host-derived volatile organic compounds on the behavior of *Dermacentor nitens* larvae – preliminary data

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Dermacentor nitens (horse-ear tick) cause both direct and indirect harm to horses. Few commercial acaricides are currently available for

its control. Allelochemicals, a type of semiochemical, are volatile organic compounds (VOCs) emitted by hosts that can either attract or repel ticks, offering a potential alternative for tick management. Donkeys are known to be non-preferred hosts for ticks, whereas horses are preferred. Previous analyses identified hexanal as predominant in donkey ear odor, ethyl octanoate as predominant in horse ear odor, and (E)-2-octenal exclusively in donkey ear odor. This study evaluated the behavioral response of *D. nitens* larvae to these VOCs at a concentration of 1 M. Rectangular filter paper strips (12 × 2 cm) were treated with 50 µL of each VOC applied to the middle third. In each of the three replicates, 10 filter papers were used. The papers were hung vertically to simulate grass, and 10 unfed larvae were released at the base of each strip. Larval behavior was classified as repellent (e.g., reverse movement during ascent upon contact), attractive (larvae arrested in the treated middle section), or neutral (uninterrupted ascent to the top). Behavioral observations were made at 10 min and every hour for 4h. Fresh larvae were introduced at each time point. Hexanal and ethyl octanoate at 1 M did not elicit either attractive or repellent behavior. However, (E)-2- octenal demonstrated significant repellency across all time points, with mean repellency rates of 98%, 82.5%, 73%, 74%, and 81.5% at 10 min, 1, 2, 3, and 4 h, respectively. These findings suggest that (E)-2-octenal acts as a repellent to *D. nitens* larvae and may function as an allomone — a chemical released by one species that adversely affects another species to the benefit of the emitter. Further studies using different concentrations are warranted to better understand the roles of these VOCs in *D. nitens* host-seeking behavior.

First report on the ovicidal activity of silver nanoparticles produced by the Nematophagous fungus *Duddingtonia flagrans* against *Toxocara canis* eggs

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Nematophagous fungi, such as *Duddingtonia flagrans*, have emerged as promising biological agents for the biosynthesis of silver nanoparticles (AgNPs) exhibiting proven biological activity against gastrointestinal nematodes of domestic animals. The failure of chemical control strategies against nematodiasis is multifactorial. This failure is largely attributed to the inability to disrupt the biological cycle, which predominantly occurs in the external environment (eggs and/or larvae). This failure perpetuates the risk of transmission. Within this context, the genus *Toxocara* merits particular attention due to its capacity to cause severe infections in canines and felines, in addition to its recognized zoonotic potential. The infective stage (embryonated eggs) of these parasites exhibits substantial resistance to climatic extremes and chemical agents. The objective of the present study was to evaluate the ovicidal potential of AgNPs synthesized by *D. flagrans* against *Toxocara canis*

eggs. *T. canis* eggs were obtained from adult nematodes. AgNPs were biosynthesized using fungal filtrates of *D. flagrans*, which were supplemented with silver nitrate solution. The biosynthesis was confirmed through ultraviolet-visible spectroscopy, and the resulting nanoparticles were characterized by transmission electron microscopy. Two experimental assays were conducted: Assay A evaluated the ovicidal activity of AgNPs–*D. flagrans* after 15 and 30 days of exposure, while Assay B assessed the inhibition of embryonic development after 30 days of exposure. In Assay A, eggs treated with AgNPs–*D. flagrans* exhibited up to 47% destruction, accompanied by significant structural alterations. In Assay B 88% of the treated eggs failed to reach the larval stage. This study signifies the inaugural documentation of the ovicidal activity of AgNPs–*D. flagrans* against *T. canis* eggs, underscoring a promising and sustainable biotechnological approach for the integrated control of parasites of zoonotic concern.

In vitro evaluation of the anti-helmintic activity of bioactive compounds derived from _Anacardium occidentale_ L.

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Haemonchus contortus is the nematode species most found in sheep farming. Plant-based products, such as the liquid from cashew nut shells (CNSL) and its derivatives, have shown potential in controlling parasitic infections. This study aimed to develop a phytotherapic from the Brazilian semi-arid region to assist in controlling gastrointestinal parasitism in small ruminants. The CNSL was extracted at the Laboratory of Natural Product Chemistry at the State University of Ceará. Two main derivatives were obtained from the CNSL: calcium anacardate (AnCa) and anacardic acid (AA). The anacardic acid was then acetylated, resulting in acetylated anacardic acid (AAAc). In the final step, AAAc was used to synthesize a copper metal complex (AACu). A mixed-breed sheep was infected with third-stage larvae of *H. contortus* via oral administration. The egg hatching test was conducted with the following treatments: CNSL and AA (0.02, 0.01, 0.008, 0.006, and 0.004 mg/mL), AnCa (0.06, 0.05, 0.04, 0.03, and 0.02 mg/mL), AAAc (0.031, 0.023, 0.019, 0.016, 0.013, 0.012, and 0.010 mg/mL), and AACu (2, 1, 0.5, 0.25, 0.12, and 0.12 mg/mL), along with thiabendazole (0.4 mg/mL) as a positive control and DMSO (3%) as a negative control. After 48 hours of incubation at 27 °C, the hatching was interrupted with a 5% lugol solution. Among the compounds evaluated, CNSL and AA showed the lowest effective concentrations for 50% inhibition (EC_{50}), with values of (0.007 mg/mL) and (0.009 mg/mL), respectively, followed by AAAc (0.015 mg/mL) and AnCa (0.03 mg/mL). AACu showed no ovicidal activity at the tested concentrations, preventing the determination of its EC_{50}. Therefore, CNSL, AA, and AAAc showed *in vitro* ovicidal potential, requiring further studies on their action in larvae and adults, followed by *in vivo* tests to assess their

effectiveness in controlling haemonchosis in small ruminants.

Impact of an ozone therapy protocol on the analytical values of dogs with leishmaniasis

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Canine leishmaniasis (CanL) is a worldwide zoonosis of increasing prevalence. To date, no cure has been found for this pathology and studies into its prevention and treatment are constantly being updated. The development of resistance to current treatments and the increase in the prevalence of the disease make it necessary to research new therapeutic approaches. As a chronic disease, leishmaniasis creates situations of oxidative stress in the body. Research into the effectiveness of ozone therapy in diseases caused by protozoa, such as canine leishmaniasis, is still recent and scarce, but that which is available reports improvements in clinical signs because it recovers immunomodulatory, antioxidant and healing activities in those infected. The present study analysed oxidative stress levels and the effect of oral supplementation with ozonated oil capsules (OXYYO3®) once a day for 30 days in animals infected with CanL. The study aimed to evaluate the impact of an ozone therapy protocol on oxidative stress and other physiological parameters in dogs diagnosed with CanL. Laboratory blood test, count, biochemistry and oxidative stress evaluation (d-ROMs, PAT and OSI redox) were performed after administration of ozonated oil capsules (OXYYO3®) once daily for a period of 30 days. Statistical analysis of the study, using one-way ANOVA tests, showed improvements in neutrophil (p=0.0491), globulin (p=0.0329) and albumin (p=0.0076) parameters in animals suffering from CanL. These results reinforce the potential of ozone therapy to modulate the immune response. These findings are consistent with the hypothesis that ozone therapy may exert beneficial effects through its antioxidant properties. By reducing oxidative stress, ozone therapy may help mitigate some of the detrimental effects associated with CanL, leading to an overall improvement in the health status of the affected animals.

***Ex vivo* and *in vivo* pharmacological interaction between antiparasitic drugs and the phytochemical monoterpenes thymol and cinnamaldehyde**

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Considering the increase of nematode resistance to the synthetic anthelmintic drugs, new control strategies are urgently needed. While many phytochemical compounds show *in vitro* antiparasitic activity, their *in vivo* therapeutic potential remains underexplored. Both the *ex vivo* and *in vivo* effects of cinnamaldehyde (CNM) and thymol (TML) on the doramectin

(DRM) and levamisole (LVM) nematodicidal response were assessed in naturally infected lambs. The interaction on intestinal absorption/secretion by CNM, TML (1.5 mM) DRM and LVM (5 µM) was assessed using a diffusion-chamber model with Rho123 (0.5 µM) as a substrate across lamb ileum tissue. Two *in vivo* trials (T1 and T2) examined the interactions between monoterpenes and the synthetic anthelmintics. In T1, CNM (100 mg/kg, orally at 0 and 24 h) was combined with DRM (0.2 mg/kg, SC), with outcomes evaluated over two years. In T2, lambs received LVM (3.75 mg/kg, SC) alone or combined with CNM or TML (80 mg/kg, SC at 0 and 3 h). Drug plasma levels were measured by HPLC and fecal egg count reduction (FECR) were used to assess efficacy. CNM and LVM decreased Rho123 efflux across lamb intestine, suggesting a drug transport-related interaction. In T1, co-administration with CNM increased DRM efficacy from 66.3% to 78.0% (first treatment year); however, no significant differences in efficacy or pharmacokinetic (PK) parameters were observed in the second year between the DRM and DRM+CNM groups. In T2, both CNM and TML enhanced LVM systemic exposure, increasing the area under the curve (AUC) by 20–50%. Despite this PK interaction, treatment efficacy remained comparable for LVM+CNM (55.5%), LVM+TML (57.8%) and LVM alone (51.4%). These results suggest that the phytochemicals CNM and TML may induce drug PK and/or pharmacodynamics interactions both *ex-vivo* and *in-vivo*. Overall, the findings offer valuable insights into the potential of phytochemicals as antiparasitic agents and on their interactions with conventional anthelmintics.

Effects of zinc oxide nanoparticle supplementation on performance and immune response in sheep naturally infected with gastrointestinal nematodes

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Gastrointestinal nematodes (GIN), particularly *Haemonchus contortus*, significantly impact sheep production. Zinc oxide nanoparticles (ZnO-NP) have demonstrated the potential to modulate immune responses against parasites. This study aimed to evaluate the effects of ZnO-NP supplementation (150 mg/day) on weight gain, hematological parameters, and immune response in sheep naturally infected with GIN. Twenty-seven sheep were divided into a Supplemented Group (n=14, 4 Santa Inês and 10 Ile de France) treated daily with 150 mg of ZnO-NP, supplied in individual capsules, and a Control Group (n=13, 4 Santa Inês and 9 Ile de France) without supplementation. Both groups grazed together on the same pasture and received concentrate (1.5% of body weight/day) and mineral supplements without Zn. Over 126 days, we monitored body weight, fecal egg count (FEC), packed cell volume (PCV), total plasma protein (TPP), eosinophil count and IgG levels. FEC remained low in Santa Ines sheep throughout the experiment (maximum 400 EPG in controls on day 56). Ile de France lambs showed peak FEC on day 56 (5,190 EPG in supplemented vs. 5,578 EPG in controls), with no significant effect of treatment on FEC ($P>0.05$). During peak in-

fection, supplemented animals showed: Higher eosinophil counts on days 70 and 84 (P<0.05); higher anti-*Haemonchus* IgG levels from day 42 (P=0.0320 on day 98); improvement in hematological parameters (PCV and TPP, P<0.05 at day 70). Significant advantage in weight gain (P<0.05 from day 70). Six supplemented and six control Ile de France lambs required anthelmintic treatment due to severe anemia (PCV ≤20%). Daily supplementation with 150 mg ZnO-NP improved weight gain and immune responses in young sheep naturally infected with gastrointestinal nematodes. However, it did not prevent anemia in severely infected animals.

Impact of grape pomace rich in tannins in the diet of lambs infected with *Haemonchus contortus*

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Gastrointestinal nematodes, particularly *Haemonchus contortus*, cause significant economic losses in sheep production. Grape pomace, a winemaking byproduct rich in polyphenols and tannins, has demonstrated anthelmintic activity *in vitro*, but its *in vivo* effects remain poorly explored. This study aimed to evaluate the impact of dietary supplementation with grape pomace on *H. contortus* infection in lambs. Eighteen Santa Inês lambs were allocated into two groups: supplemented (34.4% corn + 45.6% soybean meal + 20% grape pomace) and control (53% corn + 47% soybean meal). Both groups were artificially infected with 4,000 L3 larvae of *H. contortus*. Fecal egg counts (FEC), hematological parameters (packed cell volume, total plasma protein and blood eosinophils) and IgG levels were evaluated over 28 days. On day 29, the animals were euthanized to determine the parasite worm burden, and, size and fecundity of *H. contortus* females. Phytochemical analyses revealed that the grape pomace contained: 42.21 g/kg dry matter (DM) in total phenols; 39.06 g/kg/DM of total tannins (expressed in tannic acid equivalents) and 2.89 g/kg/DM of condensed tannins (in leucocyanidin equivalents). The supplemented group presented an average of 5167 FEC on day 21, while the control group an average of 8656 FEC (p=0.0012) and on day 28 an average of 7722 and 12311 FEC, respectively (p=0.0358). The supplemented group presented: higher levels of anti-*Haemonchus* IgG (p=0.0099); smaller females (10.8 vs. 12.2 mm, p=0.0003) and fewer eggs per female (343 vs. 585, p<0.0001). There was no differences in weight gain, hematological parameters, worm burden and parasite measurements (p>0.05). We concluded that grape pomace at 20% inclusion in the diet reduced the FEC and egg fecundity of *H. contortus* without compromising the health of lambs, suggesting its potential as a sustainable prophylactic tool in the control of haemonchosis.

Enhancement of a solid culture medium for chlamydospore production of *Duddingtonia flagrans*

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Duddingtonia flagrans is a microfungus used for the biological control of gastrointestinal nematodes in ruminants, which negatively impacts livestock production. This study aimed to evaluate chlamydospore production in different culture media and assess its impact on nematode predatory capacity. *D. flagrans* was cultured in enriched Glucose Sabouraud Agar (GSA-E) with the addition of meso-inositol (Ml) and mannitol (M) at different concentrations: GSA-E+Ml1.1%+M2%, GSA-E+Ml1.1%+M5%, GSA-E+Ml2%+M2%, GSA-E+Ml2%M5% and GSA only (control). Twelve plates per concentration were incubated for 3, 4, and 5 weeks, respectively, at 27±0.5°C and 70±5% RH. Chlamydospores were quantified under optical microscope after mycelium homogenization. Nematophagous activity was assessed in faecal cultures with faeces from naturally parasitised sheep, incubated for 15 days at 24°C. Infective larvae (L3) were recovered by baermannisation, quantified, and their reduction was calculated. Data were expressed as mean ±SEM. Normality was evaluated using the Shapiro-Wilk test. Group comparisons were performed using One-Way ANOVA and Dunnett's tests or Kruskal-Wallis and Dunn's tests. At 3, 4, and 5 weeks, respectively, the values were: GSA: 8.4×10^6 ($\pm1.1\times10^6$), 8.2×10^6 ($\pm1.4\times10^6$), 8.9×10^6 ($\pm1.2\times10^6$); GSA-E+Ml1.1%+M2%: 2.9×10^7 (±4.8×106), 3.5×10^7 (±3.4×106), 2.6×10^7 (±4.3×106); GSA-E+Ml1.1%+M5%: 3.2×10^7 (±3.6×106), 8.7×10^6 ($\pm3.0\times10^6$), 2.6×10^7 (±6.6×106); GSA-E+Ml2%+M2%: 8.9×10^6 ($\pm3.6\times10^6$), 5.4×10^6 ($\pm1.6\times10^6$), 1.7×10^7 (±5.2×106); GSA-E+Ml2%+M5%: 1.7×10^7 (±5.9×106), 4.1×10^7 (±8.6×106), 6.9×10^7 ($\pm1.3\times10^7$). The GSA-E+Ml2%+M5% medium optimised chlamydospore production at 5 weeks. The numbers of L3 recovered from Ml- and M-enriched media were 74-99.7% ($p<0.05$) lower than in the control group. Supplementation with Ml and M enhances chlamydospore production of *D. flagrans* without compromising its predatory capacity, thus optimising its production to use it in biological control against nematodes.

In vitro evaluation of a garlic-based solution (_Allium sativum_ L.) on the reproductive efficiency of _Rhipicephalus microplus_

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Resistance of the cattle tick *Rhipicephalus microplus* to chemical products has significantly reduced the control options available to livestock producers. Garlic (*Allium sativum* L.) is a phytotherapeutic agent widely studied in scientific research, with allicin and its sulfur-containing derivatives demonstrating acarici-

dal activity. The objective of this study was to evaluate, in vitro, a solution that can be prepared and applied on-farm for the control of this tick species. On March 5th, 2025, a stock solution was prepared by blending 100 g of garlic cloves with peel (white garlic variety) in 500 mL of water, to which 55 mL of alcohol vinegar (Castelo® brand) and 10 g of coconut soap (Indaiá® brand) were added. All ingredients were purchased from a local supermarket. The mixture was well crushed in a blender, filtered, and stored in a closed plastic container at room temperature in the Parasitology Laboratory of the Instituto de Zootecnia, in Nova Odessa, São Paulo State, Brazil. One week later, an adult immersion test (AIT) was performed by immersing groups of five females tick for 5 minutes in the stock solution (100%) and in dilutions with water (50%, 25%, 10%). Water was used as the control group. Each treatment was conducted in triplicate. Ten days after treatment, the eggs were weighed and the egg mass to female weight ratio (EF) was calculated. EF ratios were analyzed using the PROC GLM procedure of SAS, including the fixed effect of treatment dose. Mean differences were compared using the t test ($p < 0.05$). The estimated EF ratios for 100%, 50%, 25%, 10% and water were, respectively: 0.372 ± 0.070 AB, 0.292 ± 0.101 AB, 0.241 ± 0.104 B, 0.404 ± 0.100 A, and 0.449 ± 0.056 A. Based on the results obtained thus far, it can be concluded that the 25% dilution of the *Allium sativum* stock solution presents potential for controlling *Rhipicephalus microplus,* as it significantly reduced the oviposition of engorged females.

In vitro evaluation of a garlic-based solution (*Allium sativum* L.) on the larval mortality of *Rhipicephalus microplus*

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Resistance of the cattle tick *Rhipicephalus microplus* to chemical products has significantly reduced the available control options for livestock producers. Garlic (*Allium sativum* L.) is a medicinal plant with known acaricidal properties. The aim of this study was to evaluate, *in vitro*, a solution that can be prepared and used on-farm for the control of this tick species. On March 5, 2025, a stock solution was prepared by blending 100 g of unpeeled garlic cloves (white bulb variety) in 500 mL of water, adding 55 mL of alcohol vinegar (Castelo® brand) and 10 g of coconut soap (Indaiá® brand), all purchased from a local supermarket. The mixture was well crushed in a blender, filtered, and stored in a closed plastic container at room temperature in the Parasitology Laboratory of the Institute of Animal Science, located in Nova Odessa, São Paulo State, Brazil. Two days later, a larval mortality test was conducted using approximately 100 *R. microplus* larvae, immersed for 5 minutes in the solution and its dilutions in water (100%, 50%, 25%, 10%, and water control). Each treatment was performed in triplicate, and larval mortality was assessed after 24 hours. The results showed 100% mortality at 100%, 50%, and 25% dilutions. The 10% dilution resulted in an average larval mortality rate of 74%, while the water control showed 1.6% mortality. The same stock solution, diluted at 25%, was re-tested 13, 22, and 27 days after preparation (with water control included on each test day) on the same larval strain used previously. The mortali-

ty rates observed were 96%, 99%, and 100%, respectively. The water controls showed average mortalities of 6.5%, 14.7%, and 19%, respectively. These results indicate that the solution prepared with *Allium sativum* L., at a 25% dilution, has potential for controlling *Rhipicephalus microplus*, and that the stock solution remained effective for at least one month at room temperature.

Synergistic Effects of Natural Compounds Against an Ivermectin-Resistant Isolate of *Haemonchus contortus*

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The scenario of anthelmintic resistance has driven the search for new approaches and drugs. In previous studies, metabolomics enabled the identification of various chemical groups and their combinations within individual plant extracts. Subsequently, isolated compounds representing these chemical groups were evaluated, and lapachol (a naphthoquinone), gallic acid (a polyphenol), and gibberellic acid (a diterpene) showed greater potency against *Haemonchus contortus* and were selected for the evaluation of possible synergistic effects when combined. This study aimed to evaluate the potential synergistic effects of combining gallic acid with lapachol or gibberellic acid against an ivermectin-resistant isolate of *H. contortus*. Egg hatch tests were performed using a ivm-resistant strain. Each combination was assessed using 25-point dose-response matrices, conducted in six replicates. Drug interaction analysis was performed using four reference models: Highest Single Agent, Bliss Independence, Loewe Additivity, and Zero Interaction Potency. The optimal ratio was defined as the matrix point with the highest average synergy score across the four models. Synergistic effects were observed for all tested combinations. For the gallic acid and lapachol combination, the optimal ratio was 0.833 : 0.167 of their respective EC_{50} values. A moderate yet statistically relevant synergistic effect was seen in the gallic acid and gibberellic acid combination, with an optimal ratio of 0.333 : 0.667 EC_{50} and significant synergy in 3 out of 4 models. These findings suggest that the combination of gallic acid and gibberellic acid shows promise as a natural anthelmintic alternative against resistant *H. contortus*.

***Beauveria bassiana* and *Metarhizium anisopliae* as alternative control agents for *Amblyomma sculptum* in urban areas of the State of São Paulo, Brazil**

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to, Brazil), Leandro Bernardes da Silva Moraes (Municipal Secretariat of Health – Salto, Brazil), Paula Cristina Pereira Cabral (Environmental Management Department – São José dos Campos, Brazil), Andrea Sundfeld (Environmental Management Department – São José dos Campos, Brazil), Antônio Jorge da Silva Gomes (Municipal Secretariat of Health – Americana, Brazil), Neto Franzatto (Municipal Secretariat of the Environment – Americana, Brazil), Hamilton Humberto Ramos (Agronomic Institute of Campinas – Jundiaí, Brazil), Viviane Corrêa Aguiar Ramos (Agronomic Institute of Campinas – Jundiaí, Brazil), Priscilla Fernanda de Oliveira Ferreira (Brazilian Air Force – DCTA, Brazil), Jéssica Cascaes de Moraes Pereira (Brazilian Air Force – DCTA, Brazil), Minoru Takahashi (Toyobo do Brasil, Brazil), Guilherme Micai (Toyobo do Brasil, Brazil), José Eduardo Marcondes de Almeida (Biological Institute, Brazil)

Human activity has profoundly changed the environment in the State of São Paulo, Brazil. Agriculture promoted deforestation and eliminated predators. As urbanization progressed, farmland was absorbed by real estate development, and cattle and crops gave way to houses, lawns, and ponds. Herds of capybaras (*Hydrochoerus hydrochaeris*) multiplied, and their parasites reacted to these changes also. *Amblyomma dubitatum* was supplanted by *Amblyomma sculptum*, the vector of *Rickettsia rickettsii*, the agent of the Brazilian Spotted Fever, a deadly tick-borne disease, increasing the risk for humans. Since October 2018, the Biological Institute has partnered with Municipalities and other institutions to develop biological control strategies to mitigate such risks. The main approach is spraying wettable-powder formulations of the fungi *Beauveria bassiana* IBCB66 (Ecobass, Toyobo) or *Metarhizium anisopliae* IBCB425 (Ecometa, Toyobo), which are widely used in Crop Protection in Brazil, including organic farming. Fungi are sprayed at 2×10^13 conidia/ha monthly from October to March when adult ticks emerge. The tick population is periodically assessed with 6 – 12 fixed-position dry-ice traps, consisting of a 1.0 m x 0.7 m piece of non-woven white fabric with 200 g dry-ice on its center. Traps are removed after 40 minutes. Captured ticks are counted, and results are statistically analyzed using a mixed model with repeated measures over time. In general, both fungi reduce the tick population significantly. The current season introduced a combination strategy, with *B. bassiana* being applied on the margins of the woods and *M. anisopliae* on the lawns. This approach is expected to improve results observed so far on peri-forestal traps, where the vegetation limits the penetration of *M. anisopliae*. Over 60 ha in urban leisure areas are regularly treated and monitored in Americana, Jundiaí, Salto, and São José dos Campos. Sufficient data for regulatory approval should be available shortly.

Metarhizum anisopliae IBCB425 conidia survive the ovine gastrointestinal tract

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of São Paulo, Brazil), José Eduardo Marcondes de Almeida (Crop Protection and Animal Health Advanced Research Center, Brazil), Rodrigo Martins Soares (USP, Brazil)

The antiparasitic activity of the filamentous fungus *Metarhizium anisopliae* against arthropods and helminths has been widely reported. Studies with tracer sheep suggested its conidia might survive passage by the digestive system and infect *Haemonchus contortus* L3 in coprocultures. To confirm this hypothesis, ten naturally infected Santa Inês sheep, including seven wethers with ruminal fistulae and three rams, were allocated to treatment and control groups based on their FEC. Treatment consisted of 10 mL of an aqueous emulsion containing 10^9 *M. anisopliae* IBCB425 conidia. Control received 10 mL of distilled water with polysorbate. Treatment was administered by gavage to the rams or directly into the rumen of the fistulated sheep. Feces and ruminal fluid were collected before treatment (D1) and over the three subsequent days (D2, D3, D4). Hematological parameters were assessed on D1 and D4. On all days after FEC, individual coprocultures were prepared with 5 g feces and incubated at room temperature for 10 days. Another 5 g of feces were dissolved with distilled water, filtered with gauze, seeded on Potato Dextrose and Bengal Rose agar with penicillin, and incubated for 10 days. FEC, ruminal fluid, and blood analysis did not differ from the control. Moldy L3 were detected in coprocultures from the treated group on D2, D3, and D4 but were absent from the control. The treatment significantly altered L3 motility scores. Characteristic *M. anisopliae* colonies were recovered from fecal and ruminal samples from the treated and control groups with both media. Treated fecal samples seeded on Bengal Rose have shown a numerical UFC peak on D2, but it did not differ from the control. *M. anisopliae* is ubiquitous, and provided no moldy L3 were found in the control group's coprocultures, it might possibly relate to the presence of a non-pathogenic strain in the hay. Further investigation requires developing molecular techniques to differentiate IBCB425 from other commercial or field isolates.

Tick–Fungus–Pathogen interactions: Immune response and survival in *Ixodes scapularis*

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Ixodes scapularis, the primary vector of *Borrelia burgdorferi*, the causative agent of Lyme disease, is increasingly found in urban areas of the United States, raising concerns for public health. A sustainable alternative to synthetic acaricides is using entomopathogenic fungi for tick control. This study investigated the effects of *Metarhizium anisopliae* treatment on *B. burgdorferi*-infected and uninfected *I. scapularis* nymphs, focusing on tick survival and immune gene expression. Ticks were assigned to four groups: Ctr Buffer (pathogen-free nymphs inoculated with buffer), Bb Buffer (*B. burgdorferi*-infected nymphs inoculated with buffer), Ctr Meta (pathogen-free nymphs inoculated with *M. anisopliae* at 1×10^6 conidia mL^{-1}), and Bb Meta (*B. burgdorferi*-infected nymphs inoculated with *M. anisopliae*). Tick mortality was recorded daily for ten days. RT-qPCR was used to quantify the expression of immune-related genes: *flaB*, *myd88*, *cactus*, *dorsal*, *microplusin*, *acanthoscurin*, *dae*, *defencer*, *ctenedin*, *scap5*, *stat*, and *sting*. Ticks from the Bb Meta

group exhibited significantly higher survival than Ctr Meta. As expected, only *Borrelia*-infected ticks (treated or not) exhibited flaB upregulated, confirming *Borrelia* infection. *Borrelia*-infected ticks exhibited upregulation of *defencer* following fungal inoculation compared to infected and untreated ticks. These findings suggest that *B. burgdorferi* infection may enhance tick resistance to fungal challenges. Our results contribute to a better understanding of tick immunity and offer valuable insights into host-pathogen–fungus interactions relevant to future tick control strategies.

Analysis of parasite management and control in sheep in Caaguazu, Paraguay

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Sheep farming plays a fundamental role in Paraguay's livestock production. However, a significant number of producers operate under extensive and inefficient systems, largely due to parasitic diseases that negatively impact animal health and productivity. This study aimed to evaluate the effectiveness of qualitative (FAMACHA, body condition, diarrhea index) and quantitative (hematocrit, coprological analysis) parameters in determining the need for antiparasitic treatment in sheep at the local level. A total of 174 sheep of different ages and sexes were analyzed. Fecal samples were collected directly from the rectum, while blood samples were obtained via jugular puncture. Parasite load was determined using the McMaster method for quantifying eggs per gram (EPG) in feces. Hematocrit levels were measured using the microhematocrit centrifugation method. Qualitative parameters (FAMACHA, body condition, and diarrhea index) were assessed in situ by trained personnel. Descriptive statistics and correlation tests were used for data analysis. Of the total sampled animals, 86% were females and 14% males. Parasitic infestation was classified as mild (37%), moderate (14%), and high (48%). A total of 47% of the animals had an average of 6,540 EPG and hematocrit values below 20%. However, no statistically significant correlation was found between these variables ($p>0.05$). In contrast, qualitative parameters (FAMACHA, diarrhea index, and body condition) showed a correlation above 0.8, indicating their utility in the clinical assessment of parasite load. Animals with high FAMACHA scores exhibited lower body condition and an intermediate diarrhea index. Additionally, those with high parasite loads had decreased hematocrit levels, suggesting that this parameter could serve as a useful indicator for selecting individuals requiring antiparasitic treatment. However, further studies are needed to validate these criteria and optimize control strategies that minimize the indiscriminate use of antiparasitic treatments in sheep flocks.

In vitro anthelmintic potential of the acetone:water extract and essential oil of *Lippia origanoides* against *Haemonchus contortus*

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Haemonchus contortus is a hematophagous nematode that affects sheep and goats, generating economic losses for producers. Its resistance to conventional anthelmintics (AH) has prompted research into therapeutic alternatives, especially the use of plant extracts with antiparasitic activity. *Lippia origanoides* is a plant which can be classified in three chemotypes according to its major essential oil (EO) content, i.e, carvacrol, thymol and sesquiterpenes. It also contains other metabolites with AH potential. To evaluate the effect of the acetone:water extract and the EO of *L. origanoides* against *H. contortus* exsheathment. The leaves of *L. origanoides* chemotype thymol (500 g) were collected in Yucatan, Mexico. The hydro-distillation method was used to obtain the EO. The polyphenol extract was obtained by maceration in an acetone:water solution (70:30). The larval exsheathment inhibition tests used 1000 L3/mL incubated for 3 h at 23 °C using an extract concentration gradient at 80, 150, 300, 600, 600, 1200 µg/mL. Negative (phosphate buffer saline, PBS) and positive (levamisole) controls were included. After incubation, the L3 were washed 3 times with PBS and divided into 4 aliquots. The exsheathment was induced by the addition of a solution of sodium hypochlorite (2% v/v) and sodium chloride (16.5% w/v) diluted in PBS. The effective concentration to inhibit 50% of the exsheathment (EC50) and its confidence intervals (95%CI) were estimated. The EC50 of EO and polyphenol extract were 133.9 µg/mL (95%CI 119.77 - 147.94 µg/mL) and 326.2 µg/mL (95%CI 278.03 - 374.5 µg/mL), respectively. EO and acetone:water extract can inhibit *H. contortus* exsheathment. This is the first report of an EO blocking the *H. contortus* exsheathment. Moreover, the EO was 2.4 times more active than the acetone:water extract.

Phytochemical extracts of *Azadirachta indica* and *Clerodendrum viscosum* mediated dopaminergic, serotonergic and developmental pathways modulation in *Caenorhabditis elegans* to assess anthelmintic potency

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Target-specific resistance (TSR) and non-target-specific resistance (NTSR) surge have significantly influenced the identification of lead compounds over the past decade for the biodiscovery of new anthelmintic. Despite the rich diversity of medicinal flora in Southeast Asia, Bangladesh is far behind in utilizing the *C. elegans* model for lead discovery. This study aimed to assess the anthelmintic potency of

extracts of *Azadirachta indica* and *Clerodendrum viscosum* induced alterations and disruptions of neurotransmitters in *C. elegans*. The anthelmintic activity was assessed by examining motility (head thrashing and body bending), mortality, egg hatch inhibition (EHI) and relative gene expression, targeting *cat-1*, *ser-1*, *dat-1*, and *tba-1*. This study also optimized a robust extraction protocol, enriching the extract to yield maximum and to minimize assay interference. At 1 mg/mL, *A. indica* and *C. viscosum* induced body bending to 34.67±2.59 ($p<0.01$) and 34.67±2.59 ($p<0.01$); reduced head thrashing (71.67±1.90, $p<0.01$ for *A. indica*; 76±2.62, $p<0.01$ for *C. viscosum*). Their nematicidal efficacy was 82.34±2.027% (LC50- 0.484, $p<0.01$) and 77.33±5.46% (LC50- 0.498, $p<0.01$), respectively. Additionally, both extracts demonstrated strong EHI of 93.75±0.2197% ($p<0.01$) for *A. indica* and 97.5±3.0% ($p<0.01$) for *C. viscosum*. Gene expression analysis revealed significant downregulation of selected genes, indicating interference with dopaminergic and serotonergic pathways, along with metabolic and reproductive disruptions. Altogether, this study revealed the anthelmintic potential of *A. indica* and *C. viscosum* extracts with neuromodulatory and developmental impacts on *C. elegans*. The findings offer a foundation for further pharmacological investigations, with significant implications for combating anthelmintic resistance and advancing the discovery of novel anthelmintic agents.

Health and production of small ruminants in Paraguay: diagnosis, challenges and perspectives for the sector.

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Proper sanitary management is an essential component to improve the productivity and health of small ruminants, especially in developing countries such as Paraguay. Therefore, the effective control of gastrointestinal parasitosis is fundamental, since it significantly affects the growth, feed conversion and profitability of the animals. In order to learn about sanitary and productive management practices, surveys were conducted among small ruminant producers in Paraguay. The survey involved 74 producers from 15 departments of the country who agreed to participate in the study. One of the most relevant findings is that the average herd size is between 10-50 animals, the production is generally mixed (sheep, goats), 93.24% of the producers do not carry out control coprologies, 6.76% do not carry out an adequate rotation of antiparasitics, 72.97% control their animals through FAMACHA, 40.54% carry out paddock rotation. A 95.95% said they were open to advice and 79.73% expressed interest in being part of our project in the long term. According to the results, infestations could be associated with a lack of knowledge about the importance of parasitological diagnosis, economic limitations or lack of access to specialized veterinary services for small ruminants. The results of this survey highlight the need to strengthen training and access to diagnostic tools in the sector, promoting more efficient sanitary control strategies to improve the health and productivity of small ruminants in Paraguay.

Ovicidal effect of *Ocimum basilicum* var. 'Red Rubin' essential oil on multiresistant *Haemonchus contortus*

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Essential oil derived from *Ocimum basilicum* demonstrated different biological activities, including anthelmintic effect against *Haemonchus contortus*. However, this species comprises cultivars that are mainly distinguished by the oil chemical composition, an important factor determining of biological activity. Thus, the aim of this study was evaluated the effect of *O. basilicum* var. 'Red Rubin' oil on eggs of multiresistant *H. contortus*. The fresh leaves and flowers of this plant were collected from the medicinal plant bed of the Embrapa Agroindústria Tropical, Ceará, and the oil was extracted by hydrodistillation. Oil chemical composition was analyzed by gas chromatography coupled with mass spectrometry (GC/MS). The egg hatching test (EHT) was performed using the Kokstad isolate of *H. contortus* resistant to synthetic anthelmintic (benzimidazoles, imidatiazoles, and macrocyclic lactones). For this, approximately 100 fresh eggs were incubated for 48 h at 27 °C with the oil (0.06 to 1 mg/mL). After incubation, iodine solution was added to stop the egg hatching. Then eggs and first stage larvae were counted in microscope. The negative control was DMSO (0.5%) and the positive control was thiabendazole (0.025 mg/mL). The treatment and controls had five replicates. Results were analyzed using ANOVA and Tukey's test ($P<0.05$) by GraphPad Prism® 8.0.1. The effective concentration of oil to inhibit 50% (EC50) of egg hatching was determined by probit linear regression using SPSS® 22.0. GC/MS identified linalool (45.36%), eugenol (19.17%) and eucalyptol (14.19%) as major components. At the highest concentration tested (1.0 mg/mL), the oil efficacy was 80.69% ± 2.66. This result differed statistically from the negative (2.14% ± 0.70) and positive (92.26% ± 2.02) controls. The oil effect was dose-dependent and the EC50 was 0.69 mg/mL. Thus, the oil of *O. basilicum* var. 'Red Rubin' showed ovicidal effect, requiring further studies on their action in *H. contortus* larvae and adults.

Effect of *Ocimum micranthum* essential oil on eggs of multiresistant *Haemonchus contortus*

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The resistance to synthetic anthelmintics prompts to the search for control alternatives to gastrointestinal nematodes in small ruminants, such as plants and its derivates. The essential oil of *Ocimum micranthum* (EOOm) has different biological activities, however the anthelmintic activity of this oil yet no be searched. This study aimed to evaluate of the ovicidal activity of EOOm against multiresistant *Haemonchus contortus*. The fresh leaves and flowers of *O. micranthum* were collected from the medicinal plant bed of the Embrapa Agroindústria Tropical and the oil was extracted by hydrodistillation system. Oil chemical composition was analyzed by gas chromatography coupled with mass spectrometry (GC/MS). The egg hatching test (EHT) was performed using the Kokstad isolate of *H. contortus* resistant to benzimidazoles, imidatiazoles and macrocyclic lactones. For this, approximately 100 fresh eggs were incubated for 48 h at 27 °C with EOOm (0.06 to 1 mg/mL). After incubation, iodine solution was added to stop the egg hatching. Then eggs and first stage larvae were counted in microscope. The negative control was 0.5% DMSO and the positive control was thiabendazole (0.025 mg/mL). The treatment and controls had three repetitions with five replicates each. Results were analyzed using ANOVA and Tukey's test ($P<0.05$) by GraphPad Prism® 8.0.1. The effective concentration of EOOm to inhibit 50% (EC50) of egg hatching was determined by probit linear regression using SPSS® 22.0. GC/MS identified eugenol (79.97%), α-caryophyllene (5.96%) and γ-elemene (4.66%) as major components. At 1 and 0.5 mg/mL, the EOOm efficacy was 100% ± 0.00 and 98.01% ± 1.33, respectively. These results did not differ statistically from the positive control (96.34% ± 2.70). The oil effect was dose-dependent and the EC50 was 0.19 mg/mL. Therefore, the results indicate that EOOm demonstrates ovicidal effect against multiresistant *H. contortus* and may have potential anthelmintic.

Climbing assay for Dermacentor nitens: methodology and preliminary data to test allomones candidates

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Parasitism by *Dermacentor nitens* ("horse-ear-tick") in horses causes direct and indirect damage to the host. For example, ear drop, predisposition to myiasis and secondary bacterial infection, and transmission of *Babesia caballi*. Its control is done through acaricides, but few products are available on the market. There is a demand for new control tools where semiochemicals (allomones, volatile organic compounds identified from a non-preferred host capable of repelling tick) are included in this context. This work aimed to standardize a methodology to evaluate the behavior of *D. nitens* larvae in response to allomones in a climbing assay. The methodology developed was based on the biology and ecology of *D. nitens* larvae. Commercial DEET 6.65% (gold standard tick repellent) and trans-2-octenal 1M (allomone identified from

donkeys' ear, a non-preferred host, and repellent to *Amblyomma sculptum*) were chosen for standardization. Filter papers were cut into 12cm x 2cm rectangle pieces, and the middle third was treated with 50µL of each substance. The treated papers were hung vertically, simulating the position of the grass, and 20 larvae were released on the bottom. Their behavior was analyzed for signs of repellency, such as reversal of movement during climbing and/or hot-foot (larvae falling off from the filter paper upon touching the substance) at 10-minute intervals and every hour for the following 4 hours. New larvae were released at each evaluated time point. Preliminary tests were performed in triplicate and in each repetition 10 filter papers were used. The results confirmed DEET as a repellent, with a mean repellency of 100% at all times measured. Trans-2-octenal achieved mean repellency of 84%, 75%, 78%, 71%, and 77% for the respective time intervals. The methodology allowed observed the tick behavior and confirmed DEET as a synthetic repellent and trans--2-octenal as an allomone. This, the proposed methodology can be used for future studies with other candidates.

In vitro action of papain on _Cryptosporidium_ spp.

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Cryptosporidiosis is a parasitic disease caused by *Cryptosporidium* spp. This parasite affects epithelial cells of the gastrointestinal tract of fish, amphibians, reptiles, birds, and mammals and is an important cause of diarrhea in humans. Transmission occurs mainly through ingesting water and food contaminated with oocysts, which are highly persistent in the environment. One way to control *Cryptosporidium* is to add sodium hypochlorite to the water supply. However, the effectiveness of this treatment may be reduced with the development of resistant genotypes, making it necessary to develop new control methods. Papain (EC 3.4.22.2), a protease extracted from *Carica papaya*, may be an alternative since it is a stable enzyme over a wide range of temperatures and pH. This study evaluated the effects of papain on the biochemical control of *Cryptosporidium* spp. oocysts *in vitro*. The experiment was performed in triplicate, with repetitions of the entire procedure. For the negative control, 40 µL of a *Cryptosporidium* spp. solution with approximately 60 oocysts in 40 µL of distilled water was added. For the positive control, the oocyst solution received 40 µL of 4% (v/v) sodium hypochlorite. Finally, in the enzyme treatment, 40 µL of 15% (m/v) papain was added. The groups were incubated for 72 hours and then transferred to slides for Ziehl-Neelsen staining. Intact oocysts were counted under an optical microscope. Data were analyzed by ANOVA and t-test. Papain significantly reduced ($p<0.01$) the number of intact oocysts compared to the negative control. After exposure to the enzyme, there was a 65% reduction in the number of oocysts. These results may be related to the catalytic action of papain in the degradation of proteins present in these structures. It was concluded that papain may be, in the future, a

promising alternative for controlling oocysts in the environment.

Effect of *Haemonchus contortus* establishment on nutritional variables in hair lambs at three feeding levels

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Nutritional management of small ruminants has been identified as a key factor in achieving sustainable control of gastrointestinal nematodes (GIN). Several studies have reported that supplementing the diets of small ruminants with extra energy and protein helps control infections caused by *Haemonchus contortus*. The effect of establishment at different levels of *H. contortus* infection on the digestive physiology and productive variables of hair sheep at different nutritional levels is currently unknown. The effect of infection level during *Haemonchus contortus* establishment on intake, digestible energy (DE), digestible crude protein (DCP), nitrogen retention (NR) and energy retention (ER) was evaluated at three feeding levels. Eighteen Pelibuey lambs (male, six months old and free of GIN) were individually housed in metabolic cages, balanced by live weight (25.04±4.8 kg LW) and randomly assigned to three experimental groups (n=6): D75 diets for a daily gain of 75 g/day; D125 for 125 g/day and D200 for 200 g/day. On day 0, they were inoculated orally with 450 infective larvae (L_3) of *H. contortus*. Nutrient intake, ED, DCP, NR and ER were determined. On day 28 post-infection, all 18 lambs were humanely slaughtered to estimate the total adult parasites (TAP) in the abomasum, expressed as metabolic weight ($BW^{0.75}$). Preliminary data showed a negative correlation between TAP and NR and ER, irrespective of the feeding level, and no other variable was affected by TAP. Diets with daily gains >75g/day were not affected by the establishment of *H. contortus* in Pelibuey lambs.

Arthropods, helminths, protozoans

Establishment of a primary culture derived from embryonic cells of *Lucilia cuprina* (Diptera: Calliphoridae)

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Primary cell culture is a valuable tool for research, as it allows to realize many studies under controlled *in vitro* conditions and free from contamination. Advances in entomological studies provide models for the analysis of insecticide resistance, as well as applications in biotherapy and forensic entomology. The aim of the present study was to establish the primary cell culture of *Lucilia cuprina*. Embryonated eggs of *Lucilia cuprina* were obtained from a colony maintained at the Experimental Station for Parasitological Research William Otton

Neitz (W.O. Neitz) of the Department of Animal Parasitology at the Federal Rural University of Rio de Janeiro (UFRRJ). Specimens were identified using the taxonomic key by Carvalho and Mello-Patiu (2008). The cell culture procedures were conducted at the Cell Culture Laboratory, affiliated with the Laboratory of Parasitic Diseases of the Department of Epidemiology and Public Health at UFRRJ. For the washing of eggs and establishment of the primary culture, the protocol adapted from Lima Duarte (2022) was employed. Leibovitz's L-15 medium was adopted, supplemented with 10% tryptose phosphate broth, 20% heat-inactivated fetal bovine serum, 1% glutamine, and 1% penicillin (10,000 IU)/ streptomycin (10 mg/mL), with the pH adjusted to 6.8 and sterilized using a 0.22 µm filter. The cultures were maintained at 28°C in a climate--controlled incubator, with weekly replacement of 1.5 mL of medium. Cell propagation was monitored weekly through inverted light microscopy, revealing branched cells exhibiting pseudopods, resembling neuronal cells. The primary cell culture of tropical-origin *L. cuprina* has been successfully maintained for 20 weeks, reaching 60% confluence until the present moment. The long-term viability of cells derived from this insect encourages the future establishment of a continuous cell line, potentially serving as an invaluable resource for studies under *in vitro* conditions.

Molecular investigation and characterization of the anaplasmataceae family bacteria in rescued wild animals in the state of Rio de Janeiro, Brazil

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Hemoparasites are responsible for causing significant health damage to animals, whether they are production, companion, or free-living wild animals. Many of these pathogenic agents have zoonotic potential and represent an important topic within the One Health framework. In this context, the aim of the present study was to detect infection by bacteria of the Anaplasmataceae family in free-ranging wild animals rescued and referred to the Wildlife Rehabilitation Clinic (CRAS) at Estácio de Sá University, in the municipality of Rio de Janeiro, state of Rio de Janeiro, Brazil. Blood samples were collected from 148 birds, 52 mammals, and 20 reptiles, totaling 220 animals, between January 2019 and August 2021. The samples were properly identified and stored for subsequent processing at the Laboratory of Parasitic Diseases of the Federal Rural University of Rio de Janeiro (UFRRJ), where DNA extraction was performed. The samples were subjected to nested PCR and conventional PCR techniques targeting the 16S *rDNA*, *gltA*, and *GroEL* genes of Anaplasmataceae, as well as specific targets for the genera Anaplasma spp. (16S *rDNA* and *rpoB*) and *Ehrlichia* spp. (*Dsb*). Following all assays, Anaplasma sp. DNA was detected in a single specimen of *Coendou spinosus* (spiny tree porcupine) through amplification of the 16S *rDNA* and *GroEL* genes. Sequencing of the amplified products revealed that the detected *Anaplasma* species shared 99.4% identity with *Anaplasma* sp. (830/835) previously described in dromedaries for the *16S* rDNA gene, and 81.11% identity with *Anaplasma platys* for the *GroEL* gene. These findings demonstrate that wild animals are susceptible

to infection by agents of the Anaplasmataceae family and may act as reservoirs and maintainers of the epidemiological cycle of pathogens of significant importance to both animal and human health.

Morphological redescription of male *Amblyomma rotundatum* Koch 1844

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The tick *Amblyomma rotundatum* has been reported parasitizing cold-blooded animals and is widely distributed across Brazilian biomes. *A. rotundatum* is closely related to *Amblyomma dissimile*. Unlike *A. dissimile*, it reproduces by parthenogenesis, with males being rarely recorded. The aim of the present study was to provide a morphological redescription of the male of *A. rotundatum*. Ticks collected from *Iguana iguana* specimens were preserved in 70% ethanol for subsequent identification in the laboratory. All ticks were identified as *A. rotundatum*. Ten males were selected for morphometric measurements using Zeiss Zen software. The males of *A. rotundatum* exhibited the following characteristics: Idiosoma: length from apices of the scapulae to posterior body margin 2.87–3.90 (3.63 ± 0.32), maximum breadth 2.33–3.29 (3.02 ± 0.29). The body is outline oval, marginal grooves absent, scutum densely punctate (especially at periphery), pale iridescent patches of orange ornamentation present along scutal margins. The genital aperture possessing a flap. Gnathosoma: length of palpal apices to posterior margin 0.72–0.99 (0.91 ± 0.08). Basis capituli is subrectangular with rounded, blunt cornua. Palp length 0.72–0.90 (0.81 ± 0.06); length of palpal article I 0.06–0.21 (0.16 ± 0.04); length of papal article II 0.41–0.56 (0.49 ± 0.05); length of papal article III 0.17–0.29 (0.22 ± 0.04). Total length hypostome 0.52–0.79 (0.66 ± 0.08), length of toothed portion 0.24–0.38 (0.33 ± 0.05). Hypostome with 3/3 dental formula. Legs: Coxae each with two short, bluntly rounded spurs. Tarsus I 0.36–0.88 (0.74 ± 0.15) long, 0.17–0.28(0.22 ± 0.04) broad. Tarsus IV 0.48–0.66 (0.61 ± 0.05) long; 0.15–0.20 (0.17 ± 0.02) broad. These data contribute to the morphological redescription of males of this species, enabling more accurate identification of this developmental stage.

Susceptibility of Brazilian populations of *Rhipicephalus microplus* to fluralaner

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In 2022, a new product based on fluralaner (isoxazoline) was registered in Brazil for the control of *Rhipicephalus microplus* and other bovine ectoparasites. Two discriminating doses (DDs) were previously proposed for this new active. In this study, we evaluated the fluralaner susceptibility profile of *R. microplus* populations from all regions of Brazil using DD tests. Engorged females of *R. microplus* were collected from naturally infested cattle in 142 private properties (randomly) between December 2022 and February 2025. The engorged females were incubated for 14 days under controlled conditions (27±1°C, RH 80±10%) for oviposition and, after laying, the eggs were transferred to test tubes for hatching under the same conditions. Larvae 14 to 28 days post-ecdysis were used for the tests. A stock solution of fluralaner (50 µg/mL) was prepared from the commercial formulation Exzolt 5%® (MSD Animal Health), solubilized in 2% DMSO. From this solution, the DDs of 1.55 and 3.16 µg/mL, determined in previous studies, were prepared. The larval immersion test (LIT) was used to apply the DDs. The larvae were immersed for 3 minutes and approximately 100 individuals were transferred to filter paper packets (6 cm^2), sealed and incubated in B.O.D. (27±1°C; RH 80±10%) for 24 hours. After this period, the number of live and dead larvae was quantified. A control group was also formed with 2% DMSO. For each treatment, 10 replicates were carried out. Populations with a mortality rate of over 95% were considered susceptible. Samples from all regions of Brazil were analyzed: 15 samples from the North (AC, AM, PA, RO, TO); 13 samples from the Northeast (BA, CE, MA, PE, PI, SE); 38 samples from the Midwest (DF, GO, MT, MS); 73 samples from the Southeast (ES, MG, RJ, SP); and three samples from the South (PR and RS). For these populations, mortality ranged from 97.3 to 100% for the two DDs. We can therefore conclude that all the populations evaluated, regardless of the DD tested, were susceptible to fluralaner.

Detection of Spotted Fever Group Rickettsia in Ticks Parasitizing Humans in the Gurupi Biological Reserve, Eastern Amazon (Maranhão, Brazil)

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The growing interest in the study of accidental tick parasitism in humans in Brazil highlights the scarcity of data from the Amazon biome, where records generally originate from observations during field activities conducted by researchers and professionals. Environmental degradation tends to expand the distribution of certain tick species, many of which are vectors of pathogens, thereby increasing the risk of emerging diseases in humans. In the Amazon, hard ticks have been reported on humans, usually belonging to the genus *Amblyomma*.

Studies indicate that these ticks are associated with rickettsial bacteria such as *R. bellii* and *R. amblyommatis*. Therefore, the objective of this study was to molecularly identify the pathogens associated with *Amblyomma* ticks collected from humans during field expeditions in forest fragments of the Gurupi Biological Reserve, Maranhão. During the field collections, ticks were observed on clothing and attached to exposed skin areas of the team members. At the end of each expedition, individual inspections were carried out to detect and collect parasitic specimens. Each collected tick was stored in microtubes containing 99% alcohol, taken to the laboratory for identification and molecular analysis. The most frequent species was *Amblyomma cajennense*, with 42 records (16 males and 26 females), followed by *Amblyomma coelebs* (22 records: 12 males and 10 females) and *Amblyomma oblongoguttatum* (21 records: 7 males and 14 females). A total of 98 ticks, 38 (39%) were positive for *Rickettsia amblyommatis,* belonging to the spotted fever group. The presence of aggressive ticks carrying pathogenic bacteria underscores the need for more detailed research that highlights the importance of environmental conservation and contributes to the development of strategies for the control and prevention of tick-borne diseases.

Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* infections in goats from northern Paraná, Brazil

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Toxoplasma gondii and *Neospora caninum* are protozoa of the phylum Apicomplexa, order Eucoccidiorida, and family Sarcocystidae, with cosmopolitan distribution. *T. gondii* has mammals and birds as intermediate hosts and stands out for its zoonotic importance, which makes it capable of causing reproductive and gestational disorders. Felines are definitive hosts, shedding oocysts in their feces. *N. caninum* mainly affects ruminants, especially cattle, with canines as definitive hosts. The objective was to determine the seroprevalence of *T. gondii* and *N. caninum*, and risk factors in goats from northern Paraná, Brazil. Blood samples were collected by venipuncture from 384 goats of different ages, sexes and breeds of eight properties in northern Paraná. An epidemiological questionnaire was administered, covering the rearing system (semi-intensive or intensive), age of animals (up to 6 months or over 6 months), type of flooring in pens (dirt or slatted), and the presence of dogs and cats. Samples were analyzed by indirect immunofluorescence assay (IFA), with cut-off titers of 1:64 for *T. gondii* and 1:50 for *N. caninum*. Of the 321 goat serum samples analyzed, 110 (34.3%) were positive for *T. gondii*, 34 (10.3%) for *N. caninum*, and eight (2.5%) for both parasites. All herds had at least one animal seropositive for *T. gondii*, and 87.5% of herds had animals seropositive for *N. caninum*. A significant association was obser-

ved between the presence of cats and seropositivity for *T. gondii* ($p = 0.0114$). Intensive management was also significantly associated with *T. gondii* infection ($p < 0.0001$). No significant associations were found for *N. caninum*. The seroprevalence of *T. gondii* and *N. caninum* in goats from northern Paraná was 34.3% and 10.3%, respectively. The presence of domestic felines and intensive management practices are factors that may influence the spread of toxoplasmosis in the region.

An *in-vitro* assessment of the bioactive potential of four Brazilian plants against the exsheathment of *Haemonchus contortus* and *Trichostrongylus colubriformis*

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Plants and their secondary metabolites are proposed as natural alternatives to control anthelmintic-resistant gastrointestinal nematodes (GIN). In this study, we evaluated the *in vitro* activity of acetonic extracts from 4 tropical plant species on the exsheathment of infective third-stage larvae (L3) of *Haemonchus contortus* and *Trichostrongylus colubriformis*, two major GIN species affecting small ruminants. The plants investigated included *Blutaparon vermiculare* (L.) Mears (Amaranthaceae), *Cecropia pachystachya* Trécul (Urticaceae), *Sida acuta* Burm. F. (Malvaceae), and *Vismia guianensis* (Aubl.) Choisy (Hypericaceae). A larval exsheathment inhibition test was conducted by exposing L3 larvae to eight concentrations of each plant extract for 60 minutes. To assess the involvement of polyphenols in the observed effects, a polyphenol-binding agent (polyvinylpyrrolidone, PVPP) was included in assays at the highest extract concentration (1200 µg/mL). The effective concentration required to inhibit 50% of larval exsheathment (EC_{50}) and its 95% confidence interval (CI_{95}) were calculated for each extract. All plant extracts had EC_{50} values < 230 µg/mL against both nematode species. The extracts of *S. acuta* and *C. pachystachya* exhibited the highest efficacy against *T. colubriformis*, with EC_{50} values of 37.48 µg/mL (CI_{95}: 32.1–42.9) and 42.85 µg/mL (CI_{95}: 36.2–49.5), respectively. A similar trend was observed for *H. contortus*, with EC_{50} values of 62.56 µg/mL (CI_{95}: 53.6–71.5) for *S. acuta* and 67.41 µg/mL (CI_{95}: 59.5–75.3) for *C. pachystachya*. The addition of PVPP markedly reduced exsheathment inhibition, suggesting that polyphenols are the active compounds responsible for the observed effects. These findings suggest that the evaluated plant extracts possess promising activity at low concentrations, supporting their potential use in the development of novel phytotherapeutic agents that could be integrated to slow the progression of anthelmintic resistance.

Prophylactic anticoccidial regimen for sheep under confinement.

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Sheep coccidiosis is caused by coccidian protozoa of genus Eimeria. It affects young animals and leads to poor development and economic losses. Its spread is facilitated in confinement due to high stocking rates and direct cycle of the parasite. The control depends on management and prophylactic use of anticoccidial drugs which regimen may vary according to the production system. In a previous stage, we evaluated different concentrations of sodium monensin which compared with a control group showed better efficacy at a dose of 28 mg/kg. The dose was then adopted for use in entire sheep herd receiving the drug continuously along with ration up to the first day in the confinement. One group consisted of 64 sheep born in the farm itself separated by sex and breed into four experimental groups (N=16). A second group of 60 crossbreds purchased for fattening was separated by sex into five groups (N=12) using the same regimen. Fecal examination was performed every 15 days for oocyst counting until the end of confinement. Oocyst data (Log X+1) were evaluated by the ANAVA and Tukey's test at 0.05%. The efficacy was determined by average reductions of oocyst counts at the end of the confinement period. The prophylactic regimen adopted was successful in controlling coccidiosis in both treated groups. Even with a high initial parasite load, the farm group had oocyst counts reduced by almost 90% up to 15 days of treatment with no effect of sex or breed. Purchased from more extensive forms, the second group of sheep began confinement with a low parasite load that remained that way until the end and so the anticoccidial protocol was equally effective. It is important to mention although the protocol used was effective in both groups of animals, negative effects on the performance of animals with high oocyst counts are not ruled out and should be evaluated in a subsequent stage.

Artificial immunization with a tick paramyosin reveals the presence of immunodominant regions

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Rhipicephalus microplus is a hematophagous ectoparasite widely distributed in tropical and subtropical regions, causing anemia, reducing milk production, and acting as a vector for pathogens. Its control is mostly based on the use of acaricides, and indiscriminate use favors the emergence of resistant populations, generating environmental impacts. In this scenario, the development of vaccines is a promising alternative. Paramyosin (PRM) is a muscle protein with host immune system evasion activities, suggested to compose vaccines against different parasites. Additionally, the host immune response against PRMs has shown the presence of immunodominance of different regions of these proteins, impacting their evaluation as potential protective antigens. Therefore, this study addressed the recognition of recombinant *R. microplus* paramyosin (BmPRM) fragments by hyperimmune sera. The coding sequences of distinct regions of BmPRM were cloned into the Pet23a plasmid, as: N-terminal fragment (N-B-

mPRM), internal fragment (Int-BmPRM) and C-terminal fragment (C-BmPRM). The proteins were produced in *Escherichia coli* and purified by affinity chromatography using a nickel-loaded column. Analyses by ELISA using sera from cattle artificially immunized with rBmPRM and rabbits immunized with salivary gland extract showed the presence of IgG predominantly recognizing Int-BmPRM and C-BmPRM. These data suggest that the presence of immunodominant regions of BmPRM when immunized in a recombinant form as well as in a native form. Therefore, further studies are needed to evaluate if common immunization protocols are enough to produce an optimized immune response able to determine the BmPRM protective potential.

Microbiota composition of *Amblyomma ovale* collected from northern coastal region of São Paulo state

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The genus *Rickettsia* (family Rickettsiaceae; order Rickettsiales) includes obligate intracellular α-proteobacteria known to cause severe diseases in their vertebrate hosts. Among these diseases, spotted fever caused by *Rickettsia parkeri* (SF-Rp) has gained attention in the Americas since 2009. In Brazil, *R. parkeri* (Atlantic Forest strain) is primarily present in Atlantic Forest regions, where it is transmitted to humans by the tick *Amblyomma ovale*, which is brought into homes by domestic dogs that have access to the forest. Ticks acquire pathogens, such as *R. parkeri,* through ingestion of blood from infected hosts. After ingestion, pathogens must resist the effects of effector molecules of the tick vector's immune system to be transmitted via the tick bite during the next blood meal. In addition to effector molecules, the microbiota also plays an important role in the establishment of pathogens in ticks. Furthermore, it is known that the microbiota of arthropods, including ticks, can interfere with their vector competence. To determine the load and composition of the bacterial microbiota of *A. ovale* and its correlation to *R. parkeri* infection, ticks were collected from dogs on the northern coastal region of São Paulo. The presence of *R. parkeri* was evaluated in the midgut, salivary glands, ovaries and testes by qPCR using specific primers for the genus *Rickettsia* and confirmed by Sanger sequencing. Female and male ticks presented similar rickettsial loads in all the organs evaluated. In addition, the bacterial loads in infected and non-infected tick organs were assessed by qPCR using primers for the V2 region of the bacterial 16S rRNA gene. Female ticks presented a higher bacterial load in the ovaries followed by the midgut and salivary glands, while male ticks presented similar bacterial loads in all organs, independently on the presence of *R. parkeri*. The bacterial community composition will be analyzed using high-throughput sequencing the V3-V4 hypervariable regions of the 16S rRNA gene.

Molecular detection of *Rickettsia* spp. in ticks from birds at the Tayrona National Natural Park, in Santa Marta, Colombia

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The Tayrona National Natural Park (PNNT) is an important reservoir of biodiversity in the Caribbean region of Colombia, hosting around 600 species of birds, including resident and

migratory birds. The high diversity in the avian population makes the park a perfect scenario to study the diversity of vectors associated to the birds, potentially involved in the transmission of zoonotic diseases. The birds are an important part of the life cycle of ticks, which are vectors of diverse pathogens, including bacteria of the genus *Rickettsia*. Some of the species of this genus represent a risk for the public health and ecotourist activities. Therefore, this study aimed to identify the species of ticks that parasitize the birds of the PNNT and evaluate the presence of *Rickettsia* in ticks. Birds were captured in the Neguanje, Arrecifes and Cañaveral sectors during October, November and December of 2024, as well as in January, February and March of 2025 using mist nest. A total of 166 birds were captured, of which 29 had ticks. A total of 38 ticks were collected, distributed in nymphs (7 individuals) and larvae (31 individuals) stages. Of these, 36 were identified as *Amblyomma longirostre*, while *Amblyomma disiimile* and *Amblyomma varium* were represented by one individual each. The presence of *Rickettsia* in the collected ticks was detected by the amplification of the Glta gene using a conventional PCR. Of the 38 ticks analyzed, 2 individuals were positive for *R. colombiensi* (ON638934.1) with a 100% of identity, and 6 individuals with a 100% of identity for *R. amblyommatis* (KF702331.1). The results of this research provide updated information about the diversity of ticks and the presence of *Rickettsia* in the PNNT, representing a valuable contribution to the epidemiological monitoring and the implementation of zoonotic disease management and prevention strategies in the protected area.

First Molecular Detection of filaroids in Mosquitoes (Diptera: Culicidae) in Uruguay

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Filaroids are parasitic worms affecting vertebrates, transmitted by arthropod vectors, and are significant pathogens of both animals and humans. They belong to the Onchocercidae family, which includes genera such as *Dirofilaria*, *Onchocerca*, *Brugia*, *Dipetalonema*, and *Acanthocheilonema*. Among these, *Dirofilaria repens* and *Dirofilaria immitis* are the best-known zoonotic agents. In Uruguay, microfilariae were first reported in 1938, but no further studies on vectors or filariasis were conducted until a 2023, when an imported case of *D. immitis* was diagnosed. This has raised concerns about the local spread of filaroids, considering the presence of competent vectors and favorable climatic conditions. The aim of this study was to determine whether an epidemiological focus of filariasis exists in Maldonado, Uruguay. The objectives included assessing the ecological conditions (temperature, humidity) around the index case area, studying the diversity of mosquito vectors, and molecularly diagnosing the presence of filaroids DNA in mosquitoes. Metjods: Four BG-SENTINEL traps were placed within a 400-meter radius of the index case's home. Mosquitoes were collected for two weeks and identified under a stereoscopic microscope. Female mosquitoes were grouped into pools, preserved, and DNA was extracted using a commercial kit. PCR was performed following Vezzani et al. (2011), and amplicons were direct sequenced. A total of 1400 female mosquitoes were collected. These were grouped into 71

pools, 70 of which were Culex pipiens and 1 of Aedes aegypti. Two Culex pipiens pools resulted positive for filaroids DNA. Sequencing revealed that both samples had a 99,51% identity with *Aproctella* sp (AN: OK358942). This study represents the first molecular survey of filaroids in mosquitoes in Uruguay. Although a no zoonotic filaroid was found, the results confirm that conditions in Uruguay are suitable for the extrinsic development of filaroid larvae in mosquitoes. These findings lay the groundwork for further investigations into filariasis in Uruguay, particularly given the proximity to endemic regions like Argentina and Brazil.

Exsheathment of *Haemonchus contortus* and *Trichostrongylus colubriformis* in ruminal medium

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The research on the larval exsheathment in gastrointestinal nematodes (GIN) affecting small ruminants is limited. There is substantial evidence regarding the exsheathment process of *Haemonchus contortus* in ruminal medium (RM). In contrast, exsheathment of *Trichostrongylus colubriformis* has been thought to occur exclusively in the abomasal environment. However, the exsheathment processes of both GIN species must be compared under the same conditions of RM. To compare the exsheathment processes of *H. contortus* and *T. colubriformis* when exposed to RM. The L3 of *H. contortus* and *T. colubriformis* were incubated in an *in vitro* RM (70:30 artificial medium and rumen fluid). The L3 were incubated at various time points: 0, 1, 3, 6, 9, and 24 h, while being maintained in anaerobic conditions at 39 °C with neutral pH. At each incubation time, Lugol's solution was applied to stop the exsheathment process. The number of L3 that had exsheathed and those that retained their sheath was recorded. The exsheathed L3 were classified into four categories: A) initial (sheath rupture), B) discrete (lacking a sheath in the anterior region), C) advanced (half-sheathed larvae), and D) completely exsheathed (without any sheath). After 24 h of incubation, the majority of *H. contortus* L3 were exsheathed (74%). Most of these larvae exhibited type D exsheathment during the incubation period. In contrast, *T. colubriformis* reached a 64% exsheathment at the 24-hour mark. Among the exsheathed L3, type B exsheathment was more common in the initial hours (55% at 1 h, 47% at 3 h, and 51% at 6 h). However, in the subsequent hours, type D exsheathment became predominant, accounting for 52% at 9 h and increasing to 85% by 24 h. The *H. contortus* L3 completes its exsheathment faster in RM. Although the *T. colubriformis* L3 exsheath gradually, it was shown that part of its exsheathment occurs in the RM.

Insights from 20,000 citizen reports: enhancing tick surveillance and disease risk assessment in Sweden through the 'Rapportera Fästing' (report tick) tool

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Climate change is reshaping the distribution of ticks in Europe, including Sweden, where the incidence of tick-borne encephalitis (TBE) is increasing. Regular tick surveillance based on field collections is not feasible since it requires considerable financial efforts. To address this gap, the Swedish Veterinary Agency (SVA) launched in 2023 *Rapportera Fästing*, a web-based tool for tick surveillance. To evaluate how citizen science can contribute to increased knowledge of tick geographical and seasonal trends, and as a tool for early detection of exotic tick species. *Rapportera Fästing* enables users to report tick encounters, upload images, and provide data on the host and the finding location. From May 2023 to May 2024, 20,974 submissions were received across all Swedish municipalities, resulting in 12,773 high-quality images assessed at SVA. *Ixodes ricinus* accounted for 99% of ticks (n=12,570), followed by endemic (*Ixodes hexagonus*, *Carios vespertilionis*, *Haemaphysalis punctata*) and exotic (*Hyalomma* spp., *Dermacentor* spp., and *Rhipicephalus sanguineus*) species. Also, the first Swedish finding of *Dermacentor marginatus* was reported through the tool. Identification accuracy was highest for *I. ricinus* and lowest for *Hyalomma* spp. *I. ricinus* was active year-round and ticks were recorded even in northern regions where they were previously considered rare. This study demonstrates that citizen science can serve as a powerful tick surveillance system and increase public awareness of ticks. The tool provided information on year-round tick activity from the region of Scania to Uppland and led to the first detection of a new tick species in Sweden (*D. marginatus*). Ongoing improvements, including AI-based tick recognition, will enhance the tool's accuracy and scalability, making it an essential component of One Health preparedness strategies.

Cutaneous leishmaniasis shows different lipid composition during the life cycle compared to mucocutaneous and visceral leishmaniasis.

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Leishmania is a zoonotic parasite transmitted by sandfly bites, able of infecting mammalian hosts. It presents different clinical forms depending on the species, but the underlying biological mechanism is not yet understood. Lipids, in their synthesis and use, play a key role in modulating the host response. We compared lipid composition and variations in 3 species, *Leishmania infantum* (visceral Leishmaniasis), *Leishmania guyanensis* (cutaneous leishmaniasis) and *Leishmania mexicana* (muco-cutaneous leishmaniasis), during biological cycle. Parasite stages (procyclic promastigotes PP, metacyclic promastigotes PM and amastigotes Am) were cultured *in vitro*, with stage changes by pH and temperature variations. In total, 198 lipids were compared between the 3 stages and the 3 species by mass spectrometry. Multiple comparisons were performed

by the Kruskal-Wallis test followed by Dunn's post hoc test with Benferonni correction. Total fatty acids (FA), notably precursors of proinflammatory lipid metabolites, and triglycerides was increased in *L. mexicana* during the PP and PM stages compared to *L. infantum* and *L. guyanensis.* In contrast, free FA concentrations were not different between species but their concentrations were decreased in the Am stage compared with PP/PM, especially for precursors of anti-inflammatory lipids. In Am, total FA, phosphatidylcholine, phospahtidylethanolamine and phosphatidylinositol were increased in *L. infantum* and *L. guyanensis* compared to *L. mexicana*. The Am stage of *L. mexicana* was characterized by higher concentrations of ceramides and lower concentrations of sphingomyelins, compared to the two others species. Lipid composition during the *Leishmania* spp. cycle is species- and stage-dependent, particularly during the pathogenic stage (Am). These preliminary results show the interest of understanding the role of lipids in the physiopathology of the parasite and maintenance in reservoir hosts.

Molecular detection of Babesia/Theileria spp in ticks collected from migratory birds captured at Faunistic Observatory of the Asinara island, Sardinia, italy.

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The role of migratory birds as hosts for vectors and pathogenic microorganisms has gained increasing attention. Birds can cross geographical barriers and thus play a significant role in the dissemination of bacteria, viruses, and protozoa. Among these, hemoprotozoan parasites of the genera *Babesia* and *Theileria* are of particular concern due to their global impact on animal and human health. The aim of this study was to expand current knowledge on the potential role of migratory birds in the spread of these protozoan parasites and to assess their prevalence in ticks infesting birds during spring and autumn migrations in Sardinia, a region where such data are currently scarce. Ticks were collected from birds captured during two periods: 15 October–30 November and 15 April–31 May 2021 at the Tumbarino Bird Observatory, located on Asinara Island in northwestern Sardinia (N 41°03; E 8°16'). Birds were identified to species level and ticks were morphologically classified according to developmental stage, sex, and engorgment status. Ticks were further identified to genus and species by PCR and sequencing targeting the 16S rRNA gene. Each tick was individually homogenized and DNA was extracted. Specimens were tested for *Babesia/Theileria* infection by real- timePCR, PCR standard and sequencing with primers targeting the18S rRNA gene. A total of 188 birds including 145 European robin (*Erithacus rubecula*) (77%), 29 blackbirds (*Turdus merula*) (15%), 13 song thrush (*Turdus philomelos*) (7%) and 1 (*Lullula arborea*) (0.5%) were captured during the period 2020-2021. A total of 332 ticks were collected and identified as *Ixodes frontalis* (n=43; 2 adults, 29 larvae, and 12 nymphs), *I. inopinatus* (n=2; 2 larvae), *I. ricinus* (n=291, 6 adults, 179 larvae and 106 nymphs), and *I. ventalloi* (n=5; 4 larvae and 1 nymph) species. Fourteen ticks (5%) collected from seven robins, three blackbirds and one song thrush tested positive for *Babesia/Theileria* spp. via real-time PCR and conventional PCR. After sequencing, two larvae of *I. ricinus* collected from one robin and

one blackbird, tested positive for *B. venatorum*. One *I. ricinus* nymph tested positive for *B. capreoli,* while 4 larvae of *I.* ricinus. One *I. ventalloi* were positive for *Theileria ovis* while three larvae (*I. frontalis* showed 100% identity with *Th. equi* while *Th. orientalis* was detected in 2 larvae of *I. ricinus* and 2 of *I. frontalis* ticks. This study reports the presence of several *Ixodes* tick species and the detection of the emerging zoonotic parasite *Babesia venatorum* and the roe deer pathogen *Babesia capreoli* whose presence had not previously been reported on the island. The presence of *Theileria ovis*, *T. equi* and *T. orientalis* was also confirmed. These *Theileria* species had previously been identified in ticks collected from domestic and wild animals, as well as in the blood of both symptomatic and asymptomatic mammals (including horses, sheep, cattle and domestic pigs) in various areas of northwestern Sardinia. In conclusion, the findings of this study underscore the importance of migratory birds in the geographic spread of infected ticks and vector-borne pathogens. These results are crucial for predicting and managing the potential emergence of tick-borne diseases in the region.

Prevalence of endoparasites in dogs and cats in Ilhéus, Bahia

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The growing coexistence between humans and companion animals intensifies the challenges related to zoonoses, which include inadequate diagnosis and treatment, underreporting of cases, lack of knowledge about preventive measures, and limited access to veterinary services. The scarcity of data on the parasites circulating in these populations compromises the planning of effective control and prevention strategies. This study aimed to identify and determine the frequency of endoparasites in dogs and cats treated at the Veterinary Hospital of the Universidade Estadual de Santa Cruz (UESC). Inclusion criteria required that animals had not received anthelmintics in the last two months, with a maximum of two animals per household. From August 2023 to August 2024, samples were collected from 240 animals (114 dogs and 126 cats), and analyzed using the Mini-FLOTAC and Fill-FLOTAC coproparasitological techniques. Among the dogs, 44.74% (51/114) were males and 55.26% (63/114) were females; *Ancylostoma* spp. was the most prevalent parasite (38.89%, 42/108), followed by *Trichuris vulpis* (8.33%, 9/108) and *Strongyloides* sp. (0.92%, 1/108). Among the felines, 47.62% (60/126) were males and 52.38% (66/126) were females, with 19.05% (20/105) testing positive for *Ancylostoma* spp. and 0.95% (1/105) for *Dipylidium caninum*. These findings underscore the importance of regular parasitic control and health education programs, which are essential to reduce zoonotic exposure and promote the well-being of both animals and humans.

Prevalence of ectoparasites in dogs and cats in Ilhéus, Bahia

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The number of dogs and cats kept as companion animals has grown significantly, positivelyinfluencing physiological, therapeutic, and psychosocial aspects. However, domestic animals can serve as reservoirs of ectoparasites, which are important vectors of zoonotic diseases. This study aimed to evaluate the frequency of ectoparasites in dogs and cats treated at the Veterinary Hospital of the Universidade Estadual de Santa Cruz (UESC), in Ilhéus, Bahia. A total of 240 animals (114 dogs and 126 cats) were examined from August 2023 to August 2024. As an inclusion criterion, the animals must not have received ectoparasiticide treatment in the 30 days prior to the examination. The methodology included a detailed body inspection with a finetooth comb, skin scraping, the Scotch Tape technique, and an auricular inspection using swabs. Among the evaluated dogs, *Ctenocephalides felis* infestation was detected in 35.09% (40/114), *Rhipicephalus sanguineus* in 19.30% (22/114), *Trichodectes canis* in 4.39% (5/114), and *Otodectes cynotis* in 0.88% (1/114). In felines, there was infestation by *Lynxacarus radovskyi* in 30.16% (38/126), *Ctenocephalides felis* in 11.90% (15/126), *Rhipicephalus sanguineus* in 0.79% (1/126), and *Felicola subrostratus* in 0.79% (1/126). These results highlight the importance of continuous ectoparasite prevention and control strategies to safeguard animal health and reduce the risks of zoonoses associated with pet ownership.

Emerging tick-borne bovine theileriosis in Bangladesh and genetic diversity of *Theileria orientalis*

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Theileria orientalis, an obligate intracellular protozoan, poses a significant economic threat to the cattle industry and is widespread across Bangladesh. This study aimed to determine the nationwide prevalence, genetic diversity, and evolutionary dynamics of *T. orientalis* by analyzing bovine blood samples. The MPSP gene was analyzed through PCR for genetic profiling, haplotype network analysis and evaluation of dN/dS substitution ratios using bioinformatics tools. The MPSP-PCR detected *T. orientalis* in 63.75% (n = 800) of samples across eight divisions of Bangladesh. Sequence analysis identified three distinct clades corresponding to genotypes 3, 5, and 7, with genotype 3 reported

for the first time in Bangladesh. Among 68 sequences analyzed, high haplotype and nucleotide diversity were observed, with genotype 5 showing the greatest variability. Significant genetic differentiation suggests limited gene flow, distinct evolutionary paths, and complex expansion history. Haplotype network analysis revealed 58 haplotypes across 11 genotypes. The dN/dS ratio indicated purifying selection in most sequences, while some from Chattogram, Rangpur, and Sylhet showed signs of positive selection. These findings underscore the extensive genetic diversity in the cattle population and suggest that evolutionary pressures may contribute to the emergence of highly pathogenic *T. orientalis* lineages. This study presents the first nationwide analysis of *T. orientalis* in Bangladesh, highlighting its high prevalence and genetic diversity. The identification of genotype 3, restricted gene flow and distinct evolutionary patterns suggest complex dynamics. These findings emphasize the need for continuous surveillance and genetic monitoring to mitigate the potential economic impact of *T. orientalis* on the cattle industry in Bangladesh.

Isolation of nanoparticles from histotropic stages of *Oesophagostomum dentatum* – old school with new tools

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The parasitic nematode *Oesophagostomum dentatum*, a member of the order Strongylida, commonly infects pigs worldwide and is an established model to investigate the secretome of histotropic nematode stages. With the advent of advanced technologies for the isolation of defined nanoparticles, a deeper understanding of the production and composition of secretome products becomes possible. In this study we determined the optimal incubation conditions for the secretion of nanoparticles (NP) of histotropic stages *in vitro*. Based on the established cultivation system for *O. dentatum*, NP secretion of cultivated L3 and L4 was analyzed weekly for 21 days. At day 14 of cultivation, when the L4 development reached a plateau with about 30% developed stages the secretion of NPs was also highest (>2x1011 particles/ml). Concentration and size of NPs were determined in relation to the density of larvae/size of culture flasks, duration of incubation and incubation media. NPs were isolated by ultracentrifugation, and concentration and size distribution were analyzed and compared by Nanotracking (ZetaView). The pre-cultivated L3/L4 shed NP mainly of 50-250 nm in size with a consistent peak at 150 nm. Highest NP yields were obtained after the maximum incubation time (18 h) in RPMI medium (compared to LB and DMEM/F12) with 1x105 freshly exsheathed L3 in a 182.5 cm2 tissue culture flask. Under these conditions the AUC for 150 nm particles was 4.1x108 for the first day of cultivation, increased to 7.4x109 on day 14, and decreased slightly to 6,2x109 by day 21. Separate analysis of L3 and L4 after 14 days of cultivation showed that NP were almost exclusively (>90%) shed by L4 stages. These findings supporting previous analyses of *O. dentatum* excretory-secretory products with more modern analytical methods. In combination with *in vitro* cultivation techniques, NP of otherwise poorly accessible stages, the histotropic larvae which reside in closes contact with the host, can be analyzed in detail.

Morphological detection of parasites in soil and animal faeces in the University of Ibadan, Oyo State, Nigeria

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Parasitic infections from fecal and soil-borne organisms pose significant zoonotic risks, yet limited studies have assessed their prevalence in animal feces and the environment. This study investigated the occurrence of gastrointestinal and ectoparasites in animal feces and soil at the University of Ibadan, Nigeria, to evaluate their zoonotic transmission potential and public health implications. A total of 191 fecal samples from ruminants, pigs, dogs, lions, and chickens, alongside 130 soil samples, were analyzed using flotation, sedimentation, and zinc sulfate concentration techniques. The spatial distribution of parasites was also geo-profiled. Gastrointestinal parasites were detected in 45.55% of fecal samples, with nematodes (36.65%), trematodes (3.66%), cestodes (2.09%), and protozoans (11.52%). Notable parasites included *Haemonchus contortus* in goats (38.09%), *Fasciola gigantica* in cattle (7.02%), *Oesophagostomum dentatum* in pigs (39.29%), *Toxocara canis* in dogs (17.39%), *Echinococcus* species in lions, and *Eimeria* species in poultry (20%). Soil samples exhibited a higher parasite prevalence (76.38%), dominated by nematodes (72.31%), particularly Ascarids (67.69%), Strongyles (22.3%), and *Trichuris* species (3.08%). Ectoparasites such as mites (14.62%) and lice (4.62%) further indicated environmental contamination. The high prevalence of gastrointestinal parasites in both fecal and soil samples highlights significant zoonotic risks, especially in areas with frequent human-animal interactions. Key pathogens identified include *Toxocara canis*, *Balantidium coli*, *Dipylidium caninum*, *Ancylostoma caninum, Haemonchus contortus, Fasciola gigantica, Echinococcus* species, *Moniezia* species, *Schistosoma* species, and *Ascaris suum*, underscoring the potential for disease transmission. These findings emphasize the need for improved waste management, deworming programs, and public health education to mitigate zoonotic risks and safeguard both human and animal health.

Q fever in Brazil: evaluation of the exposure of capybaras and ticks to *Coxiella burnetii* in anthropogenic and natural areas in Brazil

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Q fever is a zoonosis caused by the bacterium *Coxiella burnetii*, of significant public health importance, endemic and widely distributed in many parts of the world, and remains neglected in Brazil. Little is known about the participation of wild animals in the maintenance of the enzootic cycle, especially the capybara (*Hydrochoerus hydrochaeris*), whose remarkable expansion through anthropized areas associated with high tick infestations, can lead to contact between pathogenic bacteria and humans. Like this rodent, the role of ticks remains poorly understood: it is believed that they act

as maintainers and dispersers of the bacterium in the environment, but with little significant vector action. The objective of this study was to evaluate the natural infection by *C. burnetii* in capybaras collected in anthropized and natural areas and in sympatric ticks (*Amblyomma sculptum* and *A. dubitatum*). As a methodology, the serum of the capybaras was submitted to IFA directed to phase I antigens – chronic (At12) and phase II – acute (Nine Mile), the DNA, after extraction by commercial kit, was submitted to qPCR directed to the IS1111 gene, as was the DNA of the ticks. To analyze the associations, Pearson's chi-square test and Fisher's exact test were used, both in the R software. In all, 392 capybaras were tested in serology, 160 (40.8%) positive for phase I, 40 (10.2%) for phase II and 29 (7.4%) for both, with a higher tendency to occur in anthropized areas ($p = 0.0009/<0.05$) and adults in phase I ($p = 0.001/<0.05$). None of the 328 capybaras sampled were positive in qPCR, as well as none of the 362 ticks. With these results, it is concluded that capybaras are intensely exposed to this bacterium, with potential environmental contamination and risk to public health, however, their ticks do not seem to act as reservoirs, nor as vectors.

Morphological identification and genetic diversities of ticks infesting sheep in Nigeria

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The arthropod ticks, are major constraints to livestock production globally. Domestic livestock, pets and wildlife are all affected and influenced by the spread of ticks through transhumance and trading activities within and between countries. The risk of transmitting tick-borne pathogens by ticks to both animals and humans are a constant worry in developing and developed nations of the world, hence, the importance of constant study, monitoring and surveillance of these economic pests. This study investigated the ticks infesting sheep and their genetic diversities in Ogun state, Nigeria. A total of 295 ticks were collected from 101 sheep. Morphologic identification of the ticks was carried out by electron microscopy, DNA extracted from the ticks and the partial region of 16SrRNA genes was amplified and sequences to identify the *Amblyoma* spp., *Rhipicephalus* spp. *and Hyalomma* spp. but Cox 1 genes sequenced to identified *Hyalomma detritum.* The obtained sequences were subjected to phylogenetic analysis. Microscopy detected three different genera of ticks including *Hyalomma* spp., *Amblyomma* spp. and *Rhipicephalus* spp. with frequency distribution of 37.3%, 41.7% and 21.0%, respectively. Molecular analysis of 16SrRNA and Cox 1 genes confirmed *Hyalomma impeltatum, H. detritum* and *Amblyomma vareigatum.* Phylogenetic analysis shows that *Hyalomma impeltatum* and *Amblyomma vareigatum* had 97.28 to 99.72% homologies with those sequences from Kenya, Algeria, Cameroon and Nigeria but *H. detritum* had 99.70 to 99.85% with those South Africa, Cameroon and Saudi Arabia. The *H. detritum* form a paraphyletic relationship with isolates from Kenya but a polyphyletic relationship with isolates from Australia, Egypt, Israel and Saudi Arabia. The molecular detection and characterization of *Hyalomma impeltatum* and *Hyalomma detritum* on sheep is the

first in Nigeria and could serve as a potential threat to tick borne diseases such as Babesiosis and Theileriosis in ruminants. These findings might be useful in developing methods for successful tick and tick-borne diseases control in Nigeria.

Insights into blood source preferences and pathogen load in *Phlebotomus neglectus* and *Ph. perfiliewi* (Diptera: Psychodidae) from Romania

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The host-feeding preferences of sand fly species on vertebrate hosts serve as epidemiological indicators of potential emerging disease foci affecting humans and animals. In this study, female sand flies of *Phlebotomus perfiliewi* and *Ph. neglectus*, collected in Romania were analyzed using molecular techniques. The presence of *Leishmania infantum* was assessed using a real-time PCR assay, while RNA of Toscana virus (TOSV) and Sandfly fever Sicilian virus (SFSV), as well as blood source preferences, were analyzed through various PCR assays. All tested sand flies were negative for pathogen presence. Regarding bloodmeal sources, a total of 52 out of 74 sequences (70.3%) were successfully identified, with 32/52 (61.5%) belonging to *Ph. neglectus* and 20/52 (38.5%) to *Ph. Perfiliewi.* In *Ph. neglectus*, the bloodmeal sources were distributed as follows: *Apodemus agrarius* (1/32, 3.1%), *Cervus elaphus* (7/32, 21.9%), *Homo sapiens* (7/32, 21.9%), *Lepus europaeus* (3/32, 9.4%), *Bos taurus* (11/32, 34.4%), *Capreolus capreolus* (2/32, 6.2%), and *Sus scrofa* (1/32, 3.1%). For *Ph. perfiliewi*, the identified bloodmeal sources included *Bos taurus* (1/20, 5.0%), *Ovis aries* (1/20, 5.0%), *Gallus gallus* (1/20, 5.0%), *Equus caballus* (16/20, 80.0%), and *Homo sapiens* (1/20, 5.0%). The diverse range of domestic and wild vertebrate hosts suggests an opportunistic feeding behavior in these sand fly species, potentially increasing the risk of pathogen transmission relevant to public health. While the local sand fly populations examined in this study did not carry any detected pathogens, these findings cannot be generalized to the entire Romanian territory or to all sand fly species present in the country. Further research is needed to gain a more comprehensive understanding of the feeding behavior and pathogen load of each sand fly species, which is crucial for assessing the potential transmission risk of diseases affecting both humans and animals.

Updates on Sandflies (Diptera: Psychodidae) in Romania

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The present study aims to compare the historical composition and geographical distribution of sand flies in Romania from 1910-1971 with current data collected during the VectorNet Project between 2013 and 2021. Historically, eight sand fly species have been recorded in Romania: *Sergentomyia minuta, Phlebotomus papatasi, Ph. alexandri, Ph. sergenti*, *Ph. longiductus*, *Ph. balcanicus, Ph. perfiliewi,* and *Ph. neglectus* (1). Between 2013 and 2021, sand flies were collected using CDC light traps, mouth aspirators, and sticky traps at 132 locations, including indoor areas, animal shelters, and natural reserves. Monthly collections were focused on two locations: Fundătura village (Vaslui County) and Canaraua Fetii natural reserve (Constanţa County), from May to October 2017 and 2020, respectively. Data on animals, shelters, and climate were recorded and analyzed, and species were identified using morphological keys, genetic tools, and MALDI-TOF protein profiling (2-5). Sand flies were found in 6% (8/132) of sampled locations, including sites in Mehedinţi, Vaslui, Giurgiu, and Constanţa counties. Of the 1868 collected sand flies, 89.5% were *Phlebotomus neglectus*, 10.15% *Ph. perfiliewi*, and smaller percentages were *Ph. papatasi*, *Ph. balcanicus*, *Ph. sergenti* sensu lato, *Se. minuta*, and the newly identified *Ph. simonahalepae*. *Phlebotomus perfiliewi* showed a single abundance peak in early August, while *Ph. neglectus* peaked in mid-July in a specific cave habitat. While six previously recorded species were confirmed, their current distribution differs from historical data. However, this seasonality data cannot be generalized across Romania. The diversity of sand flies in Mediterranean-influenced areas, poses a risk for vector-borne disease reemergence, highlighting the need for ongoing surveillance to monitor their dynamics and distribution.

Severe *Ctenocephalides felis* infestation in a calf: clinical and epidemiological findings

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Ctenocephalides felis is an ectoparasite with worldwide distribution that compromises animal health. Although it is prevalent in dogs and cats, it can also parasitize farm animals. This study aimed to report a case of *C. felis* infestation in a calf, along with its clinical complications and epidemiological aspects. A three-month-old Girolando calf (65 kg), kept in a 1.5-hectare sandy paddock, presented with apathy, fatigue, nasal discharge, respiratory distress, diarrhea, and anorexia. Clinical examination at the UFMS veterinary hospital revealed pale mucous membranes, 8% dehydration, and a severe flea infestation (thorax, abdomen,

limbs, and tail). Hematological analysis showed severe anemia (erythrocytes 2.66×10⁶/µL, Hb 2.9 g/dL, PCV 7.7%), lymphopenia (1,984/mm³), and thrombocytosis (883,000/mm³). Biochemical tests indicated hypoalbuminemia (2.2 g/dL) and hypoproteinemia (3.9 g/dL). No hemoparasites or gastrointestinal parasites were detected. Fleas were morphologically identified as *C. felis*. Treatment included blood transfusions, fluid therapy with Ringer's lactate, and fluralaner 5% (1 mL/20 kg). Anemia improved after transfusion (erythrocytes 5.40×10⁶/µL, Hb 6.7 g/dL, PCV 18.8%), and fleas were no longer observed after 48 hours. Although uncommon in production animals, *C. felis* infestation can become severe when specific risk factors are present. In this case, the calf was housed in a sandy area and in close proximity to cats, increasing the risk of infestation.

The tick gut immune response to bacterial challenge

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The importance of microbiota in the context of tick biology and vector competence has recently gained broader research attention, but the complex interactions between the resident bacteria, pathogens, and the host remain obscure. This study aimed to investigate how tick gut immune system responds to different bacteria and to identify the key immunological components that shape the gut microbiota. *Ixodes ricinus* females were experimentally inoculated either with *Staphylococcus epidermidis*, *Pseudomonas putida*, or *Borrelia afzelii*. The expression profiles of genes representing key factors of the JAK/STAT, IMD, and Toll immune pathways, free radical defense and antimicrobial peptides were analyzed using RT-qPCR. None of the immune-related genes showed significant changes in expression following tick feeding. However, ticks inoculated with *P. putida* displayed a notable increase in the expression of defensins. Infected ticks exhibited a reduced expression of the nitric oxide synthase gene, suggesting that these bacteria may suppress nitrosative stress in the tick gut to enhance their survival. While ticks successfully cleared *S. epidermidis* from their guts within 8 hours post-feeding, the colonization with *P. putida* and *B. afzelii* remained stable. *In vitro* testing of synthetic defensin revealed its inhibitory effect against *S. epidermidis*, but not against *P. putida* and *B. afzelii*. Interestingly, when exposed to lower concentrations of the synthetic peptide, *Borrelia* spirochetes exhibited changes in their morphology and viability. Our research sheds light on the intricate immune processes governing tick-bacteria interactions, uncovering the strategies ticks employ to manage infections from environmental bacteria. Future studies should investigate whether the preactivation of the gut immune system by environmentally acquired bacteria modulates the gut permissiveness to *Borrelia* infection.

Does the parasite load in different organs correlate with parasitism in sandflies submitted to xenodiagnosis in dogs with *Leishmania infantum*?

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Leishmaniasis is a group of diseases that affects millions of individuals worldwide. They are caused by protozoa of the genus Leishmania, transmitted mainly by the bite of insect vectors, the phlebotomine sandflies. Canine Visceral Leishmaniasis (CVL) can present a wide variety of clinical manifestations. Studies have shown that the clinical progression of CVL is associated with the parasite load in several organs. However, there is still a gap in the scientific literature regarding the correlation of parasitism in dogs that could influence the transmission of the vector *Lutzomyia longipalpis* to females. In this context, the study proposed to evaluate the correlation between the parasite load of Leishmania infantum in different organs of infected dogs and the parasite load in sandflies after feeding on these dogs. The objective was to identify a biomarker that correlates with the infectivity of the vector after feeding on infected dogs and that can be used to identify dogs that, even after undergoing treatment, may continue to be reservoirs of *L. infantum*. Forty-seven dogs naturally infected with L. infantum were evaluated, confirmed through the rapid DPP test and the confirmatory ELISA. The dogs underwent xenodiagnosis and bone marrow collection, submitted to euthanasia, with subsequent collection of organ samples (lymph node, spleen, and ear skin). After xenodiagnosis, qPCR was done in the sandflies to quantify the parasite load of L. infantum, as well as in the organ samples. Correlation analyses of the parasite load in the organs with the parasite load in the sandflies were performed. A positive correlation was identified between the parasite load observed in the sandflies and the parasite load in the skin; lymph node; and spleen. The highest correlation was found with the bone marrow (p: 0.026; r: 0.5092, followed by the skin (p: 0.0360; r: 0.4597) and then the lymph node (p: 0.0417; r: 0.4376). There was no correlation with the parasite load in the spleen. Thus, it suggests that dogs with a higher parasite load in organs such as bone marrow, skin, and lymph nodes may serve as biomarkers for infection by sand flies when feeding blood on naturally infected dogs. As perspective, we are determining the minimum amount of parasite load in these organs that is capable of increasing the risk of infection in sandflies.

Hard Ticks of Camels and Seroprevalence of anti-*Coxiella burnetii* Antibodies in Camels (*Camelus Dromedarius*) in Maiduguri, Borno State, Nigeria

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Coxiella burnetii is a neglected but emerging or re-emerging Gram-negative bacterium responsible for Coxiellosis, which is a cosmopolitan zoonosis with a global distribution. Ticks are haematophagous arthropods and have been implicated as a probable vector and may play an important role in the occurrence and transmission of this microorganism to several vertebrate hosts including livestock. Therefore, the aim of this study was to determine the species diversity of ticks infesting camel and the seroprevalence of C. *burnetii* in one-humped camels (*Camelus dromedarius*) in Maiduguri, Northeastern Nigeria. Blood samples (n=182) and adult ticks (n = 1353) were collected from one-humped camels (*Camelus dromedarius*) of both sexes in Maiduguri Abattoir in North-east Nigeria. Blood samples were tested for anti-*C. burnetti* antibodies using Commercial Camel *Coxiella burnetii* lgG (Q Fever lgG) ELISA Kit (SunLong, China) while ticks were identified using stereomicroscope. Findings from this study indicates that all ticks were hard ticks belonging to three genera *Hyalomma, Amblyomma* and *Rhipicephalus*. *Hyalomma dromedarii* was the most frequently detected tick species (n = 559; 41.31%) while *Amblyomma variegatum* (n = 10; 0.74%) and *Rhipicephalus evertsi evertsi* (n = 3; 0.22%) were less detected. Other tick species included *H. impeltatum* (n = 318; 23.50%), *H. rufipes* (n = 237; 17.52%), *H. truncatum* (n = 213; 15.74%), *H. impressum* (n = 10; 0.74%) and *H. Albiparmatum* (n=3; 0.22%). Overall, of the 300 camels examined for tick infestation, exactly 272 were infested with a prevalence of 90.67%. Female camel was more infested (n=165; 60.66%) than male (n=107; 39.34%). Based on age, young camels (n=242; 88.97%) were more infested than adult (n=30; 11.03%) while Camels with very good Body Condition Score (n=15; 5.51%) had the least infestation rate compared to those with poor BCS (n=103; 37.87%). Furthermore, we recorded an overall sero-prevalence of 3.29%. Highest seroprevalence was recorded in female camels (n=5; 4.20%) than male camel (n=1; 1.6%) and older camel (n=6; 5.2%) were more exposed while camels with moderate BCS (n=4; 6.06%) had a higher seroprevalence compared with those with very poor (n=1; 2.8%) and good BCS (n=1; 1.3%). In conclusion, this study reports high diversity of hard ticks infesting camels and low seroprevalence of anti *C. burnetii* antibodies in the study area.

Effect of three infective doses on the intestinal persistence of adults of *Taenia hydatigena* in dogs

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Much of the parasitological data on the species of the genus *Taenia* have been extrapolated from *T. solium* and *T. saginata*, which are the most studied due to their ability to infect humans. However, it is possible that *Taenia* species that develop in other hosts exhibit different biological behavior. To compare the effect of three infective doses of metacestodes on some parasitological parameters of *T. hydatigena* in dogs. Male dogs were distributed into 3 groups (n = 3), dogs from groups 1, 2 and 3 were inoculated with 3, 6 and 12 metacestodes of *T. hydatigena* respectively. Eggs or proglo-

ttids were searched for in feces weekly. Each dog was euthanized one month after the onset of egg or proglottid shedding in the feces. All developed adult worms (AW) were recovered and measured, the number of uterine branches was counted, and the scolexes were observed by scanning electron microscopy. All dogs (9/9) eliminated eggs and/or proglottids in feces. At necropsy, AW were found in one dog from group 1, one dog from group 2 and in all dogs from group 3, therefore, the persistence of intestinal AW was higher ($p<0.05$) in group 3 compared to the other groups. The prepatent period was 32—88 d, and a greater ($p<0.05$) number of prepatent days was observed in dogs infected with 12 metacestodes (74 ±12 d) compared to those infected with 3 metacestodes (51 ±1 d). The size of AW was not different between groups. The morphology of all recovered AW was similar to that reported for *T. hydatigena*. Dogs inoculated with the highest infective dose of *T. hydatigena* showed greater persistence of the parasite, a higher number of recovered AW and a longer prepatent period, the above suggests that —unlike what occurs with taenias in humans—, the presence of several adults of *T. hydatigena* more successfully maintains intestinal infection in dogs.

Canine somatotropin stimulates the growth and motility of *Toxocara canis*

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Toxocara canis is one of the most important intestinal nematodes in dogs. The adults of this parasite develop mainly in the small intestine of puppies (0-3 months). One of the most important physiological events in puppies, is their high levels of somatotropin (growth hormone), which coincides with their high capacity to develop adult worms. Currently, there are no studies demonstrating that *T. canis* larvae are able to recognize and utilize canine somatotropin to their advantage. To evaluate the in vitro effect of somatotropin on the length, diameter, and motility of *T. canis* larvae, and the existence of a potential receptor for the hormone. *T. canis* larvae were incubated with different concentrations of canine somatotropin (0, 0.5, 2, 4, 8, 32, 128 ng/mL), the medium was changed with fresh hormone every third day. Length, diameter and motility were measured on days 0, 5, 10 and 15 of exposure to the hormone. On the other hand, a fragment of the gene encoding the somatotropin receptor was amplified by PCR in cells of adult worms and larvae of *T. canis*, using primer sequences based on a conserved region of the somatotropin receptor gene identified in different mammals and available in GenBank. The resulting amplicon was sequenced. Overall, larvae stimulated for 5 and 10 days with 2, 8, and 32 ng/mL of somatotropin showed greater growth in length and diameter ($p<0.05$) compared to unstimulated larvae. Larvae stimulated with 2 and 8 ng/Ml for 10 and 15 days showed greater ($p<0.05$) motility than unstimulated larvae. The amplicon obtained in the PCR was 649 bp and had 99.67% similarity with the canine somatotropin receptor gene reported in GenBank. *T. canis* larvae recognized and responded to canine somatotropin and presented a 649-bp fragment highly similar to the hormone receptor gene.

Diagnostic methods and innovation

Recent advances and potential applications of electropenetrography to study the masked feeding behaviors of mosquitoes, ticks, and other medically important arthropod vectors

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Electropenetrography (EPG) is a non-invasive technique for quantifying intra-tissue host--vector-pathogen interactions and is commonly used to study plant-pathogen transmission, host plant resistance, and pesticide modes of action. Investigate the potential application of EPG to study the masked feeding behaviors of medically important blood-feeding arthropod vectors. Recently, EPG procedures were developed to characterize and quantify the unseen mouthpart movements and behaviors that mosquitoes, biting midges, and ticks perform inside host tissues during blood-feeding. Investigations in *Aedes aegypti, Culex tarsalis*, and *Culicoides sonorensis* revealed differences in the duration, count, and probability of transitioning between waveforms corresponding to behaviors such as pre-probing, penetration of the skin, search for a blood vessel/ingestion site, ingestion, putative resting, and withdrawal of the mouthparts from the skin. In addition, infection of *Ae. aegypti* or a murine host with Dengue virus significantly altered the duration, count, and probability of transitioning between many of these behaviors, demonstrating that EPG can be used to quantify the effects of pathogens on cryptic blood-feeding behaviors. Comparison of the early stages of slow--phase feeding in *Dermacentor variabilis* and *Amlyomma americanum* additionally revealed variation in putative ingestion, salivation, and resting behaviors between species. Together, these studies illustrate the value of EPG for investigating plasticity in probing and ingestion behaviors of blood-feeding arthropods. EPG can be used to quantitatively interrogate the effects of insecticides/acaricides, antifeedants, repellents, pathogens, and susceptible vs. resistant animals on specific probing behaviors responsible for tissue damage and pathogen transmission, making EPG a promising new tool with wide applications in vector and vector-borne disease research.

Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI--TOF MS) as a diagnostic tool for the rapid identification of arthropod species

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Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) is an established technology used in microbiology diagnostic labs for routine bacterial identification. The technique is not yet fully explored for parasites. Morphological identification of parasites requires high degree of expertise and is difficult to standardize. It is a timeconsuming

process, and misidentification is not uncommon due to outdated identification keys or closely related species that are hard to distinguish. We are exploring the use of MALDI-TOF MS as a rapid, scalable, and reliable method of parasite identification for routine diagnostic purposes. Known tick species (*Ixodes scapularis* and *Dermacentor variabilis*) were acquired from a breeding facility at Oklahoma State University and used for optimization of the extraction protocols and for protein spectra generation. Molecular testing of the used samples is undergoing. A total of 301 field tick samples (*Dermacentor* and *Ixodes* species) were collected as part of a bigger study and identified morphologically. These samples will be tested molecularly and with the MALDI TOF MS to assess the MALDI TOF MS as a method of identification of ticks. Protein spectra were successfully generated from ticks acquired from the breeding station and used to build MALDI-TOF MS spectra library. We are currently optimizing the protocol and generating MALDI TOF spectra from various specimens with both morphological and DNA barcoding molecular vouchers. The generated library was also challenged with spectra from other ectoparasites. MALDI TOF MS is a promising technology that has the potential to be adapted for use in parasitology diagnostic labs for parasite identification.

Haemonchus contortus isolates assessed for anthelmintic resistance using RESISTA-test©

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RESISTA-Test© is a larval development test to detect anthelmintic resistance for thiabendazole (TBZ), ivermectin (IVM), levamisole (LEV), and monepantel (MPT). Two isolates of *Haemonchus contortus* were assessed to confirm the anthelmintic status (*Echevarria1991 - HcEc91*, susceptible and *Botucatu - HcBot*, resistant) were kept in two donor lambs each. Eight lambs were artificially infected with each isolate and kept in separate paddocks (field lambs) for 160 days. To calculate the resistance factor (RF), faecal samples of the donor and field lambs of each isolate was examined using RESISTA-Test©. The results were analysed using logit dose-response by the Probit model. The RF was calculated as the LC50 values of the studied isolate divided by the respective susceptible isolate values. For the *HcEc91* isolate in the field lambs, the RF values were TIA = 0.7, IVM = 0, LEV = 1.1 and MPT = 0.9. The RF values for the *HcBot* isolate in the donor lambs were TIA = 147, IVM = 176, LEV = 931, and MPT = 53 and for the field lambs the RF values were TIA = 141, IVM = 196, LEV = 822, and MPT = 34. The results confirmed with accuracy the anthelmintic resistance status of the two isolates since the RF values for the susceptible isolate were below 3 and the contrary was observed for the resistant isolate, with RF values above 3. Moreover, RF values for the donor and field lambs infected with *HcBot* isolate were quite similar for all chemical groups demonstrating that both isolates can be kept successfully in the field as long as lambs graze different paddocks. In conclusion, RESISTA-Test© is applicable in laboratory routines to ascertain the anthelmintic

resistance status of *H. contortus* without necessitating superfluous anthelmintic treatments such as those employed in faecal egg count reduction test (FECRT). This tool would be utilised in farm's parasite management initiatives, facilitating a more precise, and sustainable control of gastrointestinal nematodes.

Evaluation of a diagnostic method for *Theileria haneyi* using the flow cytometry-based hematology analyzer XN-31

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Equine piroplasmosis (EP) is a tick-borne disease caused by the intraerythrocytic protozoa *Babesia caballi*, and *Theileria equi.* A novel species, *Theileria haneyi*, has also been reported as a causative agent of EP, and the horses infected with *T. haneyi* have been identified in North America, South America, and Africa. EP is a reportable disease according to the World Organisation for Animal Health (WOAH), and infected horses are restricted from international movement. In Japan, diagnosis of EP is conventionally performed using microscopic, molecular, and serological methods. PCR-based diagnostic assays are the only available means to confirm *T. haneyi* infection in animal quarantine; however, it is time-consuming. Recently, we reported that the hematology analyzer XN-31 (Sysmex), originally developed for diagnosing human malaria, is efficient in detecting *B. caballi* and *T. equi* infections, as well asin differentiating between the two parasites. In this study, we evaluated the diagnostic potential of the XN-31 analyzer for *T. haneyi.* Evaluate the use of XN-31 for diagnosis of *T. haneyi*. *T. haneyi* was *in vitro* cultured and measured by XN-31 (Sysmex, Kobe, Japan). The numbers of infected red blood cells (iRBC#) and ratio of infected RBCs (iRBC%) were counted by using XN-31 and by microscopic examination. XN-31 was assessed for limit of blank (LoB), limits of detection (LoD), quantitation (LoQ), and linearity. XN-31 detected *T. haneyi*-infected RBCs (iRBCs) in approximately 1 minute. To investigate the reliability of XN-31, iRBC% were compared between XN-31 analysis and microscopy examination. The correlation of iRBC% was high ($R2 > 0.9$). LoB was 0.7 cells/µl, and the LoD and LoQ were 6.8 cells/µl and 14.8 cells/µl. Linearity was good ($R2 > 0.9$). XN-31 detected *T. haneyi*-infected RBCs, reporting the infection ratio in approximately 1 minute. These findings indicate that XN-31 would be useful for EP diagnosis, especially for the monitoring of infected horses and for screening tests.

Antigens of *Neospora caninum* for the development of immunochromatographic and biosensor assays

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Neosporosis significantly impacts cattle production, resulting in annual losses of $1.29 billion. There are no economically viable treatments or commercial vaccines available, making diagnosis key for animal management. This work aimed to characterize recombinant antigens as potential biorecognition molecules to develop

point-of-care diagnostic tests. The study analyzed *N. caninum* surface proteins NcSAG1 and NcSRS2. Homology modelling (using Modeller) and epitope prediction were performed. Their genes were cloned into the pET28a(+) and expressed in *E. coli* BL21(DE3) for heterologous expression. Protein expression was induced using IPTG for NcSAG1 and autoinduction for NcSRS2, followed by Ni-column affinity purification. Polyclonal antibodies against recombinant NcSAG1 and NcSRS2 were generated in BALB/c mice. Antigenicity of antibodies and antigens was assessed via ELISA (iELISA), Western blot (WB) and invasion assays. Structural analysis revealed a dumbbell-shaped structure with 14 (rNcSAG1) and 10 (rNcSRS2) predicted epitopes. Both proteins were soluble (rNcSAG1: 20 kDa, rNcSRS2: 15 kDa) in 20 mM Tris-HCl, 250 mM NaCl, pH 7.3. iELISA detected antisera reactivity at 1:16,000 (rNcSAG1) and 1:8,000 (rNcSRS2). WB analysis of rNcSRS2 confirmed the presence of the monomeric, while rNcSAG1 confirmed the presence of the monomeric and dimeric forms, consistent with previous reports. In the invasion assay, the combined use of anti-NcSAG1 and anti-NcSRS2 antibodies (1:50), resulted in significant inhibition ($p = 0.0002$). The recombinant antigens were successfully produced in water-soluble form, and their antigenic potential was confirmed, supporting their use in immunochromatographic tests and biosensor assays based on plasmonic signal. Therefore, the next execution steps will be carried out, including crosslinking of antigens, characterization, and conjugation with plasmonic gold nanorods as part of the biosensor's development steps.

Assessing the reactivity of TRX and DUF148 antigens for detection of Guinea worm (*Dracunculus medinensis*) infection in dogs

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Guinea worm (GW, *Dracunculus medinensis*) is a nematode that causes a painful and debilitating neglected tropical disease in humans. The GW Eradication Program has been successful at decreasing human infections by >99% over the last 40 years. However, GW has recently been detected in several animal hosts, especially dogs, which has hampered eradication efforts. Currently, there is no method for diagnosing GW infection during the prepatent period, before the emergence of adult female worms. Previous work has identified two GW proteins, thioredoxin-like protein (TRX) and a domain of unknown function protein 148 (DUF148), as immunoreactive antigens with GW-positive human sera. In this study, we developed and validated enzyme-linked immunosorbent assays using each antigen alone or in a combination of both antigens. Using serum and plasma samples from experimentally exposed dogs, TRX and DUF148 showed reactivity at 9- and 12-weeks post-exposure, respectively. TRX showed cross-reactivity with sera of dogs experimentally infected with *Brugia malayi* and *Brugia pahangi*. In contrast, DUF148 alone

or in combination with TRX showed no cross-reactivity with sera of dogs experimentally infected with these filarial nematodes. We further screened sera of dogs from Chad, a GW-endemic country, suspected of GW infection and dogs from Texas as a non-endemic region. DUF148 showed significantly higher reactivity with Chad dog sera compared to Texas dog sera. However, DUF148 also showed cross-reaction with several Texas dog sera. To mitigate this cross-reaction, we produced 3 peptides of DUF148. Peptide 3 showed better sensitivity and specificity in comparison with DUF148 whole protein and other peptides. Our findings could facilitate the development of diagnostic methods for early detection of GW infection in dogs in endemic countries.

+Lugar platform as a tool for collaborative mapping of geohelminthiases and Aedes infestation: an educational and epidemiological field study

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Collaborative mapping platforms are increasingly valuable for epidemiological surveillance, integrating public health, education, and digital technologies. The +Lugar platform enables participatory georeferencing of zoonotic and environmental risk factors. To evaluate the educational and epidemiological potential of +Lugar in mapping geohelminth infections and *Aedes* spp. breeding sites. Eighteen undergraduate students participated in the SummerClass Field Epidemiology course (Dec 2024–Jan 2025) at UEFS. Field collections were conducted within the university campus and the neighboring Campo Limpo district. Fecal samples from dogs were collected in public areas and analyzed for geohelminths and protozoa. PneuTrap3D ovitraps were deployed to detect *Aedes* spp. eggs. All data were georeferenced using +Lugar, analyzed in Epilnfo 7.2, and scanned for spatial clusters using SaTScan 10.2.5. The +Lugar platform allowed real-time mapping and visualization of sample locations, aiding in the identification of spatial patterns and environmental predictors. Clusters of canine fecal samples with positive results were detected within specific sectors of the UEFS campus. The presence of potential mosquito breeding sites was also

geolocated, including water tanks, bowls, and containers. The +Lugar platform proved to be a powerful, low-cost, and educational tool for participatory epidemiological mapping. Its integration into teaching, fieldwork, and laboratory diagnostics fosters interdisciplinary learning while enhancing the surveillance of neglected tropical diseases.

No more use of experimental animals: Using an in vitro cultivated *Trypanosoma evansi* antigen in agglutination test and indirect immunoabsorbent assay (iELISA)

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Trypanosoma evansi is a flagellate protozoa parasite responsible for trypanosomosis in many hosts and specifically in cattle impacts animal health and production and leads to economic loss. In Thailand, *T. evansi* infections in livestock often present with non-specific clinical signs, complicating diagnosis and hindering effective disease control. Asymptomatic carriers play a crucial role in disease transmission within herds. The indirect ELISA (iELISA) using whole-cell lysate antigens for identifying carrier status, recommended by the World Organisation for Animal Health (WOAH), is traditionally produced using experimental animals. However, this process is complex, costly, and raises ethical concerns. Thus, there is also a need for a rapid, accurate, and applicable point-of-care diagnostic test for trypanosomosis. Recently, in vitro cultivation of the *T. evansi* Thai strain has become available for antigen production. However, there is lack of *T. evansi* diagnostic test available in Thailand. This study aims to develop in vitro cultivated antigen and utilize an in vitro cultivated *T. evansi* antigen to measure the seroprevalence of *T. evansi* infection in cattle Thailand. *T. evansi* TEDC 953 Thai strain was grown in vitro. Freeze-dry *T. evansi* antigen produced in vitro was developed and used for validation on the agglutination test using 374 cattle serum samples. Crude *T. evansi* antigen produced in vitro was developed and validated on iELISA using 65 cattle serum samples. Cross-reactivity of in vitro produced crude antigen and freeze-dry antigen were performed with reference serum samples positive for *Lieshmannia* spp., *Babesia bovis*, *Babesia bigemina*, *Anaplasma marginale* and *Theileria oreintalis.* Sensitivity and specificity of agglutination test and iELISA using in vitro cultivated *T. evansi* antigen were calculated. Seroprevalence of *T. evansi* infection in cattle in Thailand were measured. *T. evansi* freeze-dry crude antigens produced in vitro were successfully developed. Sensitivity and specificity of the agglutination test using in vitro-produced freeze-dry *T. evansi* antigen were 87.10% and 96.70%, respectively. The iELISA using in vitro produced antigen method achieved a sensitivity of 93.5% (95% CI: 86.5–97.6%) with 43 true positives and 3 false negatives, and a specificity of 67.5% (95% CI: 58.3–75.8%) with 77 true negatives and 37 false positives. No cross-reactivity against four 29 blood parasites was detected. The overall seroprevalence was 62.03%, (CI 53.72–62.28%; 232/374), 30 with the seroprevalence in each province in the range 22.75–100.00% measured by agglutination test. The overall seroprevalence of *T. evansi* infection in cattle in Thailand was 20.05% measured by iELISA. In vitro produced *T. evansi* can be useful to produce antigen for both agglutination test and iELISA. This new procedure and can be replace the traditio-

nal procedure of produced *T. evansi* antigen *in vivo*.

Development of a multiplex quantitative PCR assay for detecting *Theileria bicornis* and *Babesia bicornis* in rhinoceroses

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Black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceroses in Southern Africa face significant threats from poaching, habitat loss, and translocation-related stress, which has been linked to mortalities associated with *Theileria bicornis* and *Babesia bicornis* infections. Current molecular diagnostic assays, though effective in detecting subclinical infections, are not well-suited for routine surveillance or large-scale screening. In this study, we report on developing a multiplex quantitative real-time PCR assay to detect and distinguish between *T. bicornis* and *B. bicornis* infections in rhinos. Primers and TaqMan minor groove binder (MGM™) probes were designed based on full-length 18S rRNA sequences from GenBank. Both simplex and multiplex formats of the assay were evaluated, demonstrating high efficiency, specificity and reliability in detecting either *T. bicornis* or *B. bicornis* infections from field samples. This novel multiplex qPCR assay represents a significant advancement in the molecular detection of these parasites, facilitating early infection screening before translocation thus improving routine diagnostics. By enhancing epidemiological surveillance, this tool strengthens conservation strategies for rhinoceros populations, reducing mortality risks and contributing to the long-term sustainability of these endangered species.

Epidemiology, modeling, and machine learning

Isolation of *Toxoplasma gondii* in wildlife animals from Minas Gerais, Brazil – Preliminary results

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Human activity, mainly due to urbanization, causes the degradation of forests, causing a significant environmental impact and increasing the number of wild animals being run over. This human action also favors contact between wild animals and humans and domestic animals, which can result in the risk of transmission of zoonotic diseases, including toxoplasmosis, an infectious disease caused by the intracellular protozoan *Toxoplasma gondii*. Due to its high

genetic variability, studies with wild animals contribute to a better understanding of the molecular epidemiology of toxoplasmosis in the wild-urban interface. Thus, the present study aimed to isolate *T. gondii* in wildlife from Minas Gerais state, Brazil. During September 2024 to January 2025, dead wild animals from Minas Gerais state that were sent to the Veterinary Pathology Department for necropsy were included in the study. Tissue samples (brain, heart, lung, liver, skeletal muscle) from each animal were collected during necropsy. The tissue samples were digested using a pepsin solution and inoculated into mice to biologically verify the presence of tissue cysts. During the period studied, tissue samples were collected from 18 wild animals, with 55.6 % (10/18) mammals and 44.4 % (8/18) birds. The main species collected was the *Callithrix penicillata* (black-tufted marmoset), with three animals. In the mouse bioassay, no mice showed clinical signs of infection after inoculation, and no cysts or tachyzoites were observed after euthanasia. The next step of the study includes the serological analysis of mice, molecular analysis of each tissue, and more sample collections to isolate the parasite. Based on the results so far, this study indicates that *T. gondii* is not circulating among wild animals from the region studied.

Epidemiology of *Eimeria* spp. in goats from northern Paraná, Brazil

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Coccidiosis is a parasitic disease responsible for significant economic losses in goat farming. The disease is caused by the infection of different species of *Eimeria*; however, there are no studies describing the prevalence of the parasite in northern Paraná, Brazil. Thus, the present study aimed to evaluate the presence, identify *Eimeria* species, and evaluate epidemiological aspects associated with the infection in goat herds in northern Paraná, Brazil. Fecal samples from 384 animals were collected from eight goat farms. The variables (age, breed, sex, rearing system, type of floor in the pen) were collected to evaluate infection-associated factors. The McMaster technique was used to detect and count *Eimeria* spp. oocysts. Positive samples were submitted to sporulation using a potassium dichromate ($K_2Cr_2O_7$) solution at a concentration of 2%. After sporulation, species identification was based on morphometry of oocysts and sporocysts. In total, 82.3% (316/384) of the samples were positive, and most of them (63%; 199/316) had oocysts per gram (OPG) less than 1,000. Significant factors associated with infection were the semi-intensive production and the facilities with dirt floors. Eight *Eimeria* species were identified: *E. arloingi* (23.9%), *E. apsheronica* (19.7%), *E. ninakohlyakimovae* (14.3%), *E. alijevi* (12%), *E. caprina* (10%), *E. jolchijevi* (9%), *E. hirci* (6.9%), and *E. christenseni* (4.2%). This study showed a high prevalence

and diversity of *Eimeria* species in goats in northern Paraná, Brazil.

Computational model for predicting the number of generations of the bovine tick *Rhipicephalus (Boophilus) microplus* in brazil

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Rhipicephalus (Boophilus) microplus is a significant ectoparasite affecting livestock health and productivity in Brazil. It transmits pathogens like *Babesia bovis*, *B. bigemina* and *Anaplasma marginale*, resulting in substantial economic losses (~3.2 billion USD/year). Abiotic factors, such as temperature, influences multiple life stages of this tick and impacts the number of annual generations across different Brazilian regions. The aim of this study was to develop a model to predict the number of annual generations of *R. (B.) microplus* in Brazil. By searching for data on the biology of the non-parasitic phase of the tick, a bibliographic review was carried out in the PubMed, Scielo and Google Scholar databases. Subsequently, prediction equations by third-degree polynomial nonlinear regression models were performed, with temperature data as the predictor variable and the biological parameters pre-laying and egg incubation periods as the response variables, considering $R^2 \geq 0.70$ and $p < 0.05$, significant. Brazil was then divided into 136 quadrants (2.5° x 2.5° resolution) using a geostatistical exponential method. Climate data (2020–2024) from meteorological stations in each quadrant were sourced from the AGRITEMPO database to feed the model. Statistical robustness was assessed using RMSE, MAE and R^2. As a result, the mathematical equations for predicting the pre-laying period ($R^2 > 0.70$, RMSE = 2.67) and incubation ($R^2 > 0.80$, RMSE = 11.80) showed a negative correlation ($r < 0$) as a function of the climate variables and predictions differ from the real values by 1.93 days (MAE) for the pre-laying period and 9.21 days (MAE) for egg incubation. Model outputs estimated tick generations ranging from 2.92 (South region) to 6.18 (North region) per year. These results support the development of region-specific tick control strategies by providing insights into population dynamics, aiding in the timing of interventions and improving pasture management.

Presence of zoonotic gastrointestinal nematodes in parks of major cities in Colombia

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Gastrointestinal nematodes affecting dogs and cats are a public health concern, as humans may be at risk of exposure to various infectious stages of these parasites, either through

contact with canine and feline feces or through the contaminated soil. In Colombia, few studies have been conducted to determine the presence of zoonotic gastrointestinal parasites in parks from the main cities, which can be a thread for public health, especially for children. The aim of the study was to determine the presence of geohelminth eggs and zoonotic larvae in soil samples collected from public parks from 7 cities in Colombia, to highlight their potential impact on public health and the well-being of animals and their owners. Seven main cities in Colombia were sampled (Bogotá, Medellín, Cali, Barranquilla, Bucaramanga, Ibagué and Villavicencio) during April 2025 (rainy season). From each city, 10 parks were sampled. A pool of soil sample and two fecal samples were collected in each park, for a total of 70 soil samples and 140 fecal samples (n = 210). Samples were transported to the laboratory of Veterinary Parasitology, Faculty of Agricultural Sciences, University of Antioquia, Medellin-Colombia. Samples were processed and analyzed following standard parasitological procedures for larval migration test, flotation with Sheather's solution and McMaster technique. At least one soil sample per city showed contamination with some parasitic stage. Hookworm eggs were present in all cities. Among the larvae, *Strongyloides stercoralis* was the most prevalent. The presence of parasitic structures indicates fecal contamination in public parks in Colombia, posing a threat to the health of both humans and animals. Enhanced measures must be implemented to prevent soil contamination by intestinal parasites, particularly in public parks.

In silico study of eugenol in complex with enzyme phosphoethanolamine N-methyltransferase of *Haemonchus contortus*

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Eugenol exhibits anthelmintic activity against gastrointestinal nematodes of small ruminants. However, its mechanism of action remains undefined. Phosphoethanolamine *N*-methyltransferase (PMT), a di-domain enzyme (PMT-1 and PMT-2), is critical for the survival of *Haemonchus contortus* and absent in mammals, representing a promising target for anthelmintic development. Thus, the study's objective was to determine the interaction of eugenol against *Hc*PMT enzyme. For molecular docking, the *Hc*PMT structure was selected from the Protein Data Bank (PDB ID: 4KRG e 4KRH) using the *H. contortus* model. In the target preparation step, residues were removed, polar hydrogens were added, and *Kollman* and *Gasteiger* charges were calculated using the AutodockTools™. A redocking procedure was performed to validate the docking simulations using the following co-crystallized inhibitors: *S*-adenosylmocysteine (SAH) and *S*-adenosylmethionine (SAM), natural substrates of *Hc*PMT1 and *Hc*PMT2, respectively. Fifty docking simulations were performed, each generating 20 poses evaluated by Root Mean Square Deviation (RMSD $\leq$ 2.0 Å) and affinity energy (< -6.0 kcal/mol). The interaction between eugenol and *Hc*PMT-1 showed

RMSD value of 1.264 Å and affinity energy of -7.0 kcal/mol. The active site of the *Hc*PMT1 domain is formed by 11 hydrophobic interactions, six hydrogen bonds and one π-stacking. However, the eugenol did not bind to the same catalytic site as SAH. The interaction between eugenol and *Hc*PMT-2 showed RMSD value of 1.089 Å, affinity energy of -5.5 kcal/mol and six hydrophobic interactions and five hydrogen bonds. Despite presenting a weaker interaction with *Hc*PMT-2, the molecule bound to the same region as SAM. This study revealed interactions between the eugenol and the catalytic domains of *Hc*PMT2, suggesting a potential mechanism involving the disruption of phosphatidylcholine biosynthesis. *In silico* studies involving other *H. contortus* targets should be performed.

In silico identification of nematicidal compounds derived from edible mushrooms _Pleurotus ostreatus_

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The edible mushroom *P. ostreatus* produces a wide variety of secondary metabolites with potential bioactive properties, particularly as nematicidal agents. This approach represents a more sustainable alternative to conventional anthelmintics, whose effectiveness in controlling agricultural nematodes has significantly declined1. This study focuses on the *in silico* assessment of bioactive compounds from *P. ostreatus* using bioinformatics tools to predict molecular docking and potential interactions with target proteins. Bioinformatics tools and specialized digital platforms were used for searching and analyzing structural data. These included PubChem to obtain the SMILES code of the compounds, as well as the *Natural Products Atlas*, which provides important information for protein modeling. Additionally, *SwissTargetPrediction* was used to predict molecular interactions, and Avogadro was employed for structure optimization. Finally, molecular docking was performed using *Autodock Tools*. Several compounds with potential nematicidal activity were identified, including linoleic acid, pentadecanoic acid, and palmitic acid. These compounds exhibit structural similarities, suggesting a possible shared mechanism of action. Notably, linoleic acid demonstrated affinity for potential molecular targets, such as the *Fatty Acid-Binding Protein in Adipocytes* and the *PPAR-γ receptor*. It is important to emphasize that these findings are based on computational predictions of molecular target interactions. The results suggest that linoleic acid and pentadecanoic acid possess potential nematicidal activity by interacting with key proteins in nematodes.

Anti-_Neospora caninum_ and anti-_Toxoplasma gondii_ antibodies in cattle intended for human consumption in the State of Espírito Santo, Brazil: Prevalence and associated factors

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Neospora caninum and *Toxoplasma gondii* are apicomplexan protozoa of major veterinary importance. In cattle, *N. caninum* is a leading cause of reproductive losses, particularly abortion, resulting in significant economic impacts. *T. gondii*, although less frequently associated with clinical disease in cattle, is a zoonotic agent capable of infecting humans through the consumption of contaminated meat, posing a potential public health risk. Despite their relevance, data on the prevalence and associated factors of these infections in cattle populations in the state of Espírito Santo, Brazil, remain scarce. The objective of this study was to describe the seroprevalence and factors associated with *Neospora caninum* and *Toxoplasma gondii* infections in cattle in the state of Espírito Santo, Brazil. Serum samples were collected from 600 slaughtered cattle and destined intended for consumption, originating from 27 municipalities. The samples were analyzed using the Indirect Immunofluorescence Test, with cut-off points for anti-*N. caninum* antibodies at 1:200 and for anti-*T. gondii* antibodies at 1:64. Positive samples were titrated until negative. Epidemiological questionnaires were applied to gather information about the properties and herds. The seroprevalence for *N. caninum* was 13.2% (79/600), with antibody titers ranging from 1:200 to 1:6400, and for *T. gondii* it was 12.5% (75/600), with titers ranging from 1:64 to 1:1024. In the multivariate analysis, the factor associated with *N. caninum* infection was a history of abortion (OR 6.483; CI 6.082-6.884; $p < 0.01$), and for *T. gondii*, contact with cats (OR 7.683; CI 7.172-8194; $p < 0.005$). The seroprevalences for *N. caninum* and *T. gondii* in cattle slaughtered for human consumption in Espírito Santo are significant, with a history of abortion being a factor associated with *N. caninum* infection and contact with cats associated with *T. gondii* infection.

Usage Patterns and Concentration-Dependent Analysis of Macrocyclic Lactones Formulations in Farm Animals

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In Uruguay, macrocyclic lactones (MLs: ivermectin, moxidectin, doramectin, abamectin and eprinomectin) are the cornerstones of livestock antiparasitics, but their unmonitored use risks development of resistance and food security issues (residues). We evaluated the deployment of ML through pharmacoepidemiological drug sales data (DSD: tones/year active ingredient) integrating national information from five years. Between 2016 and 2021, MLs represented 26 per cent of the total ectoparasites indicated for tick control (average of 1.25 tonnes/year; range 0.51 to 1.73 tonnes). Within MLs, Ivermectin dominated 85% (from 0.4 to 1.4 tons), followed by Doramectin 7% (from 0.07 to 0.12 tons), Eprinomectin 4% (from 0.01 to 0.16 tons), Abamectin 3% (from 0.03 to 0.05 tons) and Moxidectin 0.8% (from 0.003 to 0.024 tons). The data analysed from 622 administrative areas by Geographically Weighted Summary Statistics (GWSS) revealed hot spots in the northwest regions and secondary clusters in the southeast. With regard to concentration-dependent patterns, we

compared 1% to 3.15 % of ivermectin and doramectin formulations. In the case of Ivermectin, the highest consumption was the formula of 3.15%, which represents 70% of the volume consumed. Doramectine showed a reverse behavior, with 1% being the most used formulation (70%). The highest use of the formulation of 3.15 % was particularly visible in the endemic tick zone of Uruguay (for example, in the north and south-east
regions). This study is a pioneer in a specific concentration-based risk assessment for MLs. The results call for the improvement and regular implementation of ML drug use monitoring systems with on-site support, which will enable regional management of integrated parasitic control, optimal resource use, and food security.

Exposure Patterns of Veterinary Ectoparasiticides in Uruguay: A Pharmacoepidemiological Approach

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In Uruguay, ectoparasiticides are essential for the control of livestock parasites, but unmonitored use could lead to the emergence of resistance and chemical residues. The exposure to veterinary ectoparasiticide agents using sales data must be related to the total amount sold in a given area in a given year (volume of the prod-uct in mg of active ingredients) and the size of the animal population in the same area. We assessed the effectiveness of pharmaceuticals for livestock by developing novel indicators that integrate: Drug Sales Data (DSD) in mg of active ingredients/year; Types of Medicines (TM) including ectoparasiticide formulations; Effective Time Period (ETP) representing minimum days between applications as recommended by sanitary authorities; and Livestock Units (LU) standardized for 380 kg breeding cattle. National ectoparasiticide sales data (2016-2021) from 622 administrative areas were analyzed. Indicators compared the application frequency for five therapeutics: ivermectin (subcutaneous), fluazuron (pour-on and subcutaneous), fipronil (pour-on), amitraz (topical), and ethion/cypermethrin (4:1 combination, topical). Geospatial regression (GWSS) identified high-risk areas. Uruguay maintains an average of 11.3 million LU and 40.6 tons of ectoparasiticides per year. The northwestern regions showed the highest application density, with GWSS revealing additional hotspots in the southwest and southeast. Ivermectin showed the highest us-age (peaking in 2021), followed by fipronil, ethion/cypermethrin, fluazuron, and amitraz. Spatiotemporal indicators (DSD + TM + ETP + LU) visualized through choropleth maps allowed identification of risk zones and enabled targeted management suggestions. Seasonal peaks suggest treatment misalignment, while underuse may indicate access barriers or lack of evidence-based integrated management. This framework supports One Health policies to mitigate parasite resistance, vector-borne diseases, and food safety concerns.

Seroprevalence of canine leishmaniasis in Paraguay, diagnosed by the National Program of Zoonoses Control and Rabies National Center, 2022-2024.

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Visceral leishmaniasis is a chronic parasitic disease caused by *Leishmania infantum*, transmitted by infected sandflies that affects humans and its urban reservoirs dogs. The purpose of this work was to determine the seroprevalence of canine leishmaniosis by immunochromatographic rK39 test in serum samples obtained for routine exam requested by dog's owners and veterinarians, active surveillance in areas of silence transmissions and control of human cases notified for the National Service of Eradication of Vector-borne diseases (SENEPA) to the National Program of Zoonoses Control and Rabies National Center (PNCZyCAN) since 2022 to 2022. A total of 7.899 blood samples were analyzed by immunochromatographic rK39 (Kalazar Detect Rapid Test, canine. lnbios®, Seattle, USA), in the laboratory of Leishmaniasis of the PNCZyCAN, from 11/17 departments of the country. The 4.274 canine blood samples proceeding for routine exam from Asunción (capital city), Amambay, Central, Concepción, Cordillera, Guairá, Itapúa, Ñeembucú, Paraguarí, Presidente Hayes and San Pedro departments, showed 1.032/4.274 positive serum samples with a prevalence of (24.1%).The active surveillance showed that 99 out of 672 samples had a positive result with a prevalence of (14.7%).From the focus of human visceral leishmaniasis was observed that 307 out of 2.953 canine samples were positive with a prevalence of (10.4%). A global seroprevalence of (18.2%) of canine leishmaniasis was observed. Euthanasia procedures were carried out in 294 positive dogs (20.4%) from routine exam, focus of human visceral leishmaniasis and active surveillance in areas of silence transmission of visceral leishmaniasis. The high prevalence of canine visceral leishmaniasis shows the compelling need to continue a strict epidemiological surveillance, sanitary education and community participation by the Ministry of Public Health and Social Welfare in the control of this disease in Paraguay.

Simplifying Tick Engorgement Assessment: Novel Predictive Models Based on Linear Dimensions of *Amblyomma variegatum*

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Accurate measurement of blood engorged female ixodid tick weight is crucial in veterinary parasitology research, yet current methods relying on expensive ultra-electronic balances are often tedious, require specialized laboratory settings, and are inaccessible in resource-limited environments. To address these limitations, this study investigated the potential of using readily obtainable linear body dimensions measured with vernier calipers (length, width, and height) to reliably estimate the weight of female *Amblyomma variegatum*. Live adult female ticks at varying engorgement levels were collected from naturally infested cattle, and their weight and linear dimensions were recorded (n=121).Data analysis involved fitting sim-

ple linear and regular polynomial regression models to predict weight (and its log10-transformation) based on each dimension (and their log10-transformations). Comprehensive model evaluation, including residual analysis and assessment of regression assumptions, identified five robust models. The top-performing model, log10(weight) = -1.8633 + 1.8829*log10(height) + 0.2102*log10(height)^2, exhibited the highest R-squared (0.974) and lowest AIC (-251.42), indicating exceptional predictive accuracy. A second highly effective and simpler model, weight = -2.7456 + 0.2512*length + -0.0046*length^2 (R-squared = 0.9738, AIC= -249.99), also demonstrated strong predictive capabilities without requiring variable transformation.The findings reveal a significant polynomial relationship between tick weight and linear dimensions, particularly height and length. These findings suggest that linear body measurements obtained with inexpensive vernier calipers can serve as a reliable and practical alternative for estimating the weight of engorged female *Amblyomma variegatum*. These predictive models offer a cost-effective and field-friendly approach for veterinary parasitology research, particularly in settings where sophisticated laboratory equipment is unavailable, thereby facilitating broader and more accessible investigations into tick biology and control.

Abattoir survey of dairy sheep and goats' haemonchosis in Greece and associated risk factors

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Epizootiological studies provide a good understanding of the seasonal effects on the annual of *Haemonchus contortus* burdens for a range of environments, to set the basis for the effective application of control programmes on a case-specific basis. The objectives of this study were to determine the prevalence of *H. contortus* infections, in dairy sheep and goats in continental and insular Greece, based on an abattoir survey and to evaluate potential host-related risk factors including the age, sex, the altitude, the management system, the co-existence of goats and sheep, the season and the anthelmintic treatment, on the occurrence of haemonchosis. 1004 abomasa of small ruminants were examined to evaluate the prevalence of *Haemonchus* spp. infection. A questionnaire was used to obtain relevant information regarding animal and farm characteristics. *Haemonchus*-like helminths were collected from the abomasa and used for the molecular species identification. The prevalence of mono-species *H. contortus* infection of small ruminants was 37.2%. For sheep, multivariable analysis revealed the anthelmintic treatment (treatment with pro/benzimidazoles), the age (lambs under 2 months old) and the management system (intensive management system) as significant factors for preventing *H. contortus* infection. Likewise, the management system (intensive management system), the anthelmintic treatment (treatment with macrocyclic lactones and their combination with pro/benzimidazoles), the altitude of the farms (farms located over 300 meters above sea level) and the season (spring and summer) were significant risk factors in preventing *H. contortus* infection in goats.

Host selection and resilience

Complement and coagulation cascades activation regulates the early inflammatory mechanism of resistance of suckling lambs against *Haemonchus contortus*

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Haemonchus contortus is a highly pathogenic blood-sucking nematode from the abomasum of small ruminants, globally known for its multidrug resistance and for causing high economic losses in sheep industry. Understanding the biological mechanisms involved in the immunity of sheep against *H. contortus* is crucial for developing effective strategies to prevent the severe infection caused by this nematode. This study investigated the molecular mechanisms related to the complement and coagulation cascades in resistant (Santa Ines) and susceptible (Ile de France) lambs experimentally infected with *H. contortus*. Naïve 14 days of age suckling lambs (n=4 per group) were orally infected with 5,400 infective larvae (L3) of *H. contortus* every two days until the age of 66 days, following an infection protocol divide within 27 infections as follows: 1) 9 infections with 100 L3 each, 2) 9 infections with 200 L3 each, and finally, 3) 9 infections with 300 L3 each. At day 68, lambs were euthanized, and abomasal tissue was collected for mRNA extraction and RNA-sequencing. At the end of the experimental period, Santa Ines lambs had a mean eggs per gram of faeces (EPG) of 1,200 EPG, while Ile de France lambs averaged 11,075 EPG. Enrichment analysis identified 32 differentially expressed genes associated to the complement and coagulation pathways, 29 of which were upregulated in Santa Ines. Genes such as C3, F3, F5, CFB, and CFI were linked to the resistance phenotype. Upregulation of F3 and F5 in Santa Ines suggests enhanced activation of coagulation in response to tissue damage. C1S was also upregulated in Santa Ines, and protein-protein interaction analysis indicated its role in classical complement pathway activation. The data suggest that resistant Santa Ines lambs exhibit a polygenic overexpression pattern regulating complement (classical, lectin, and alternative pathways) and coagulation (extrinsic pathway) cascades, contributing to early protection against *H. contortus* infection.

Host switching is the main driver of coevolution between Hepatozoon parasites and their vertebrate hosts

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Hepatozoon spp. are apicomplexan parasites with heteroxenous life cycles. These parasites infect a wide variety of wild and domestic vertebrates causing sub-clinical infection or mild to severe clinical manifestations, depending on the parasite species and vertebrate host. Each *Hepatozoon* spp. has a specific host range suggesting a close host-parasite coevolutionary

relationship, therefore, we evaluated whether this follows a cophylogenetic signal. We reconstructed host and parasite phylogenies using 18S rDNA and *cyt*B sequences, respectively. Subsequent analyses were stratified according to host vertebrate orders, Carnivora, Rodentia and Squamata, and the corresponding sequences of their *Hepatozoon* parasites. Then, Procrustean Approach to Cophylogeny (PACo) and ParaFit were employed to assess their global cophylogenetic relationship, and eMPRess was used to estimate the most probable co-evolutionary events, such as host switch, duplication, sorting or cospeciation. Global assessments of congruence between phylogenies of carnivore, rodent and squamate hosts and those of their *Hepatozoon* parasites were significant (PACo all $p < 0.001$; ParaFitGlobal Statistic all $p < 0.007$, all Procrustes R2 > 0.25), but it was not for the association between *Hepatozoon* spp. and invertebrates (PACo $p < 0.001$; ParaFitGlobal Statistic $p = 0.124$, R2 = 0.37). The most significant links occurred between *Hepatozoon felis* and felid hosts or *H. canis* and canid hosts, but not between *Hepatozoon americanum* and domestic dog or coyotes. Moreover, eMPRess showed that the coevolutionary history between *Hepatozoon* spp. and vertebrate host phylogenies was mainly explained by host switching and less frequently by cospeciation. These findings highlight the ability of *Hepatozoon* spp. associated to certain vertebrate orders to infect new sympatric hosts, which helps to understand how hepatozoonosis can emerge in susceptible hosts by spillover events.

Establishing a relationship between body growth, behavior, and fecal egg count in naturally infected thoroughbred foals in southern Brazil

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The prevalence of gastrointestinal parasites remains high among foals raised under pasture-based systems, particularly cyathostomins. Parasite control often relies on routine treatment, increasing anthelmintic resistance. This study aimed to evaluate the relationship between body growth, behavior, and fecal egg count (FEC) in Thoroughbred foals. We calculated an FEC repeatability coefficient for each animal. Twenty-five 1–10 months old Thoroughbred foals were used from a stud farm in southern Brazil. The animals were monitored monthly for body weight (BW), withers height (WH), and FEC. Animals' behavior was checked daily by resident veterinarians. Foals showing clinical signs or altered behavior were selectively treated with ivermectin (1%) and praziquantel (13%). Statistical analyses included generalized mixed linear models and Odds Ratio. The data revealed a high correlation between BW and age ($R^2 = 0.89$) and between WH and age ($R^2 = 0.91$) with no significant differences between sexes ($p > 0.05$). No significant correlation was found between FEC and BW. A weak correlation between FEC and age was detected for females ($R^2 = -0.025$) and males ($R^2 = 0.168$). Only four foals showed clinical or behavioral signs and were treated. These foals had 16 times more chance to present FEC > 1000. The FEC repeatability coefficient by age and sex was low (0.206), while individual variability was mode-

rate to high (0.794). The data confirm horses' resilience to initial and up to 10-month parasite challenges. Despite natural exposure, foals were under effective farm management, including balanced nutrition, large pasture areas, and constant health checks. In addition, the integration of FEC, body growth measurement, and frequent behavioral assessments allowed for effectively monitoring parasite-related risk, even the decision for treatment. Selective anthelmintic treatment should incorporate these values to promote equine health, minimize drug use, and maintain parasites in refugia.

Living with parasites: tolerance to nematodes in sheep

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Productivity loss caused by gastrointestinal nematode (GIN) infection is a major problem in the livestock industry. Management of GIN infections has traditionally relied on anthelmintic drugs, but the evolution of anthelmintic resistance means this is unsustainable and alternative means of controlling GIN, including through genetic selection, are needed. Broadly, there are two ways individuals cope with infections: resistance and tolerance. Resistance reduces parasite burden through an immune response which directly targets the parasite, while tolerance is the maintenance of productivity despite increasing parasite burden. The overall effect of both resistance and tolerance is termed resilience. Breeding for resistance to GIN is a common strategy, but selection for tolerance has not been widely implemented. A potential drawback of selecting for resistance is a trade-off with productivity, but such concerns do not apply to tolerance. While breeding for tolerance could be a useful tool in mitigating the impact of GIN, we first need to establish whether variation in tolerance exists. To do this, we monitored 200 Romney ram lambs from 16 different sires, 10 bred for resilience and 6 from unselected lines. Following weaning, faecal egg count (FEC) and body weight were measured fortnightly for 4 months. Using random regression models, we estimated tolerance as the slope of weight on FEC and quantified between-individual and between-sire variation. The overall relationship between weight and FEC was negative, but the slope of this relationship varied by individual and sire. Our models suggested that the least tolerant individuals experienced a check in their growth 10 times greater than that of those most tolerant. Further, individuals bred from resilient lines were heavier and treated later than those from unselected lines. These differences indicate the potential for selective breeding for tolerance to nematodes in sheep, reducing anthelmintic use.

Assessing the immune response of different β-globin haplotypes and breed in *Haemonchus contortus* infected sheep

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Sheep exhibit two distinct β-globin haplotypes, Hb-A and Hb-B. During anemic conditions, Hb-A animals can express juvenile βC, which displays a higher affinity for oxygen, an ability absent in Hb-B animals. β-globin haplotypes have been associated with variable sheep resistance/resilience to gastrointestinal nematode infection. This research aimed to compare the adaptive immune response in the abomasum of lambs of different breeds and β-globin haplotypes artificially infected by *Haemonchus contortus*. Twenty-one male lambs, comprising 12 Santa Inês (SI), 5 Texel (TX), and 4 White Dorper (DO), with six months old, were selected from the Embrapa Southeastern Livestock experimental farm based on their β-globin types (AA, AB, BB) through blood DNA analysis. Subsequently, those lambs were dewormed and received a single dosage of 4,000 *H. contortus* L3. Twenty-eight days post-infection, they were slaughtered, and the fundic region of abomasum tissue was formalin-fixed and paraffinembedded for immunohistochemistry analysis of MHC II (HLA-DR) and T lymphocytes (CD3) cells. Positive cell counting was performed in ten random fields under a 40x objective with a 1 mm^2 graticule. There was no effect of haplotype in MHC II+ ($p = 0.980$) (AA: 248±60.6; AB: 234±47.1 and BB: 254±121.8) and in CD3+ means ($p = 0.140$) (AA: 1122±304.9; AB: 976±820.3 and BB: 520±595.5). Similar results were observed for breed effect in MHC II+ ($p = 0.130$) (DO: 162±77.7; SI: 263±83.3 and TX: 280.6±114.5) and CD3+ means ($p = 0.787$) (DO: 1360±278.9; SI: 1233±778.2 and TX: 1473±497.4). The limited presence of AA animals in the flock may have influenced the absence of differences among haplotypes. This similar immunological response is being better investigated with a larger number of animals, as there seemed to be a tendency for AA animals to have a higher average of CD3+, along with a breed effect in MHC II+ of SI and TX compared to DO.

Parasites of swine and poultry

A novel way for Eimeria inoculum production

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Coccidiosis is one of the most economically devastating diseases of poultry industry in which difficulties remain to maintain an efficient program (vaccination, anticoccidials, etc.) to control infections. Registration and approval of such products requires in vivo trials demonstrating efficacy against artificial *Eimeria* infections. In latter, a mix of the most common and pathogenic chicken Eimeria species (*E. tenella*, *E. maxima* and *E. acervulina*) is often desired, yet restricted by regulation to recently collected strains, not replicated more than three times, with sufficient virulence as demonstrated by intestinal lesions. Replication of such field isolates to obtain ample oocysts for a trail however favours *E. acervulina* overgrowth due to its higher fecundity. This renders the inocula only adequate for *E. acervulina* on requirements, yet not for *E. tenella* or *E. maxima*. Hence to gain control over the inocula composition, the standard inoculum production method was revised. A novel method was created, circumventing risks of missing out sufficient lesion induction on minor *Eimeria* species. This enhances predictability in the infected untreated control thus improves predictability in the overall trial.

lmportantly, inocula production remains within the maximum of three passages starting from the original field strain, as required by regulation. Moreover, as for sole research purposes, this novel method allows to selectively in- or exclude specific species to focus only on species-of-interest, rarely possible using field strains. Consequently, these inocula can assist in the development of better scoring systems for *Eimeria* species in other than chickens and turkeys, like quails, partridges, rabbits, ...

Poster Presentation
Day 20

Poster Presentation - Day 20

Education and extension programs

@Parasinsta_UFRRJ: the use of social media as a tool for parasitology education

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The presence of technology and social media in academic routines has impacted teaching methods across all fields of knowledge, generating new educational approaches. The profile @parasinsta_ufrrj, created in 2018 on Instagram, is a practical example of this transformation. Characterized as a Studygram, it serves as a complementary tool for teaching Parasitology. The page has already offered more than 280 feed posts, hundreds of stories, and highlights (which function as folders and can always be accessed by the virtual community). The posts include various types of content, ranging from morphological images and explanatory videos on specimens important to One Health to quizzes and live sessions with specialists, making learning more dynamic and accessible for students and professionals. During the COVID-19 pandemic, the profile stood out for maintaining close connections in education, functioning as an extension of courses and a space for exchange. It is also the official channel for promoting the extension project Rede Parasitologia Online, announcing public calls and courses focused on the use of digital tools in the field, emphasizing how the project can overcome physical barriers by connecting UFRRJ with other institutions. Today, with approximately 3,060 followers, @parasinsta_ufrrj attracts national and international attention. The profile makes posts aimed at teaching parasitology, refutes misleading posts about the subject, and helps students with their studies. An analysis of the profile's metrics from March 29 to April 27, 2025, shows that its posts garnered more than 15,000 views, with 46.5% of these coming from feed publications. Its largest audience is Brazilian (85.1%), but it also has followers in other countries such as Iran (1.4%), Italy (1.1%), and Mexico (1%). The visibility generated by the profile has strengthened UFRRJ's reputation and demonstrated the educational potential of social media. In conclusion, @parasinsta_ufrrj represents a modern, effective, and accessible tool for teaching Parasitology, promoting meaningful, engaging, and digitally aligned learning for today's students.

REDE PARASITOLOGIA ON LINE: strengthening collaborative learning in parasitology

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The REDE PARASITOLOGIA ONLINE was created in 2020 from the initiative of 3 professors from different institutions, aiming to establish

an accredited network of professors and researchers to contribute to the teaching-learning process during Covid-19 pandemic, with resources that could later facilitate the integration of active strategies in the teaching of parasitology. Currently, the network is registered as an interinstitutional extension project and has the collaboration of professors and researchers, with the active participation of graduate students, undergraduates, and others interested in the field. The network features a virtual platform that organizes and records each stage of the project, along with a public curation of teaching materials aimed at enhancing connections between professors, researchers and students. Strategic actions have been carried out, such as the production of video lessons, teaching materials, online courses, live sessions on social media, and a mapping of parasitology laboratories in Brazil. Additionally, the project's website offers supporting materials for organizing the routines of teachers and students, such as monthly and weekly planners. In 2024, the first parasitic photography exhibition was held during the Brazilian Congress of Veterinary Parasitology and a calendar was made with selected images in partnership with the Brazilian College of Veterinary Parasitology, launched during the event. Thus, the REDE remains a pioneer, and potentially the first collaborative learning community for higher education, helping, since its creation, to disseminate original content related to parasites and the teaching and learning of parasitology. It is increasingly becoming a reference in this field, both for human parasitology and veterinary parasitology. As a new challenge for the network, the goal is to pursue internationalization by expanding the collaborative network through the integration of new accredited members.

Evaluating the Educational Impact of Video Tutorials on Coproparasitological Diagnostic Techniques in Veterinary Parasitology: A Cross-Sectional Study

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Coproparasitological techniques are fundamental in veterinary medicine for diagnosing intestinal parasitic infections and form a core part of clinical training. Due to their procedural nature, teaching these techniques can benefit from scalable, visual tools that support skill acquisition and self-directed learning. This study aimed to evaluate the impact of instructional videos on students' understanding and perceptions of coproparasitological methods. After receiving a grant from DGPA-PAPIME through project PE205623, a cross-sectional study was conducted with 110 veterinary students who viewed instructional videos covering 11 coproparasitological techniques. Their knowledge was assessed using a 17-item multiple-choice exam. Additionally, a structured opinion questionnaire was used to gather student feedback on the clarity and usefulness of the videos. Fourteen of the seventeen exam items were answered correctly by more than 80% of participants, with one item reaching 96.4% accuracy. Regarding perceptions, 94% of students rated the videos as "very clear," and 94% as "very useful," highlighting strong acceptance and satisfaction. Instructional videos significantly supported students' comprehension and were perceived as effective learning tools. Their integration into veterinary parasitology curricula is recommended to reinforce technical training, improve learning outcomes, and address limita-

tions in access to hands-on instruction, especially in resource-limited educational settings.

Environmental health education as a strategy for preventing neglected and emerging diseases in the North of Minas Gerais

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Neglected and emerging diseases, such as arboviral infections, leishmaniasis, and Chagas disease, pose significant public health challenges, particularly in vulnerable communities. This study aimed to train teachers, healthcare agents, and students in recognizing, preventing, and controlling these diseases through interactive methodologies fostering community engagement. Educational interventions were carried out in public and quilombola schools in Porteirinha, Riacho dos Machados, and Pai Pedro, Minas Gerais, involving 510 students from Elementary Schools I and II. Pre- and post-intervention questionnaires addressed transmission, treatment, diagnosis, and prevention of diseases like schistosomiasis, taeniasis, cysticercosis, and arboviral infections. Activities included board games, puzzles, memory games, and a "day in the laboratory" where students observed vectors and pathogens under microscopes. Before the interventions, prior knowledge varied: in Porteirinha, Elementary School I had 38% accuracy, and Elementary School II, 47%. In Pai Pedro, Elementary School II had 50% accuracy, and Elementary School I, 42%. In Riacho dos Machados, Elementary School I ranged from 0% to 90%, with errors exceeding 75% on complex issues like cysticercosis disease and schistosomiasis. After the interventions, accuracy increased by an average of 35% in Porteirinha—Elementary School I reached 75% accuracy, and Elementary School II, 85%. Unanswered questions decreased by 53% in Elementary School I and 60% in Elementary School II, indicating greater engagement. Post-intervention evaluations for Pai Pedro and Riacho dos Machados are still pending. Teachers and healthcare agents showed a 40% improvement in knowledge after training. Playful methodologies proved effective in engaging students, particularly in early education, and the integration of schools, health services, and the environment strengthened the actions' impact, suggesting the model's scalability for other regions.

Integrated and holistic management

Targeted selective treatment for gastrointestinal nematode control in sheep: two decades of research and application

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This abstract will detail research findings and experience of over two decades in the practical application and refinement of Targeted Selective Treatment (TST) protocols. Former studies in commercial flocks showed that FAMACHA System (F) could reduce anthelmintic (AH) treatments. Over one-year period, the proportion of sheep that did not require AH treatment ranged from 12,2 to 68,3% and even the entire flock. Sheep evaluation practice revealed that high temperature and evaluator training level could cause F-score misclassification. Subsequently, 47 training sessions involving 1004 participants completing 20,080 classifications demonstrated a significant reduction in average error from 2.5 to 0.56 by the 20th animal ($p<0.05$), with a mean error reduction of 0.0713 per sheep, determined by linear regression. This underscores the importance of evaluator training for accurate sheep classification. Post-project, 69% adopted the system and 83% performed regular assessments. Consistent monitoring showed F's importance as a TST indicator in sheep, but in some conditions, it is important to associate other indicators. A treatment methodology was validated for ewes with $F \geq 3$, $BCS \leq 2.0$ (if $F = 2$), or submandibular edema. Treated ewes in the validation had significantly lower hematocrit (19.2% vs 29.3%; $p<0.001$) and higher fecal egg count (8,747 vs 1,163; $p<0.001$) than untreated ewes. This methodology identified 13% more cases needing AH treatment than using only the F score. Process mining revealed adult ewes spent an average of 78.5 days (d) at F1 and 25 d at F2. Progression from F1 to F2 took 59 d and from F2 to F3 took 26.3 d. More susceptible ewes remained at F1 and F2 for shorter durations (34 and 20 d, respectively), transitioning from F2 to F3 in 19.7 d. These results suggest F-score evaluations should occur every 15 d in late-pregnant/lactating ewes and every 30 d otherwise. This research underscores the significant role of TST in optimizing AH use in sheep.

Defining and discussing principles of anthelmintic stewardship

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The cattle nematodes *Ostertagia ostertagi, Cooperia oncophora* and *Dictyocaulus viviparus* and the trematode *Fasciola hepatica* can cause severe problems for cattle health, welfare and production. Veterinarians and farmers have excessively relied on anthelmintics to control infections and disease. As anthelmintic treatment of cattle has adverse effects on the ecosystem and selects for anthelmintic resistance, "anthelmintic stewardship" (AHS) is needed for the sustainable control of helminth disease. This review aims to provide principles that work towards anthelmintic stewardship, which we defined as follows: "Anthelmintic stewardship is a systematic approach to op-

timize the management on farms of helminth infections while minimizing the use of anthelmintics, minimizing the consequences of helminth disease, reducing the development of anthelmintic resistance and ensuring environmental safety." The principles that anthelmintic stewardship should aim to provide step-by-step guidance for farmers and veterinarians in managing helminths sustainably. As farms vary in their management options, they should be able to incorporate a wide variety of tools. The four principles supporting anthelmintic stewardship are: 1st Predict helminth infection in cattle, using diagnostics and prediction tools. 2nd Prevent helminth disease in cattle using (management) methods aligned with prediction and disease susceptibility. 3rd Diagnose helminth disease in individual diseased animals using disease specific parameters. 4th Exclusively treat diseased animals with helminth infections with anthelmintics. Currently available tools can be leveraged to work towards anthelmintic stewardship. Scientific research should focus on the implementation of these tools, in addition to developing complementing tools and addressing the existing knowledge gaps.

A novel integrated tick management practice: association of strategic acaricide treatments and "back-and-forth of paddocks" ("bate-volta de potreiro") to achieve a sustainable cattle tick control in Southern Brazil

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Control of the cattle tick *Rhipicephalus microplus* relies heavily on chemical acaricides, but their overuse has led to the emergence of multi-resistant tick populations. Therefore, developing integrated and sustainable control practices is essential. This study presents and validates integrated tick management (ITM) protocols that combine strategic acaricide use with a novel model of pasture management practice. We conducted three field trials over nine months on cattle farms in Rio Grande do Sul, Brazil. In each farm, one group of animals was subjected to the experimental protocol, while another group served as a non-treated control. Initially, the experimental groups received different acaricide treatments (two or three treatments based on the result of previous in vitro bioassay), and after 98 days, they were moved to a second paddock (P2) for 42 days, leaving the original paddock (P1) without animals. Before use, the P2 paddocks were used for sheep grazing, or rice cultivation. Upon returning (42 days later, on day 140), the experimental groups were free of ticks. In spite of animals being further reinfested when they returned, their tick load remained low. The experimental groups presented tick infestation 70 - 100% lower than control groups during the study. In all cases, the ITM protocols allowed a successful tick control using only two/three acaricide treatments in a period of 245 days. The 42 days of spelling was significatively shorter than other protocols, which can facilitate its adoption. Also, it makes use of common situations in Southern Brazil, such as farms with cattle and sheep, and rice-livestock integration. The ITM strategy presented here proves to be effective to allow a sustainable tick control in field situations in Southern Brazil. The ITM protocol was named "back-and-forth of paddocks" (or "bate-volta de potreiro"). We are expanding the evaluation

of this protocol in other farms from Southern Brazil.

Parasites of companion animals & exotic pets – Helminth

National survey of gastrointestinal helminths in rural dogs from Uruguay

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Several gastrointestinal helminths in dogs pose a risk to humans, as many of them are potentially zoonotic. In rural areas of Uruguay, dogs are mainly used for livestock tasks, and veterinary supervision is, therefore, not the norm. Traditionally, deworming was only carried out when government officials visited farms during the hydatid disease control program. Since 2023, the government deworming program has been halted to reassess the hydatidosis situation in the country. While *Echinococcus granulosus sensu lato* is of great importance in Uruguay, there is no information regarding the distribution of other zoonotic helminths such as *Toxocara canis* and *Ancylostoma* spp. An accurate diagnosis of the prevalence of zoonotic parasites in dogs is essential for implementing effective and efficient control measures, particularly in the highest-risk areas. The aim of this study was to carry out a national survey to establish the prevalence of helminth parasites present in dog feces from rural areas. The country was divided into 6 zones. Subsequently, in each zone, farms were randomly selected using a geographic information system. The feces were collected in sterile containers and labeled with an internal code. In the laboratory, the feces were first frozen at -80°C for at least 48 hours before processing. A flotation technique was performed, and parasitic eggs were recorded. A total of 527 farms were analyzed, resulting in 438 positive for at least one type of helminth, while the remaining 89 farms were negative. Eggs from the families Taeniidae and Ancylostomatidae, as well as from the genera *Spirometra*, *Alaria*, *Toxocara*, and *Trichuris*, were identified. This is the first national survey reporting gastrointestinal helminths in Uruguay. These findings provide a starting point for designing strategies to control these parasites, especially the zoonotic ones.

Biogeographic patterns of zoonotic gastrointestinal helminths in stray dogs in Peru: environmental and sociocultural drivers

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The presence of stray dogs infected with gastrointestinal parasites is considered one of the leading public health problems in Peru, a country characterized by its environmental diversity across its different ecoregions and diverse sociocultural habits associated with dog ownership, characteristics that constitute zoonotic risk factors. A search strategy based on bibliographic data and reports from our research team was used. Analyzing the three engagement biogeographic ecoregions, the main zoonotic helminths reported in dogs in the Pacific coast ecoregion are: *Toxocara* spp., *Toxascaris leonina*, *Ancylostoma/Uncinaria* spp., *Trichuris vulpis*, *Dipylidium caninum*, *Adenocephalus pacificus* y *Corynosoma australe*; in the Tropical Andes ecoregion are: *Toxocara* spp., *T. leonina*, *Ancylostoma/Uncinaria* spp., *Echinococcus granulosus*, *Taenia multiceps* y *T. serialis*; and in the Amazon Forest ecoregion are: *Ancylostoma/Uncinaria* spp., *Toxocara canis*, *T. vulpis*, *Strongyloides* sp. y *Dioctophyme renale*. These findings lead us to conclude that some helminths are distributed throughout the national territory and have adapted to the different climatic characteristics of the different ecoregions. In contrast, the presence of others depends on the survival capacity of eggs or larvae or is directly related to the distribution of intermediate or paratenic hosts, domestic and/or wild. Their distribution is also involved in popular beliefs about their diet (for example, cattle viscera infected with *E. granulosus* cysts to make dogs more aggressive and thus improve their herd guarding skills) or with the food they have access to (marine fish viscera with plerocercoid larvae of *A. pacificus* y/o cystacanths/juveniles de *C. australe*, on the marine-coast ports).

Efficacy of a single monthly dose of a topical formulation of selamectin and sarolaner (Revolution® Plus) against transmission of Dipylidium caninum by Ctenocephalides felis fleas

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Two laboratory studies were conducted to confirm the efficacy of Revolution® Plus in preventing *Dipylidium caninum* infection in cats for one month through killing of the flea intermediate host, *Ctenocephalides felis*. These studies were compliant with local regulatory, animal welfare, and legal requirements. Two groups of 10 cats proven to be suitable hosts for *C. felis* were used in each study. On Day 0, cats were treated with either the placebo or Revolution Plus at the minimum recommended dose of 6.0 mg/kg selamectin and 1.0 mg/kg sarolaner. Cats were infested with 100 (±5) unfed, *D. caninum*-infected fleas on Days 0 (after treatment), 7, 14, 21, and 30. Live flea counts were conducted on Day 33 (72±2 hours after Day 30 infestation). All cats were euthanized on Day

58, and necropsies were performed to enumerate *D. caninum* scoleces from the gastrointestinal tract of each cat. Placebo-treated cats maintained adequate flea infestations of 22–176 fleas throughout the duration of each study and had infections of 0–255 cestodes, accounting for at least 60% of control cats having ≥ 50 *C. felis* fleas on Day 33 and at least 80% having ≥ 2 *D. caninum* scoleces at necropsy. Only 2/20 placebo-treated cats were cestode-free at the time of necropsy vs. 17/20 of the Revolution Plus-treated cats. Significantly lower mean flea counts were recorded for Revolution Plus-treated cats compared to placebo-treated cats ($P \leq 0.0001$), and efficacy based on arithmetic mean flea counts was 100% (Study 1) and 94.3% (Study 2) on Day 33. Scolex counts in Revolution Plus-treated cats were also significantly decreased compared to placebo-treated cats in both studies, and the efficacy of Revolution Plus based on least squares mean scolex counts was 97.1% (Study 1) and 99.3% (Study 2).

Detection of *Eucoleus* spp. in dog feces from Uruguay

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Capillarids (Nematoda: Enoplea: Capillariidae) are parasitic nematodes that infect a wide range of vertebrate hosts, including companion animals. Depending on the species, they may parasitize the gastrointestinal, respiratory, or urinary tracts. In Uruguay, the genus *Eucoleus* (= *Capillaria*) has been reported in domestic animals through coprological examinations and is generally identified as *Capillaria* spp. To date, species-level identification in Uruguay has only been documented in felines, based on adult and egg morphology, with specimens classified as *Capillaria aerophila*. To detect eggs of *Eucoleus* species in naturally infected dogs using coprological analysis. Dog feces were collected from both public and private areas in Barros Blancos, Canelones, Uruguay. Samples were placed in sterile containers and, for biosafety purposes, were inactivated at –80 °C for at least 72 hours. Willis's flotation technique was performed using a sucrose-salt solution with a specific gravity of 1.33. Eggs were morphologically identified under a light microscope. Images and measurements were obtained using a Dino-Lite AM4025X and DinoCapture software. Quantitative data were analyzed using the Quantitative Parasitology 3.0 software, and 95% confidence intervals (CIs) were calculated using Sterne's exact method. A total of 200 canine fecal samples were examined. Of these, 11.5% (23/200) tested positive for *Eucoleus* spp. (95% CI: 7.69–16.69%). Two distinct egg morphotypes were identified: one with a densely striated shell and a network of anastomosing ridges, consistent with *Eucoleus aerophilus*; and another with a pitted eggshell surface, consistent with *Eucoleus boehmi*. These findings represent the first morphological record of *Eucoleus boehmi* in dogs from Uruguay. Further studies employing molecular techniques are needed to confirm species identity and enhance understanding of its epidemiological significance.

Prevalence of infectious and parasitic agents in domesticated cats in the metropolitan region of Niterói, RJ, Brazil

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The increase in the number of domesticated cats contributes to a higher risk of transmission of diseases of veterinary and zoonotic relevance. In order to investigate the prevalence and risk factors associated with infectious and parasitic agents in the metropolitan region of Niterói, blood and fecal samples from 105 domestic cats were analyzed. According to the ELISA SNAP Feline Triple (Idexx®) test, 0.95% (1/105) were positive for Feline Leukemia Virus (FeLV), 3.81% (4/105) for Feline Immunodeficiency Virus (FIV), and none for *Dirofilaria immitis*. Thirty of 105 (28.5% samples were positive for at least one parasitosis using either the Sheather technique orthe Mini-FLOTAC, while seven (6.66%) were positive at the Baermann technique. Regardless of the technique used, either mono or polyparasitism, among the 105 samples, 18.10% (19/105) were positive for hookworms, 9.52% (10/105) for *Toxocara cati,* 5.71% (6/105) for *Cystoisospora* spp., 3.81% (4/105) for *Platynosomum fastosum* and 1.90% (2/105) for *Trichuris campanula*, *Strongyloides* spp., *Aelurostrongylus abstrusus* or *Toxoplasma gondii*. Among the positive fecal samples, 30% (9/30) were from neutered females and 44.44% (12/27) from non-neutered, 30.77% (8/26) from neutered males and 31.82% (7/22) from non-neutered males. Regarding age, 38.77% (19/49) were up to 1 year old, 34.09% (15/44) were 1-8 years old, and 16,66% (2/12) were over 8 years old. About food consumption, 32.98% (31/94) consumed only kibble and 45.45% (5/11) kibble and homemade food. Regarding hunting habits, 32.20% (19/59) had hunting habits and 34,78% (16/46) did not. About treatment, 30.51% (18/59) of the infected cats were dewormed in the last 12 months and 39,13% (18/46) were not. Regarding housing, 40.84% (29/71) of infected cats lived at home and 20,59% (7/34) in apartments. The data obtained can contribute to the control of the infectious and parasitic agents found in the study area and show the need constant of monitoring in feline populations.

Occurrence of endoparasites in healthy and immunocompromised cats

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FIV and FeLV are the primary viral diseases causing immunosuppression in cats. Gastrointesti-

nal parasites continuously encounter immune system defenses as the hosts trives to eliminate them. Clinical signs are generally nonspecific, including soft stools, bloody diarrhea, anemia, anorexia, vomiting, and dehydration. The severity of these signs depends on the parasite species, parasite load, and the host's immune status. The objective of this study was to identify endoparasites through fecal examination in felines with FIV and/or FeLV. The study population consisted of healthy felines undergoing routine visits or hospitalization at HV-UEL. Blood and spontaneously defecated fecal samples were collected from 30 felines. The IDEXX SNAP® Feline Triple Test was used to detect *Dirofilaria immitis* antigen, feline leukemia virus (FeLV), and antibodies against feline immunodeficiency virus (FIV). Coproparasitological examinations included the Mini-FLOTAC and Hoffman techniques. FIV/FeLV-positive cats were 33.3% (10/30) in the sample analysis. No patients tested positive for *Dirofilaria immitis*. 20% (6/30) of the cats were positive in coproparasitological examination, with 3 of these also being FIV/FeLVpositive. The identified parasites included *Platynosomum* spp. (2/30), *Ancylostoma* spp. (2/30), *Giardia* spp. (1/30), and *Cystoisospora felis* (1/30). In this study, felines with FIV/FeLV showed a higher positivity rate in coproparasitological examination. However, it is necessary to increase the sample size to conclude the result.

Occurrence of endoparasites in dogs detected by Mini-Flotac and Hoffman

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Dogs are constantly identified as reservoirs of parasites, some with zoonotic potential. Gastrointestinal pathogens establish a parasitic relationship with the host to collect nutrients,delaying the development of the parasitized animal and potentially causing gastroenteritis. The objective was to identify endoparasites in healthy dogs using Mini-Flotac and Hoffman. Sixty healthy dogs were selected based on anamnesis, physical examination, complete blood count, and serum biochemistry. Additionally, fecal samples were collected to perform Miniflotac and Hoffman sedimentation technique. The dogs had not received anthelmintic treatment for at least 3 months. Mini-flotac identified 6/60 (10%) positive dogs, while the Hoffman technique identified 7/60 (11.6%). In the identification of eggs, oocysts, and larvae, *Cystoisospora* spp. (5%), *Ancylostoma* spp. (3.3%), and *Trichuris vulpis* (1.6%) were found using Mini-Flotac. For the Hoffman technique, *Ancylostoma* spp. (6.6%), *Cystoisospora* spp. (1.6%), *Toxocara* spp. (1.6%), and *Trichuris vulpis* (1.6%) were detected. It is concluded that, despite the techniques having different principles, it was possible to identify similar parasites in both. Even healthy dogs without clinical signs and with normal blood tests can present endoparasites. Mini-Flotac detected more *Cystoisospora* spp., while Hoffman obtained more positives for *Ancylostoma* spp. Both achieved

the same percentage for *Trichuris* spp., and only Hoffman detected *Toxocara* spp.

Persistence of zoonotic nematode egg shedding in dogs and cats living in an urban area of northeastern brazil and its importance for one health

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Introduction: Although dogs and cats exhibit a range of interactions with humans, they are frequently affected by gastrointestinal parasites of zoonotic importance as *Ancylostoma* spp and *Toxocara* spp and the correct diagnosis is key to treating animals and reducing the risk of environmental contamination of these pathogens among humans. Objective: The objective of this study was to assess the persistence of zoonotic gastrointestinal parasites in domiciled dogs and cats in an urban area of Northeastern Brazil. Methods: This study was conducted with permission from the Ethics Committee on the Use of Animals of ARIS2Lab. In total, 220 fecal samples (110 from dogs and 110 from cats) from January 2024 to November 2024 were collected by spontaneous defecation and processed for search of ova of nematodes by the Mini-FLOTAC® technique. Results: Of the 220 animals, 9,09% (20/220) were positive. *Ancylostoma* spp. was the most frequent parasite identified in dogs, with 6.4% (7/110), followed by *Trichuris vulpis* 1.8% (2/110). Among cats, 3.6% (4/110) of analyzed samples were positive for *Ancylostoma* spp. and 1.8% (2/110) were positive for *Toxocara cati*. Conclusion: The persistency of nematode eggs shedding in pets (dogs and cats) along with the *interaction* of the multi species families, particularly children, increase the risk of transmission of visceral larva *migrans* and cutaneous larva *migrans*, reinforcing the importance of owner's education by veterinarians. This study was sponsored by Boehringer Ingelheim Animal Health.

Epidemiological aspects of endoparasites and vector-borne pathogens in domestic dogs in the metropolitan region of Niterói, RJ, Brazil.

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The domestic dog is host to a wide variety of pathogens, including zoonotic agents. The objective of this study was to evaluate the prevalence of endoparasites and vector-borne pathogens in 108 owned dogs in the metropolitan region of Niterói, RJ, Brazil, by hematological and coproparasitological tests, using the techniques of Mini-FLOTAC, Sheather, DPP (Dual Path Platform) test for *Leishmania* and IDEXX 4DX Plus for *Dirofilaria*, *Anaplasma*, *Ehrlichia* and *Borrelia*. Overall 12% (13/108) of the fecal samples were positive for some intestinal para-

site using the Mini-FLOTAC technique, and 11.1% (12/108) using the Sheather technique. Considering both techniques, *Ancylostoma* spp. was detected in 10.2% (11/108), and *Toxocara canis, Cystoisospora* spp., *Ancylostoma* spp. + *Uncinaria stenocephala* + *Trichuris vulpis* in 0.93% (1/108). Of the 108 blood samples analyzed, 36.1% (39/108) were positive with the IDEXX 4DX Plus test, as follows: 20.4% (22/108) for *Ehrlichia* spp., 6.5% (7/108) for *Ehrlichia* + *Anaplasma*, 2.8% (3/108) for *Ehrlichia* + *D. immitis*, 5.6% (6/108) for *D. immitis*, 0.9% (1/108) for *Anaplasma*, 0.9% (1/108) for *Leishmania* spp. No sample was reactive for *Borrelia burgdorferi*. Among the infected dogs, 5% (2/40) were mixed-breed and 17.5% (10/57) were of defined breed. 6.7% (3/45) of the positives were females and 17.3% (9/52) males, with 26.7% (4/15) castrated and 13.5% (5/37) non-castrated. According to age, 23.1% (3/13) of the positives were less than 1 year old, 10.2% (6/59) were between 1-8 years old, and 12.0% (3/25) were older than 8 years. 10.2% (6/59) of those infected were fed with kibble and 15.8% (6/38) with homemade food. 12.1% (8/66) lived at home, and 12.9% (4/32) lived in an apartment. The present study contributes to the updating of data on endoparasites and vector-borne pathogens in dogs and highlights the relevance of monitoring and surveillance of these infections for the implementation of therapeutic, prophylactic and control measures, aiming at the well-being and health of the human and animal population.

Efficacy of NexGard® Combo (esafoxolaner-eprinomectin-praziquantel) against natural *Mammomonogamus ierei* infections in cats

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Mammomonogamus ierei is a parasitic nematode that infects the nasal cavities of cats in limited geographic areas. Despite reports of prevalence reaching up to 45% in cats on St. Kitts, no large-scale study has evaluated effective treatment options, highlighting the need for a validated therapeutic approach. The objective of this study was to assess the efficacy of eprinomectin, as part of the topical combination product NexGard® Combo (esafoxolaner-eprinomectin-praziquantel), in treating naturally acquired *M. ierei* infections in cats. This was a randomized, negative control, continuous enrollment field study. Based on pretreatment fecal egg counts, cats were assigned to the treatment (20 cats) or untreated control (6 cats) group. Fecal samples were collected from day of treatment (day 0) up to day 8 and analyzed via double centrifugation using Sheather's flotation solution. Study participation for treated cats ended after obtaining three consecutive negative fecal results. Control cats were monitored for up to 8 days and then treated. 81 cats were screened to identify 26 *M. ierei* positive cats. Following treatment, *M. ierei* fecal egg counts became negative within 5 days. Other parasites present included *Ancylostoma* (all enrolled cats), *Trichuris* (16 treated and 5 control cats) and *Physaloptera* (1 treated cat). Fecal egg counts for *Ancylostoma* were negative within 7 days and there was a

>98% reduction in *Trichuris* within 8 days. Untreated control cats remained positive. In the cat positive for *Physaloptera*, adults passed in feces 4 days post treatment. This is the first study to demonstrate the 100% efficacy, based on fecal egg counts, of a macrocyclic lactone, administered topically as a single dose, against *M. ierei* in naturally infected cats. The results support the use of NexGard® Combo as an effective and practical treatment option, particularly suitable for feral or difficult-to-handle feline populations.

Specific gravity determination of helminth eggs from cats using sugar gradient centrifugation

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Sensitivity of fecal flotation, the most commonly used method for diagnosing helminth infections, depends on the specific gravity (SPG) of the flotation solution in comparison to the SPG of the helminth eggs. However, data on the SPG of feline helminth eggs are limited, particularly for parasites prevalent in tropical and subtropical regions. Knowing the SPG of feline helminth eggs could improve diagnostics, enabling better treatment and control programs to protect feline health. This study aimed to estimate the SPG of *Ancylostoma tubaeforme*, *Mammomonogamus ierei*, *Trichuris felis*, and *Platynosomum fastosum*. Six sugar solutions (SPG 1.01–1.31), colored for visibility, were layered in 15 mL conical centrifuge tubes to obtain a consistent density gradient. Egg suspensions (0.25–0.5 mL containing ~115,000 *A. tubaeforme*, 19,900 *M. ierei*, 87,400 *T. felis* or 43,000 *P. fastosum* eggs) were layered on top (3 tubes per parasite) and tubes were centrifuged at 800 × g for 20 minutes. At the end of centrifugation, eggs of each parasite species formed a macroscopically visible band within the gradient, corresponding to the level at which the eggs reached neutral buoyancy. Aliquots were collected using a pipette from three distinct locations: just above the band, within the band itself, and just below the band. The specific gravity of these fractions was determined using a handheld refractometer. Based on the consistent formation of visible bands across replicates, the following SPG ranges were determined: *A. tubaeforme* 1.050–1.0611; *M. ierei* 1.1057–1.1168; *T. felis* 1.1244–1.1487; and *P. fastosum* 1.2065–1.2207. These findings indicate that standard flotation solutions should be effective for detecting *M. ierei*, *A. tubaeforme* and *T. felis* eggs. However, the higher SPG of *P. fastosum* eggs suggests that higher-density flotation solutions are necessary to ensure reliable detection.

Circulation of Vector-Borne and Gastrointestinal Pathogens Among Brazilian Army Military Dogs

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Surveillance of working dogs can help identifying regions of transmission of pathogens. Since the military dogs are exposed to outdoors, where vectors, wild reservoirs and different soils mingle, they can be efficient sentinel of the circulating pathogens. Dogs maintained at Military Organizations (MOs) were included. Samples of 274 dogs were received during 2023. All dogs had blood samples examined at TECSA- Laboratorio, Minas Gerais and 268 of them had fecal samples examined at Laboratório de Parasitologia, Universidade Federal Fluminense. Blood samples were tested for *Dirofilaria immitis* antigen, *Ehrlichia canis*, *Anaplasma platys* and *Leishmania infantum* antibodies. *Babesia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Hepatozoon* spp., *Mycoplasma* spp., *L. infantum* and *Leptospira* spp. were tested by PCR. *D. immitis* was detected in 17 dogs (6.2%), antibodies against *E. canis* in 43 (15.7%), *A. platys* in 18 (6.6%) and *L. infantum* without infection confirmation in 8 (3%). Older dogs (>7 years) presented higher risk of developing antibodies against *E. canis* than the younger (odds ratio 7.4; <0,001). Ten dogs were infected by *L. infantum* (3.7%). One young dog presented *Anaplasma* spp. positive PCR with no antibody detected. Fecal samples were examined using coproparasitological microscopic techniques. Although only 24 (8.9%) dogs were infected, 16 (6%) were from MOs at Northern or Central regions (odds ratio 3.6; p<0.01). The intestinal parasite most identified was *Ancylostoma* spp. 20 (7.4%); *Cystoisospora* spp. 3 (1.1%) and *Toxocara canis* 1 (0.4%). The two flying insects vectored pathogens were less detected in known endemic areas, suggesting that perception of risk in those areas is high, making stakeholders prone to provide preventive measures to dogs. On the other hand, in areas where the risk perception is lower, those measures were less enforced. Therefore, these results must improve awareness of the community to improve prevention.

Histiocytic Enteritis Associated with Srongyloidiasis and Gut Dysbiosis in a Puppy

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Enteritis in puppies is caused by infectious and parasitic diseases. Helminthiasis is usually an acute disease. Strongyloidiasis is an uncommon, zoonotic parasitosis and is underdiagnosed due the difficult diagnosis with conventional techniques. *Strongyloides stercoralis* is one of the smallest nematodes and gained global importance due to its association with severe and sometimes fatal conditions. Dysbiosis is an emergent topic, as the microbiome can be also known as a “new organ”. The relatioship between parasitic diseases and deworming treatments and the induction of dysbiosis still lacks total comprehension. This case was about parasitism associated with unusual histologi-

cal classification and dysbiosis. A 7-month-old Yorkshire Terrier puppy was presented for evaluation with a history of chronic diarrhea. At the physical examination, the puppy growth was evidently impaired and the previous clinical records did not found any parasites in fecal parasit screening. But, the patient was treated with Praziquantel and Fenbendazole as a tentative. Endocospy and colonoscopy was indicated, with na additional evaluation of fecal dysbiosis index and parasite screening. The samples collected at the endoscopy and colonoscopy exhibited parasitic images at the histopathological exam, hystiocitic infiltration and villous atrophy. After that was maked Baermann-Moraes fecal protocol. The new fecal screening was able to identify the presence *Strogyloides larvae*. The treatment consisted in 3 cycles of Ivermectin at 0.5mg/Kg/day that resulted in clinical remission of the diarrhea and its consequences. The dysbiosis index of new fecal analysis was normalized. This case shows the possibility of causing severe structural damages to the gut mucosa, secondary dysbiosis and Ivermectin did not impair the microbiota recovery, in contrast to antibiotics.

Successful treatment of canine roundworm infections with a single administration of a fluralaner, moxidectin and pyrantel chewable tablet in a non-terminal study design

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In the development program for a chewable tablet containing fluralaner (FLU, 10 mg/kg), moxidectin (MOX, 0.025 mg/kg) and pyrantel (PYR, 5 mg/kg), a non-terminal study design was successfully implemented to determine anthelminthic efficacy. Two non-terminal dose confirmation studies were conducted to investigate efficacy of a FLU, MOX and PYR chewable tablet against *Toxascaris leonina* (study 1) and *Toxocara canis* (study 2). Study 1 was a single-site study in dogs inoculated with L3 of *T. leonina*. Using fecal egg counts (FECs), dogs were blocked randomized to groups (10 per group). Study 2 was a multi-site study in 110 naturally hookworm or roundworm infected animals. Dogs with at least 100 eggs per g feces for hookworm and/or *T. canis* were eligible for inclusion. On day 0, dogs were treated once orally, with either FLU, MOX and PYR chewable tablet, a chewable tablet with FLU (10 mg/kg) and milbemycin oxime (0.75 mg/kg) or were left untreated. Two weeks after treatment, all groups were treated orally with Profender® tablets for dogs as diagnostic dewormer (DDW). The primary efficacy objective was determined by counts of expelled worms recovered after DDW treatment. Copro Antigen-ELISA (PetCheck™ IP), PCR and FECs were completed at key time points. Adequacy of infection was demonstrated in the control group in study 1 and in study 2 in the control animals for *T. canis*. Relative to the control groups, statistically significant reductions of mean worm counts were found in FLU, MOX and PYR chewable tablet groups in both studies (99.4% *T. leonina*, 97.5% *T. canis*; $p<0.05$). FECs and findings from molecular methods were confirmatory of the conclusions from worm counts. A single dose of FLU, MOX and PYR chewable tablet is effective in the treatment of canine infections with adult *T. leonina* and *T. canis*. Non-terminal stu-

dies present a valid means of determining the anthelminthic efficacy against roundworms in dogs.

Emerging *Strongyloides stercoralis* infections in dogs from Buenos Aires: a case series with molecular and therapeutic insights

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Strongyloides stercoralis is a nematode affecting multiple species, including dogs and humans. While Buenos Aires City was considered non-endemic area, a recent case was reported in a 7-month-old dog with no travel history. *S. stercoralis* mitochondrial gene *cox1* haplotype 20 (HP20) was identified, responding correctly to moxidectin therapy. This led to the inclusion of the Baermann technique in our diagnostic protocol for puppies with intestinal and/or respiratory signs. The aim of this study was investigated *S. stercoralis* infection in puppies under 12 months through parasitological and molecular diagnosis, parasite genetic typing, and therapeutic response evaluation. Between December 2023 and December 2024, first-stage larvae were detected in three puppies from Buenos Aires, all with persistent diarrhea and from different kennels. Molecular analysis, including 18S rRNA gene amplification (18S-PCR) and *cox1* typing, was performed to confirm the diagnosis and determine genetic variants, respectively. Following diagnosis, a single oral dose of moxidectin (0.2 mg/kg PO) was administered. Treatment success was assessed based on clinical improvement and the absence of larvae in Baermann after seven days. If initial treatment failed, fenbendazole (50 mg/kg PO for 5 consecutive days) was administered, with follow-up evaluation using Baermann. Genetic analysis identified haplotype HP16 in two cases and HP20 in one. The HP20 case responded to moxidectin, while both HP16 cases required fenbendazole. This is the first clinical case series of *S. stercoralis* in dogs in the Americas beyond molecular characterization. Our findings support the routine use of the Baermann technique in the diagnostic workup of dogs with clinical signs. Moreover, our data suggests that the infecting *S. stercoralis* haplotype could possibly be linked to treatment response, warranting further investigation. Long-term follow-up of these cases will provide additional insights into treatment outcomes.

Parasites of ruminants and horses

Ivermectin pharmaco-parasitological evaluation on different beef cattle anthelmintic resistance scenarios in Italy and Argentina

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Beef cattle farming plays a crucial role in the agricultural economies of both Argentina and Italy. Although both countries have grazing systems of meat production, the heterogeneity of these systems leads to different parasitological scenarios regarding epidemiology and anthelmintic resistance. In this context, considering ivermectin (IVM) is the most widely used anthelmintic for treating gastrointestinal nematodes in cattle, the current study evaluated the efficacy and the pharmacokinetic profile after the subcutaneous (SC) administration of ivermectin (IVM) given both to young calves and adult cows in ten commercial cattle farms from Italy (A to E) and Argentina (F to J). While in Italy adult cows and young calves were included in the study, in Argentina only calves aged 8-14 months old were selected to perform the efficacy trial. On each farm fifteen (15) calves/cows naturally infected with gastrointestinal nematodes were treated with IVM (0.2 mg/kg). The efficacy was determined at 14 days after treatment by the FECRT. Six (6) adult cows and eight (8) young calves treated with IVM were randomly selected to perform the PK study with blood samples being taken from these animals at regular intervals. Drug concentrations were measured by HPLC. Similar IVM PK trends were obtained for both adult and young cattle. The IVM systemic exposure (expressed as AUC) obtained for adult cows (380±158 ng.d/mL) was similar to that observed in young calves group (313±85.5 ng.d/mL). No statistical differences between both categories were observed for the most representative PK parameters (P>0.05). Surprisingly, while in Argentina IVM resistance was present in all the farms (90% CI = 0%-95%), in Italy a susceptible nematode population was presented in three farms (90% CI = 96%-100%) and a low level of IVM resistance in the rest of the farms (90% CI = 92%-98%). *Cooperia* spp. and *Haemonchus* spp. were the main genera resistant to IVM. In conclusion, IVM can be effectively used in both young cattle and adult cows. While its efficacy remains notably high in Italy, its use should always be preceded by a diagnostic assessment of the nematode population's resistance status.

Prevalence of *Cryptosporidium* spp. infections on dairy farms in Northeastern Brazil

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Cryptosporidiosis is caused by *Cryptosporidium* spp. and affects a wide range of hosts. It is an important cause of diarrhea in both humans and animals, transmitted through ingestion of oocysts. In cattle, it leads to significant economic losses, especially in calves, and is common-

ly associated with species such as *Cryptosporidium parvum*. To determine the prevalence of *Cryptosporidium* spp. and *C. parvum* in dairy farms located in the states of Paraíba, Ceará, and Rio Grande do Norte, Northeastern Brazil. A total of 270 fecal samples were collected directly from the rectal ampulla of calves up to 21 days of age: 120 from Paraíba, 53 from Ceará, and 97 from Rio Grande do Norte. Samples were processed using the acid flocculation method followed by centrifugation to concentrate the oocysts. Subsequently, 10 freeze–thaw cycles in liquid nitrogen were performed. Genomic DNA was extracted using a commercial kit according to the manufacturer's instructions. Nested PCR (nPCR) was carried out to detect *Cryptosporidium* spp. and *C. parvum*. PCR products were separated by electrophoresis on 1.5% agarose gel at 80 V for 40 minutes and visualized under UV light. In the state of Paraíba, 15% (18/120) of the samples tested positive for *Cryptosporidium* spp. and 5% (6/120) for *C. parvum*. In Ceará, the positivity rates were 39.6% (21/53) for *Cryptosporidium* spp. and 20.7% (11/53) for *C. parvum*. In Rio Grande do Norte, 10.3% (10/97) of the samples were positive for *Cryptosporidium* spp., and 5.1% (5/97) for *C. parvum*. Overall, *C. parvum* was detected in 8.14% (22/270) of the total samples. *Cryptosporidium* spp. is present in the cattle herds studied, and the detection of *C. parvum* in different states highlights the importance of this protozoan as an enteric pathogen in calves and its zoonotic potential.

Molecular diagnosis of *Giardia duodenalis* in dairy calves from Northeastern Brazil

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Giardiasis is a significant disease that affects cattle and has high zoonotic potential, being one of the most common parasitic infections in humans. Among the species of the Giardia genus, *Giardia duodenalis* is the only one capable of infecting both humans and animals. The prevalence of this parasite varies depending on different management systems and production strategies, and it is particularly common in dairy farming. To investigate the occurrence of *G. duodenalis* in calves from dairy farms in the state of Paraíba, Brazil. A total of 38 dairy farms located in 12 municipalities of Paraíba were visited. Fecal samples were collected from 115 calves aged up to 30 days. Cyst concentration was performed using the acid flocculation method, followed by cycles of freezing in liquid nitrogen and thawing in water at 56 °C to disrupt the cyst wall. For Polymerase Chain Reaction (PCR), a protocol developed in collaboration with the Moredun Research Institute (MRI) was used. DNA was extracted using the Macherey-Nagel NucleoSpin® Tissue kit. Detection of *G. duodenalis* was carried out using nested PCR (nPCR). PCR products were subjected to electrophoresis in 1.5% agarose gel at 80 V for 40 minutes and visualized under UV light. Of the 115 calves analyzed, 17 (14.8%) tested positive for *G. duodenalis* by nPCR, showing amplification of a 511 bp fragment of the β-giardin gene. Of the 38 farms assessed, 14 (36.8%) had at least

one positive animal. Among the 17 positive calves, 13 (76.5%) were aged ≥ 20 days. This study identified the presence of *G. duodenalis* in calves in the state of Paraíba, highlighting its zoonotic potential and epidemiological relevance. Infected animals may act as reservoirs for the parasite, posing a risk to both human health and livestock.

Evaluation of Anti-*Cryptosporidium parvum* hyperimmune igy therapy in calves with experimentally induced cryptosporidiosis

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Cryptosporidium parvum is one of the most common and significant enteropathogens responsible for diarrhea in neonatal calves. In the absence of highly effective drug therapies, passive immunotherapy using immunoglobulin Y (IgY) derived from hyperimmune egg yolks has emerged as a promising alternative. This study evaluated the efficacy of anti-C. parvum IgY, produced from hens immunized with sonicated C. parvum oocysts, in neonatal calves with experimentally induced cryptosporidiosis. Eighteen newborn calves were randomly assigned to two groups (n = 9 each): an IgY-treated group (GIgY) and an untreated control group (GC). From 12 hours after birth until day 7, GIgY calves received 10 mL of anti-C. parvum IgY concentrate twice daily, while GC calves were fed the same diet without supplementation. On day 3, all animals were orally challenged with 1×10^6 C. parvum oocysts. Fecal samples were collected daily until 32 days of age. On the third day of diarrhea onset, a 24-hour total fecal collection was performed to determine dry matter content. Outcomes assessed included oocyst shedding (using a Neubauer chamber), duration and severity of diarrhea (based on fecal scoring), and acid–base status (via blood gas analysis) at inoculation and on diarrheic days 1, 3, 5, and 7. Calves in the GIgY group shed fewer oocysts overall and for a shorter duration. Peak oocyst shedding occurred on day 5 post-challenge in GIgY calves (1.55×10^5 oocysts/g), compared to day 7 in GC calves (7.22×10^5 oocysts/g). Diarrhea persisted for 6 days in the GIgY group versus 13 days in the GC group, with lower average fecal scores (2 vs. 3). Fecal dry matter was significantly higher in the GIgY group (9.03%) compared to the GC group (6.02%), reflecting reduced fecal volume and weight. Additionally, the GC group showed a greater base deficit on diarrheic day 5, which remained elevated until clinical resolution. In summary, oral supplementation with specific anti-C. parvum IgY significantly reduced oocyst shedding and shortened the duration and severity of clinical diarrhea. These results demonstrate the effective local action of the hyperimmune IgY and support its potential as a promising, low-cost immunothe-

rapeutic strategy for managing cryptosporidiosis in neonatal calves.

Molecular prevalence of *Trypanosoma vivax* in dairy cattle herds in the Brazilian Semiarid region: Report and monitoring of an outbreak

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Trypanosoma vivax is a protozoan parasite that causes clinical disturbances and mortality in cattle. In South America, where its biological vector (*Glossina* spp.) is absent, transmission occurs mechanically via hematophagous flies or through shared needles and syringes. In the Brazilian Semiarid region, the infection is not endemic and typically occurs as sporadic outbreaks, often severe and economically significant. To determine the prevalence of *T. vivax* in dairy cattle herds in the states of Paraíba and Ceará (Brazilian Semiarid region) and to report the occurrence of a trypanosomiasis outbreak. A total of 393 blood samples were collected from cattle in Ceará and Paraíba using EDTA tubes. Genomic DNA was extracted with a commercial kit following the manufacturer's instructions. PCR was performed using primers TviCatL-F and DTO155-R under the following thermal cycler conditions: initial denaturation at 94 °C for 10 minutes; 35 cycles of denaturation at 94 °C for 1 minute, annealing at 65 °C for 1 minute, and extension at 72 °C for 1 minute; followed by a final extension at 72 °C for 10 minutes. Amplicons were separated by electrophoresis on a 1.5% agarose gel at 80 V for 40 minutes and visualized under UV light. Positive samples yielded a 177 base pairs (bp) product. Of the 393 samples, 1.02% (4/393) tested positive for *T. vivax*, all from a single farm in Orós, Ceará. At the time of collection, repeated abortions and estrus recurrence were reported in the herd. Recently introduced animals and reuse of needles and syringes in the herd were also reported. The outbreak occurred during the dry season, in the absence of mechanical vectors. The prevalence of *T. vivax* in cattle from the Brazilian semiarid region is low. However, its capacity to cause outbreaks underscores the need for surveillance and preventive measures in the region.

Perspectives of Brazilian veterinarians in the control of parasitic infections in horses and the relationship to One Health

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Parasitic infections in horses are a global concern, affecting animals from their first weeks of life. Routine control methods include, anthelmintic use, fecal egg count (FEC), drug efficacy testing, and pasture management. Anthelmintics are sold without prescription in Brazil. This study aimed to investigate (1) the use of antiparasiticides by equine veterinarians, the correlation with One Health, and (3) their perception of a prescription-only practice in Brazil using an open questionnaire. The project was approved by the Research in Animal Ethics Committee of UFPR, n099067/2021. The document had 26 questions divided in: demographic data, perspectives on the interaction between humans, animals, and the environment, and adopted parasite control protocols and the perception of the necessity for a veterinary prescription for dewormers. The survey included 84 professionals from 16 states. The majority (82%) of the respondents had more than 5 years of experience. Ivermectin was the most used antiparasitic (52%), followed by moxidectin (33%) and praziquantel (27%). Most veterinarians (79%) reported using predefined treatment intervals. 54% of professionals rotate chemical classes, while 27% base their decision on efficacy tests. Furthermore, 52.4% of veterinarians reported evaluating the efficacy of the prescribed anthelmintics. About One Health, 92.9% agreed that animal and human welfare are directly related. Veterinarians agreed (84.5%) that horse health can impact human health (workers, vets) and vice versa. Regarding the regulation of antiparasiticides through veterinary prescriptions, only 6% of respondents were opposed or had no opinion on the matter. There is a need to integrate regular FEC and drug efficacy test for appropriate parasite control, preventing anthelmintic resistance. Prescription only may be an important step to maintain horse, human and environmental health, renovating the welfare indicators (phenotypic markers) as a step for One Health.

Lungworm Infections in Cattle from the Brazilian Amazon: The Influence of Seasonality and Climatic Factors

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Lungworm infections caused by *Dictyocaulus* species have a significant impact on livestock health and productivity. In cattle, the epidemiology of dictyocaulosis is well studied in temperate climates but remains poorly understood in tropical regions. We investigated the seasonality of *Dictyocaulus* infection in beef cattle from the Brazilian Amazon and evaluated the influence of environmental conditions on infection prevalence.A longitudinal study was conducted on a commercial farm in Maranhão State, northeastern Brazil, monitoring two groups of untreated Nellore heifers: Group 1 (16–18 months; n=102; June 2022–August 2023) and Group 2 (9–10 months; n=87; December 2022–August 2023). Fecal samples were collected at 15-day intervals and analyzed using a modified

Baermann technique, multiplex PCR, and next-generation sequencing.Climatic variables were recorded and statistically analyzed.A marked seasonal pattern was observed. In Group 1, prevalence peaked during the dry season (28.7%) and decreased thereafter. In Group 2, infection prevalence was significantly higher during the rainy season (41.2%) than in the dry season (12.8%), with an odds ratio of 4.78 (95% CI: 2.74–8.63). Molecular analyses confirmed the presence of *Dictyocaulus* at the genus level, although some sequences indicated nonspecific amplification or unidentified species. Infection prevalence was positively correlated with rainfall and humidity. Younger animals were more susceptible, likely due to immunological immaturity. These findings emphasize the role of seasonality in lungworm transmission in tropical regions and support the need for age- and season-specific parasite control strategies. Further research using species-specific molecular diagnostics is essential to accurately identify the *Dictyocaulus* species involved and guide more effective interventions.

Evaluation of the reproductive biology and histological analysis of the ovaries of engorged *Rhipicephalus microplus* females recovered from cattle treated with fluralaner

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In this study, we evaluated the effects of fluralaner on the reproductive biology and ovarian morphology of engorged *Rhipicephalus microplus* females. For the study, three calves were artificially infested every three days with approximately 5,000 larvae and kept in stalls. On day 0, the animals were treated with a commercial formulation containing fluralaner (Exzolt 5%® - 2.5 mg/kg, MSD Animal Health). Before the acaricide was applied, engorged *R. microplus* females were collected to constitute the control group (10 for biological analysis and 5 for histological analysis). One hour after the commercial formulation was applied, the stalls were washed. Naturally detached engorged females were recovered at intervals of 1-6, 6-12, 12-24 and 24-48 hours after treatment (10 engorged females/period) to assess reproductive biology, and between 12-24 and 24-48 hours (5 engorged females/period) for histological assessment of the ovaries. In terms of reproductive biology, the treatment had an impact on egg laying and the percentage of hatching, resulting in control percentages of 98.5, 97.5, 99.9 and 100% for ticks recovered at intervals of 1-6, 6-12, 12-24 and 24-48 hours, respectively. Females recovered in both treated groups produced lower quantities of type V oocytes and females recovered between 24-48 hours showed an increase in the number of type I oocytes, exhibiting lower viability when compared to oocytes from the control group ($p < 0.05$). The changes observed in the ovaries of the treated females were: irregular oocyte shape, presence of vacuoles, less vitelline and a reduction in the number of type V oocytes ($p < 0.05$). Thus, we can conclude that fluralaner affected the reproductive

biology of engorged *R. microplus* females in all evaluation periods, with a control percentage of over 98%. We correlate this effect with the reduction in the number of mature oocytes and the morphological changes that were possibly caused by fluralaner.

Effect of acaricide treatment on the reproductive parameters of the cattle tick subjected to immersion and spraying with different droplet sizes under laboratory conditions

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Rhipicephalus microplus is a major ectoparasite affecting lactating cows. Due to increasing tick resistance, optimizing acaricide treatments is essential. While spraying equipment influences efficacy, the role of droplet size—well-documented in crop protection—remains unclear in tick control. This study evaluated the impact of droplet size on acaricide efficacy and tick reproductive parameters under laboratory conditions. Experimental infestations with *R. microplus* larvae produced engorged females, which were divided into homogeneous groups of 10 ticks. Treatments were applied using a pressurized power sprayer (Yamaho®, 100 psi) and included: C – control, I – immersion, MD – medium droplets (200–400 µm), and UCD – ultra-coarse droplets (>400 µm). The acaricide used was a combination of organophosphate and pyrethroid (Flytion® EC50). Treatments were conducted in triplicate, with a 3-minute exposure. Post-treatment, ticks were individually placed in well plates and kept in a climate-controlled BOD chamber (27±1°C, 85±5% RH). After 18 days, egg masses were individually weighed and incubated under the same conditions for an additional 18 days until larval hatching. Treatment efficacy for groups I, MD, and UCD was 52%, 52%, and 60%, respectively. No significant differences in oviposition weight were observed among the groups: 0.1121 g (C), 0.0670 g (I), 0.0667 g (MD), and 0.0630 g (UCD). However, larval hatching rates differed significantly ($p<0.05$): 56% (I), 46% (MD), and 32% (UCD). This is the first study to assess droplet size effects on acaricide application under laboratory conditions. Further research is needed to evaluate other spraying parameters and pressure variations in field conditions for improved tick control in dairy cattle.

Evaluation of treatment protocols for equine oxyuriasis

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Parasitic resistance, driven by the indiscriminate and empirical use of antiparasitic drugs, remains a persistent concern in commercial

livestock operations. In horses, the strong research focus on Strongylidae often leads to the neglect of other parasites and a lack of reports on the broad efficacy of available antiparasitic treatments. *Oxyuris equi* is a nematode commonly found in equid populations which, despite its low pathogenic potential, causes notable economic losses and compromises animal welfare. This study aimed to evaluate the efficacy of different antiparasitic drugs, doses, and routes of administration for the control of *O. equi*. Fifty-one adult not dewormed for at least three months prior to the study were selected. The Graham Test was used to detect and quantify eggs, with animals presenting at least one egg considered positive for *O. equi.* Horses were randomly allocated into eight treatment groups: oral ivermectin (ol; n=6), transrectal ivermectin (tl; n=7), combined oral and transrectal ivermectin (otl; n=7), oral ivermectin with transrectal piperazine (oltP; n=7), oral ivermectin with rectal trichlorfon (oltT; n=6), oral piperazine (oP; n=6), oral trichlorfon (oT; n=7), and a double dose of oral ivermectin (ol2; n=5). Doses were based recommendations according to body weight. The Graham Test was repeated on days 7 and 21 post-treatment, and animals were considered positive if eggs were found in either evaluation. A total of 23 out of 51 horses remained positive, distributed across all groups. Briefly, lo=3/6 (positive/treated), lt=5/6, lto=1/7, loPt=3/7, loTt=4/6, Po=3/6, To=2/7, lo2=2/5. The findings indicate that none of the tested treatments achieved 100% efficacy, even with variations in administration routes, drug combinations, or dosage. It is concluded that the performance of drugs commonly used to control of *O. equi* remains insufficient, even when using modern and widely adopted macrocyclic lactones such as ivermectin.

Study model to measure the efficacy of a pharmaceutical product for the control of chorioptic mange in cattle

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Bovine chorioptic mange is a rare disease in tropical regions of the world. Infestation with *Chorioptes* mites causes irritation, dermatitis and pruritus, negatively impacting animal welfare and milk production. Evaluating the efficacy of antiparasitic products against chorioptic mange in cattle is challenging since no artificial infestation experimental model exists, so using naturally infested animals are mandatory. Asymptomatic infections, natural and seasonal fluctuation in the number of mites on the animals, irregular distribution of mites in the lesions, the small size of the lesions in most of the affected animals that may limit weekly scrapings for mite counts and impact parasite total recovery midterm, are key concerning points. We report a successful efficacy study model for *Chorioptes texanus,* using naturally infested lactating adult Holstein cows from a commercial dairy farm in Brazil. Two 2x2cm skin areas on the border of mange lesions were scraped before treatment and at Days 7, 14, 21, 28, 42 and 60. Mites were counted immediately after sampling with the use of a stereomicroscope. Twenty-four cows with more than 30 mites (47 to 1798) were included in the study, ranked in 12 replicates of two animals, and within replicates randomly allocated into treated and untreated

groups. Arithmetic means of mite counts were above the minimum number for the inclusion and maintenance criteria in the control group (respectively 458.2, 284.7, 113.1, 113.7, 115.4, 63.3 and 41.9) and mites were recovered from at least eight animals at all study timepoints. In the treated group, mean mite counts were 424.7, 1.2, 0.2, 0.1, 4.3, 0.00 and 0.00, with only three to one positive cow detected per day between days 7 and 28. Even though there was a decrease in mite counts in the controls, an adequate number of mites and positive cows were available throughout the 60 days of the study, allowing a proper efficacy evaluation of the investigational veterinary product.

Molecular analysis of congenital and primary infections by *Anaplasma marginale* in calves from Minas Gerais, Brazil

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Anaplasma marginale, an obligate intracellular gram-negative rickettsia, is responsible for bovine anaplasmosis. Endemic in Brazil, the disease can result in major economic losses in livestock. Transmission of *A. marginale* is associated with the presence of ticks, considered biological vectors, in addition to mechanical vectors, such as flies, mosquitoes, and contaminated fomites, as well as congenital transmission. The present study aims to evaluate the dynamics of congenital transmission of *A. marginale* through molecular analyses and the epidemiological impacts. The research was carried out on a farm in Minas Gerais, where, of the 205 calvings performed, 86 Holstein calves were randomly selected for material collection. Blood samples were collected in EDTA tubes on the day of birth and analyzed by nested-PCR for the detection of *A. marginale*. Of these calves, 24.42% (21/86) tested positive at birth. After the first screening, 19 initially positive calves (group 1) and 19 negative calves (group 2) were monitored by nested-PCR every 4 weeks until 24 weeks of age. At the 4th week, eight calves (42.1%) from group 1 and one (5.3%) from group 2 were positive. At the 8th week, 15 animals (78.9%) from group 1 and eight (42.1%) from group 2 were positive. From the 12th to the 24th week, all 19 animals from both groups tested positive. The low positivity at the 4th week in group 1 can be explained by a decrease in rickettsemia in these animals, which were no longer detected as positive by the molecular test. The increase in the frequency of positive animals in the 8th and 12th weeks in group 1 is due to possible reinfections by *A. marginale* at the property, which also occurred simultaneously in group 2. Therefore, it can be concluded that congenital transmission of *A. marginale*, although significant, was not a determining factor in the prevalence of infection, since all animals tested positive at the end of the study. However, animals in group 1 received fewer treatments compared to group 2 because they presented less intense clinical signs of anaplasmosis and lower rickettsemia. Further studies should be carried out on congenitally transmitted strains in herds in relation to protection against possible reinfections by other circulating strains or even the intensity of disease manifestation.

Dynamics of nematode infection in sheep managed in an ILPF Caatinga system.

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Integrated systems allow optimizing the use of land with benefits for nutrient recycling, animal welfare, and the environment. The areas undergo a resting period which affects surveillance of nematode larvae reducing contamination and the need for treatments. Planning a parasite control in the field requires understanding the dynamics of infection within animal categories throughout the production cycle. In 2024, a sheep herd of 120 sows grazing the same area of 12 hectares area of iLPF Caatinga system were monitored. Grazing was alternated in three paddocks with 20m strips of bushland interspersed with 20m strips of cultivated *Panicum maximum* Massai grass and sorghum. The animals were kept on native pasture during the rainy season, having access to the iLPF areas only during the dry season, where they spent 90 days until weaning. Sires (N=10) and lambs (N=100) were kept indoors. All animals were examined monthly by egg counts (EPG) and coprocultures over a 12-month period. Animals with Famacha > 3 received monepantel at a 2.5 mg/kg dose as salvage. Egg counts data (Log X+1) were evaluated per month and for a 12-month period by ANAVA with Tukey's test at 0.05%. Despite the monthly variations observed, overall parasite load of the herd remained relatively low throughout the year, with a significant increase only in the sixth month of evaluation (August/2024) at 2,400 EPG (~60% *H. contortus*). The category responsible for this increase was young females that presented up to 22,000 EPG with no deaths in the period. Anyway, when considering total monitoring period the most infected sheep category was male breeders. These data indicate that although the iLPF system contributes to maintaining a relatively low parasite load, there are situations that require early intervention which also require close monitoring in order to keep the parasite control and animal health in this system.

Cyathostomin larval morphotype distribution after ivermectin treatment in equotherapy horses

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Equines are affected by a wide variety of gastrointestinal endoparasites with cyathostomins being the most prevalent. This group includes more than 60 species subdivided into morphotypes that are distinguishable through the identification of infective third-stage larva (L3). The aim of this study was to report the variation in L3 of cyathostomins present before and

after 2% ivermectin (IVM) treatment on four adult mixbreed equotherapy horses in Fazenda Rio Grande, Brazil. Individual fecal samples were collected before deworming (G0), and at 12h, 36h, 60h, 13 (D13), and 48 days (D48) after treatment. Each sample was analyzed using a standard fecal egg count (FEC) method . Fecal culture (coproculture) and the L3 identification was made according to the literature . On G0, FEC ranged from 250 to 1725 with all samples showing strongyle-type eggs. In the identification, 75% of the animals showed a predominance of morphotypes A (52–65%) and D (30–43%), along with a small presence of types C, E, F, G, and H. The the other group presented a prevalence of types A (37%) and D (46%). At 12h after IVM treatment, the animals continued to shed eggs; however, the average egg hatch was only 0.038%, possibly by IVM egg suppression or residual eggs in feces. At 36 and 60h, a progressive decrease in FEC and egg hatch was observed, and by D13, no eggs were found (100% efficacy of IVM). By D48 (6.8 weeks) after treatment, FEC values varied considerably among the animals, ranging from 250 to 1725 with only strongyle-type eggs observed. L3 identification showed 90% of types A and D. Thus, IVM demonstrated full efficacy, eliminating parasites and rendering eggs nonviable for a few weeks. However, egg reapearance period of almost 7 weeks for cyathostomin returned to pre-treatment levels, including morphotype distribution.

Morphological identification of adult cyathostomins using diagnostic deworming in adult horses

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Cyathostomins are the most prevalent gastrointestinal nematodes in equines, which includes more than 60 species. The eggs are morphologically similar, placing a major challenge for their differentiation. Species identification is more clear when using adult parasites. The aim of this study was to report the cyathostomin species at adult stage through morphology using four mix-breed adult horses used for equotherapy. Animals were housed at an “Inclusion Assoc.” in Fazenda Rio Grande, Brazil. The animals were treated with 2% ivermectin (IVM) for fecal collection (diagnostic deworming) during three days. Homogenized fecal (1 kg) samples were collected individually at 12, 36 and 60 h after treatment using the subsampling (quarter) method of 12 samples. The adult parasites were manually collected from fresh feces and preserved in 5% glycerin alcohol. Fecal egg count was conducted to determine IVM efficacy after two weeks. Six parasites from each animal were selected (n = 24) and identified through morphological comparison. We recovered 1358 parasites from all animals. Parasite elimination were 3, 74 and 23% from day 1, 2, and 3, respectively. The species found were: Cylicocyclus nassatus (29%), Cyathostomum pate-

ratum (29%), Cy. catinatum (17%), C. ashworthi (13%), C. radiatus (4%), C. ultrajectinus (4%), and Posteriostomum imparetentatum (4%). No eggs were found on day 13, indicating 100% efficacy of IVM. This is the first report of cyathostomin identification at species level in the State of Paraná, south of Brazil, demonstrating a series of commonly reported parasites worldwide.

Prevalence and risk factors associated with the parasitic fauna in horses in Trinidad, West Indies

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Horses are susceptible to a range of endoparasites and ectoparasites that can negatively impact their health. To determine the prevalence of ectoparasites and endoparasites in horses in Trinidad and to investigate any association between host factors (e.g., sex and sex) and external factors (e.g., deworming schedule and tick control) with parasite prevalence. A total of 144 horses (56 adult males, 76 adult females and 12 foals) were examined and sampled from 13 farms throughout Trinidad. Data on demographics and farm management practices were also collected using questionnaires. Faecal samples were evaluated using standard floatation techniques, while ectoparasites collected were examined under a dissection microscope and identified based on morphological keys. Endoparasites were detected in the faeces of 71 (49.3%) horses, including strongyles (n=71; 49.3%), *Strongyloides* spp. (n=17; 11.8%), *Parascaris equorum* (n=5; 3.5%), and *Dictyocaulus* spp. (n=5; 3.5%). Faecal egg counts ranged from 0 to 13,800 eggs per gram. The hard tick, *Dermacentor nitens* (n = 263), was the only ectoparasite detected on 28 of 144 (19.4%) horses examined. Overall, endoparasites were more prevalent in foals (10 of 12; 83.33%) compared to adult horses (61 of 132; 46.2%). Age and sex ($p<0.05$) were significant host risk factors for the presence of endoparasites only. These findings underscore the importance of implementing more effective parasite control strategies to enhance the health and well-being of horses in Trinidad.

Dynamics of *Eimeria* spp. in sheep holobiont artificially infected with *Haemonchus contortus*

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The concept of a sheep holobiont underscores the notion that the health and welfare of the organism is contingent on the regulated interaction between its macrobiota, microbiota, biotic and abiotic factors. Throughout their life, particularly prior to weaning, sheep contribute to the enrichment of their intestinal ecosystem by exposure to diverse environments. These environments can introduce organisms such as *Eimeria* spp. in the context of housed production and *Haemonchus contortus* in the context of grazing. Recently, the function of these organisms in the behaviour of other symbionts has

been called into question. The objective of the present work is therefore to analyse variations in oocysts and eggs per gram of faeces using the McMaster technique, with a view to discussing the microbiota-macrobiota dynamics of the ovine holobiont. Fourteen 6-month-old sheep from Topilejo, Mexico City were utilised for the experimental infection. The animals were infected naturally with *Eimeria* spp. and artificially with *H. contortus* by two oral doses of 8000 larvae. The establishment of infection was corroborated on D26 with an average release of 2318 EPG. From -D15 to D30 post-infection, the McMaster technique was used to obtain oocyst and egg counts per gram of faeces, which were subsequently sorted and plotted. The analysis of the results obtained a correlation coefficient *r* of 0.1385, p-value of 0.0209 ≤ ⍺= 0.05, indicating a statistically significant weak positive correlation. This enables us to conclude that the particular dynamics of these two inhabitants of the holobiont are related, although not strongly. Understanding these interactions allows diseases to be studied from a holistic perspective, considering as part of the holobiont dynamics not only the pathogens or pathobionts (within the pathogenic potential), but also that their behaviour depends on aspects related to the animal and its environment.

Beyond the Usual Suspects: Investigating *Oesophagostomum radiatum* Infection in Beef Cattle

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Gastrointestinal nematodes pose a significant challenge to the beef cattle industry worldwide. *Oesophagostomum* is an important genus that affects calves and induces severe disease. However, it has been overlooked, possibly due to its reduced incidence and prevalence relative to other nematode species. This trial aimed to assess the worm burden and evaluate the local immune response in the large intestine of Nellore calves naturally infected by *O. radiatum*. Blood and faecal samples were obtained from twenty-three Nellore calves for packed cell volume (PCV), total plasma protein (TPP), faecal egg counts (FEC), and faecal cultures. The *O. radiatum* FEC was calculated based on total FEC counts and the proportion of L3 in faecal cultures. Worm burden and mucosal thickness, counts of mast cells and eosinophils, and CD3+ were performed after euthanization at 13 months of age. Semi-liquid faeces were observed for the whole experimental period as a clinical sign of intestinal nematode infection. *O. radiatum* burden average was 725, ranging from 50 to 2363, and the last FEC mean was 96, ranging from 0 to 342. Significant correlations were observed between *O. radiatum* burden and FEC (r=0.7741, R2=0.5992; P<0.0001), and between *O. radiatum* burden and PCV (r=-0.6106, R2=0.3728; P=0.002). No significant correlations were observed between *O. radiatum* burden and FEC related to mucosal thickness, eosinophil, mast cells, and CD3+ count in the large intestine mucosa. *O. radiatum* induces anaemia, a clinical manifestation frequently associated with *Haemonchus* infection. In mixed infections, their cumulative impacts must

be taken into account when assessing the reduction of PCV and TPP values. These results highlight the necessity for innovative research aimed at finding answers and advancements in the management of gastrointestinal nematode infections in cattle.

Zinc concentration in the feces of lambs infected with *Haemonchus contortus* and supplemented with zinc oxide nanoparticles

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Zinc is an essential micromineral for animals, playing multiple physiological roles and requiring daily inclusion in the diet. In sheep farming, its use in the form of zinc oxide (ZnO) nanoparticles has gained prominence due to their higher bioavailability and absorption. One of the main challenges in sheep production is endoparasitic infections, with *Haemonchus contortus* being one of the most significant species. This study aimed to evaluate the effects of ZnO nanoparticle supplementation in sheep infected with *H. contortus* by measuring the zinc content in their feces. The experiment was approved by the Ethics Committee for Animal Use in Experimentation of CENA/USP (Protocol No. 001/2021). A 3x2 factorial design was used, with three types of zinc supplementation and two health statuses. Twenty-six eight-month-old Santa Inês sheep were used, divided into six experimental groups: two control groups without zinc supplementation (with and without *H. contortus* infection), two groups supplemented with 50 mg of ZnO microparticles/animal/day (with and without infection), and two groups supplemented with 50 mg of 40 nanometer ZnO nanoparticles/animal/day (with and without infection). Infection began after 30 days of supplementation, with animals receiving 1200 *H. contortus* L3 larvae once per week. Feces were lyophilized and, after microwave digestion, analyzed by inductively coupled plasma mass spectrometry to determine zinc concentration. Animals that received nanoparticles showed higher zinc levels in their feces ($p < 0.05$). No significant differences were observed regarding infection status ($p > 0.05$). However, a significant interaction ($p < 0.05$) between supplementation and infection was found between the infected and non-infected nanoparticle groups. The animals supplemented with ZnO nanoparticles showed higher zinc content in their feces, with a significant difference observed only between the nanoparticle groups.

Evaluation of zinc oxide nanoparticle supplementation through fecal egg counts and body performance in lambs infected with *Haemonchus contortus*

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Zinc is a fundamental micromineral for animals, playing a key role in the immune system and overall development. Zinc oxide (ZnO) nanoparticles have been studied as an alternative to conventional zinc supplementation due to their higher bioavailability and absorption. In sheep farming, one of the main challenges is *Haemonchus contortus*, a blood-feeding parasite that causes anemia and can lead to death in cases of severe infection. This study

aimed to evaluate the effects of ZnO nanoparticle supplementation in sheep infected with *H. contortus* by assessing the number of parasite eggs in the feces and the animals body weight. The experiment was approved by the Ethics Committee for Animal Use in Experimentation of CENA/USP (Protocol No. 001/2021). The experimental design was completely randomized in a 3x2 factorial arrangement, with three types of zinc supplementation and two health status. Sixty Santa Inês lambs were used, divided into six treatments: two control groups without zinc supplementation (with and without *H. contortus* infection), two groups supplemented with 50 mg of ZnO microparticles/animal/day (with and without infection), and two groups supplemented with 50 mg of 40 nanometer ZnO nanoparticles/animal/day (with and without infection). After 30 days of supplementation, the animals in the infected groups received 1200 *H. contortus* L3 larvae in 2 mL, once a week. Animals were weighed and fecal samples collected for egg counts (EPG) every two weeks. The animals in the infected nanoparticle group showed a lower EPG compared to the other treatments ($p < 0.05$), with a significant interaction between time and treatment ($p < 0.05$). No differences in body weight were observed among the treatments ($p > 0.05$). Animals supplemented with zinc oxide nanoparticles showed a lower level of *H. contortus* infection, with no effect on body performance observed across all treatments.

Effects of dietary protein supplementation on gastrointestinal nematodes and host responses in goats around parturition

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Periparturient relaxation of immunity (PPRI) to gastrointestinal nematodes (GIN) in small ruminants is linked to nutrient scarcity. Sheep supplemented with metabolisable protein (MP) have a decreased PPRI, but studies in periparturient goats and exploring individual responses to dietary interventions are limited. Here, we studied the effects of MP supplementation on GIN infections and host responses of goats around parturition. Styrian Pied goats (n=11), naturally infected with GIN, were stabled and synchronically mated. At Day (D) –28 before kidding, two groups of similar faecal egg counts (FEC) were randomly allocated to either a control diet (CD; n=5) or a protein-supplemented diet (PD; n=6), covering 100% or 140% of their MP requirements, respectively. Experimental feeding started on D–24 until D39, with kidding as D0. Individual FEC were assessed twice weekly (eggs per gram [EPG]). Group larval cultures, individual packed cell volume (PCV) and serum anti-*Haemonchus* IgG were performed weekly. Individual whole blood transcriptome was analysed at D39 by RNA-seq. Data was evaluated with generalised linear models (FEC) or repeated measures. FEC increased in both groups but with significantly higher EPG in the CD animals from D18 until D39 ($P<0.001$; D39 mean EPG±SD CD = 4447±3596 vs PD = 1974±1656). The proportion of *Haemonchus* L3 increased in both groups from ~20% (D–24) to 82% and 78% at D39 in CD and PD, respectively. Anti-*Haemonchus* IgG were affected by time but not by diet, with high variability within groups. Goats with ≥ 2000 EPG at D39 (PD n=3

[PCV = 21-27) vs CD n=4 [PCV = 14-25]) had a distinct IgG shift based on different MP intakes in the last 14 days of the trial, with 53-62% higher IgG in PD animals compared with CD goats ($P<0.05$). RNA-seq revealed 48 differentially expressed genes between CD and PD animals (FDR $P<0.05$). MP supplementation moderates the PPRI in goats and leads to variable individual host responses that are being further characterised.

An outbreak of babesiosis and a strategic treatment protocol for young naïve calves in Ponta Grossa, Brazil

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Babesiosis is a vector-borne disease transmitted by the *Rhipicephalus microplus* tick to cattle. Its prevalence varies according to climate and geographical location. The south of Brazil has 24 million head of cattle and is considered an area of enzootic instability for babesiosis. This study aims to describe an outbreak of *Babesia bovis* in neonatal Black Angus calves from Ponta Grossa, Paraná. It was possible to implement a treatment protocol to secure other animals in the herd. Animals (cows and calves) were supposed to be moved to a safe pasture (low tick presence), but dry weather and poor pasture availability prevented it. The cases developed during the high tick season (June and July) 2024, and animals were infected during the first weeks of life. Babesiosis resulted in the death of 13 calves (13/35; 6 females and 7 males) aged between 17–34 days. The diagnosis was based on clinical signs and the presence of ticks on the cows and calves. *B. bovis* was confirmed through blood analysis. Symptomatic calves (fever, hemolytic anemia, incoordination, no abortion) were treated with half a dose of imidocarb dipropionate. Ticks were controlled using a combination of fluazuron and eprinomectin. Emergency treatment aimed to reduce tick numbers and pathogen invasion, giving calves more time to support disease development. Although babesiosis in calves of this age group is rare, we believe that calves were not protected by immunoglobulins ingested through colostrum. The farm is located in an enzootic instability zone, which resulted in low immunity levels even in adult animals, compromising passive immunity. Moreover, the outbreak can also be explained by the large tick numbers in the contaminated pasture. We identify that the climatic, geographical, and management factors, including high exposure to the vector, contributed to this atypical disease outbreak of *B. bovis* in young animals. Herd health was back to normal after treatment.

Health and body development of black angus cattle under the crop-livestock-forest integration system

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Integrated crop-livestock systems for cattle management need to be studied due to the complex environmental conditions imposed on the animals. Parasite prevalence is a challenge in different microclimate conditions. This study was conducted from August 2023 to May 2024 and evaluated the relationship of crop-livestock-forest systems and helminth infection in 23 one-year-old Angus and crossbred heifers (196.5 kg). The study was located at the Center for Technological Innovation in Agriculture, NITA, UFPR. The systems livestock (L), livestock forestry (LF), crop-livestock (CL) and crop-livestock-forestry (CLF) were analyzed. Monthly weighing and parasitological analyses (EPG and coproculture) were performed. The EPG showed a significant difference between systems ($p<0.001$), with the LF having the highest mean and differing from the others systems (L vs. LF, $p=0.013$; CL vs. LF, $p=0.001$, CLF vs. LF, $p<0.001$). The data showed high numbers of eggs and larvae in LF and CLF system. The CL and CLF systems had the lowest average EPG (36 and 17), suggesting that crop component reduced larval viability in the soil. Coproculture revealed the prevalence of *Oesophagostomum* spp. (0–34%), *Cooperia* spp. (0–72%), *Ostertagia* spp. (0–37%), *Trichostrongylus* spp. (6–77%), and *Haemonchus* spp. (3–84%), with a massive predominance of the latter two by the end (Jan-Mar, 2024) of the experiment. The average daily weight gain (DWG) varied over the months ($p<0.001$), possibly influenced by pasture quality. In descending order of live weight gain and average DWG, the results were: CL (199.0 kg; 0.713 kg), L (198.5 kg; 0.711 kg), CLF (196.2 kg; 0.703 kg), and LF (179.2 kg; 0.642 kg). When correlating EPG and DWG, the LF group showed a negative-low correlation ($r = -0.33$; $p = 0.025$). The results indicate that systems with crops reduce EPG, while the forestry component, despite microclimatic benefits, may increase parasite persistence in the environment, negatively influencing DWG.

Snail hosts and fluke infections in cattle: new parasitological findings from Southern Italy

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Liver fluke (*Fasciola hepatica*) and rumen fluke (*Calicophoron daubneyi*) are among the most prevalent trematodes affecting ruminant livestock in Europe. While *F. hepatica* is highly endemic in western European countries, *C. daubneyi* has recently emerged as a significant parasitic threat. In the Mediterranean regions of Italy, *F. hepatica* occurs at low prevalence, whereas *C. daubneyi* is considerably more widespread. Despite their economic and veterinary relevance, data on the intermediate snail hosts responsible for sustaining these parasitic cycles in southern Italy remain scarce. The present study aimed to identify snail species inhabiting cattle-grazed pastures, assess the presence of *F. hepatica* and *C. daubneyi* DNA in the snails, and genetically characterize *F. hepatica* specimens recovered from infected cattle. Fieldwork was conducted on 11 cattle farms in a Mediterranean area of southern Italy. A total of 319 snails were collected and identified using morphological criteria and ITS-2+ se-

quencing, revealing *Galba truncatula* (56.7%) and *Physella acuta* (43.3%) as the predominant species. Molecular screening of 130 snails, grouped into pools of ten, detected *F. hepatica* DNA in *G. truncatula* and *C. daubneyi* DNA in *P. acuta*. BLAST analysis confirmed two isolates of *G. truncatula* (99.75% identity) and one of *P. acuta*. Adult *F. hepatica* flukes were recovered from nine cattle on seven farms and characterized morphologically and genetically, revealing three closely related isolates (99.40–99.79% identity). The concurrent detection of trematode eggs in cattle feces and parasite DNA in gastropods indicates ongoing parasite maintenance within these farming systems. These findings highlight the need for expanded malacological surveillance to elucidate the ecological roles of local snail species, particularly *P. acuta*, in sustaining trematode infections in Mediterranean environments.

Optimization of a new protocol to evaluate the efficacy of diclazuril and toltrazuril in sheep farms

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Ovine coccidiosis, caused by protozoan of the *Eimeria* genus, is usually controlled by anticoccidial drugs, such as diclazuril and toltrazuril from the triazine family. Loss of efficacy is sporadically suspected in farms, but no consensus protocol is available to date. We have developed a protocol for assessing the efficacy of anticoccidials in sheep farm, with the aim of making it simple and cost-effective. We estimated the ideal time post-treatment for evaluating efficacy, the minimum number of animals per groups and the method for calculating this efficacy. In two volunteer farms, ewe lambs naturally infected with *Eimeria* spp. were divided into three groups of 20, balancing in terms of age and weight. The first group was treated with diclazuril (1 mg/kg bodyweight) and the second with toltrazuril (20 mg/kg bodyweight). The last one was an untreated control group. Individual fecal samples were taken from all ewe lambs on the day of treatment (D0) and 5 (D5), 7 (D7) and 10 days (D10) post-treatment. *Eimeria* oocysts were counted individually using a McMaster method. Wilcoxon tests were used to define the best post-treatment time for evaluating the efficacy of the two molecules. Three methods of calculating efficacy were tested, and a bootstrap simulation approach was used to define the ideal number of animals per group. The ideal time between treatment and control is within the range D5 and D7 for diclazuril, and D7 only for toltrazuril. The best way to calculate efficacy is a simple one, allowing each animal to be compared before and after treatment, thus dispensing with the need for a untreated control group. Finally, the estimated minimum number of animals per group is between 10 and 15. This new protocol for evaluating the efficacy of anticoccidial drugs now needs to be tested in a large number of farms.

Are eprinomectin resistant *Haemonchus contortus* isolates more pathogenic than the susceptible ones?

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Haemonchus contortus is a highly pathogenic nematode of sheep due to its blood-sucking mode of nutrition. In French dairy sheep farms, many populations of *H. contortus* have developed resistance to eprinomectin (EPR), the only anthelmitic with a zero withdrawal time for milk. Studies have shown that some parasites are more pathogenic when they are resistant to antiparasitics, but this has not yet been evaluated for EPR-resistant *H. contortus* isolates. This study aims to compare the pathogenicity of EPR-susceptible and EPR-resistant isolates of *H. contortus* in experimental infections. Six groups of ten three months old female-lambs of the same breed and coming from the same farm were equally infected (5000 infective larvae) either with EPR-Susceptible isolates (2 groups) or EPR-Resistant isolates (4 groups). These isolates have been obtained recently from the same geographical area in Southwest of France. A seventh non-infected group of ten ewe-lambs provided reference values. Changes in body weight and several blood parameters were monitored weekly from experimental infection's day to necropsy, 49 days later. No growth retardation was recorded in the infected groups compared to the control one. A significant decrease of red blood cells was observed in all infected groups in comparison with the non-infected one, without any difference between infected groups. Animals infected with three R-isolates showed significantly higher mean corpuscular volumes and red blood cell distribution width (RDW). One S-isolate showed higher RDW. Mean hemoglobin concentration was significantly higher in two R-isolates compared to the other groups. The loss of haematocrit per worm was significantly higher in one R-isolate compared to the other infected groups. Although there was no difference in hematocrit reduction between resistant and susceptible isolates, resistant isolates often induced intense regeneration of red blood cells.

Fitness cost of eprinomectin resistance for *Haemonchus contortus*: a legend?

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Anthelmintic resistance is being reported everywhere. Many solutions are proposed to delay its spreading, including the targeted selective treatment (TST) of animals in order to preserve a refuge population. The proportion of non-treated animals depend directly on the fitness cost of resistance, the lower the cost of resistance, the larger the refuge must be. For parasitic nematodes, fitness can be defined as the number of L3 of second generation obtained from one L3 of the first generation. There are very few data concerning fitness of *H. contortus* resistant to macrocyclic lactones

and none regarding eprinomectin (EPR), which is frequently used in French dairy sheep farms due to its zero withdrawal period for milk. We compared the fitness of EPR-resistant (R) or susceptible (S) isolates of *H. contortus* with a single experimental infection on naïve sheep. Six groups of ten three months old female-lambs of the same breed and the same origin were equally infected either with EPR-Susceptible isolates (2 groups) or EPR-Resistant isolates (4 groups). These isolates have been obtained recently from the same geographical area. Life traits like fecal egg excretion, worm's establishment rate, females' fertility and length, eggs to L3 development rate or survival of larvae to desiccation were measured. Finally, we calculated the overall fitness for each isolate. Differences of life traits were observed between isolates, some of them having higher egg excretions (2 R-isolates) while other having longer females (2 S-isolates). One R-isolate and one S-isolate had significant better survival to dryness than the others. The isolate with the higher overall fitness was a resistant one as was the one with the lowest fitness. Finally, no clear cost of resistance was identified. In the absence of fitness cost for R-isolates, the "size" of the refuge should be high in a TST strategy and the hope to reversion could be very limited.

Molecular Detection of *Trypanosoma vivax* in Cattle from Southern Sergipe

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Bovine trypanosomiasis caused by *Trypanosoma vivax* is a disease of economic and sanitary importance in livestock production, potentially leading to a range of clinical manifestations. In Brazil, the prevalence of this infection and its impacts on production remain insufficiently investigated in the Northeast region. This study aimed to determine the occurrence of *T. vivax* in cattle from the southern region of Sergipe through molecular analysis. Blood samples were collected from 245 cattle on 16 properties across 10 municipalities (Boquim, Estância, Arauá, Pedrinhas, Umbaúba, Itabaianinha, Tomar do Geru, Salgado, Santa Luzia do Itanhi, and Indiaroba) between August 18 and November 26, 2024. To ensure representative sampling, approximately 20 to 25 animals were targeted per municipality, selecting properties of varying sizes in order to reach the total of 245 animals. Blood was obtained via jugular venipuncture, labeled, refrigerated, and subjected to DNA extraction. Detection of *T. vivax* was performed by polymerase chain reaction (PCR) using species-specific primers. The parasite was identified in five municipalities, with the following distribution of positive cases: Boquim (2), Pedrinhas (6), Arauá (1), Umbaúba (13), and Tomar do Geru (1), totaling 23 positive samples (9.38%). Furthermore, the samples were sequenced and, in a preliminary analysis, showed 97% to 100% similarity to *T. vivax*. Most animals did not exhibit evident clinical signs, although some presented reduced body condition scores. No significant reductions in milk production or

reproductive performance were reported, possibly due to the lack of systematic zootechnical monitoring. These findings confirm the circulation of *T. vivax* in the region, underscoring the need for further epidemiological studies to assess the parasite's distribution, as well as the implementation of surveillance and control strategies to mitigate its dissemination and potential impacts on cattle production.

Antibody-based response diagnostics in horses with anoplocephalosis and associated risk factors

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The pathogenic nature of equine tapeworms and insufficient attention to tapeworm control highlight the necessity of a proper and accurate diagnostic approach. The study aimed to detect the tapeworms *Anoplocephala* spp. by serum- and saliva-antibody detection and coprological methods, as well as to analyze the risk factors among horse facilities in Slovakia. Fecal (n=401), serum (n=424), and saliva (n=295) samples were collected from 31 farms in Slovakia. Fecal samples were assessed using double centrifugation/combined sedimentation-flotation technique. Serum and saliva samples were analyzed by serum-ELISA (Horse Serum Tapeworm ELISA) and saliva-ELISA (EquiSal® Tapeworm Saliva Test) to determine the IgG(T) antibody levels against 12/13 kDa excretory/secretory *Anoplocephala* spp. antigens. A questionnaire survey (yard management and anthelmintic preventive practices) was performed for risk factor analysis. Cestode eggs were detected in 1.99% of fecal samples (FP=12.90%), serum-ELISA revealed 39.39% positive horses (FP=83.87%), while EquiSal® testing showed a positivity of 56.95% (FP=96.77%). The effects of variables associated with an individual horse were tested for the positive result of saliva-ELISA. The GLMM analysis revealed four meaningful predictors that significantly impacted the likelihood of tapeworm presence in horses: horse age, pasture size, access to pasture and anthelmintic treatment. The first report of antibody detection against equine tapeworms in Slovakia revealed considerably higher positivity rather than coprological analysis. Increased antibody levels in young horses with unlimited access to pasture grazing on large pastures and treated with anti-tapeworm treatment could consider this category at higher risk of disease with indication of reduced anticestodics treatment efficacy.

Equine Piroplasmosis in aborted fetuses and neonates

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Equine Piroplasmosis (EP) is a tick-borne disease caused by the protozoans *Babesia caballi* and *Theileria equi*. Common symptoms in young and adult animals are fever, anemia, loss of appetite, edema, jaundice, hepatomegaly, and splenomegaly. EP affects the equine industry causing economic losses related to treatment costs, and death. Given the high prevalence of EP in Brazilian equine herds, the present study aimed to diagnose these two pathogens as causative agents of abortion and natimortality by multiplex PCR, in 110 aborted equine fetuses and neonates. The samples were related to convenience sampling sent to the Biological Institute (IB) to perform the differential diagnosis of EP. Thirty-four individuals underwent necropsy, where aliquots of organs were fixed in 10% buffered formalin for histopathological evaluation (paraffin embedding and hematoxylin and eosin staining). Aliquots of organs were kept at -20°C for multiplex PCR analysis targeting *Babesia caballi* and *Theileria equi* through primers aimed at the 18S ribosomal RNA gene sequence. Molecular biology results indicated transplacental transmission of *T. equi* in only one neonate. It´s blood smear, stained using the rapid panoptic method, showed the parasite within the cytoplasm of red blood cells. The necropsy revealed hydropericardium, splenomegaly, and enlarged thymus and lymph nodes, along with a hemorrhagic adrenal gland and congested brain. Histopathological examination of these organs demonstrated thromboembolism in the liver, heart, lung, lymph nodes, adrenal gland, thymus, and brain. Additionally, coagulation necrosis was observed in the convoluted tubules of the kidneys, along with pleural edema and congestion, lymphoid reactions in the lymph nodes and thymus, and moderate hyperplasia in the white pulp of the spleen. Multiplex PCR confirmed *T. equi* transplacental transmission, suggesting EP inclusion among the differential diagnosis of equine reproductive diseases.

Molecular detection of Bartonella spp. in horses from the Baixada Maranhense (Maranhão Lowlands), Brazil

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The Baixada Maranhense region (Maranhão Lowlands) is a typical wetland region in northeastern Brazil, characterized by seasonal flooding, rich biodiversity, and traditional horse rearing. Horses in this region are frequently exposed to ticks and fleas in a humid environment, increasing their risk of infection by *Bartonella* spp. The region's flooding cycles, combined with the overlap of wildlife, livestock, and vectors, create ecological conditions that may

exacerbate the risks of *Bartonella* transmission. This study aimed to detect the presence of *Bartonella* DNA in blood samples from naturally exposed horses raised in the Baixada Maranhense, Brazil. This epidemiological study utilized nested PCR-based targeting fragment of the citrate synthase gene (gltA) specific for Bartonella sp. using four primers GLTAF1, GLTAR1, GLTAF2, GLTAR2, according to Gil et al, 2010. Blood samples were collected on EDTA--containing vials from horses sampled in 21 municipalities within the Baixada Maranhense, with nine animals sampled per property in each municipality. Molecular detection revealed that 22 samples tested positive for *Bartonella* spp. The region's flood-prone wetland ecosystem serves as a reservoir for pathogen proliferation, facilitating the infection of grazing horses. This environmental overlap amplifies zoonotic risks for animal handlers and compromises herd health, underscoring the public health implications of *Bartonella* transmission in this unique biome. These findings highlight the vulnerabilities of livestock health and underscore the need for continued surveillance in flood-prone tropical regions. Future research should investigate species-specific transmission dynamics and explore mitigation strategies to protect animals in ecologically sensitive areas.

Molecular detection of *Rickettsia* spp. in horses from the Baixada Maranhense (Maranhão Lowlands), Brazil

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The Baixada Maranhense region (Maranhão Lowlands) is a seasonally flooded wetland in northeastern Brazil that provides an ideal ecological niche for arthropod vectors such as *Amblyomma sculptum* ticks, known carriers of *Rickettsia* spp. These obligate intracellular bacteria are responsible for zoonotic diseases, posing significant risks to both animal and human health. Grazing horses are among the hosts for these bacteria. This study aimed to detect the presence of *Rickettsia* spp. DNA in blood samples from horses raised in the Baixada Maranhense region of Brazil. This epidemiological study employed nested PCR-based molecular techniques to detect the presence of the bacteria in blood samples targeting the intergenic space 23S-5S rRNA, gltA genes such as primers named CS 239 and CS 1069 according to Pacheco et al., 2011. Blood samples were collected on EDTA-containing vials from horses across 21 municipalities within the Baixada Maranhense, with nine animals sampled per property in each municipality. A total of 90 DNA samples were subjected to analysis. Molecular detection revealed that four samples tested positive for *Rickettsia* spp. in horse blood from the Baixada Maranhense. Despite the region's seasonal flooding and wetland ecosystem, it serves as a reservoir for pathogen proliferation, facilitating infections in grazing horses. This environmental overlap increases the zoonotic risk to animal handlers and compromises overall herd health. These findings underscore the zoonotic threats faced by handlers and the vulnerability of equi-

ne herds in the region. The molecular detection of *Rickettsia* spp. in horses from the Baixada Maranhense highlights the interplay between ecological dynamics and zoonotic risk in this flood-prone wetland area. The identification of *Rickettsia* DNA in equine blood samples confirms the circulation of these pathogens in the region.

A wakeup call for roundworm biosecurity in UK cattle

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Gastrointestinal nematodes (GIN) are often overlooked by cattle farmers, despite impacting on production and growing reports of anthelmintic resistance (AR). Improved biosecurity could slow the spread of AR, particularly in the early stages (as is assumed to be the case in UK cattle GIN). This study explored a) the risk of GIN importation posed by traded cattle, b) on-farm practices for GIN biosecurity and c) farmer perceptions of industry current parasite control advice. A questionnaire and three workshops assessed biosecurity practices and opinions on current advice of UK cattle farmers. Faecal samples were collected at auction markets for faecal egg count (FEC) recovered eggs were assessed for species composition (ITS2) and prevalence of benzimidazole-resistance (BZ-r) markers (β-tubulin). Cattle GIN were typically a low priority for farmers. They recognized AR in the UK cattle industry but few felt it was an important problem for their own business. Quarantine practices varied (44% treated and 78% isolated incoming stock; 2-56 days), and farmers eluded to further variation dependent on the type of cattle and time of year they were introduced. Of the 395 herds sampled from 8 markets, 67% had positive FEC; mean 18±2.8 epg (range 0-747). Molecular analysis of 225 GIN populations found *Ostertagia ostertagi* and *Cooperia oncophora* as the commonest species (42 and 43% respectively), with co-infections observed in 87% of positive herds. BZ-r markers (F200Y/E198A/F167Y) were identified in several species, including *O. ostertagi.* A novel variant (R156C) was identified in several *C. oncophora* populations, the function of which is unknown but may be associated with BZ-r. Findings highlight the need for effective quarantine of incoming cattle. AR is often associated with sheep, but the lack of anthelmintic classes licensed for cattle and AR identified in multiple GIN species limits future control options for cattle.

Nemabiome of gastrointestinal strongyles in wild and domestic herbivores in Romania: implications for the European bison (*Bison bonasus*) conservation efforts

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Nemabiome of gastrointestinal strongyles in wild and domestic herbivores in Romania: The European bison (*Bison bonasus*) was upgraded from “Vulnerable” to “Near threatened” by IUCN in 2019, highlighting the progress in its conservation. Once extinct in the wild, its population has recovered to over 10,000 individuals across Europe, including more than 200 reintroduced in Romania since the 2010s. However, the population bottleneck, resulting in loss of their native parasites and their low genetic variability makes them vulnerable to parasitic diseases. This study aimed to assess the impact of multi-host parasites on captive and reintroduced European bison and examines the correlation between gastro-intestinal (GI) strongylid parasite diversity in bison and sympatric herbivores and potential transmission between hosts. Between 2023 and 2024, faecal samples (n=442) were collected from European bison, cattle, sheep, horse, donkey, red deer, roe deer, wild boar, and chamois across three reintroduction sites (Vânători-Neamț Nature Park, Făgăraș Mountains, Țarcu Mountains), as well as from bisons from captive (Hunedoara Zoo) and semi-captive (Acriș) populations. Samples were analyzed using fecal flotation, and the 336 which tested positive for strongyle eggs were further processed in pools, using Next-Generation Sequencing tools. After DNA isolation, a two-step PCR was performed to generate high-throughput sequencing libraries, sequenced on the MGI platform. Identified GI strongyles belonged to 26 species, revealing different host associations and highlighting transmission risks. These findings contribute to strategic parasite surveillance and control measures for the conservation efforts of the European bison in Romania.

The development of novel molecular diagnostic tests for cattle lice based on the detection of ectoparasite DNA on the bovine skin surface

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Parasitic lice cause animal welfare and production problems for the beef and dairy cattle industries, articularly in colder climates, causing

economic losses of ~$120 million annually in North America. Control depends on blanket macrocyclic lactones and pyrethroids treatment, raising concerns about drug resistance and environmental impacts. Current detection relies on visual inspection, which is time-consuming and insensitive, making more targeted use of parasiticides difficult. Objective 1: Detection of louse DNA on the bovine skin surface through PCR and its characterization using short-read amplicon sequencing. Objective 2: Development of Loop-Mediated Isothermal Amplification (LAMP) assays to detect louse DNA on cattle skin swabs as rapid diagnostic tools. Adult lice specimens and DNA skin swabs were collected from beef and dairy herds in Western Canada and Northern USA. We undertook PCR amplification and metabarcoding of the 18S rDNA gene from genomic DNA extracted from lice and skin swabs. Amplicon Sequence Variants (ASVs) were mapped to reference databases to identify and assess the relative abundance of lice species. We also developed real-time fluorescence LAMP (Rt-LAMP) assays. We successfully identified *Linognathus vituli* and *Bovicola bovis* using 18s PCR metabarcoding on DNA skin swabs. We also developed several Rt-LAMP assays that can detect louse DNA (18s & COX-1) from the skin swabs using primers: PE-1 which was specific for *L. vituli*, PE-2 which was specific for *B. bovis*, and PE-3 which targeted multiple lice species. Sensitivity assessments revealed limits of detection of about 1,000 DNA copies/µL for PE-1 and about 10 DNA copies/µL for PE-2 and PE-3 primers. We have provided proof of concept that louse DNA can be detected on the surface of cattle skin by either PCR-based metabarcoding or Rt-LAMP. We have developed both species-specific and pan-louse Rt-LAMP assays that we plan to transition to colorimetric platforms as rapid on-farm tests.

Using applied and molecular parasitology techniques to characterise nematode populations in wild and farmed deer in the United Kingdom

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Deer and livestock contact is increasing in the UK, either deliberately through the use of rotational grazing strategies on mixed animal farms or unintentionally through co-grazing due to increasing wild deer populations. The question is what impact these interactions may have on the dissemination of livestock parasites and anthelmintic resistance, now and in the future. To quantify, and characterise, the nematode populations in wild and farmed deer in the UK using applied and molecular parasitology techniques. Faecal egg counts and deep amplicon sequencing were used to quantify and characterise nematode species composition (ITS) and benzimidazole-resistance (BZ-r) markers (β-tubulin) of parasite material obtained from faecal samples collected from wild and farmed deer. Faecal samples were collected from wild (Red, Roe, Fallow and Sika) and farmed (Red) deer and gDNA was examined from extracted nematode eggs. A total of 986 (n=645 wild & n=341 farmed) faecal samples were examined for egg counts; 77% (wild) and 89% (farmed) of samples had positive strongyle egg counts with an average ~30 eggs per gram [EPG; range 0–2376].

The commonest species/genera identified were *Oesophagostomum venulosum* and *Ostertagia leptospicularis* in both the farmed and wild deer. Diversity was greater in the wild deer with at least 12 different species being identified with *Ostertagia ostertagi, Trichostrongylus axei and T. colubriformis* predominating the additional species. Egg count and species composition differed between deer species and may reflect their grazing preferences. Genes for BZ-r were only detected in a small number of populations, but in a number of different species. The large parasite diversity in wild deer suggests they could disseminate multiple nematode species to livestock. However, they currently appear to play only a limited role in the spread of BZ resistance and may actually provide a source of refugia.

Red Blood Cell Characteristics in Texel Sheep: Implications for Anemia Diagnosis in Haemonchus contortus infection

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Parasitic infections are the main cause of losses in sheep production, especially due to the occurrence of severe anemia. Therefore, its accurate and rapid identification is crucial for handling the clinical infection. The aim of this study was to compare different methods to detect anemia in White Dorper (DO), Texel (TX) and Santa Inês (SI) sheep breeds. 151 lambs (42 DO, 44 TX and 65 SI) were raised in an endemic area for *H. contortus* infection. The lambs were monitored for faecal egg counts (FEC) and packed cell volume (PCV) from 63 to 189 days of age. At 105 and 189 days of age, complete hemogram was assessed. The criteria for anthelminthic treatment (AHT) were PCV ≤ 24% and FEC ≥ 10,000, or PCV ≤ 22%, and for anemic status were PCV < 24% or red blood cell counts (RBC) < 8 million/mm3. The correlation between PCV by microhematocrit and complete hemogram (Ht) was 0.85. However, PCV and Ht were 0.88 and 0.95 correlated to RBC. 56 lambs were considered anemics based on RBC, but 12 (7 TX – 58.3%) and 9 (6 TX – 66.7%) presented globular volume >24% by Ht and PCV, respectively. Among these divergent measurements, we observed 2 TX with 25% and 30% PCV at D105, but 21% (criteria for AHT) at D126. TX animals presented corpuscular volume means (VCM) and hemoglobin concentration means (CHM) higher ($p<0.001$), but concentration of corpuscular hemoglobin mean (CHCM) lower ($p<0.001$), compared to SI and DO breeds. To our knowledge this is the first report pointing out for differences in erythrocyte sizes among sheep breeds. Since TX present higher VCM compared to the other breeds, the identification of anemic animals through globular volume, as PCV or Ht, may fail, and consequently some animals requiring AHT may be missed. Therefore, the cut-point of globular volume for

this breed may require a review, aiming to avoid death due to parasitic infections.

Survival analysis of lambs from different breeds after worm replacement with a susceptible isolate of *Haemonchus contortus*

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Multiple anthelmintic resistance of the *Haemonchus contortus* populations occurs worldwide. The objective of this study was to evaluate the effect of worm replacement on survival of White Dorper (DO), Santa Inês (SI) and Texel (TX) lambs. Ewes from the three breeds in the final third of gestation, and naturally infected with resistant *H. contortus*, were divided into three groups: Control (C), Partial Replacement (PR) and Total Replacement (TR). The PR and TR ewes were dewormed and artificially infected with 3,000 L3 of a *H. contortus* susceptible isolate (Echevarria1991), and divided into two paddocks: one naturally contaminated with resistant parasites (PR) and another free of contamination (TR). Group C was not submitted to worm replacement or anthelmintic treatment, and animals were allocated into a naturally-contaminated pasture. Lambs born in each group in two birth seasons (85 C, 67 PR, and 75 TR, totaling 227 animals) were phenotypically monitored for fecal egg count (FEC), packed cell volume (PCV), weigh gain and anthelmintic treatments from day 42 to day 189, every 21 days. Lifesaving treatment with albendazole and levamisole was applied to animals with FEC $\geq$ 10,000 and PCV $\leq$ 24%, or with PCV $\leq$ 22% independently of FEC. Kaplan-Meier survival analysis was performed considering the number of lifesaving treatments within each group and breed. Throughout the 189 day-period, the survival curve analysis revealed significantly lower ($p<0.001$) number of lifesaving anthelmintic treatments in lambs from PR and TR compared to C group and in SI compared to DO and TX breeds. The proportion of the lambs surviving at 189 days post-lifesaving anthelmintic treatment were 7%, 12%, and 14% in the C, PR, and TR groups, respectively. At the same time point, survival among breeds were DO: 2%, SI: 41% and TX: 6%. Thus, these results reinforce the potential uses of worm replacement and Santa Inês breed as sustainable strategies for controlling *H. contortus* infections in sheep.

Characterization of gastrointestinal nematode egg shedding in dairy goats from Mexico

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Nematodosis can affect the health and productivity of dairy goats. Control is challenging

due to the need to avoid anthelmintic (AH) residues in milk. Currently, AHs are used rationally through targeted selective treatment strategies. The selection of individuals is based on thresholds such as eggs per gram of faeces (EPG), body condition, FAMACHA, diarrhoea, weight gain and milk production. This study aimed to characterize a dairy goat flock based on EPG shedding by analyzing the fortnightly distribution of parasite egg elimination. This approach was adopted to promote the rational use of AH. The present study was conducted in a dairy goat flock in Tequisquiapan. Queretaro, Mexico. Over a period of 14 months, the gastrointestinal nematode burden of 54 French Alpine goats in production was evaluated fortnightly. The fecal samples were processed using a modified McMaster quantitative technique with a sensitivity index of 50 EPG. EPG values were analyzed using measures of central tendency. Goats were classifed into three groups: low (<Q1), moderate (Q2 and Q3), and high shedders (>Q3). The Kruskal-Wallis test was followed by the Wilcoxon test with Holm's correlation for multiple adjustments in order to test for differences between groups. Fourteen animals were identified as low, 33 as medium, and 10 as high shedders. The low maintained at a level of <1000 EPG, while the moderate were maintained at >1000 EPG during high parasite burden months (September to January). The high shedders demonstrated a mean of 4000 EPG. A significant difference was found between the groups ($p<0.05$). Characterizing EPG shedding helps identify susceptible, resilient, or resistant animals. This facilitates the implementation of selective deworming strategies, optimizes the rational use of AH, reduces costs and minimizes residues in milk and the environment through sustainable parasite control.

Ecological niche of Cyathostomins and *Strongylus* spp. in a horse farm in Mexico

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Equines are natural hosts for gastrointestinal nematodes, with the subfamily Strongylinae being the most common. These are divided into migratory (Strongylinae) and non-migratory (Cyathostominae) species, with the former being more pathogenic. In practice, veterinarians have targeted anthelmintic treatments against Strongylus spp, which may have led to a decrease in their prevalence. The aim of this study was to identify gastrointestinal nematode species in a horse farm for evidence of the presence of cyathostomins through monthly larval culture and morphometric examination of infective larvae. The study period was 27 months, from September 2022 to December 2024. The study was conducted in a horse farm in the Avandaro, State of Mexico. The parasite burden of an average of 285 horses (+44 animals) of different gender and ages, ranging from two--month-old foals to geriatric animals over 25 years old, was evaluated monthly. Larval cultures were performed to identify 100 infective larvae (L3) on a monthly basis. Morphometric examination included total larval length (TLL), sheath tail length (STL), and gastrointestinal cell number. A total of 2,700 L3 were identified, and 100% of the species isolated from the larval cultures were found to belong to the cyathostomin group. The mean TLL was 800 µm, while the STL was 310 µm. All larvae were found to have eight gastrointestinal cells. This study established an ecological niche characterized by the absence of Strongylus spp. Routine larval

culture is a fundamental tool for the development of parasite control programs in horses, as the pathology of cyathostominosis differs from that of strongylosis caused by Strongylus spp. Molecular studies are required to accurately identify the more than 50 cyathostomine species involved.

Molecular identification of *Trypanosoma theileri* in cattle from north and west of Iran

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Trypanosoma theileri is a flagellated hemoprotozoan parasite infecting different species of the Order Artiodactyl, especially cattle, and buffaloes. It has a worldwide distribution and is transmitted mainly by Tabanidae. It has been considered a nonpathogenic parasite; however, recent studies show that *T. theileri* is an opportunistic parasite that can cause anemia, fever, swollen lymph nodes, and lower hemoglobin concentration. In Iran, this parasite has been reported only thrice, in 1978, 1995, and 2020. This preliminary study aimed to investigate the prevalence of *T. theileri* in cattle populations from two provinces of Iran with different climatic conditions using molecular tools. During 2024, blood samples were collected from healthy cattle of both sexes aged 10 months to 10 years in different regions of Mazandaran province in the north with Mediterranean climate (n = 87) and Kermanshah province in the west, bordering Iraq, with semi-arid climate (n = 50). Genomic DNA was extracted from a 100 µL aliquot of whole
blood using a commercial kit and tested with a species-specific primer pair (TthCATL1/DTO155) targeting a fragment of the cathepsin L-like gene of *T. theileri* with conventional PCR assay. Positive samples were Sanger sequenced. 25.5% of examined cattle scored positive *i.e.*, 28% in the west, and 24.1% in the north. Infected cattle were of both sexes and different ages from 10 months old. No statistical significance was observed between the infection and the region, sex, or age. Nucleotide sequences of the amplified products were identical to *T. theileri* isolates of cattle and water buffalo in Asia and South America. This study presents the molecular identification of *T. theileri* for the first time in the north and west of Iran. Considering the substantial prevalence of *T. theileri* and the opportunistic nature of this parasite, test-and--treatment of infected hosts is recommended.

Efficacy of a recommended and double dose of pour-on version of eprinomectin on small ruminant farms

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The macrocyclic lactone eprinomectin (EPN) is the only anthelmintic drug with a zero-day milk withdrawal period approved for use in goats. The same dose has been established for sheep and goats, although a double dose is usually recommended for goats. The main aim of the study was to compare the efficacy between recommended and double dose of EPN on sheep

and goat farms. The study was conducted on six goat farms and three sheep farms. A total of 120 goats and 70 sheep were included into the survey. The goats and sheep on each farm were divided into two groups. The first group was administered with EPN (EPRINEX Multi®) at the recommended dose for sheep and goats (1 mg/kg body weight) and the second group was treated with double dose of EPN. Third-stage larvae of gastrointestinal nematodes were harvested from faecal samples collected before and 14 days after treatment and were assigned to species/genus level based on their morphological characteristics. The efficacy of EPN on sheep detected by faecal egg count reduction test farms ranged for single and double dose from 73.1 to 94.2% and from 80.2 to 99.2 % respectively. A percentage reduction after application of double dose was higher of 5 – 16%. The efficacy of EPN on goat farms ranged from 51.4 to 86.8% for single dose and from 74.7 to 95.8 % for double dose. Higher efficacy of double dose was recorded on each farm (5 – 24%). *Haemonchus contortus* and *Teladorsagia circumcincta* were the predominant species before the treatment on each farm. *Haemonchus contortus* was the predominant species after the treatment on the farms with the lowest percentage reduction and with the minimal differences in efficacy between the both doses. The recommended dose of pour-on version of EPN should be higher for both species. The efficacy of EPN was mainly influenced by the proportion of *Haemonchus contortus* on the farm.

Does disease in early life impact future ewe productivity?

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Livestock encounter numerous challenges in early life, but we do not yet understand how these affect the long-term productivity of the animal. Our aim is to determine the impact of disease in early life on later performance in ewes. We hypothesise that ewe-lambs experiencing higher levels of infection and disease in their first two years will have reduced productivity when they mature. The births of 101 females were recorded in 2022 and information including birthing ease and litter size collected. They were monitored fortnightly throughout their first two grazing seasons (May 2022-October 2023): we collected data on body weight, faecal egg count (FEC) for a range of natural gastrointestinal nematodes (GIN), faecal oocyst count (FOC) for coccidia, other opportunistic diseases, welfare measures and any veterinary medicines. In 2024 our cohort were two years old and gave birth to their own lambs. We then monitored the health and welfare of 97 of their lambs during their first grazing season (May-October 2024). The average birth weight of the 101 females in 2022 was 5.2kg (range 2.5-7.25). Mean strongyle FEC during their first grazing season was 186epg (range 0-2421) and mean *Nematodirus* FEC was 44epg (range 0-468). Their lamb's average birth weight was 5.3kg (range 3-8.25), their mean strongyle FEC was 98epg (range 0-1107) and mean *Nematodirus* FEC was 34 epg (range 0-2196). Dag score was the only variable welfare measure across both groups with females averaging a score of

1 and their lambs 2. Preliminary analysis suggests that females with higher body condition score produce heavier lambs at finishing. Females with higher average *Nematodirus* FEC and coccidia FOC produce lighter lambs, and females with higher average dag score produced lambs with higher dag scores. Our results suggest that disease and welfare challenges experienced during a ewes first grazing season can negatively impact her growth and welfare in addition to her own lambs.

Does disease in early life impact future ewe productivity?

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Livestock encounter numerous challenges in early life, but we do not yet understand how these affect the long-term productivity of the animal. Our aim is to determine the impact of disease in early life on later performance in ewes. We hypothesise that ewe-lambs experiencing higher levels of infection and disease in their first two years will have reduced productivity when they mature. The births of 101 females were recorded in 2022 and information including birthing ease and litter size collected. They were monitored fortnightly throughout their first two grazing seasons (May 2022-October 2023): we collected data on body weight, faecal egg count (FEC) for a range of natural gastrointestinal nematodes (GIN), faecal oocyst count (FOC) for coccidia, other opportunistic diseases, welfare measures and any veterinary medicines. In 2024 our cohort were two years old and gave birth to their own lambs. We then monitored the health and welfare of 97 of their lambs during their first grazing season (May-October 2024). The average birth weight of the 101 females in 2022 was 5.2kg (range 2.5-7.25). Mean strongyle FEC during their first grazing season was 186epg (range 0-2421) and mean *Nematodirus* FEC was 44epg (range 0-468). Their lamb's average birth weight was 5.3kg (range 3-8.25), their mean strongyle FEC was 98epg (range 0-1107) and mean *Nematodirus* FEC was 34 epg (range 0-2196). Dag score was the only variable welfare measure across both groups with females averaging a score of 1 and their lambs 2. Preliminary analysis suggests that females with higher body condition score produce heavier lambs at finishing. Females with higher average *Nematodirus* FEC and coccidia FOC produce lighter lambs, and females with higher average dag score produced lambs with higher dag scores. Our results suggest that disease and welfare challenges experienced during a ewes first grazing season can negatively impact her growth and welfare in addition to her own lambs.

Effect of zinc oxide nanoparticles supplementation on the hematological profile of lambs infected by *Haemonchus contortus*

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Zinc (Zn) participates in various reactions of animal cellular metabolism and is essential for physiological functions such as immunity and growth. Deficiency of this mineral can impair the immune system, as the circulating Zn levels in the body are limited and do not have extensive reserves. Therefore, daily supplementation is necessary to ensure its proper function. In sheep, helminth infections primarily affect young animals, whose immune systems are still developing. *Haemonchus contortus* stands out as a parasite due to its high pathogenicity and drug resistance. Consequently, proper nutrition, including mineral supplementation, plays a fundamental role in immune response and infection control. This study evaluated the effect of zinc oxide nanoparticle supplementation in lambs by analyzing their complete blood count. The lambs were weaned at 60 days of age, at which point their supplementation began with a daily ZnO amount of 50 mg/lamb/day. At 90 days of age, the controlled infection was initiated with 1200 *H. contortus* L3 per lamb/week until they reached 8 months of age. Cell counts were performed using a hematology analyzer, and data were analyzed using PROC Mixed SAS ($p<0.05$). Infected animals had lower hematocrit levels, higher hemoglobin production with nanoparticle treatment, and the highest red blood cell production at 15 and 30 days of inffection, with greater variation between microparticle and nanoparticle treatments. It was concluded that the supplementation with zinc oxide nanoparticles influenced the metabolic profile of lambs throughout the study period, suggesting a positive impact on the animals immune responses.

Occurrence of *Cryptosporidium* spp. in Goats from Northern Paraná, Brazil: Preliminary Results

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Cryptosporidium spp. are protozoan parasites with zoonotic potential, commonly found in the gastrointestinal tract of various domestic animals, including goats. These infections are associated with diarrhea, reduced weight gain, and economic losses, particularly in young or immunocompromised animals. Data on this parasite occurrence in small ruminants in Paraná and Brazil remain limited. The objective of this study was to evaluate the occurrence of *Cryptosporidium* spp. in goats from different management systems in northern Paraná, Brazil. A total of 228 fecal samples were collected from the rectum of goats in farms located in the northern region of Paraná State (cities of Apucarana, Londrina, Maringá, Ortigueira, and Paiçandu). An epidemiological questionnaire was used to assess factors related to the rearing system (semi-intensive or intensive), the age of the animals (up to 6 months or over 6 months), and the type of flooring in the pen (dirt or slatted). DNA extraction was performed using NucleoSpin Tissue® (Macherey-Nagel). Detection of

DNA was carried out using nested-PCR (nPCR) targeting the 18S rRNA gene. PCR products were visualized by 1.5% agarose gel electrophoresis and stained with SybrSafe®. Out of the 228 samples processed, four tested positive by nPCR. Positive samples were detected in goats from the cities of Apucarana, Ortigueira, Paiçandu, and Londrina, and were limited to adult animals. Three positive samples were from animals in semi-intensive systems, and one was from an animal in an intensive production system. Two of the positive animals were housed on slatted floors, while the other two were kept on dirt flooring. The detection of *Cryptosporidium* spp. in goat herds from northern Paraná highlights the presence of this protozoan in caprine populations in Brazil. Further analysis with additional samples and molecular characterization of the isolates are ongoing to better understand the epidemiology and zoonotic potential of these infections.

Parasite control practices in horses in Mexico: questionnaire survey

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Common parasitic control strategies in horses have been mostly based on the continuous and indiscriminate administration of anthelmintics (AH), to eradicate the presence of parasites, which has favored the development of anthelmintic resistance (AHR), compromising the welfare of the horse. The objective of this study was to identify the risk practices employed in the control of parasites in horses in Mexico in order to determine factors of loss of effectiveness of AH through surveys of veterinarians. A survey was designed with 28 questions on the parasitic management employed, obtaining 89 responses. A logistic model was employed, with α=0.05. A p-value $\leq$0.05 was considered significant. The analysis sought to identify an association between two categorical variables: the presence of clinical parasitic problems and AH effectiveness, considered as dependent variables, while each question was treated as an independent variable. Fourteen models were executed using the *glm* function of the R 4.0.2 statistical software. A dependence on the use of AH was observed, with p-values of 0.009, 0.02, 0.05 and 0.04, due to the use of frequent deworming practices without prior diagnosis and limited knowledge about other alternatives oriented to preserve the effectiveness of AH. A reduction in drug effectiveness was related due to exclusive use of a calendar as a deworming strategy (p 0.04) and the reuse of drugs already administered (p 0.04). This study offers pivotal insights to optimize parasite control strategies in horses. It underscores the professional guidance of a veterinarian specialized in equine parasitology, being fundamental to promote responsible Equine Integrated Parasite Management for an integral welfare, encompassing both ecosystem and the public health.

Seventeen species of cyathostomins identified in Mexico

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Pastured yearling are commonly present gastrointestinal nematodes, with strongylids being the most common. These are diverse in species and are divided into migratory and non-migratory, the former being considered the most pathogenic. AH treatments have focused on the control of *Strongylus* spp, which has reduced their prevalence. On the other hand, the population of Cyathostominae has increased significantly, as has AHR. More than 50 cyathostominae species have been identified, of which the pathogenesis, lesions and even the coexistence between them are unknown, making species identification an important task. The objective of this study was to identify the cyathostominae species present in a fecal sample from a yearling by systematic analysis to demonstrate the coexistence of at least five species. Seventy-one specimens found in a fecal sample were collected for morphological identification using taxonomic keys, including characteristics such as color, total length, tail length, buccal capsule, oesophagus, number of teeth, rays and sex. Each specimen was also photographed and the species found catalogued. Seventeen different species were identified, the most important being *Cylicostephanus longibursatus* (30%), *Cylicocyclus insigne* (11%), *Hsiungia pekingensis* (10%), *Cc. ashworthi* (7%), *Cc. radiatus* (7%) and *Cyl. hybridus* (7%). Other species found were *Coronocyclus sagittatus, C. coronatus, C. labiatus, Cc. elongatus, Cc. nassatus, Cc. leptostomum, Cyl. calicatus, Cyl. goli, Cyl. bidentatus, Poteriostomum imparidentatum and P. ratzii.* This is evidence of the coexistence of more than five species in a single horse. This work provides guidance for future molecular research in Mexico on cyathostominae multi-species infections and their coexistence, as there are few studies on this topic. It is a contribution to future epidemiological studies, especially those aimed at countering the effects of AHR strains and sustainable equine parasite management.

Molecular detection and characterization of *Theileria parva* in selected buffalo populations in Zambia

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The form of bovine theileriosis, Corridor disease caused by buffalo-derived *Theileria parva,* is neglected in Zambia and many other African countries where East Coast fever (ECF) caused by cattle-derived *T. parva* is endemic. African buffalo (*Syncerus caffer*) are reservoir hosts of *T. parva,* an intracellular protozoan parasite transmitted by the vector tick, *Rhipicephalus appendiculatus*. Although Corridor disease is fatal, there is no record of the molecular occurrence and characterization of *T. parva* in Zambian buffalo populations. The efficacy of the infection and treatment method (ITM) vaccine stocks, Chitongo and Katete, used to immunize cattle against ECF in Zambia, is yet to be evaluated against Corridor disease. Therefore, this study used molecular tools to detect *T. parva* DNA from buffalo blood samples collected from selected provinces of Zambia, and to characte-

rize the parasites, with comparison to cattle associated parasites and the ITM vaccine stocks. Majority of buffalo samples (>75%) tested positive for *T. parva*. p67, Tp1 and Tp2 antigen gene and protein sequences revealed extensive genetic and antigenic diversity in buffalo associated parasites compared to those detected from Zambian cattle. Median-joining networks revealed a close antigenic relationship of some of the sequences from buffalo samples with those from cattle, especially those related to the Chitongo vaccine stock. Based on microsatellite markers, genomic populations analyzed in this study were generally in linkage disequilibrium. The findings in this study suggests that the cattle populations in Zambia are at risk of infection with Corridor disease. The close antigenic relationship of some of the buffalo-derived parasites with the Chitongo vaccine stock, suggests that this vaccine stock may provide protection against infection with some of the buffalo associated parasites. However, the extensive diversity in the latter remains a concern as the current vaccine stocks may not provide protection against all buffalo derived parasites.

Sero-positivity of *anti-Toxoplasma gondii* & *anti-Neospora caninum* antibodies and their biochemical and immunological influences among cattle and buffaloes from Delta of Egypt

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Due to the scarcity of data on the sero-positivity of anti-*Toxoplasma gondii* & anti-*Neosporoa caninum* antibodies and the worldwide importance of these protozoans in Egypt, our research aimed to discover the sero-prevalence of *T. gondii* & *N. caninum* in buffaloes & cattle from Delta of Egypt. Our study was extended to clarify the hematological, biochemical & immunological influences of these diseases on the examined animals. Both IgG & IgM against these protozoans were detected using specific recombinant surface Ag 2 of *T. gondii* (*Tg*SAG2) & surface Ag 1 of *N. caninum* (*Nc*SAG1) based ELISA. Then Among positive and negative bovine samples liver enzymes activity, cholesterol, triglycerides, urea, IL-5, IL-6 & blood picture were detected. The overall sero-positivity of anti-*N. caninum* & anti-*T. gondii* in cattle from Gharbia, Beheira and Menoufia provinces were (22.19% & 16.88% for IgM) and (38.13% & 31.88% for IgG), respectively. In buffaloes, sero--positivity of anti- *N. caninum* & anti- *T. gondii* from Gharbia, Damietta and Menoufia provinces were (6.82% & 7.27% for IgM) and (15.45% & 16.82% for IgG), respectively. This study showed significant differences regarding the age; gender & season. Infection with these parasites showed significant changes in the AST and ALT activity, cholesterol, triglycerides and urea. Significant increase in the IL-5, IL-6, total WBCs & relative differential (lymphocytes & eosinophil) count associated with a significant decrease in the red blood corpuscles count, hemoglobin concentration, platelets count and neutrophil relative count in the infected bovines compared to the negative animals. Toxoplasmosis and neosporosis are prevalent in cattle and buffaloes in the examined Provinces of the Delta of Egypt. Both diseases had negative influences on the bovine animal health, which may reflect a harmful impact on the animal performance and productivity.

Molecular characterization and phylogenic analysis of Trypanosoma evansi drug resis-

tance-associated TevAT1 gene from bufalloes in Ngawi district, Indonesia

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Resistance case to the *diminazene aceturate* (DA) drug was a major problem to control *Trypanosoma evansi*. Commercialized more than half a century ago, the drug was ineffective, as the spread of trypanocidal drug resistance was currently reported in several regions in Indonesia. Resistance was the result of DA uptake that was lacking by parasites. Reduction of DA uptake due to changes in the TevAT1 gene encoding adenosine transporters 1. Cases of resistance to DA drugs in Indonesia should be concerned. However, there was no genetic information available about the TevAT1 gene, so that rapid detection with molecular for DA drug resistance had never been conducted. Therefore, it was necessary to conduct research in gene mapping on *T. evansi* that played a role in drug resistance in the adenosine transporter TevAT1 gene. In vivo tests were performed to 25 mice: BALB/c, male, two-month-old, weighing ± 25-30 grams. The genomic DNA was isolated from *T. evansi*. Polymerase chain reaction (PCR) was carried out to amplify TevAT1 gene using the designed primers. The PCR product was cloned into pTA2 plasmid vector and transformed into Escherichia coli DH5α cells. The plasmid was isolated from E. coli and was sequenced. The sequence was analyzed and submitted in Genbank and phylogenetic analysis. BLAST results showed that the TevAT1 gene from Indonesia had a high level of similarity (99%) to all adenosine transporter 1 genes in GenBank for isolates that were sensitive and resistant. TevAT1 *T. evansi* gene isolates from Indonesian buffalo (MK276881) were sensitive to DA. Furthermore, alignment results with *T. brucei* (TbAT1) gene TbAT1r (AF152370) presented 14 points of nucleotide base differences and nine differences at amino acid level. There were points in common of TevAT1 gene from the differences in nucleotides and amino acid bases as a result of the comparison of TbAT1 and TbAT1r. It was the first information about the TevAT1 gene from Indonesia and can be as candidate points for rapid identificaton of DA-resistant stocks of *Trypanosoma evansi* by PCR–RFLP.

Comparison of *in vitro* and *in vivo* resurrection success of three ovine gastrointestinal nematode species following different cryopreservation strategies

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Nematode infective larvae (L3) of veterinary importance have classically been cryopreserved in liquid nitrogen (LN2) to minimize the need for continued passage through live animals. Health and safety concerns, along with increasing pressures to reduce energy costs have led to exploring alternative preservation techniques. Super-cold (-150°C) freezers have been used for long term storage of cell lines, but no published data were available for L3 of

livestock gastro-intestinal nematodes (GIN). To test three GIN species (*Teladorsagia circumcincta* (MTci2), Trichostrongylus *colubriformis* (MTco1) and *Haemonchus contortus* (MHco3 and MHco18)) *in vivo* and *in vitro* survivability following three cryopreservation storage methods. Fifty thousand L3 were exsheathed and cryopreserved using one of three methods; snap frozen in LN2 before storage at -150 °C (LN2/-150°C); stored directly at -150°C or stored in LN2. *In vitro* survivability of L3 (dead vs alive) in phosphate buffered saline were assessed between 1 and 23 months post -150°C and LN2/-150°C storage. Larvae were defrosted, left in PBS overnight at 39.6°C and 10% CO2 prior to assessment. An *in vivo* study was undertaken with L3 following 4 months of storage compared to fresh L3 to assess life history traits of the species. Tubes stored directly in -150°C have consistently shown ≥90% *in vitro* survivability for all isolates, whereas LN2/--150°C showed inter species variability (range: 7-63%). The *in vivo* assessment demonstrated a significant difference in establishment with overall group mean establishment ranged from 9% of the LN2/- 150°C larval challenge to 62% of the fresh larvae, with the -150°C and LN2 groups establishing 25% and 10% respectively. -150°C freezer storage is a viable alternative to LN2 storage for infective larvae of sheep and has the advantage of being relatively safe, costs less to run and results in more consistent recovery of larvae of veterinary importance.

Influence of bioactive plants (*Cichorium intybus*) and zinc oxide nanoparticles on the fecundity of *Haemonchus contortus* females parasitizing lambs

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The gastrointestinal nematode *Haemonchus contortus* is one of the most pathogenic parasites of small ruminants. Also, this parasite has developed a strong resistance to all anthelmintics registered worldwide. Therefore, the development of non-chemical control methods is urgently needed. Applying bioactive plants and zinc oxide nanoparticles (ZnO-NPs) as food supplements for small ruminants is a promising control method. We investigated the egg productivity of *H. contortus* females parasitized lambs when bioactive plant chicory (*Cichorium intybus*) and ZnO-NPs were introduced into the lamb's diet. Thirty lambs 3–4 months old were divided into 3 groups: control (10), lambs grazing on pasture with 25% of chicory (10), and lambs supplemented with ZnO-NPs (10). All lambs were orally infected with ~5,000 *H. contortus* infective larvae. On Day 107, the lambs were humanely slaughtered and necropsied; *H. contortus* adults were collected. Later, 147 mature females from the control (50), chicory- (47), and ZnO-NPs (50) groups were collected,

and the number of eggs in their reproductive systems was calculated. The number of eggs in *H. contortus* females varied from 87 to 907 and significantly differed between the three groups of lambs (Kruskall-Wallis test; $p<0.001$). In the control group, the average egg number was 416 (208–906; median 397); in the chicory group, 285 (87–492; median 293); in the ZnO-NPs group, 332 (96–881; median 325). Also, the length of *H. contortus* females was significantly higher in the control (19.4±3.0 mm) than in the chicory (16.2±2.0 mm) and ZnO-NPs (16.4±2.1.mm) groups. Our study demonstrated the significant reduction of fecundity of *H. contortus* females influenced by the chicory and ZnO-NP food supplements in lambs.

Intraspecific sequence variation in Parascaris spp. from domestic horses analyzed using selected nuclear and mitochondrial genes

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The ascarids of the genus *Parascaris* are considered the most pathogenic parasites infecting juvenile equids globally. Nowadays, two morphologically almost identical species, *Parascaris equorum* and *P. univalens*, are recognized in wild and domestic equids. Both species were regularly found in domestic horses in the previous century; however, only *P. univalens* has been documented during the last decades. The purpose of the study was to provide information on the genetic variability in horsederived *Parascaris* from central/eastern Europe by analyses of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*), small subunit of rRNA (12S rRNA) genes, and the internal transcribed spacer region (ITS1) of nuclear rRNA. A total of 56 specimens of *Parascaris* were collected from domestic horses in Slovakia, Ukraine, Poland, and Hungary, originating from 17 geographical locations. PCR reactions generating products for sequencing were conducted following von Samson-Himmelstjerna et al. (2021). All isolates showed genetic characteristics that can be assigned to *P. univalens*. Gene diversity estimated by indices of nucleotide diversity (π) and haplotype diversity (Hd) in *cox1* and 12S was highest in Ukrainian samples (π: 0.015, Hd: 0.60), followed by Slovakia and Poland. From Fst indices, it was evident that the Ukrainian population was the most distinct (Fst values from 0.065 to 0.071) among the populations defined by country. The population structure of *P. univalens* in Poland, Slovakia, and Hungary was more homogeneous compared to Ukraine, likely due to closer geographical distance and higher gene flow among populations. A few locally adapted isolates seem to have emerged in Ukraine, some of which have an affinity to Chinese equid samples.

Clinical and parasitological diagnosis of *Metarhabditis* sp. in lactating Gir cows during a treatment program on a farm in Paraopeba, MG, Brazil

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Parasitic otitis is an inflammatory process in the ear canal of cattle caused by *Metarhabditis* sp., commonly seen in Gir breed. The parasitism may cause external ear lesions that may lead to bacterial infection on the central nervous system and death. There is little information about this parasite, and the treatments evaluated to date have shown little or no efficacy. Thus, the objective of this study was to evaluate the natural infestation by *Metarhabditis* sp. in the ear canal of Gir cattle at a farm in Paraopeba-MG, during a treatment program. Fifty-four lactating cows were used. They have been treated for two years at 30-45 day intervals, by cleaning and application of anti-inflammatory, antibiotic and antiparasitic topically to the ear. Three collections were performed from each animal using saline solution instilled into each ear canal that was taken to the laboratory for parasite quantification. Clinical diagnosis consisted of direct visualization of the parasite in the ear canal. The percentage of positive animals gradually decreased from the first to the third collection, being 62.6, 52.4 and 37.5%, while a similar profile was observed for positive ears, which were 26, 50 and 31.7%. Most animals (53.7%) presented varied diagnoses among the three collections, while 47.3% always presented the same diagnosis (5.6% always negative and 40.7% always positive). Regarding ears, the majority (61.5%) presented similar results throughout the three analyses, with 12.9% always negative and 48.6% always positive. In general, the correlation between clinical and parasitological evaluations was 0.64 (moderate correlation), but it increased to 0.92 (very strong) when only negative or animals with high parasitism were included. The results suggest that: animal and ear components determine infestation over time; treatment measures promoted a gradual reduction in the helminth prevalence; the clinical diagnosis presents moderate correlation with the parasitological diagnosis.

Prevalence of *Metarhabditis* sp., by animal classes, in a Gir cattle herd at the Getúlio Vargas Experimental Field - EPAMIG Oeste, Uberaba, MG, Brazil.

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Gir cattle in Brazil have a high prevalence of *Metarhabditis sp.*, a helminth that causes otitis. It is estimated that 90% of the herds are affected. There are also reports in Indubrasil cattle, which reinforce the hypothesis that this predilection may be related to the morphology of the ears. The more frequent reports of severe cases of chronic otitis in older animals suggest that age-related factors may contribute to the worsening of symptoms. Progression to death is not uncommon in such situations due to central nervous system involvement. The aim of this study was to identify infestation by *Metarhabditis sp.* in a Gir cattle herd and to as-

sess its prevalence by animal classes. For this purpose, samples were collected from flushing both ear canals with 20 ml of saline solution from all of 266 Gir cattle from EPAMIG Oeste. Each animal had to be individually restrained in a trunk and, when necessary, a nasal immobilizer was used. The washing liquid was collected in a sterile plastic bottle, using a funnel. The diagnosis was made by assessing a drop of sediment from the collected material added to a drop of 2% Lugol's solution on a slide and coverslip, under an optical microscope and a 10x objective. The data were evaluated using descriptive statistics. Among the 84 cows, 55 (65%) were positive, 41 in both ears and 14 in only one of them. Among the 72 heifers aged one to three years, only 13 over two years old (18%) were positive, 50% in both ears. Among the 39 calves up to one year old, both male and female, none were positive, as were the six bulls over 4 years old. Among the 65 males aged one to four years old, only one presented infestation. The results reinforce the possibility of age- plus sex-related effects on *Metarhabditis sp*. prevalence. However, it is important to consider management aspects, especially those related to population density, which could explain higher prevalences in cows than to adult males.

Detection of Trypanosoma evansi in water buffaloes (*Bubalus bubalis*) from Minas Gerais, Brazil: implications for emerging health risks

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The breeding of buffaloes (*Bubalus bubalis*) has gained importance in Brazil due to their adaptability to diverse biomes and management systems. However, health issues such as hemoparasite infections remain poorly studied in this species. Among these agents, *Trypanosoma* spp. are notable—blood parasites with a global distribution and a life cycle involving both vertebrate and invertebrate hosts. This study aimed to detect *Trypanosoma vivax* and *T. evansi* using molecular techniques and blood smear examination in 251 buffalo calves up to 12 months old in the Bom Despacho microregion, Minas Gerais, Brazil. *Trypanosoma evansi* was found in 0.8% (2/251) of the animals, which did not show clinical signs; *T. vivax* was not detected. Neither species was observed in the blood smear examination. One positive sample in the molecular test, amplified using the RoTat 1.2 VSG gene, was sequenced and analyzed via BLASTN against GenBank. The top matches were known *T. evansi* VSG gene sequences, showing 95.14–95.39% identity. The highest similarity was with *T. evansi* CJ-1 (India), followed by RoTat1.2 (Kenya) and another partial VSG gene (India). The identification of *T. evansi* is concerning, as it causes Surra, a disease with variable clinical signs depending on the host and region. Detection in Minas Gerais, a non-endemic area, suggests possible geographic expansion of this pathogen. The presence of *Trypanosoma* spp. in buffaloes also raises

concern for the dairy cattle industry, given the potential for production losses in both sectors. Thus, epidemiological monitoring, vector control, and good sanitary practices are essential to limit parasite spread and reduce its impact on Brazil's growing buffalo industry.

Molecular Detection of Vector-Borne Pathogens in Llamas (*Lama glama*): Overcoming Diagnostic Challenges

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South American camelids hold great cultural and economic significance for Indigenous and rural communities throughout South America, being used for transport, wool, meat, and leather production. However, information regarding health and issues related to parasitic diseases in camelid farming is scarce. Therefore, this study aimed to investigate the presence of vector-borne pathogens in llamas admitted to the Veterinary Hospital of the Federal University of Minas Gerais, Brazil. The farm of origin for these llamas had reported issues related to anaplasmosis, diagnosed through blood smear examination on-farm, which resulted in the death of some animals. A total of 10 blood samples from llamas were collected and screened for bacteria of the Anaplasmataceae family, hemotropic *Mycoplasma* spp., piroplasmids, and *Trypanosoma* spp., using molecular methods. Due to the suspicion of anaplasmosis, the samples were tested for *Anaplasma marginale*, but all were negative. On the other hand, 50% (5/10) tested positive for hemotropic *Mycoplasma* sp., with sequencing revealing similarity to *'Candidatus* Mycoplasma haemolamae', 60% (6/10) for piroplasmids, with sequencing showing similarity to *Babesia bovis*, and 10% (1/10) for *Ehrlichia* sp., with sequencing revealing similarity to '*Candidatus* Ehrlichia regneryi'. However, no samples tested positive for *Anaplasma* sp., *T. vivax, T. evansi,* and *T. cruzi*. The genetic characterization of the detected pathogens will be conducted later. Given the farm's history of anaplasmosis and the absence of *A. marginale*, associated with the identification of *'Ca.* M. haemolamae', a probable diagnostic error is suggested, possibly due to the morphological similarity between these bacteria. This highlights the need for enhanced staff training to improve diagnostic accuracy. The observed clinical signs and mortality may have been associated with hemoplasma infection, which, when combined with other pathogens, can lead to severe disease outcomes.

Special topic - Tick resistance

Scanning Electron Microscopy and Energy-Dispersive X-ray Spectroscopy (SEM/EDS) of *Rhipicephalus microplus* cuticle suggest structural marker associated with cypermethrin resistance

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This study aimed to characterize the elemental composition of the larval cuticle in *Rhipicephalus microplus* populations from various regions of Brazil and to investigate its association with susceptibility to commonly used synthetic acaricides. Larvae from 13 populations were analyzed, including a susceptible reference strain (POA) and a resistant strain (MGl). Susceptibility to cypermethrin, chlorpyrifos, fipronil, doramectin, and moxidectin was evaluated in the remaining 11 populations using the larval packet test (LPT). Elemental analysis of the dorsal and ventral cuticle regions at 7, 14, and 21 days of age was performed to references tick strains using Scanning Electron Microscopy coupled with Energy-Dispersive X-ray Spectroscopy (SEM/EDS). The tick populations exhibited varying resistance profiles, particularly to cypermethrin, chlorpyrifos, and fipronil, while showing universal susceptibility to doramectin and moxidectin. SEM/EDS revealed a predominance of carbon in the cuticle, with significant differences among populations and between dorsal and ventral regions. In 9 out of 11 populations, the dorsal region exhibited higher carbon content than the ventral. A strong, statistically significant positive correlation was found between dorsal carbon content and cypermethrin resistance ($r = 0.7728$, $p = 0.0081$). Although positive, correlations with chlorpyrifos and fipronil were not statistically significant. No relevant correlation was observed between ventral carbon content and acaricide resistance. Doramectin and moxidectin were excluded from the correlation analysis due to the consistent susceptibility observed across populations. This is the first study to investigate the elemental composition of the larval cuticle in *R. microplus* and its potential association with resistance to synthetic acaricides.

Effect of metabolic synergists on the reduction of *Rhipicephalus microplus* resistance to ivermectin

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Infestation by the tick *Rhipicephalus microplus* represents a major challenge for livestock production in Brazil due to climatic conditions favorable to its reproduction. This compromises herd productivity and facilitates the transmission of pathogenic agents such as *Babesia spp.* and *Anaplasma spp*. Parasite control is primarily carried out using chemical products such as ivermectin. However, the continuous and widespread use of this drug has favored the emergence of resistant populations. Resistan-

ce may be related to the action of detoxifying enzymes such as cytochrome P450, esterase, and glutathione S-transferase. The objective was to evaluate, through the larval immersion test (LIT), the efficacy of ivermectin alone and in combination with the metabolic inhibitors piperonyl butoxide (PBO), triphenyl phosphate (TPP), and diethyl maleate (DEM) on a population of *R. microplus* from the municipality of Icó, Ceará, aiming to identify the resistance mechanisms involved and determine which enzymes play the most significant role in ivermectin detoxification in resistant populations. Approximately 100 engorged female ticks were collected from cattle without recent acaricide treatment and incubated for oviposition. The hatched larvae were subjected to LIT using high-purity ivermectin, both alone and in combination with the inhibitors. Data analysis was performed using Probit analysis. The results showed that the studied population exhibited high resistance to ivermectin (LC50 = 483.006 ppm) compared to the susceptible POA population (LC50 = 25.225 ppm). The combination with PBO was the most effective, reducing the LC50 to 141.872 ppm and showing a synergistic factor of 3.405, indicating that cytochrome P450 plays a key role in ivermectin detoxification. TPP showed a moderate effect (LC50 = 264.774 ppm; SF = 1.824), suggesting lower esterase involvement. DEM did not show significant synergism (LC50 = 607.882 ppm; SF = 0.794), indicating limited efficacy in this population. It is concluded that combining ivermectin with PBO represents a promising strategy for controlling resistant populations. However, further studies with other synergists and tick populations are needed to improve control strategies against *R. microplus.*

Standardization of adult immersion test in ixodid ticks using fluralaner

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Adult immersion test (AIT) is one of the methods used to monitor tick susceptibility to acaricides. The aim of this study was to standardize an AIT protocol using fluralaner against females of *Rhipicephalus microplus*, *Rhipicephalus linnaei*, and *Dermacentor nitens*. The assays were conducted at the Laboratorio of Quimeoterapia Experimental in Parasitologia Veterinária/UFRRJ. Initially, a 1% fluralaner stock solution (10,000 ppm) was prepared, followed by a pre-dilution to 0.1% (1,000 ppm) and then to 100 ppm, with all dilutions made in DMSO. For the AIT, Initial solution with 4 ppm from 1.6mL of the 100 ppm pre-dilution, diluted with 10% DMSO to a final volume of 40mL. Subsequently, additional concentrations were obtained thorough serial diluicions using 10% DMSO, down to 0.007 ppm. A solution containing 10% DMSO was used as negative control. The AIT was performed using 20 females immersed in each of the test solutions for 5 minutes. After immersion, the ticks were dried, weighed, and individually stored at 27 ± 1°C and 80 ± 10% relative humidity. After 21 days, the egg masses were weighed individually. The weights of females and eggs laid were used to calculate the Index of fertility and, based this, the percentage of mortality. Mortality data were utilized for probit analyses whit 95% confidence interval (95% CI). Mortality rates in-

creased whit concentration, reaching 100% at 2 ppm for D. nitens, 1 ppm for *R. microplus*, and 4 ppm for *R. linnaei*. The lethal concentrations 50 (LC_{50}, 95% CI, in ppm) were 0.391 (0.326 to 0.471) for *D. nitens* and 0.762 (0.629 to 0.928) for *R. linnaei*. For *R. microplus*, it was not possible to determine the LC_{50} due to the rapid increase in mortality at the concentrations tested. This study demonstrated the in vitro susceptibility of different tick species to fluralaner, with *R. microplus* being the most susceptible and *R. linnaei* the least susceptible.

Characterization of the Resistance of the Tick *Rhipicephalus* (Boophilus) *microplus* in Three Regions of the State of São Paulo

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The cattle tick *Rhipicephalus* (Boophilus) *microplus* is considered one of the main parasites affecting cattle farming. Its control largely depends on the use of acaricides. However, the frequent and often inadequate application of these products has significantly contributed to the emergence and spread of resistance to the various active ingredients used in control. This study aimed to evaluate the resistance of *R. microplus* ticks from three regions of the State of São Paulo by exposing larvae to the diagnostic dose of the active ingredients chlorpyrifos, cypermethrin, flumethrin, and fipronil. Based on the average mortality (%) for each acaricide in the West, Northwest, and Southeast regions, the level of resistance (LR) was assessed according to the pre-established criteria. In the West region, a high level of resistance (A) was observed for cypermethrin (10.55%), chlorpyrifos (39.75%), and flumethrin (15.18%). Meanwhile, fipronil exhibited intermediate resistance (I) with 65.37% mortality. In the Northwest region, all the active ingredients analyzed showed a high level of resistance (A) with the following results: cypermethrin (4.70%), chlorpyrifos (23.31%), fipronil (16.36%), and flumethrin (18.78%). In the Southeast region, cypermethrin (8.90%) and flumethrin (17.59%) also presented high-level resistance (A). On the other hand, chlorpyrifos (44.55%) and fipronil (65.37%) showed intermediate resistance (I). The results indicate the prevalence of acaricide resistance in the three regions studied, particularly to cypermethrin and flumethrin. It is important to highlight that only fipronil and chlorpyrifos exhibited cases of intermediate resistance. Moreover, there were no cases of very low resistance (VL) in the areas studied, as none of the active ingredients achieved an average mortality above 95%.

Innovative therapeutic strategies for Fluazuron and diagnostic resistance in *Rhipicephalus microplus*: A pharmaco-parasitological research

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The management and eradication of *Rhipicephalus microplus* in agricultural settings throu-

gh the strategic application of Fluazuron (FLU) for generational tick control pose a significant sanitary challenge in Uruguay. The objective of this study was to elucidate, using a pharmacological and parasitological approach, the parasitological efficacy of *R. microplus* in generational control using FLU application. In a strategic generational control scheme, four doses of FLU (2.5 mg/kg, pour-on, 2.5%, Acatak®, Elanco, Uruguay) were administered repeatedly to 40 Hereford cows in the final third of gestation, at 42-day intervals during gestation and lactation. Blood and milk samples were collected from all identified animals (dams and calves). From a parasitological perspective, individual evaluations were conducted to determine infestation levels (presence/absence of adult female ticks). Plasma, milk, and tick samples were collected for quantification of concentration levels through a validated analytical methodology utilizing LC-(ESI)-MS/MS. The moving average concentrations (overdosing period) in plasma (80 µg/kg) and milk (500 µg/kg) exhibited a cumulative behavior of FLU in cows and calves, indicating that calves tend to accumulate significantly during the first dose period, with a subsequent decrease and an inversion in cow/calf concentrations observed in the last dose. The presence of teleogynous ticks with oviposition and hatching capacity was identified, along with concentrations at the teleogynous level (10 to 56 µg/kg) indicating exposure to FLU throughout its parasitic cycle. Our findings demonstrated the existence of tick populations exposed to FLU with low sensitivity under natural infestation conditions. Clinical monitoring, coupled with the implementation of new complementary sensitivity diagnostic tools, should be routinely employed to optimize the sustainability of antiparasitics and exposure to natural parasite populations in strategic control plans.

Farmer perceptions and detection of acaricide resistance in *Rhipicephalus microplus* ticks in Burundi

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Acaricide resistance in cattle ticks has emerged as a critical challenge for livestock production across sub-Saharan Africa. High prevalence of resistant tick populations has been documented in countries such as Uganda and Tanzania, with specific single nucleotide polymorphisms (SNPs) associated with acaricide resistance identified previously. Despite widespread farmer concerns regarding declining acaricide efficacy in Burundi, empirical evidence confirming acaricide resistance has been lacking. This study aimed to assess the efficacy of commonly used acaricides in Burundi and characterize the genetic markers associated with acaricide resistance in field populations of *Rhipicephalus microplus* ticks. A cross-sectional survey was conducted from October to December 2017 in Bubanza, Cibitoke, and Bujumbura provinces, involving 395 farms distributed across 26 districts. A structured questionnaire was administered to farmers to gather data on their knowledge, perceptions, and experiences regarding acaricide use and efficacy. Simultaneously, ticks were systematically collected from cattle, and engorged female *R. microplus* ticks were specifically targeted for larval packet tests. Due to their one-host lifecycle and frequent exposure to acaricides, often applied weekly, *R. microplus* ticks rapidly develop resistance. Therefore, these ticks were selected for acaricide resistance assessment. Genomic

DNA from collected ticks was subjected to PCR amplification targeting genes previously linked to acaricide resistance, namely the octopamine/tyramine receptor and the voltage-gated sodium channel. SNP detection was performed via sequence similarity analyses, and phylogenetic relationships were established using Maximum Likelihood algorithms. The study identified 13 commercial acaricide brands in use, with most farmers (90.6%, 348 out of 384) applying amitraz up to twice weekly. Approximately 60% (230 out of 384) of farmers rated acaricide efficacy as "Very Good," and 35% (136 out of 384) rated it as "Good," indicating that 95% of farmers were satisfied with acaricide performance. Larval packet tests revealed that tick populations were resistant to at least one of the five amitraz molecules and exhibited multi-acaricide resistance—contradicting farmers' positive perceptions. While tick populations remained susceptible to synthetic pyrethroid chemicals in laboratory tests, genetic analysis revealed a concerning mutation. A non-synonymous mutation in the domain II S4-5 linker region of the para-sodium channel gene was found in one field sample. This mutation, previously linked to acaricide resistance in other countries, indicates that ticks in Burundi are developing resistance to synthetic pyrethroids. Frequent acaricide application has led to the emergence of acaricide-resistant *R. microplus* in Burundi. While most farmers perceive their tick control as effective, our findings highlight early signs of resistance. These results underscore the need for integrated tick management strategies – including rotation of acaricide classes, farmer training in proper use, and alternative control measures – to sustain effective tick control and mitigate tick-borne diseases.

Validation of a New Artificial Intelligence-Based Technology for Predicting Acaricidal Efficacy against *Rhipicephalus microplus*

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This study aimed to validate a new artificial intelligence-based technology for predicting the efficacy of acaricidal products against *Rhipicephalus microplus*. Engorged females from 20 different populations were subjected to the adult immersion test, being divided into 15 experimental groups (n = 10): one untreated control group and 14 treated groups with different acaricides, applied at single and specific concentrations as indicated by the manufacturers. After the incubation period, 0.1 g samples of eggs with 14 days of oviposition were collected from each treatment and population for automated efficacy analysis. The eggs were placed in Petri dishes and photographed using a smartphone coupled to a binocular stereoscopic magnifying glass. The images obtained were used to estimate the percentage of larval hatching and calculate the control percentage (C%) through the automatic model. The validation consisted of comparing the C% automatically generated by the software with those obtained manually in the adult immersion tests, through the analysis of three statistical metrics: RMSE (Root Mean Square Error), MAE (Mean Absolute Error), and R^2 (Coefficient of Determination). The obtained RMSE was 20.76, the MAE was 16.28, and the R^2 reached a value of 0.74, indicating that the

model was able to explain 74% of the variability of the real data. The results demonstrate that the deep learning model shows satisfactory performance and potential for use as an auxiliary tool in estimating tick control, being considered validated for preliminary use. However, the expansion of the database is necessary to increase the robustness and applicability of the predictive system. This new method accelerates result acquisition by up to 16 days, half the time required by the traditional method.

Evaluation of a Rapid Alternative for Resistance Diagnosis in *Rhipicephalus microplus*

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The cattle tick *Rhipicephalus microplus*, a vector of pathogens in bovines, causes estimated annual losses of R$ 350 million to the livestock industry in Rio Grande do Sul, Brazil. The increasing resistance to acaricides worsens the situation, making effective control more difficult and costly. Resistance diagnosis through bioassays such as the Adult Immersion Test (AIT) is essential for guiding treatment strategies. However, the standard AIT takes 42 days, due to the need to wait 14 days for oviposition and 28 additional days for larval hatching. This study aimed to evaluate whether results based solely on egg mass production at 14 days differ significantly from the full AIT, potentially allowing resistance diagnosis to be anticipated by up to four weeks. For each acaricide formulation - cypermethrin, deltamethrin, cypermethrin+chlorpyrifos, and amitraz - the efficacy variables based on oviposition (EIP) and fertility (EIF) were compared using Pearson correlation analysis. Additionally, agreement between the standard and alternative tests was assessed using Cohen's kappa statistic based on efficacy categories (high, moderate, or low). The overall Pearson correlation between EIP and EIF was 0.9273, indicating a strong correlation. High correlations were also observed individually: cypermethrin (r=0.8832), deltamethrin (r=0.9457), amitraz (r=0.8988), and cypermethrin+chlorpyrifos (r=0.8932). In terms of kappa agreement, cypermethrin (k=0.7922) and deltamethrin (k=0.7832) showed moderate agreement between the two methods, while amitraz (k=0.8233) and cypermethrin+chlorpyrifos (k=0.8088) showed strong agreement. These results demonstrate that diagnostic evaluations based on egg mass production at 14 days can serve as a reliable proxy for the full AIT. This approach significantly benefits livestock producers by reducing the time needed for diagnosis, improving responsiveness, and supporting more effective tick control strategies

A new larval packet test protocol for field screening of fluralaner resistance in *Rhipicephalus microplus*

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Rhipicephalus microplus is considered the most important bovine parasite worldwide. Tick control relies primarily on chemical aca-

ricides, in a scenario of widespread multiple resistance. Fluralaner, an isoxazoline compound, is the most recent acaricide introduced to the market. Therefore, it is essential to standardize testing methods to establish baseline susceptibility values for both reference strains and field populations. These standardized tests serve as diagnostic tools for early detection and surveillance of emerging resistance in the field. This study aimed to develop a larval packet test (LPT) using technical grade and commercial fluralaner to determine baseline susceptibility in *R. microplus* populations from Brazil. LPTs were conducted with ticks from one susceptible and two resistant reference strains, as well as nine field populations from Rio Grande do Sul state, southern Brazil. Mortality data were analyzed by probit analysis to determine the median lethal concentration (LC50) for each sample. Field efficacy trials with fluralaner pour-on were also conducted on naturally infested cattle from two sampled farms. LC50 values for technical fluralaner were significantly lower than those for the commercial formulation in the susceptible strain POA ($P < 0.001$). LC50s in resistant colonies were up to 3.2-fold higher than in POA. LC50 ratios in field populations ranged from 0.642 to 1.511. Field efficacy reached 100% for up to 49 days in both farms (lethal dose ratios: Farm 1 = 1.5; Farm 3 = 1.2). The observed LC50 variation likely reflects the natural biological variability of *R. microplus* populations rather than established resistance, as field efficacy remained high. This study contributes to resistance surveillance by supporting the early detection of fluralaner resistance in tick populations.

Larval packet test evaluation of chlorpyrifos and cypermethrin: mixture effects vs. isolated compounds

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Rhipicephalus microplus is the most important bovine parasite in tropical and subtropical regions. Control of cattle ticks mainly involves the use of chemical acaricides. However, resistant ticks have been selected due to their indiscriminate use. Resistance to organophosphates (OP) and synthetic pyrethroids (SP) are described since 1972 and 1989, respectively, in tick populations from Rio Grande do Sul (RS) state, Brazil. Resistance to mixtures of OP+SP was described for the first time in 1995, in the same region. The aim of this work was to assess the resistance of OP, SP, and a mixture of OP+SP in tick populations from cattle farms in RS. Additionally, the association and correlation between those tests was analyzed. Larval packet tests (LPT) were performed with previously established discriminating doses for cypermethrin (CYP, 1%), chlorpyrifos (CLO, 0.08%), and a mixture of CYP and CLO (CYP+CLO, 0.015%+0.025%). Resistance intensity was classified in four levels (1 to 4), according to the mortality of larvae. Using those levels, the association of resistance between CYP and CYP+CLO, and CLO and CYP+CLO were performed by Chi-square test, as well as the correlation. Tick samples from 206 cattle farms were included in this study. Resistance (mortality < 95%) to CLO, CYP, CYP+CLO, and to all compounds was observed in 92%, 100%, 98%, and 92% of the samples,

respectively. Most of the populations of ticks demonstrated resistance level 4 to CYP (55%), and to CYP+CLO (45%). For CLO, resistance was predominantly level 1 (36%). The association between resistance to CYP and CYP+CLO, and CLO and CYP+CLO were both statistically significant (p value≤.001). The only positive correlation observed was between CLO and CYP+CLO (R2=.5288). Probably, resistance to CYP+CLO is determined through the resistance to CLO. Resistance to these acaricides classes is spread in Rio Grande do Sul. These results show the possibility of performing LPT with a mixture of compounds.

The potential for use of haematological and anti-IgE humoral responses as phenotypic markers for tick resistance in cattle

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Approximately 80% of the global cattle population is at risk of infestation and infection by ticks and tick-borne diseases (TTBDs). The economic losses from animal mortality, lost production, the cost of vector control and animal treatment are substantial especially in Africa, hence the need for integrated tick control strategies. Breeding for host tick resistance is a scalable TTBD control especially in cattle. However, the gold standard method for phenotyping tick resistance in cattle remains bodily tick counting which is very laborious and subjective. Better methods for phenotyping tick resistance that are objective, faster and scalable are urgently needed. We aimed at assessing the correlation between haematological cellular profiles and immunological responses (immunoglobulin E, IgE) and full body tick counts in herds of *Bos indicus* and *Bos taurus* following artificial tick challenge with *Rhipicephalus decoloratus* larvae. Fifty-four Friesian and Ayrshire (*Bos taurus*) and 52 East African Zebu (*Bos indicus*) calves were each infested with ~2500 larvae. Near-replete adult female ticks (≥ 4.5 mm) were counted daily from Day 20–25. Blood and serum samples were obtained from each animal on Days 0 and 23 for cellular blood and IgE titre analysis, respectively. The indicine cattle were refractory to *R. decoloratus* infestation in comparison with the taurine breed ($P < 0.0001$). Repeated measurements of blood components pre-infestation revealed a significant ($P < 0.05$) association with tick count in IgE and red blood cells, haematocrit, and haemoglobin post-infestation. There was also a strong positive correlation between the tick counts and red blood cell numbers, haemoglobin, haematocrit, and IgE concentration ($P < 0.0001$) following tick challenge. The utility of this approach to phenotype host resistance needs to be validated using higher cattle numbers and with different tick species. Our results contribute to the search for suitable phenotypes for quantifying genetic resistance to ticks in cattle.

Poster Presentation
Day 21

Poster Presentation - Day 21

Antiparasitic drugs and drug development

Comparative speed of kill provided by lotilaner (Credelio), sarolaner (Simparica Trio), and afoxolaner (NexGard) to control *Amblyomma americanum* infestations on dogs

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Canine acaricides with rapid onset and sustained activity can reduce pathogen transmission risk and enhance pet owner experience. This randomized, complete block design, investigator-masked study compared the speed of kill of *Amblyomma americanum* provided by three monthly-use isoxazoline-containing products. Study groups (8 beagles/group) were treated (day 0), per label, with sarolaner (combined with moxidectin and pyrantel, Simparica Trio™), afoxolaner (NexGard™), or lotilaner (Credelio™), or remained untreated. Infestations (50 adult *A. americanum*/infestation) were conducted on days - 7, - 2, 21, and 28, and tick counts were performed on day - 5 (for blocking), and at 4, 8, 12, 24, 48, and 72 h following treatment and re-infestations. Efficacy calculations were based on geometric mean live tick counts with a linear mixed model used for between-group comparisons. On day 0, only lotilaner significantly reduced an *A. americanum* infestation by 12 h (43.3%; $P = 0.002$). On day 21, at 12 h postinfestation, lotilaner efficacy (59.6%) was significantly different from sarolaner (0.0%) ($P < 0.001$) and afoxolaner (6.3%) ($P < 0.001$). On day 28, at 12 h postinfestation, lotilaner efficacy (47.8%) was significantly different from sarolaner (17.1%) ($P = 0.020$) and afoxolaner (9.0%) ($P = 0.006$). At 24 h, lotilaner efficacy (92.3%) was significantly different from sarolaner 4.9% ($P < 0.001$) and afoxolaner (0.0%) ($P < 0.001$). Speed of kill for sarolaner and afoxolaner, but not lotilaner, significantly declined over the study period. Only lotilaner achieved ≥ 90% efficacy by 24 h post-treatment and 24 h postinfestation on days 21 and 28. Lotilaner has a more rapid onset of acaricidal activity against *A. americanum* throughout the dosing period compared to sarolaner (combined with moxidectin and pyrantel) and afoxolaner.

In vitro evaluation of the acaricidal activity of *Illicium verum* essential oil against unfed instars of *Rhipicephalus sanguineus* sensu stricto

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The species *Rhipicephalus sanguineus sensu stricto*, of medical veterinary relevance, serves as a vector of pathogens, primarily transmitting diseases to and parasitizing dogs. Conventional control mostly uses chemical acaricides, but their use has led to the emergence of resistant populations. One alternative that has been studied is the use of essential oils (EOs), due to their natural acaricidal properties. Additionally, EOs offer a more sustainable approach with lower environmental impact. In this context, a study was conducted to evaluate the *in vitro* acaricidal activity of star anise (*Illicium verum*) EO against unfed larvae and adults of *R. sanguineus s.s.*. The specimens originated from a colony maintained at the Laboratório de Quimeoterapia Experimental em Parasitologia Veterinária at UFRRJ (CEUA IV/UFRRJ No. 1268101223). The packet test was performed in triplicate using filter papers impregnated with serial concentrations of the EO, ranging from 6.25 to 100mg/ml. Approximately 100 larvae and 8 adults were placed in each packet. The packets were kept in BOD incubators at 27 ± 1 °C and 80 ± 10% relative humidity for 24 hours. Mortality was assessed after this period. The mortality data were utilized to calculate the lethal concentration 50 (CL50) per probit analysis with a 95% confidence interval (CI 95%). Larval mortality reached 100% from 50 mg/ml onward, while adult mortality peaked at 82.7% at 75 mg/ml. The LC50 was 33.33 mg/ml (25.39–39.64) for larvae and 46.89 mg/ml (31.72–60.85) for adults, indicating that larvae were 1.4 times more susceptible. It is concluded that *I. verum* EO has acaricidal potential against *R. sanguineus*, with greater effectiveness against larvae.

In vitro evaluation of the insecticidal activity of cinnamaldehyde on *Haematobia irritans* adults

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Cinnamaldehyde is an aromatic compound found in cinnamon essential oil, commonly used in the food and cosmetic industries. Recently, it has attracted attention for its bioactive properties, including insecticidal effects. Given the need for alternatives to conventional insecticides, this study aimed to evaluate the insecticidal activity of cinnamaldehyde against Haematobia irritans and determine its lethal concentrations (LC50 and LC95). Flies were collected in the morning from naturally infested cattle at the experimental farm of the Laboratório de Quimioterapia Experimental em Parasitologia Veterinária, Universidade Federal Rural do Rio de Janeiro-Seropédica, using an entomological net and were immediately placed in modified polypropylene cages. The flies were then exposed to filter paper discs impregnated with varying concentrations of cinnamaldehyde diluted in acetone (0.8, 3.9, 5.9, 6.7, 7.9, 9.8, 11.8, and 15.7 µg/cm^2). Each concentration was tested in triplicate, with an average of 15 flies per replicate. Mortality was assessed at 2- and 4-hours post-exposure, and the data were used to calculate LC50 and LC95 values using Polo-Plus software, with a 95% confidence interval ($p \leq 0.05$). At the 2-hour evaluation, mortality generally increased with concentration, except at 7.9 µg/cm^2, which showed 5% lower mortality than the previous concentration (6.7 µg/cm^2). Complete mortality was only observed at 15.7 µg/cm^2. At the 4-hour evaluation, complete

mortality was observed from 7.9 µg/cm². The LC50 values were 7.4 (6.9–7.9) µg/cm² and 4.7 (4.3–5.1) µg/cm², while the LC95 values were 13.3 (11.8–15.9) µg/cm² and 6.9 (6.3–7.9) µg/cm² at 2 and 4 hours, respectively. These findings confirm that cinnamaldehyde exhibits insecticidal activity against H. irritans, supporting its potential as an alternative control method for this species.

In vitro evaluation of the insecticidal activity of eugenol on _Haematobia irritans_ adults

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Eugenol is a volatile phenolic compound and the major constituent of clove essential oil. It exhibits several biological properties, including insecticidal activity by affecting the insect nervous system, making it a promising candidate for eco-friendly pest control strategies. Despite its recognized insecticidal potential, no previous studies have evaluated its activity against *Haematobia irritans*. This study sought to evaluate the insecticidal activity of eugenol against *H. irritans* and determine its lethal concentrations (LC50 and LC95). Flies were collected in the morning from naturally infested cattle at the experimental farm of the Laboratório de Quimioterapia Experimental em Parasitologia Veterinária, Universidade Federal Rural do Rio de Janeiro-Seropédica, using an entomological net and immediately placed in adapted polypropylene cages. They were exposed to filter paper discs impregnated with different concentrations of eugenol diluted in acetone (0.8, 3.9, 7.9, 11.8, 15.7, 23.6, 31.4, 39.3 µg/cm²). Each concentration was tested in triplicate with an average of 15 flies per replicate. Mortality assessments were conducted at 2- and 4-hours post-exposure and the data were used to determine LC50 and LC95 values using PoloPlus software with a 95% confidence interval ($p \leq 0.05$). No mortality was observed at the lowest concentration at either evaluation time. At 2 hours, 84.39% mortality was observed at 11.8 µg/cm², while 78.68% mortality was recorded at the next higher concentration (15.7 µg/cm²), unlike the 4-hour evaluation, where mortality rates were proportional to increasing concentrations. Complete mortality was achieved at 31.4 µg/cm² at 2 hours and at 23.6 µg/cm² at 4 hours. LC50 values were 10.5 (8.8–12.3) µg/cm² and 8.2 (6.9–9.5) µg/cm², and LC95 values were 18.4 (15.9–23.2) µg/cm² and 15.4 (13.4–18.7) µg/cm² at 2 and 4 hours, respectively. Eugenol showed insecticidal activity against *H. irritans*, indicating its potential as an alternative control method.

Evaluation of the in vitro activity of fipronil against _Amblyomma sculptum_ and _Amblyomma cajennense_

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Among the main ticks of veterinary importance is the genus *Amblyomma* (Ixodidae). Due to its low parasitic specificity, it can infest several species, including humans, and is a vector of pathogens of public health importance. Understanding how members of this genus respond *in vitro* to acaricidal compounds is important to establish control strategies. Thus, the aim of this study was to evaluate the *in vitro* activity of fipronil against nymphs and unfed adults of *A. cajennense* and *A. sculptum*. Specimens from laboratory colonies maintained at the Laboratório de Quimioterapia Experimental em Parasitologia Veterinária of the UFFRJ (CEUA IV/UFRRJ n° 1268101223) were used. The *in vitro* evaluation was performed in triplicate using the larval packet test with fipronil concentrations ranging from 7.8125 to 1000 ppm for nymphs and 78.125 to 5000 ppm for adults. Approximately 50 nymphs and 8 adults were placed in each package and kept at 27°C ± 1°C and 80% ± 10% relative humidity for 24 hours to assess mortality. The mortality data obtained were analyzed via probit to determine the lethal concentration 50 (LC50) with a 95% confidence interval (95% CI). The LCs50 (95% CI, in ppm) for *A. sculptum* nymphs was 25.07 (21.26–29.43) and for *A. cajennense* it was 24.67 (20.29–29.15). For adults, the LCs50 (95% CI, in ppm) were 149.92 (119.80 -183.96) and 333.11 (228.39 - 467.49) for *A. sculptum* and *A. cajennense*, respectively. The ratio between the LC50 values for nymphs was 1.02, indicating similar susceptibility to fipronil between the two species. In contrast, adults *A. cajennense* was 2.2 times more tolerant than *A. sculptum*. These results suggest that the susceptibility of the evaluated species to fipronil shows little variation.

Between larvae, nymphs and adults: tolerance of *Amblyomma sculptum* and *Amblyomma cajennense* to fluralaner in vitro

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Amblyomma sculptum and *Amblyomma cajennense* sensu stricto are two of the six species that comprise the *Amblyomma cajennense* complex. These ticks exhibit a broad parasitic capacity and can infest various species, including humans, making them significant to public health. Evaluating the response of both species in *in vitro* assays can aid in developing control strategies, monitoring acaricide susceptibility, and enabling the early detection of resistance. The objective of this study was to evaluate the unfed stages of *A. sculptum* and *A. cajennense* when exposed *in vitro* to different concentrations of fluralaner. Both species used are kept in a laboratory colony (CEUA IV/UFRRJ n° 1268101223) at the Laboratório de Quimioterapia Experimental em Parasitologia Veterinária of the UFFRJ. The package test essays were performed in triplicate whit serial concentrations of fluralaner, ranging from 400 to 3.125 ppm for larvae and nymphs, and from 6,400 to 50 ppm for adults. Each test package contained approximately 50 larvaes and nymphs and eight adults. The data were analyzed using pro-

bit analysis to determinate the lethal concentration 50 (LC_{50}) whit 95% confidence intervals (95% CI). The LC_{50} values (95% CI; in ppm) for larvae, nymphs and adults were 28.49 (32.27-36.99), 28.39 (19.73-40.86) and 174.226 (135.49-220.24) for *A. sculptum* and 47.28 (28.23-70.77), 20.81 (18.84-22.62) and 260.40 (196.74-337.83) for *A. cajennense*, respectively. Nymphs of *A. sculptum* were 1.36 times more tolerant to fluralaner than *those of A. cajennense*, while *A. cajennense* larvae and adults were 1.65 and 1.49 times more tolerant, respectively, compared to *A. sculptum*. In conclusion, there were few variations between the two species analyzed, with tolerance to fluralaner showing only minor differences depending on the phase of the life cycle.

Pharmacokinetic assessment of a novel Albendazole-Levamisole combined formulation in cattle: patterns of tissue/parasite accumulation

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The use of anthelmintic combinations is a recognized strategy to optimize parasite control and delay resistance. In this context, a new oral formulation combining albendazole and levamisole was evaluated in cattle. The studies included: (i) assessment of plasma drug profiles following administration of each drug separately or in combination; and (ii) analysis of tissue and parasite concentrations of the main analytes in the gastrointestinal system. Plasma pharmacokinetics showed that systemic exposure to each active principle was maintained without adverse interactions. Enhanced concentrations of target analytes were detected in gastrointestinal tissues and parasites following administration of the combined formulation. The highest concentrations were measured within adult nematodes after the treatment with the new combined formulation, with concentrations within target nematode parasites ranging from 8.1 to 11.9 µg/g. These concentrations levels where higher than those recovered after the single treatment (between 0.55 and to 5.3 µg/g). These findings support the potential of this novel combination as an effective alternative for the control of resistant parasites in cattle.

Evaluation of anthelmintic efficacy of Biopersol Forte M.V. administered subcutaneously to naturally infected cattle

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The majority of cattle in Brazil are parasitized by several species of helminths at the same time. The economic losses caused by cattle parasites can be measured by the negative effects of parasitism on cattle productivity, such as lower milk production, reduced weight gain, growth retardation, predisposition to other diseases, reduced response to vaccines and even death

of young animals. The aim of this study was to evaluate the anthelmintic efficacy of Biopersol Forte M.V. (levamisole hydrochloride 17,76%) in naturally infested cattle. The studies only began after approval by the UFRRJ Animal Ethics Committee and were carried out in accordance with Portaria 48 of the Ministry of Agriculture and Livestock (MAPA). For the anthelmintic study and the efficacy study against *Dicyocaulus viviparus*, EPG and coproculture evaluations were carried out prior to treatment. The animals were randomized into control and treated groups according to the mean values of the EPG counts. The animals on the control groups received no treatment, while the animals on the treated groups received Biopersol Forte M.V. at a dose of 1 mL/50kg bodyweight subcutaneously, corresponding to a minimum dose of 3,55 mg levamisole hydrochloride/kg bodyweight. After treatment, feces were collected on D+2, D+4 and D+6 for coproculture and EPG evaluations. On D+7 all animals were euthanized, and parasitological necropsies were carried out to collect and identify the helminths in order to assess the product's efficacy. Biopersol Forte M.V. had efficacy over 98% for *Dicyocaulus viviparus*, *Haemonchus placei*, *Haemonchus similis* and *Oesophagostomum radiatum* and efficacy over 92% for *Trichostrongylus axei*, *Cooperia pectinata*, *Cooperia punctata*, *Bunostomum phlebotomum* and *Trichuris discolor.* According to the data presented, Biopersol Forte M.V. is an excellent treatment option for cattle affected by both gastrointestinal and pulmonary helminths.

Evaluation of the therapeutic efficacy of Stand up® against *Babesia bovis* and *Babesia bigemina* in experimentally infected cattle

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Babesiosis is a hemolytic tick-borne disease caused by intraerythrocytic protozoal parasites of the genus *Babesia*. The disease can be transmitted biologically by *Rhipicephalus microplus*, considered endemic in Brazil due to the tropical and subtropical climate conditions. The aim of this study was to evaluate the therapeutic efficacy of a product containing Imidocarb 12% (Stand Up®), administered subcutaneously at a dose of 1 mL/100 kg of body weight, against *Babesia bovis* and *Babesia bigemina* on experimentally challenged cattle. Before the experimental phase, the protocol was analyzed and approved by the ethics committee of Nowavet under number 382. The experimental design was entirely randomized with four groups distributed in 8 subgroups of 2 animals each, in accordance with Portaria 48 (Mapa). Sixteen clinically healthy cattle aged between 9 and 10 months were selected. From days D-4 to D0, eight cattle were randomly selected to undergo immunosuppression with dexamethasone. Treatment was given to each animal when parasitemia of more than 0,5% was detected in the *Babesia bovis* group and 2% in the *Babesia bigemina* group, in addition to hyperthermia (temperature higher than 39,2°C) and a drop in globular volume (less than 24%). All the immunosuppressed animals developed alterations on white blood cells count and all animals showed clinical signs of babesiosis. The symptoms star-

ted on D+7 until D+31 after babesiosis challenge, with some animals showing temperature above 40°C and globular volume less than 10%. Eight animals received Stand Up treatment and were monitored for at least 10 days. After treatment, body temperature and globular volume returned to normal, parasitemia was negative with 12 hours and all animals were PCR negative. According to the evaluations and results found in this study Stand Up® was effective against *Babesia bovis* and *Babesia bigemina* parasitizing experimentally infected cattle.

Evaluation of the effectiveness and preventive efficacy of the product Forbox® FT against the tick *Rhipicephalus microplus* in experimentally infested cattle (traditional and modified pen trial)

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Cattle ticks cause major economic losses worldwide including Brazil and are predominantly controlled with acaricides, especially when animals are heavily infested. The objective of this study was to evaluate the tick efficacy of a product based on cypermethrin 4%, imidacloprid 4% and fluazuron 3% (Forbox® FT) on treatment and prevention of *Rhipicephalus microplus* in cattle using the traditional and modified pen trial. This study was approved by the Ethics Committee for the Use of Animals (CEUA IV UFRRJ 2355230819) and began only after approval. The design was carried out in accordance with Portaria 48 (1997) of the Ministry of Agriculture and Livestock (MAPA). On day -34, 16 animals were acclimatized in pens and on day -1, 12 cattle were selected. The animals were randomized into two groups of 6 animals each (Control and Treated Group) by means of a list, in descending order, of the average values of the teleogynes released on days -3, -2 and -1. At thirteen different times during the pre-treatment period, infestations occur with 2,500 to 5,000 *Rhipicephalus microplus* larvae. Treatment was made on day 0 for the Treated Group using the product Forbox® FT at a dose of 1 mL/10 Kg of weight by pour on via. Infestations with 10,000 larvae took place at weekly intervals from day +7 to +70 after treatment. Post-treatment ticks counts were taken daily until the end of the study. Carrapaticidal efficacy (D+1 until D+23) was 95.6%, meeting the requirements of Portaria 48. There was also an evaluation of the reproductive inhibition showing an average efficacy of 88,3% considering D+1 until D+23. Preventive tick efficacy was 97,8% (D+7); 98,5% (D+14); 98,4% (D+21); 97,6% (D+28); 97,6% (D+35); 98,5% (D+42); 97,9% (D+49); 98,8% (D+56); 81,2% (D+63) and 50,2% (D+70). Forbox® FT showed tick efficacy above 95% during 23 days after treatment on the traditional pen trial, as well as protection against reinfestation at weekly intervals up to 56 days after treatment.

Larvicidal activity and synergistic effects of diaryl dichalcogenides against *Haemonchus contortus* in combination with ivermectin

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Gastrointestinal nematode (GIN) infections significantly impact small ruminant production worldwide, with *Haemonchus contortus* being the most pathogenic nematode species. The widespread multidrug resistance in nematodes has pushed the search for alternative therapies. The present study aimed to investigate the anthelmintic effect of diacetal ditelluride (LQ07) and diacetal diselenide (LQ62), individually and in combination with ivermectin (IVM), against *H. contortus*. Third-stage larvae (L3) were obtained from naturally infected sheep at the Sheep and Goat Production and Research Center, UFPR, Brazil. L3 were treated for 24 h at 28 °C, then placed on 24-well plates fitted with 25 µm mesh. Migrated L3 were counted after mesh removal. Drug combination assays were performed using the fixed IC50 of each compound in combination with IC10, IC30, and IC50 of IVM, and vice versa. For cell death analysis, L3 were exposed to the IC90 of each compound for 24 h at 28 °C and subsequently stained with propidium iodide (PI). Representative images were captured using confocal microscopy. LQ62 exhibited the most potent larvicidal activity. The IC50 of LQ07 and LQ62 for inhibiting L3 migration was 2.21 mmol L-1 and 0.90 mmol L-1. The combination of diacetal organochalcogen and IVM results in a significant drug interaction, achieving greater than 90% larvicidal activity at all tested concentrations. The additive effect ranged from 10.7% to 33.9%, inhibiting L3 migration. Images of PI-stained L3 demonstrated that LQ62 triggered general PI labeling, revealing cellular death in multiple cells and organs. In contrast, LQ07 yielded cellular death in intestinal cells and nerve rings at the anterior and posterior end of the cells. Our data contribute to novel blending-based therapeutic strategies using innovative organochalcogen compounds with distinctive mechanisms of action to control multidrug-resistant parasites of small ruminants.

Inhibitory effect of Ebselen in combination with ivermectin against *Haemonchus contortus*

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Gastrointestinal nematode infections (GIN) constitute a significant challenge in pasture-based livestock systems. *Haemonchus contortus* is the predominant Strongylid parasite in tropical and subtropical regions, leading to high mortality of young animals. The emergence of multidrug-resistant parasites has intensified the search for new and safe compounds. This study evaluated the anthelmintic effect of Ebselen, a selenium-based organochalcogen, against eggs and third-stage larvae (L3) of *H. contortus*. The inhibitory effect of Ebselen in combination with ivermectin (IVM) was also tested. Eggs and L3 were obtained from natu-

rally infected sheep at the Sheep and Goat Production and Research Center of UFPR, Brazil. Approximately 200 eggs per well were treated with Ebselen for 48 h at 28 °C. Lugol's iodine solution was added to halt egg hatching. The number of unhatched eggs and first-stage larvae was quantified. For the larval migration inhibition assays, around 200 L3 per well were treated with Ebselen for 24 h at 28 °C, then washed and placed on 24-well plates fitted with 25 µm mesh. Migrated L3 were counted after mesh removal. Drug combination assays used the fixed IC50 of Ebselen combined with IC10, IC30, and IC50 of IVM, and vice versa. The data revealed that Ebselen exhibited inhibitory efficacy ranging from 30.9% to 83.4% on eggs, and from 22.8% to 89.9% against L3. The IC50 of Ebselen and IVM for inhibiting egg hatching and L3 migration was 0.48 mmol L-1 and 0.44 mmol L-1, and 1.56 mmol L-1 and 0.91 mmol L-1, respectively. The combination of Ebselen and IVM showed a promising drug-drug interaction, achieving >90% larvicidal activity at all tested concentrations. An additive effect >30% was observed when the IC10 of Ebselen was combined with the IC50 of IVM, and vice versa. These findings describe the ovicidal and larvicidal effect of Ebselen. The drug interaction is a promising therapeutic strategy to manage *H. contortus* infections in small ruminants.

Larvicidal Activity of Fluralaner Against *Dermacentor nitens*

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The tick *Dermacentor nitens* preferably parasitizes equines; however, there are few acaricidal products specifically registered for use in this animal species, which often leads to the off -label application of formulations developed for cattle. This scarcity of therapeutic alternatives encourages the investigation of compounds with acaricidal activity that can be used in horses, such as isoxazolines. In this context, the aim of this study was to evaluate the response of *D. nitens* larvae exposed in vitro to fluralaner. The analyses were conducted at the Laboratory of Experimental Chemotherapy in Veterinary Parasitology, affiliated with UFRRJ. For the larval packet test (LPT), filter papers impregnated with fluralaner in serial concentrations ranging from 3.125 to 400 ppm were used. The LPT was performed in triplicate, with approximately 100 unfed *D. nitens* larvae, about 21 days old and from a laboratory colony (CEUA IV/UFRRJ no. 4653140723), placed in each packet. The packets were then stored in climate-controlled chambers at 27 ± 1°C and 80 ± 10% relative humidity to assess mortality after 24 hours. Mortality data were used to calculate lethal concentrations by probit analysis with 95% confidence intervals (95% CI) using the Polo Plus software. A progressive increase in mortality was observed, reaching 100% from 50 ppm onwards. The estimated LC_{50} and LC_{95} (95% CI, in ppm) were 17.72 (13.53–23.30) and 54.39 (28.43–76.90), respectively. These results indicate that high concentrations of the compound are not required to achieve significant mortality rates, suggesting that a fluralaner-based formulation developed

for administration in horses could be an effective tool for the control of *D. nitens*.

A From Larvae to Adults: How *Rhipicephalus sanguineus* sensu stricto and *Rhipicephalus linnaei* Ticks Respond to Fluralaner.

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Two species of the *Rhipicephalus sanguineus* complex, *R. sanguineus* sensu stricto and *R. linnaei*, are among the main parasites of dogs in Brazil and are commonly controlled using acaricides such as isoxazolines. Understanding how both species respond to in vitro assays can aid in diagnosing resistant populations and developing targeted control strategies. The objective of this study was to evaluate the unfed stages of *R. sanguineus* s.s. and *R. linnaei* when exposed in vitro to fluralaner. A strain of each species (RsPA and RsRJ, respectively) are maintained in a laboratory colony (CEUA/IV 9812271021) at the Laboratório de Quimioterapia Experimental em Parasitologia Veterinária at UFRRJ. RsRJ was originally isolated in Seropédica, RJ, in 2008, while RsPA was isolated in Eldorado do Sul, RS, in 2022. Species confirmation was performed through sequencing of the 12S rRNA gene. Using the packet test, the lethal concentration 50 (LC_{50}) and the 95% confidence intervals (CI 95%) were determined for the unfed larvae, nymph, and adult stages. The LC_{50} values (CI 95%; in mg/mL) for larvae, nymphs and adults were 16.4 (11.5–17.4),19.13 (17.8–20.5), and 180.0 (138.9–257.1) for *R. linnaei* and 3.6 (3.4–3.8), 6.6 (5.9–7.3), and 29.5 (19.6–41.4) for R. sanguineus respectively. *R. linnaei* larvae were 4.5 times more tolerant to fluralaner than *R. sanguineus* s.s. larvae, while nymphs and adults were 2.8 and 6 times more tolerant, respectively. It is concluded that the LC_{50} increases with each developmental stage evaluated, and that RsRJ strain of *R. linnaei* is more tolerant to fluralaner than the RsPA strain of *R. sanguineus s.s.*.

***In vitro* synergistic activity of the growth disruptors pyriproxyfen and fluazuron against engorged females of *Rhipicephalus sanguineus* sensu lato**

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Insect growth disruptors are a class of unconventional ectoparasiticides that promote control by disrupting the development of these organisms. In addition to being safer for vertebrates, they also assist in controlling the developmental stages present in the environment, complementing the efficacy of these products. This study aimed to evaluate the synergistic effect of pyriproxyfen combined with fluazuron on engorged females of the tick *Rhipice-*

phalus sanguineus sensu lato. The ticks used were obtained from a laboratory colony approved by CEUA/IV.UFRRJ under protocol number 9812271021. After natural detachment from the hosts and sanitization, the teleogines were immersed in solutions of pyriproxyfen, fluazuron, and their combination at concentrations of 250, 500, and 750 µg/mL. Immersion lasted 10 minutes, followed by drying and incubation in Petri dishes for 21 days in a climate-controlled chamber at 27°C and 80% relative humidity. Subsequently, egg masses were individually weighed and incubated for another 21 days, at which point the larval hatching percentage and reproductive efficiency (RE) were determined. For the control group, the teleogines were immersed only in the diluent. Each treatment was performed in four replicates with 10 teleogines each. Data were tabulated, and the combinatory effect was evaluated using the Compusyn software. No significant differences in egg mass weight were observed between treated groups and the control. The efficacy on RE was 38.5%, 75.4%, and 81.9% for pyriproxyfen; 49.8%, 69.1%, and 78.4% for fluazuron; and 86.1%, 89.2%, and 96.4% for the combination at the three tested concentrations. The combination indices (CI) obtained were 0.39, 0.63, and 0.46, indicating a synergistic effect between pyriproxyfen and fluazuron on engorged females of *R. sanguineus* sensu lato. Thus, it is possible to conclude that the combination of these two compounds presents a promising strategy for the effective control of this ectoparasite.

In vitro synergistic activity of the growth disruptors pyriproxyfen and fluazuron against fed nymphs of *Rhipicephalus sanguineus* sensu lato

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Due to the negative parasite-host interaction and its wide geographical distribution, *Rhipicephalus sanguineus* sensu lato is considered one of the main ectoparasites of veterinary importance. Given the need for safer molecules for its control, the insect growth disruptors fluazuron and pyriproxyfen represent a promising strategy due to their lower toxicity profiles. The aim of this study was to evaluate the synergistic effect of the combination of these compounds against engorged nymphs of *R. sanguineus* sensu lato. The ticks were obtained from a laboratory colony, approved by the CEUA under protocol number 9812271021. The concentrations used were 100, 250, and 500 µg/mL for each compound, both individually and in combination. After collection, the nymphs were immersed in 10 mL of solution for 10 minutes, dried, and stored in adapted 3 mL syringes, then incubated for 21 days in a climate-controlled chamber at 27 °C and 80% relative humidity. After this period, the number of live and dead nymphs and adults was recorded, and ecdysis inhibition (EI) was calculated. Based on the EI results, the combination index (CI) for each concentration was determined using Compusyn software. The EI percentages were 28.7%, 35.1%, and 34.4% for pyriproxyfen; 17.2%, 51.7%, and 54.9% for fluazuron; and 61.7%, 58.7%, and 68.2% for the fluazuron + pyriproxyfen combinations, respectively. The CI values obtained were 0.2, 0.55, and 0.61, indicating a synergistic effect at all evaluated concentrations. Additio-

nally, morphological changes were observed in adult specimens, such as the absence of one or two legs, limb shortening, and body size reduction. In conclusion, the combination of fluazuron and pyriproxyfen enhances their effects on ecdysis inhibition in engorged nymphs of *R. sanguineus* sensu lato, making it a promising alternative for controlling this arthropod, especially in resistant strains, due to the potential for multi-target action.

In vitro synergistic activity of the growth disruptors fluazuron and piriproxifen against engorged larvae of *Rhipicephalus sanguineus* sensu lato

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The search for associations of acaricidal compounds allows us to observe actions previously unseen and/or reduce the dosage in treatments. Thus, the study aimed to evaluate in vitro the synergistic activity of the fluazuron (FLU) and piriproxifen (PIR) association against fed larvae of *Rhipicephalus sanguineus* s.l. After pilot tests, dilutions of FLU were prepared at concentrations of 0.25; 0.5 and 1 µg/mL, PIR at concentrations of 0.5; 1 and 2 µg/mL, and the FLU+PIR association at concentrations of 0.25+0.5; 0.5+1 and 1+2 µg/mL. For each replicate, 30 engorged larvae of *R. sanguineus* s.l. from a laboratory colony (CEUA-UFRRJ n° 9812271021) were immersed in 10 mL of each solution for 10 minutes. Subsequently, they were dried, placed in adapted 3 mL syringes, and incubated in a climate chamber at 27±1°C and 80±10% humidity for 21 days. Each concentration had six replicates, with a control group and a placebo. After 21 days, the number of larvae that underwent ecdysis was recorded to calculate the ecdysis inhibition percentage (IE) using the following formula: IE (%) = [(number of ticks that did not undergo ecdysis / total ticks)× 100]. To verify the interactions between PIR and FLU, the ecdysis inhibition data were calculated using the CompuSyn 1.0 program. The results were classified according to the combination index (CI): synergism (<0.70); moderate synergism (0.70–0.90); additive (0.90–1.10); moderate antagonism (1.10–1.45) and antagonism (>1.45). As results, while the control and placebo groups had IE lower than 4%, FLU at concentrations of 0.25; 0.5, and 1 µg/mL showed IE of 21.35; 45.25, and 67.48%, respectively. PIR at concentrations of 0.5; 1, and 2 µg/mL showed IE of 7.19; 5.81, and 6.40%, respectively. When testing the synergism of FLU and PIR at concentrations of 0.5 + 0.25; 1 + 0.5; and 1 + 2 µg/mL, the IE was 36.99%, 51.19%, and 74.01%, with CI of 0.7; 0.7, and 0.8, respectively. It is concluded that the association of fluazuron and piriproxifen shows moderate synergism against fed larvae of *R. sanguineus* s.l.

Evaluation of in vitro acaricidal activity of benzoylphenylureas on engorged *Amblyomma sculptum* larvae

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Amblyomma sculptum is an ectoparasite of relevance to One Health, controlled by acaricides such as benzoylphenylureas (BPUs), which exhibit selectivity toward arthropods and greater safety in vertebrates. In this context, the aim of the study was to evaluate the acaricidal effect of fluazuron (FLU), novaluron (NOV), teflubenzuron (TEF), and triflumuron (TRI) on engorged larvae of *A. sculptum*. Starting from a stock solution, serial dilutions of each BPUs were prepared in a solvent mixture composed of 0.8% DMSO, 0.04% Triton X, 0.081% N-methylpyrrolidone, 2.5% acetone, and distilled water q.s.p., to obtain 10 concentrations ranging from 0.009–10 µg/mL for FLU and 78.125–40,000 µg/mL for NOV, TEF, and TRI. For each replicate, 30 engorged larvae from a laboratory colony of *A. sculptum* maintained on rabbits (protocol no. 1268101223 from CEUA-IV-UFRRJ) were immersed in 10 mL of each solution for 10 minutes. After immersion, the larvae were dried, stored in adapted 3 mL syringes, and incubated in a climate chamber at 27±1°C and 80±10% relative humidity for 21 days. Each concentration was tested in eight replicates, alongside a control and a placebo group. After the incubation period, the number of nymphs was quantified under a stereomicroscope. Ecdysis inhibition (EI) was calculated using the formula: EI (%) = {[(Total nymphs in control group – Total nymphs in treated group) × 100] / Total nymphs in control group}. The 50% inhibitory concentration (IC_{50}) was determined based on the mortality percentages for each concentration using the RStudio statistical software. As results, an EI of 97.57% was observed for FLU at 10 µg/mL and 100% for NOV at 40,000 µg/mL, whereas TEF and TRI did not significantly affect larval ecdysis. The estimated IC_{50} was 0.66 µg/mL (0.55–0.79 µg/mL) for fluazuron and 11,462.10 µg/mL (8,076.20–13,141.60 µg/mL) for novaluron, indicating that the IC_{50} of novaluron is 17,366.82 times higher. It is concluded that fluazuron and novaluron, at the tested concentrations, exhibit inhibitory activity on the ecdysis of engorged *A. sculptum* larvae.

Long-Term Efficacy of BRAVECTO® 365 (fluralaner 150 mg/ml suspension for injection) against *Rhipicephalus sanguineus* (sensu lato) Ticks on Client-Owned Dogs in Brazil

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The study evaluated the efficacy of BRAVECTO® 365 against *Rhipicephalus (R.) sanguineus* (*sensu lato*) ticks in naturally infested dogs in Minas Gerais, Brazil. Client-owned dogs naturally infested with at least five *R. sanguineus* ticks were treated once subcutaneously with BRAVECTO® 365 at a dose of 0.1 ml/kg body weight (BW) (15 mg fluralaner/kg BW). The required dose was based on the body weights of each dog determined on the day before treatment (D-1). Tick counts confirmed infes-

tation (D-8 and D-3) and were conducted post-treatment on D+2, D+7, D+14, D+30, D+60, D+90, D+160, D+180, D+250, D+270, D+336, and D+365. Results were compared to pre-treatment data to assess efficacy. No untreated control group was included due to animal welfare concerns. Forty mixed breed dogs, aged 7 to 32 months and weighing 5.6 to 16.3 kg, were included. The average tick infestation before treatment was 8.8 on D-8 and 11.5 on D-3. BRAVECTO® 365 treatment resulted in a 100% reduction of live ticks by D+7, maintaining this efficacy until D+160. From D+180 to D+365, efficacy was 99.8%, with only one dog having one tick on each counting day. All study animals completed the trial, with no drug-related adverse events recorded. BRAVECTO® 365 was efficacious and safe when administered subcutaneously at the recommended dose to dogs infested with *Rhipicephalus sanguineus* (*sensu lato*) ticks, ensuring protection against this tick species for 365 days post-treatment.

The Effectiveness of BRAVECTO® 365 (fluralaner 150 mg/ml suspension for injection) for the Control of Fleas on Naturally Infested Client-Owned Dogs in Brazil

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This study evaluated the efficacy of BRAVECTO® 365 against *Ctenocephalides (C.) felis* fleas in naturally infested client-owned dogs in Minas Gerais state, Brazil. Client-owned dogs were included in the study if they had at least five *C. felis* fleas in both pre-treatment counts conducted on D-8 and D-3. Body weights (BW) of study dogs were recorded on D-1 to determine the required dose of BRAVECTO® 365. Treatment was administered to each enrolled dog subcutaneously on D0 at a dose of 0.1 ml/kg BW (15 mg fluralaner/kg BW). Following treatment, flea counts were performed on D+2, D+7, D+14, D+30, D+60, D+90, D+160, D+180, D+250, D+270, D+336, and D+365. Results were compared to pre-treatment data to assess efficacy. The study did not include an untreated control group due to animal welfare considerations. A total of forty mixed-breed dogs, aged 7 to 40 months and weighing between 3.4 kg and 24.5 kg were included in the study. Mean pre-treatment flea counts were 12.3 on D-8 and 15.9 on D-3. BRAVECTO® 365 treatment achieved 100% reduction of live fleas by D+10, maintaining this level of efficacy until D+180. From D+210 to D+365, efficacy remained equal to or above 99.8%, as only one dog had one or two fleas on each counting day. All study animals completed the trial, and there were no treatment-related adverse events throughout the study. The results demonstrate the efficacy and safety of BRAVECTO® 365 when administered at the recommended dose to dogs infested with *Ctenocephalides felis* fleas, ensuring protection for 365 days post-treatment.

Clinical Evaluation of Fluralaner chewable tablet for Treating Naturally Infested Dogs with Otodectic Mange

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The objective of this study was to evaluate the effectiveness of a fluralaner chewable tablet, against *Otodectes cynotis* mites in naturally infested dogs in São Paulo, Brazil. Dogs were selected for enrollment based on otoscopic evaluation, which confirmed mite infestations in both ears. The dogs were randomly assigned to two treatment groups: an untreated control group and a fluralaner chewable tablet treatment group. Dogs in the fluralaner group received treatment orally on Days 0 and +28 at a dose of 10 – 20 mg fluralaner/kg body weight. Post-treatment otoscopic evaluations were conducted on Days +3, +7, +14, +21, +28, +42, +49, and +56 to evaluate treatment efficacy. Twelve dogs, aged 40 to 157 months and weighing 10.8 to 26.5 kg, were included. Six dogs were treated with fluralaner, while the other six remained untreated. At Day +42, a 100% reduction in *Otodectes cynotis* infestation was observed in the fluralaner treated group, whereas all animals in the untreated group were still infested with mites. No adverse events related to fluralaner chewable tablets were recorded. The results demonstrated the safety and efficacy of fluralaner 5.46% (w/w) chewable tablet when administered at the recommended dose twice, 28 days apart, for treating dogs infested with *Otodectes cynotis*.

Evaluation of a Chewable Tablet Containing Fluralaner + Moxidectin + Pyrantel for the Treatment of Demodectic Mange in Dogs

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This study evaluated the effectiveness of a tablet containing fluralaner, moxidectin and pyrantel against *Demodex canis* mites in naturally infested dogs in São Paulo, Brazil. Dogs that tested positive for *Demodex canis* mites in two skin scrapings taken at different time points within a week of starting were enrolled. All dogs were treated orally with the combination tablet on Days 0 and +30, at the recommended dose of 10-20 mg fluralaner/kg BW, 0.025-0.05 mg moxidectin/kg BW, and 5-10 mg pyrantel/kg BW based on body weight determined prior to treatment. Mite counts were determined on Days +10, +20, +33, +37, +44, +51, +58, +65, and +90. This study did not include an untreated control group due to animal welfare. A total of twelve dogs, aged 3 to 96 months and weighing 5.1 to 16.7 kg, were included in the study. On Days +10, +20, and +33, the treatment demonstrated mite count reduction of 16.6%, 86.8%, and 99%, respectively. From Days +37 to +90, a mite count reduction of 100% was observed. No adverse events related to the treatment were noted throughout the study. The combination chewable tablet (containing fluralaner, moxi-

dectin, and pyrantel) was safe and effective for the treatment of dogs with demodectic mange when administered twice, 30 days apart, at the recommended dose.

Evaluation of BRAVECTO® 365 (fluralaner 150 mg/ml suspension for injection) for the Treatment of Demodectic Mange in Dogs

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This study evaluated the effectiveness of BRAVECTO 365 against *Demodex canis* mites in naturally infested dogs in São Paulo state, Brazil. Dogs which were positive for *Demodex canis* mites in two skin scrapings performed at two different timepoints were enrolled in the study. All enrolled dogs were treated subcutaneously with BRAVECTO 365 at a dose of 0.1 ml/kg body weight (BW) (15 mg fluralaner/kg BW) based on body weight determined on D0 prior to treatment. Mite counts were determined on D+3, D+7, D+14, D+21, D+28, D+35, D+42, D+56, and D+63 following treatment. The study did not include an untreated control group due to animal welfare reasons. A total of ten dogs, aged between 6 and 132 months and weighing between 6.7 and 26.0 kg, were included in the study. However, eight dogs completed the study as two were removed at the request of their owners. On days D+14 and D+21, BRAVECTO 365 demonstrated efficacy rates of 94.8% and 96.6%, respectively. From D+28 to D+63, 100% reduction in *Demodex canis* infestation was observed in all treated animals. No adverse events related to the treatment were noted throughout the study in any treated dogs, including the two removed from the study by their respective owners. BRAVECTO 365 when administered at the recommended dose, was safe and effective for the treatment of dogs with demodectic mange.

Clinical Evaluation of BRAVECTO® 365 (fluralaner 150 mg/ml suspension for injection) for Treating Naturally Infested Dogs with Otodectic Mange

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This study evaluated the effectiveness of BRAVECTO 365 against *Otodectes cynotis* mites in naturally infested dogs in Sao Paulo state, Brazil. Dogs confirmed to be infested with mites in both ears, based on otoscopic evaluations, were selected for enrollment. The dogs were randomly assigned to two treatment groups: an untreated control group and a BRAVECTO 365 treatment group. The BRAVECTO 365 group received a subcutaneous injection at a dosage of 0.1 ml/kg body weight (BW), equivalent to 15 mg fluralaner per kg BW. Post treatment otoscopic evaluations were conducted on Days +3, +7, +14, +21, +28, +42, +56, and +63 to evaluate treatment efficacy. Sixteen dogs, aged 13 to 119 months and weighing 10.2 to 42.2 kg, were included. Eight dogs were treated with BRAVECTO 365, while the other eight remained untreated. At Day +63, a 100% reduction in *Otodectes cynotis*

infestation was observed in the treated group, whereas all animals in the untreated group were still infested with mites. No adverse events related to BRAVECTO 365 were noted throughout the study. The results demonstrate the safety and efficacy of BRAVECTO 365 administered at the recommended dose for treating dogs infested with *Otodectes cynotis* mites.

Elucidating the Dose-Response of Ivermectin 3.15% on Tick Burden Using a PKPD Model

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Pharmacometrics is a crucial tool in modern veterinary medicine, as it enables the optimization of treatments and addresses challenges such as parasitic resistance. This study combined pharmacokinetics (PK) and pharmacodynamics (PD) to model the relationship between drug exposure and its therapeutic effect, enhancing decision-making in the control of parasites such as *Rhipicephalus microplus*, one of the primary concerns in livestock production. The utilization of population dynamics knowledge in conjunction with Ivermectin pharmacokinetics (PK) information can facilitate the implementation of strategic control measures that reduce the need for acaricide applications. To develop a PK/PD model establishing the relationship between ivermectin (IVM) exposure and tick control. The experimental design included 10 adult bovines infested over 100 days with 2,000 tick larvae (*R. microplus*) daily, commencing 4 weeks prior to administering IVM treatment (630 µg/kg, 3.15%, Ivomec Gold®, Boehringer Ingelheim). Throughout the study, key data such as daily engorged female fallen tick counts and plasma ivermectin concentration were recorded. PK data were modelled and fitted to a one-compartment with effect compartment model (with proportional error) plus covariates (Fat level, recorded by ecographical images) (kapop=0.07 day-1; Vpop=147 L; beta_Vpop-Fat= 2.42 L; Clpop=102 L/day;), while PD data were analysed using a probability mass function (negative binomial model) (lambda=120; betaOnmicro=2.13, IC50=0.1 ng/mL). Simulations demonstrated how to adjust the dosage (single *vs.* multiple dosing or missing doses) according to parasitic sensitivity (resistance strains [IC50]), optimizing therapeutic efficacy. This innovative approach addresses current demands in animal health, offering a solution that balances the efficacy of antiparasitic treatments and the sustainability of their long-term use.

Multicenter study on tick control in cattle using different pharmacological tools

Diego Robaina (Universidad de la República, Uruguay), Gonzalo Suárez (Universidad de la República, Uruguay)

Rhipicephalus mircoplus is the main ectoparasite in cattle. There are numerous pharmacological alternatives (neurotoxic [ivermectin IVM] and non-neurotoxic [fluazuron FLU]) to control this ectoparasite. The objective was to assess for field efficacy of different therapeutic schemes for the pharmacological control of natural infestations of *R. microplus* in Uruguay. Three farms were used: A (70 Hereford cows), B (27 Hereford cows), and C (77 Hereford cows), following a similar treatment strategy. Group FLU (FLU 2.5%, 2.5 mg/kg, pour-on); Group

IVM+FLU (IVM 1%, 0.2 mg/kg + FLU 12.5%, 2.5 mg/kg, subcutaneous); Group IVM1 (IVM 1%, 0.2 mg/kg, subcutaneous); and Group IVM3.15 (IVM 3.15%, 0.63 mg/kg). From day 0 to day 14, engorged female tick (EFT) presence was ranked as null (no presence), low (less than 20), medium (between 20 and 50), and high (more than 50). Ranked data were analyzed by cumulative link models using the ordinal package (Ritz *et al*., 2015) for R Statistical Software (version 4.4.1). The best-fitted model was selected by the goodness of fit criteria and AIC. Time was handled as a continuous variable and group as a categorical variable. Estimated odds ratios (OR) and 95% confidence intervals (CI95) were calculated. A p-value < 0.05 was considered statistically significant. The effectiveness of each product is contingent upon the sensitivity status of each farm. In field A, the FLU treatment was the sole intervention that reduced the parasite load compared to the IVM+FLU, IVM1, and IVM3.15 treatments (OR 2.4 CI95 0.4-18.9). In field B, the IVM+FLU treatment was effective in comparison to IVM1 and IVM3.15 (OR 0.013 CI95 0.001-0.014). In field C, IVM3.15 emerged as the most effective treatment relative to FLU and IVM+FLU (OR 0.38 CI95 0.19-0.73). These findings underscore the importance of conducting diagnostic assessments at the farm level prior to the implementation of sanitary control plans.

Is there bioequivalence for Ivermectin 1% when administered as single drug or combined with flauzuron 12.5% on cattle?

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Ivermectin (IVM) is the first Macrocyclic Lactone developed for veterinary applications. Variations in formulation influences plasma kinetics and parasite exposure. The prevalence of commercially available IVM formulations prompts an inquiry into the behavioral similarities of these products. Efficacy on tick control could be affected by drug delivery between formulations. The objective of this study was to assess for IVM bioequivalence using two formulations. Twelve Hereford heifers were randomized into two groups. Group A (317 ± 59 kg) received Ivermectin (IVM 1% [0.2 mg/kg]), while Group B (338 ± 41 kg) received a combined formulation of IVM (1%, 0.2 mg/kg) and Fluazuron (12.5%, 12.5 mg/kg); administration was subcutaneous on the neck as single dose. Blood samples were collected up to 29 days following the administration. Pharmacokinetic data were processed with PKanalyx (PKanalix version 2024R1, Antony, France: Lixoft SAS) for noncompartmental analysis. To assess relative bioequivalence, a one-way ANOVA was applied to Cmax, AUC, and the ratio Cmax/AUC; Mann-Whitney U test was applied for Tmax analysis (R Core Team, 2024). Bioequivalence was established when the 90% confidence intervals (CI90%) for the parameter´s ratio was within the equivalence range of 0.8 to 1.25 (Martinez et al., 2002). A p-value of less than 0.05 was deemed statistically significant in all assessments. Non-compartmental approach showed bioequivalence for IVM between formulations, with Cmax ratio of 1.1 (CI90: 1.00 – 1.21, p = 0.077) and AUC0-t ratio of 0.99 (CI90: 0.93 – 1.05, p = 0.85) with Tmax (2.5 ± 2.3 and 1.6 ± 0.8 days [Mann-Whitney U test, p = 0.79] for A and B, respectively). We conclude that both IVM 1% formulations (alone or combined with fluazuron 12.5%), are bioequivalent in cattle. Carrying out bioequiva-

lence studies are of great importance to ensure interchangeability between formulations.

Efficacy of 1% Fipronil Pour-On Against *Bovicola equi* Infestation in Horses

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The infestation by chewing lice *Bovicola equi* (*Werneckiella equi*) is a common ectoparasitosis in horses, causing intense pruritus, dermatitis, and compromised welfare. Transmission occurs primarily through direct contact or fomites, and control is challenging due to the limited availability of licensed products and insufficient efficacy information. This study evaluated the efficacy of 1% fipronil pour-on against *B. equi* infestation in horses admitted to a veterinary hospital. Two adult, castrated, mixed-breed horses (Horses 1 and 2) were treated at the Veterinary Clinics Hospital of the Santa Catarina State University (HCV-CAV-UDESC) between 2022 and 2023. Both exhibited intense pruritus, especially in the head, withers, and croup regions, and were diagnosed with severe *B. equi* infestation in the clinical examination and morphological identification of the ectoparasites. The treatment consisted of a single topical application of 1% fipronil pour-on (1 mg/kg) on the dorsal line of the animals. Efficacy was assessed by daily lice counts in demarcated areas (20×20 cm for Horse 1; 10×10 cm for Horse 2) on the left costal region, delimited by the seventh thoracic vertebra and tuber coxae, until complete parasite elimination. Infestation was resolved within eight days (Horse 1) and twelve days (Horse 2), with no recurrence observed over six months. Although parasitological clearance was achieved, Horse 1 exhibited transient residual pruritus. The results demonstrate that 1% fipronil pour-on effectively eliminated *B. equi* under the study conditions, offering advantages such as minimized stress during application, precise dosing, and enhanced handler safety. Adoption of preventive measures, including proper sanitary management and regular monitoring, is crucial to prevent reinfestation.

Evaluation on different strategic control protocols against *Rhipicephlus microplus* on the dairy heifers raised in the tropical climate.

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The objective of this study was to evaluate different schemes of strategic control against *Rhipicephalus microplus*, in cattle infested and kept on pasture, in a tropical climate region. Thirty heifers ¾ Holstein and ¼ Gyr were divided into three groups of 10 animals each, based in tick counts, and body weight. The formulations used were a spray combination containing

Chlorpyrifos 500ppm + Cypermethrin 200ppm (Ciclorfós® – Pearson Saúde Animal), doramectin 200µg/kg injectable subcutaneously (Doramec® – Pearson Saúde Animal) and fluazuron 2.5mg/kg + abamectin 500µg/kg, pour-on (Proatac® – Pearson Saúde Animal). T01 received tick-killing treatment via spraying on days 0, 28, 56 and 77 after the start of the study. T02 received spraying + injectable doramectin on day 0; on D42 the pour-on combination; and on D70 the same pour-on formulation + the injectable product. The animals in T03 were kept as a negative control. The study lasted 133 days, and tick counts were performed weekly. Every 30 days the animals were weighed, and at the beginning and end of the study egg counts per gram of feces were performed. The tick counts in the treated groups were lower ($p \leq 0.05$) than those in the control group from D7 to D126. From D7 to D112, the average effectiveness of the strategic control scheme in T01 was 91.9%. The strategic control scheme used in T02, with the pour-on and injectable formulation, was 93.9%. The average EPG load of T01, T02 and T03 at the beginning of the study was 13.64, 27.27 and 18.18 EPG, respectively. The average weight of animals in T01, T02 and T03 at the beginning of the study was 339.7 kg, 339.9 kg and 339.9 kg, respectively. On D+126, animals in T01, T02 and T03 weighed on average 387.8 kg, 395.2 kg and 381.8 kg, respectively. At the end of the experimental period, the different strategic treatment schemes against cattle ticks carried out in T01 and T02, provided a difference in weight gain of 6.0 kg and 13.4 kg more in relation to the control group, respectively.

Evaluation of the strategic control protocols against *Rhipicephlus microplus* on the beef heifers raised on the tropical climate

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The objective of this study was evaluated a strategic control protocol against the *Rhipicephalus microplus* on the beef cattle naturally infested and kept on the pasture, in the tropical climate region. Twenty Canchim heifers were divided into two group with 10 animals each, based in ticks counts and body weight. T01 received treatment on the D0 of the study (fluazuron 2.5 mg/kg and abamectin 500 µg/kg, pour-on – Proatac Pearson Saúde Animal + doramectin 200 µg/kg injectable subcutaneously - Doramec® – Pearson Saúde Animal), day 42 (fluazuron 2.5 mg/kg and abamectin 500 µg/kg, pour-on - Pearson Saúde Animal) and on day 77 (cypermethrin 5% and chlorpyrifos 7% and piperonyl butoxide 5% and geraniol 2% - Bovecto® via pour-on - Pearson Saúde Animal + doramectin 200 µg/kg injectable subcutaneously - Pearson Saúde Animal) and the animals of T02 were kept as control group. The study lasted 133 days, and tick counts were performed weekly. Every 30 days the animals were weighed, and at the beginning and end of the study, egg counts per gram of feces were performed. Tick counts in the group submitted to strategic control were lower than those in the control group ($p \leq 0.05$) throughout the post-treatment period (D7 to D133). From D7 to D126, the average efficiency of the T01 strategic control scheme was 90.1%. The mean FEC load of T01 and T02 at the beginning of the study was 40.91 and 27.27 FEC, respectively. The mean weight of T01 and T02 animals at the beginning of the study was 285.5 kg and 285.1 kg, respectively. On D+126, T01 and

T02 animals weighed on average 368.7 kg and 349.5 kg, respectively. At the end of the study, the strategic control scheme adopted against the cattle tick in T01 provided a difference in weight gain of 19.2 kg more in this group, in relation to the control group.

Efficacy of Selenated and Tellurated compounds *in vitro* against *Haemonchus* spp. from naturally infected cattle and sheep

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Gastrointestinal nematode (GIN) infections severely affect the health of ruminants. Excessive use of anthelmintics has contributed to the development of drug resistance. Selenium (Se) and tellurium (Te)-based compounds have emerged as therapeutic pharmacological innovations. This study aimed to determine the effects of diphenyl diselenide $(PhSe)_2$, phenylselenyl chloride $(4\text{-Cl-PhSe})_2$ and trichotellurate (ammonium O,O'-diethylentellurate; AS-101) on third-stage larvae (L3) and eggs of nematodes collected from cattle and sheep. The larval migration inhibition tests (LMIT) and egg hatch tests (EHT) were conducted at concentrations ranging from 0.10 to 16.00 mmol L^{-1}. Ivermectin (IVM) was used as a positive control and for drug combination. Among the helminths identified, *Haemonchus* spp. was the most prevalent in sheep (76%) and cattle (64%), followed by *Trichostrongylus* spp. (16 and 24%, respectively). *Oesophagostomum spp.* and *Cooperia* spp. were less frequent (6 and 2% in sheep and 9 and 3% in cattle). The results revealed a concentration-dependent inhibition by $(PhSe)_2$, $(4\text{-Cl-PhSe})_2$, and AS-101. AS-101 showed the highest larvicidal activity. AS-101 achieved a 50% inhibitory concentration (IC_{50}) of 0.976 mmol L^{-1} in sheep and 1.035 mmol L^{-1} in cattle. $(PhSe)_2$ exhibited the strongest ovicidal effect, with IC_{50} = 1.801 mmol L^{-1} for sheep GIN and 1.845 mmol L^{-1} for cattle GIN. The compound $(4\text{-Cl-PhSe})_2$ also demonstrated significant activity, though at higher concentrations. Dilutions were performed using the IC_{50} of $[(PhSe)_2]$ and varying IVM doses (IC_{10}, IC_{30}, and IC_{50}) and vice versa. Similar experiments were performed with AS-101. Combinations of $(PhSe)_2$ and AS-101 with IVM showed additive effects of up to 13.33% against L3s. The data highlights the larvicidal effect of these molecules.

Target animal safety, in relevant cattle categories, bioequivalence and withdrawal period in edible tissues, of a novel pour-on fluralaner ectoparasiticide, NexLaner (Ourofino Saúde Animal Ltda.)

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Fluralaner, the only ectoparasiticide compound available for cattle not yet affected by resistance, is an isoxazoline highly efficient against insects and acari. NexLaner (NXL) is an alternative formulation with fluralaner for cattle. The studies here presented, all GCP-compliant trials adhering to international standards and applicable laws, evaluated its: margin of safety in young cattle, pregnant females and reproductively-sound males; margin of safety in healthy young adults compared to Exzolt® (EXZ – MSD Animal Health); bioequivalence with EXZ; and depletion in edible tissues for determining the withdrawal period. All safety and margin of safety trials adhered to specifications/recommendations of VICH GL43, with adequate numbers of animals, randomization procedures and variables evaluated. The bioequivalence trial randomized 24 animals in two statistically equivalent groups, treated with NXL or EXZ. After pharmacokinetic parameter calculations, bioequivalence was defined based on VICH GL52. The last trial, based on VICH GL48/49 and the EMA guide for withdrawal periods in edible tissues, euthanized 25 bovines treated with NXL in five distinct days post-treatment (DPT), to quantify fluralaner on tissue samples. Based on ANVISA's MRL, withdrawal period was determined using the EMA software. Safety of NXL was confirmed in healthy young adults and in three-month-old heifers, for up to 5x the recommended dose, as well as in pregnant females and reproductively sound-males, for up to 3x the dose. The bioequivalence of NXL and EXZ was statistically confirmed. The withdrawal period of NXL in edible tissues of cattle is inferior to EXZ, with no quantifiable concentrations of fluralaner after 21 DPT. Conclusion: NXL, administered on its recommended dose, 1 mL/10 kg (2.5% fluralaner; 2.5 mg/kg), is safe in all relevant cattle categories, for up to 5x the recommended dose, being bioequivalent and with an inferior withdraw period to the other fluralaner product for cattle.

The potential of phenothiazinium dyes against *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular parasite related to disorders in humans and animals, mainly in pregnant women and/or in immunosuppressed patients. Pyrimethamine (Pyr) or trimethoprim combinations with sulfonamides or atovaquone are the main forms to control toxoplasmosis in patients. However, cases of resistance against Pyr have been reported in malaria patients, indicating the need for alternative drug strategies. Thus, new compounds with anti-*T. gondii* activities are required. In this study, we tested the phenothiazinium dyes Methylene Blue (MB), New Methylene Blue (NMB), Toluidine Blue O (TBO) and 1,9 - Dimethyl Methylene Blue (DMMB) against *T.*

gondii in *in vitro* and *in vivo* models. We also evaluated the ROS formation (DCFDA) and the mitochondrial potential (JC-1) in infected cells, egressed and purified tachyzoites by flow cytometry. The phenothiazinium dyes inhibited the *T. gondii* tachyzoites proliferation in nano-molar concentrations, demonstrating low toxicity in Vero and human fibroblasts (MTT and NR). The dyes (except DMMB) also improved the formazan formation in cells. Acutely infected mice were partially protected after treatment with DMMB alone (60%) or combined with Pyr (80%). Possible mechanisms of the dyes in *T. gondii* were proposed, involving the action of the dyes in the ROS production and decrease of the mitochondrial potential in infected cells, egressed and purified tachyzoites. Thus, our study initiates the use of phenothiazinium dyes against *T. gondii*, following the promising advances from *Plasmodium* and *Neospora caninum*. The use of phenothiazinium dyes against *T. gondii* contributes to the development of new strategies based on a well-established family of compounds.

Anthelmintic effectiveness of the oral administration of ivermectin + praziquantel + fenbendazole against intestinal helminths in fighting birds

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Intestinal helminths are an important impediment in poultry production. With the increasing breeding of "cage-free" production systems or cages in contact with the ground, the incidence of parasitosis by intestinal helminths has increased in poultry production systems. Because the available anthelmintics do not act on all classes of helminths, the combination of anthelmintics can be used to cover the broad spectrum of action and to delay the development and spread of anthelmintic resistance. The objective of the study was to evaluate in vivo the effectiveness of the combination of ivermectin + praziquantel + fenbendazole (I+P+F) against intestinal nematodes and cestodes in fighting birds. Stool samples from 181 birds were taken to estimate the frequency of intestinal helminths. For the evaluation of anthelmintic efficacy, the birds were randomly distributed into the following groups (10 to 19 birds/group) according to the anthelmintic evaluated: ivermectin (I:0.4 mg/kg orally), praziquantel (P:10 mg/kg orally), fenbendazole (F:16 mg/kg orally), and the combination of I+P+F (ivermectin 0.4 mg/kg + praziquantel 10 mg/kg + fenbendazole 16 mg/kg, orally). The effectiveness of the treatments was measured on days 0, 7, 14, and 21 post-treatments (PT). Feces samples were taken and analyzed using Centrifugal Flotation and McMaster techniques. 33.7% (61/181) of the birds were positive for intestinal helminths, these being *Heterakis* spp. (55.7%), *Capillaria* spp. (68.8%) and *Raillietina* spp. (54.0%). The I and F groups showed efficacy of 86-100% and 66-100% against nematodes, respectively. The P group presented 90-100% efficacy for controlling *Raillietina* spp. The combination of I+P+F (Endovet Gallos®) showed anthelmintic efficacy (100%) from 7 up to 21 days PT against *Capillaria* spp., *Heterakis* spp., and *Raillietina* spp. This formulation was

more effective than the individual administration of the evaluated anthelmintics in naturally infected fighting birds.

Evaluation of *in vitro* anthelmintic activity against *Haemonchus contortus* adult stage, nonspecific cytotoxicity and proteomic analysis of imidazoheterocyclic structures.

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Haemonchus contortus is a pathogenic nematode that affects small ruminants, causing anemia, decreased wool and meat quality, and potentially death. Chemotherapy is the primary treatment, but increasing drug resistance demands new anthelmintics. In this work, we employed the multicomponent Groebke-Blackburn-Bienaymé (GBB) reaction using green conditions to generate a library of imidazoheterocyclic compounds as privileged scaffolds in medicinal chemistry. Over 30 GBB compounds were tested *in vitro* for activity against *H. contortus* adults in a motility assay and for nonspecific cytotoxicity in human macrophage-like cells (phorbol ester differentiated THP-1 cells). In the 72-hour activity assay, six compounds impaired worm motility at 72 h and two showed significant activity from 24 h, outperforming Albendazole at 25 µM. The active compounds showed no significant cytotoxicity. In the proteomic analyses of adult worms treated with the most potent compound, **8**, applying shotgun LC–MS/MS, a total of 1378 proteins were identified, and among the differentially expressed proteins (DEPs), 67 were significantly upregulated and 78 were significantly downregulated, in comparison to no treated adult worms. Bioinformatic analysis of the DEPs, through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment, indicated that downregulated DEPs were mainly involved in tricarboxylic acid cycle (TCA), malate metabolism, oxidoreductase activity and nucleotide metabolism, suggesting mitochondrial dysfunction and interference with DNA synthesis. Upregulated DEPs were related to translation, protein synthesis and fructose metabolism, possibly reflecting response to stress, protein misfolding and energy imbalance produced by the alteration of the TCA, respectively. These findings support imidazoheterocycles as promising anthelmintic scaffolds, with compound 8 as a strong candidate for further *in vivo* studies, thus contributing to the development of new anthelmintics.

Pharmacokinetics of eprinomectin in lactating and dry dairy goats after administration of Eprecis® 20 mg/mL at the label dose

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Physiological status (lactation) may alter eprinomectin (EPN) performances, particularly in goats, with a strongly reduced Area Under the Curve (AUC) in lactating goats compared with dry goats when EPN is used topically [1, 2]. To date, no pharmacokinetic study is available to evaluate if this is occurring when EPN injectable is used. The objective of the study was to determine the pharmacokinetics of EPN in lactating and non-lactating goats when EPN injectable is used at the label dose (0.2 mg/kg). Eight lactating and eight non-lactating non-pregnant French Alpine goats aged 2 to 6 years with a BW of 45 to 73 kg and a BCS of 2.5 to 3.5 originating from a single commercial farm were included. Each animal's general health status was observed daily from arrival until the end of the study. On D0, all included goats were administered Eprecis® 20 mg/mL (Ceva) subcutaneously at a dose of 0.2 mg/kg. Blood samples were collected at scheduled time-points for 21 days after administration. Assay of EPN in plasma was performed using Solid Phase Extraction for sample preparation followed by LC-MS/MS detection. Individual plasma concentration-time profiles were submitted to a non-compartmental analysis using the Phoenix® WinNonlin® 8.3.5 software. No significant differences in main pharmacokinetic parameters of EPN were observed between the two groups ($P \geq 0.198$). The mean observed maximum concentration (Cmax, 14.831 ng/mL) and mean AUCINF (54.83 day.ng/mL) in lactating animals were very similar to the mean Cmax (15.843 ng/mL) and AUCINF (53.17 day.ng/mL) observed in non-lactating animals. No correlation was evidenced between animals' characteristics and main pharmacokinetic ($R2 \leq 0.2096$). In this population of Alpine dairy goats, we found no difference in the pharmacokinetic profiles of EPN between lactating and non-lactating goats after subcutaneous administration of injectable EPN (Eprecis® 20 mg/mL) at the label dose of 0.2 mg/kg.

Effectiveness of Afoxolaner (NexGard®) and Esafoxolaner (NexGard® COMBO) for the treatment and control of *Amblyomma maculatum* ticks in experimentally infested dogs and cats

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Amblyomma maculatum, the Gulf Coast tick, infests a wide range of vertebrate species including livestock, dogs, cats and humans. It is a species of significant veterinary and public health importance, especially as a vector of diseases, for instance the American canine hepatozoonosis or the tidewater spotted fever. It also commonly causes severe inflammatory reactions at attachment site. The Gulf Coast tick preferably thrives in coastal lands, woodlands, and more xeric environments. It is endemic in Mexican Gulf USA states, parts of Central and South America, and it is expanding to Northeast and Midwest USA. Three experimental studies following a masked, randomized and negative controlled design were conducted to verify the efficacy of the isoxazolines afoxolaner in dogs (2), or esafoxolaner (the active (S)-enantiomer of afoxolaner) in cats (1), against induced infestations with *A. maculatum*. Each study used an untreated control group and a

treated group, dosed once with the minimum label dose on Day 0. Animals were infested with 50 adult *A. maculatum*, on Day -2, then weekly for 5 weeks (or every other week for 4 weeks in the second dog study). The efficacy calculations were based on comparison of the numbers of live ticks found in each group, 72 hours after treatment and after the subsequent infestations. At all evaluations, the numbers of live ticks found on the untreated animals exceed 13 (25%), which demonstrated vigorous tick population and adequate study model. Inclusive of the three studies, the curative efficacy against established infestations, 72 hours after dosing, was at least 98.7%, and the preventive efficacy, 72 hours after post-treatment infestations, ranged from 94.6% to 99.4%, for 4 or 5 weeks ($p<.0001$ at all timepoints). Significant and consistent reductions exceeding 94.6% of live *A. maculatum* after induced infestations were demonstrated for afoxolaner and esafoxolaner, for 4 to 5 weeks.

Efficacy of an oral combination of afoxolaner and milbemycin oxime for the prevention of transmission of *Babesia canis* by *Dermacentor reticulatus* ticks to dogs

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Babesia spp. are major blood parasites of vertebrates. Their pathological effect is caused by the invasion and destruction of erythrocytes through replicative cycle. The numerous known canine *Babesia* species are variable in term of geographical distribution, vector, clinical signs, severity, and treatment sensitivity. *Babesia canis*, the agent of canine babesiosis is transmitted by *Dermacentor reticulatus* ticks in Europe. Itis a disease of significant veterinary importance, causing systemic inflammatory reactions and hemolysis-related symptoms such as febrile syndrome, anemia, hemoglobinuria, jaundice, lethargy, organ failure. An experimental study was conducted to verify the efficacy of NexGard Spectra®(IVP), an oral combination of afoxolaner and milbemycin oxime, to block the transmission of *B. canis* by *D. reticulatus* to dogs. Three groups of eight dogs were used, one group treated on Day 0 with the IVP at the minimum recommended dose and two untreated control groups. On Day 1, dogs from the IVP group and from the control group 1 were infested with 50 *D. reticulatus* adult ticks infected with *B. canis* at a 23% rate. On Day 28, dogs from the IVP group and from the control group 2 were similarly infested. Ticks were removed 6 days after each infestation. Seven to nine days after each infestation, all control dogs displayed clinical signs of canine babesiosis, (lethargy, and/or dark urine, and/or>39.5°C) and were confirmed infected by blood smear examination and PCR. These dogs were rescue treated and were further confirmed positive by IFA, two or three weeks after infestation. None of the IVP-treated dogs expressed any clinical sign of canine babesiosis following each of the two infestations and until Day 56, and repeated IFA and PCR analyses remained negative. Therapeutic solutions for canine babesiosis are challenging and limited, the blocking of transmission shown in this study may provide an interesting preventive strategy.

Efficacy of NexGard® Plus in dogs naturally infested with *Sarcoptes scabiei*

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Canine sarcoptic mange, caused by *Sarcoptes scabiei*, is a highly contagious and intensely pruritic skin disease in dogs. It is prevalent worldwide and zoonotic. This is why effective treatments is critical to safeguard public health and animal welfare. This clinical field study aimed to confirm the efficacy of NexGard® Plus in treating dogs naturally infested with *S. scabiei*. It was a blinded, randomized, single-centered, negatively controlled efficacy study with parallel group designed. Twenty dogs, naturally infested with *S. scabiei*, were included, and divided into two groups: a treatment group receiving NexGard® Plus on Day 0 and Day 26/28 at label dose, and an untreated control group. Skin scrapings were conducted once from Day -6 to 0, then on Day 26 to 28, and 56 for mite counts and assessment of clinical symptoms. In this study, a significant reduction of 100% of the mites count was observed in the NexGard® Plus treated group ($p<0,001$), in comparison to the untreated control group after two monthly treatments. The percentage of mite free dogs in the treated group (100%) was significantly higher than in the control group (0%) ($p<0,001$). Treated dogs had no pruritus, papules or crusts and clear evidence of hair re-growth recorded by Day 56, unlike the dogs in the control group. This study has demonstrated an important and significative reduction of *Sarcoptes scabiei* mites count with clear improvement of clinical signs in naturally infested dogs treated with NexGard® Plus.

Nanoemulsion-Based Azithromycin: A Novel Approach for Treating Acute Toxoplasmosis

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Toxoplasma gondii is an intracellular parasite that can cause serious complications in immunocompromised individuals. The standard therapy using pyrimethamine and sulfadiazine often leads to significant side effects, highlighting the need for alternative therapies. Azithromycin, a macrolide antibiotic, has been promising in the prophylaxis of congenital toxoplasmosis but its activity against T. gondii remains limited. Nanoemulsion-based drug delivery systems offer an attractive approach for enhancing drug bioavailability and therapeutic efficacy. The aim of this study was to compare the effectiveness of azithromycin nanoemulsion (NE-AZT) for the treatment of acute toxoplasmosis with its suspension formulation (S-AZT). NE-AZT was prepared using the spontaneous emulsification method with soybean oil, polysorbate 80, ethanol, and DMSO. The formulation was characterized for stability, particle size, zeta potential, and viscosity. Toxicity was assessed in BALB/c mice over three weeks by monitoring body weight, liver function, and histopathological changes. For efficacy evaluation, mice were infected with *T. gondii* tachyzoites and divided into treatment groups receiving PBS, S-AZT, NEAZT, or a drug-free nanoemulsion. Survival rates and parasite loads were measured, and

posttreatment tachyzoite virulence was examined in secondary infections. The characterization of NE-AZT using the Nano Zetasizer confirmed its optimal physicochemical properties, including a stable particle size and zeta potential. Stability tests indicated no significant changes in color, turbidity, or phase separation over one month, and particle size remained consistent after freeze-thaw cycles. TEM imaging further validated the spherical morphology of the nanoemulsion particles. These results support the stability and suitability of NE-AZT for further therapeutic evaluation. *In vivo* toxicity of NE-AZT indicated no behavioral and physical changes in all treated mice. Moreover, histopathological results did not show any significant abnormalities in the liver and spleen tissues examined from mice in the treatment group. These results confirmed that nanoemulsion could be applied as a safe drug delivery system. In acute experimental toxoplasmosis, mice treated with NE-AZT showed a significant reduction in tachyzoite count in the peritoneal cavity and a prolonged survival time ($P \leq 0.05$). The mean survival time was 14 days in the NE-AZT group compared to 10 days in the S-AZT group ($P < 0.005$). In contrast, mice in the control group and those receiving the drug-free nanoemulsion succumbed within 7 days. Additionally, tachyzoites isolated from treated mice exhibited reduced virulence, leading to extended survival in secondary infections. These findings highlight the superior efficacy of NE-AZT in controlling *T. gondii* infection. Nanoemulsions provide a promising strategy for enhancing drug delivery and efficacy. Encapsulation of AZT in a nanoemulsion improves its stability, bioavailability, and anti-*T. gondii* activity, increasing its effectiveness against the acute phase of infection. Their controlled release, biocompatibility, and ability to enhance drug solubility further underscore their potential as an advanced therapeutic approach for toxoplasmosis treatment.

Comparing short versus long persistent efficacy of anthelmintics: Impacts on dairy sheep production

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Gastrointestinal nematode (GIN) infections pose a significant challenge to the health and productivity of dairy ewes, leading to reduced milk yield and quality. Effective anthelmintic treatments are essential for controlling these infections while maintaining optimal dairy production. Albendazole, a commonly used short-persistent efficacy anthelmintic, has shown effectiveness in parasite reduction, but its persistent efficacy remains limited. In contrast, eprinomectin, available in pour-on and injectable formulations, offers prolonged protection against GIN. The objectives of this study were to compare the short (i.e., albendazole) and long (i.e., eprinomectin) persistent effect of these anthelmintics and to determine these effects on milk yield and quality in naturally infected with GIN dairy ewes. From each farm, 40 selected ewes were divided into four similar groups based on their faecal egg counts; namely Group 1: control group, Group 2: albendazole treated group, Group 3: pour-on eprinomectin treated group, Group 4: injectable eprinomectin treated group, on Day 0. Faecal egg counts and coprocultures were performed on Days 0, 15, 30, 45, 60 and 75. The milk yield and milk quality were estimated on the forementioned occa-

sions. Eprinomectin outperformed albendazole in treating gastrointestinal nematode infections in dairy ewes. Both pour-on and injectable eprinomectin formulations provided long-lasting protection, by reducing faecal egg counts. Furthermore, eprinomectin treated ewes exhibited increased daily milk yield and improved milk composition, in terms of fat and protein concentrations and somatic cell counts. These findings highlight the benefits of long persistent efficacy of eprinomectin as a preferred anthelmintic treatment for lactating dairy ewes, offering enhanced productivity and milk quality, while addressing parasite resistance concerns.

Effect of administration interval on the efficacy of combination anthelmintic treatments against multi-resistant gastrointestinal nematodes in goats

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Reports of anthelmintic resistance are increasing worldwide. In some regions, including Sudan, resistance has spread across multiple anthelmintic-drug classes, complicating parasite control. This study evaluated the efficacy of albendazole, ivermectin and levamisole as well as the unregistered drug moxidectin in Sudan. Conducted on 580 goats in South Darfur naturally infected with multi-resistant strongyle nematodes, the study used the faecal egg count reduction test (FECRT) to assess drug efficacy. The anthelmintics were tested alone and in combination (except for moxidectin), administered either once or consecutively after 24 hours with different application orders. A total of 15 treatment schemes were used, each involving at least 25 animals. A parametric FECRT model with individual efficacy, alongside parametric and non-parametric models with common efficacy, identified moxidectin as effective against strongyle communities. The models calculated a lower 90% credible limit (CrL) range of 99.6 – 100% and an upper CrL range of 99.8 – 100%. However, all three models classified albendazole, ivermectin and levamisole as ineffective when used alone (upper 90% CrL range of 73.7 – 97.6%). Notably, combining two or all three drugs significantly improved efficacy, whether administered simultaneously or consecutively. Consecutive administration after 24 hours was more effective than simultaneous dosing. Combinations including levamisole were particularly effective under the consecutive 24-hour scheme, reporting a lower 90% CrL range of 95.1 – 99.8% and an upper 90% CrL range of 97.4 – 100% across the three models. Combination treatments, particularly those including levamisole with consecutive administration, were more effective against resistant strongyles and overcame resistance to individual drugs. Moxidectin presents a viable alternative for treating multi-resistant strongyle communities in Sudan.

Deeper into knowledge of eprinomectin: pharmacokinetics, efficacy, cross-resistance

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Eprinomectin is widely used in veterinary medicine for controlling parasitic nematodes. However, its transdermal absorption and efficacy require optimization. This study aimed to develop and evaluate novel pour-on formulations of eprinomectin, assessing their pharmacokinetic properties and anthelmintic efficacy against *Haemonchus contortus* in sheep. The primary objective was to formulate new eprinomectin pour-on compositions and compare their pharmacokinetics and efficacy with commercial references in preclinical trials. Several formulations were developed using self-emulsifying microemulsions containing surfactants, co-surfactants, and penetration enhancers. In vitro permeation studies were conducted using Franz diffusion cells with synthetic membranes and porcine skin. Two optimized formulations (F1, F2) were selected for in vivo pharmacokinetic and efficacy studies in sheep. Drug absorption was evaluated through plasma concentration-time profiles (Cmax, Tmax, AUC). Efficacy was assessed using the faecal egg count reduction test (FECRT). Pharmacokinetic analysis showed that Cmax values for F1 (4.4 ng/ml) and F2 (4.8 ng/ml) were comparable to the reference product (4.4 ng/ml), with AUC values of 667 and 735 ng h/ml, respectively. Despite adequate drug exposure, FECRT results indicated suboptimal efficacy (F1: 0.0%; F2: 23.1%) compared to the reference product (46.1%). Molecular analysis confirmed *H. contortus* resistance to eprinomectin, likely due to previous ivermectin treatments in the original sheep flock. Although the novel formulations achieved bioequivalent pharmacokinetic profiles, their clinical efficacy was compromised by anthelmintic resistance. Future studies should explore alternative formulations or combination therapies to address resistance challenges in parasitic nematodes.

Equine strongyle control using selective anthelmintic treatments: observations in a horse farm in Argentina during a three-year period

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Small strongyles are the most prevalent horse parasites worldwide and widespread of drug resistant is driving the application of selective treatments (ST) based on treating only those animals that exceed a certain threshold egg in the feces. To evaluate a ST program in a horse farm in Argentina (31°13′50″ S; 64°18′58″ W) and to compare the cost of ST versus deworming all animals at fixed intervals over a three-year period. Historically horses were treated 4 times/ year with a combination of moxidectin

+ oxfendazole + praziquantel. Individual faecal samples from 60 horses (>2 years) were collected on each season (2022-2024) and analyzed using a modified McMaster technique (minimum detection 10 EPG: egg per gram). Only horses with ≥ 300 EPG were treated with drug combination (2022-2023) and ivermectin (IVM) (2024). Efficacy of the anthelmintic (AH) combination was 100% and, based on egg excretion, it was recommended to deworm 21% of the horses the first two years and 34% using IVM. On this farm (low or moderate parasite transmission) no horse needed more than two treatments/year using the combination. Using IVM, 5 horses received 3 treatments. Economic analysis revealed that ST represented a 54% savings compared to mass deworming at fixed intervals, with the AH combination being the highest costs (44%). However, using IVM the highest cost was the laboratory fecal egg count (35%), In this region ST should be supplemented with an annual mass prophylactic treatment for large strongyles and tapeworms control. AH drugs to control small strongyles could be significantly reduced when ST are performed instead of routine treatments. Under Argentinian conditions, ST could reduce costs and help prolonging the lifespan of AH drugs. Education and extension will be needed to aim a successful implementation of these programs as an essential step towards evidence-based veterinary medicine.

Effectiveness of NexGard® and NexGard Spectra® on the treatment of dogs artificially infested with nymphs of *Amblyomma sculptum*.

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Amblyomma sculptum is the most widely distributed tick species among Brazilian states and is recognized as being a tick of great importance for animal and public health, as it is the main vector for *Rickettsia rickettsii*, causative bacterial agent for Brazilian Spotted Fever, which affects humans. Horses, capybaras and tapirs are considered the main hosts for all parasitic stages of this tick species. However, due to its low parasitic specificity, especially in the immature stages, other mammalian species may also serve as hosts for this ectoparasite, including dogs and humans. This laboratory study was conducted according to the Good Clinical Practices. The objective of study was to evaluate the efficacy of a single oral administration of NexGard® (afoxolaner) and NexGard Spectra® (afoxolaner plus milbemycin oxime) against nymphs of *A. sculptum* in artificially infested dogs. This study was conducted in Brazil and was evaluated and approved by Ethics Committee for Animal Use from Federal Rural University of Rio de Janeiro. Twenty-four health dogs, weighing between 9.05-18.05 kg and 22-96 months of age, were included in the study. The dogs were infested with approximately 200 viable unfed nymphs of *A. sculptum* ticks on days -1, 5, 12, 19, 26 and 33. Tick

counts were performed out on days 2, 7, 14, 21, 28 and 35. The dogs were allocated on Group 1 (Untreated), Group 2 (NexGard®) and Group 3 (NexGard Spectra®). The dogs were once treated orally on day 0, according to label dose. The efficacy against *A. sculptum* based on arithmetic mean live and attached tick counts on days 2, 7, 14, 21, 28 and 35 was 99.8%, 99.4%, 98.9%, 96.4%, 98.4%, and 99.2%, respectively for NexGard®, and 99.8%, 99.4%, 98.5%, 99.7%, 98.3% and 99.1%, respectively for NexGard Spectra®. The results obtained in the study showed that a single oral treatment with NexGard® or NexGard Spectra® was highly efficacious against adult *A. sculptum* for 35 days.

Effectiveness of NexGard® and NexGard Spectra® on the treatment of dogs artificially infested with nymphs of *Amblyomma cajennense*

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Amblyomma cajennense is only found in the Amazon region and is restricted to the states of Pará, Rondônia, Roraima and Tocantins. This tick is recognized as being a tick of great importance, both for animal health and for public health, as it is the main vector for *Rickettsia rickettsia*, causative bacterial agent for Brazilian Spotted Fever, which affects humans. Horses, capybaras and tapirs are considered the main hosts for all parasitic stages of this tick species. However, due to its low parasitic specificity, especially of immature stages, other mammalian species can also serve as hosts for this ectoparasite, including dogs and humans. This was a laboratory study conducted according to the Good Clinical Practices. The objective of study was to evaluate the efficacy of a single oral administration of NexGard® (afoxolaner) and NexGard Spectra® (afoxolaner plus milbemycin oxime) against nymphs of *A. cajennense* in dogs artificially infested. This study was conducted in Brazil and was evaluated and approved by Ethics Committee for Animal Use from Federal Rural University of Rio de Janeiro. Twenty-four health dogs, weighing between 8.45-18.95 kg and 11-108 months of age, were included in the study. The dogs were infested with approximately 200 viable unfed nymphs of *A. cajennense* on days -1, 5, 12, 19, 26 and 33. Tick counts were performed out on days 2, 7, 14, 21, 28 and 35. The dogs were allocated on Group 1 (Untreated), Group 2 (NexGard®) or Group 3 (NexGard Spectra®), based on gender and tick count on day -7. The dogs were treated orally on day Zero, according to label dose. The efficacy of NexGard® against *A. cajennense* on days 2, 7, 14, 21, 28 and 35 were respectively 99.6%, 99.9%, 100%, 99.3%, 98.9%, and 99.7% and for NexGard Spectra® were 99.9%, 99.9%, 98.4%, 99.4%, 99.3% and 100%, respectively. The results obtained in the study showed that NexGard® and NexGard Spectra after a single oral treatment were efficacy against *A. cajennense* for 35 days.

Pharmacokinetic Modeling and Therapeutic Simulation of 1% Ivermectin in Tabapuã Cattle for the Control of *Rhipicephalus microplus* and Gastrointestinal Nematodes

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Ivermectin (IVM) is widely used for parasite control but raises concerns about residues in animal-derived products and environmental impacts. This study characterized the pharmacokinetic profile of 1% IVM in Tabapuã cattle using computational modeling to simulate therapeutic protocols and optimize antiparasitic efficacy against *Rhipicephalus microplus* (RM) and gastrointestinal nematodes (GN). Plasma concentration-time curves were analyzed using a nonlinear mixed-effects model in Monolix 2023 R1 (Lixoft SAS)®, and the most robust model was applied in population simulations (1000 animals/protocol) via Simulx (Lixoft SAS)®. Monte Carlo simulations evaluated three protocols against RM, considering 8 ng/mL as the minimum effective concentration: (1) a single 200 µg/kg dose; (2) two 200 µg/kg doses at a 3-day interval; (3) three doses (200, 100, 100 µg/kg) at 3-day intervals. For NG, three protocols were simulated using 2 ng/mL as the minimum effective concentration: (1) a single 200 µg/kg dose; (2) two 200 µg/kg doses at a 10-day interval; (3) three doses (200, 100, 100 µg/kg) at 10- and 8-day intervals. The Therapeutic Efficiency Index (TEI) for RM was calculated, and environmental elimination estimated based on 90% fecal excretion in a 450 kg animal, while for GN, it was based on the duration serum concentrations remained above 2 ng/mL. The modeling adjusted to an extravascular model, with no lag time, first-order absorption, one-compartment distribution, and linear elimination, considering weight and body condition score as covariates. At 200 µg/kg, 95% of the population maintained serum concentrations above 8 ng/mL until day 3. The three-dose protocol sustained effective levels for ≅11.2 days, while the two-dose protocol lasted ≅9.6 days. For GN, a single dose maintained levels above 2 ng/mL until day 10. The two-dose protocol sustained levels for ≅21 days, and the three-dose protocol for ≅26 days. The TEI of the three-dose protocol was 20% higher for RM and 27.8% higher for GN. Simulations indicate greater efficacy of this protocol, ensuring more rational IVM use with lower environmental impact, though *in vivo* confirmation is needed.

Pharmacokinetics of Ivermectin in Cattle – A Model-Based Meta-Analysis

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The pharmacokinetics (PK) of ivermectin (IVM) has been extensively studied in various animal species, but its variability depends on both pharmaceutical and physiological factors. These complexities highlight the need for more precise approaches to predict plasma concentrations and optimize antiparasitic protocols, ensuring efficacy and reducing resistance. In this context, model-based meta-analysis (MBMA) emerges as a promising tool, allowing

the integration of aggregated data from multiple studies to develop a population pharmacokinetic (PopPK) model and support decision-making. Thus, the objective of this project was to create a PopPK model using IVM kinetic data in cattle published in the literature, following the principles of MBMA. Monolix 2024R1 Lixoft ® software was used for nonlinear mixed-effects modeling. Mean plasma concentrations from 17 studies in different herds were used. Variables such as weight, breed, sex, age group, category and absorption profile were considered. The one-compartment model with linear elimination best described the kinetics of IVM. The estimated PopPK parameters were (inter-individual variability): absorption constants (Ka), 0.2/day (14.6%), compartment volume (V), 0.34 L/kg (25.9%), clearance (Cl), 0.25 L/kg/day, considering a bioavailability (F) of 33%. Animal weight was shown to be a significant covariate influencing the compartment volume. This model can be used to simulate different therapeutic protocols with ivermectin in order to ensure greater therapeutic efficacy.

Pharmacokinetic Modeling of Ivermectin Premix in Swine

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Ivermectin (IVM) is widely used in swine for controlling gastrointestinal and pulmonary worms, as well as mites. IVM premix for swine accounted for 38% of off-label uses, highlighting the need for stricter control. This study developed a pharmacokinetic (PK) model of IVM administered via premix to predict plasma concentrations and simulate dosing regimens. Published PK studies were analyzed to build the model using Monolix2023R1, Lixoft ® to optimize dosing regimens. Different models were tested: with first-order or zero-order absorption, with one-, two- or three-compartment distribution, and with linear or Michaelis-Menten elimination. Different error models were evaluated to select the one that best described the variability of residuals. The most appropriate pharmacostatistical model was selected and evaluated based on the criteria of decrease in the objective function (calculated by importance sampling) and BIC and also on the visual inspection of the diagnostic graphs, also called diagnostic plots (Observed vs. predicted, individual weighted residuals - IWRES, normalized prediction distribution errors - NPDEs and visual predictive verification - VPC). This model was then used as a basis to generate a virtual population of 10 animals with 1000 replicates with the Lixoft® Simulx Software and a Monte Carlo simulation was performed with oral administrations of ivermectin via premix to observe the pharmacokinetic behavior in a population aspect. The best-fit model included oral/extravascular or intravenous administration, with no delay, first-order absorption, a two-compartment distribution, linear elimination, and clearance parameterization. The model showed a good fit between observed data and predicted curves for both oral and intravenous administration. The PK modeling of IVM premix in swine revealed high absorption variability, influenced by irregular feed intake, reinforcing the need for dose adjustments to ensure efficacy and reduce resistance. Future studies should

establish PK/PD indices to improve evidence-based antiparasitic strategies.

Development of a nanocarrier pharmaceutical formulation containing metronidazole for the topical treatment of bovine trichomoniasis

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Trichomonosis is a venereal disease of cattle caused by the flagellated protozoan *Tritrichomonas fetus*. In males, the protozoan is found in the mucosa of the preputial cavity, and in older animals, the increase in the size of the preputial crypts creates a microenvironment conducive to the survival of the pathogen. Thus, dose-dependent antiprotozoals, such as metronidazole, may have their systemic use compromised. The use of topically administered mucoadhesive nanoparticles is a promising strategy to promote increased intracrypt drug retention. Chitosan nanoparticles cross-linked with sodium tripolyphosphate (TPP) were chosen because they interact easily with the metronidazole molecule and have high mucoadhesion. Pre-formulation stoichiometric studies were carried out (3:1 chitosan:TPP); quality control of chitosan by acid-base titration (95% deacetylation) and solubility tests (1% acetic acid). Tests were carried out with different means of incorporating metronidazole in the formation of the nanostructure, to find the conditions of maximum encapsulation efficiency and stability. The scope of an already validated analytical method for the quantification of metronidazole by UPLC-MS/MS was expanded, following the ICH guide. The hydrodynamic characterization was carried out using dynamic light scattering (DLS) to measure the size and Zeta potential of the particle with metronidazole (191.4 nm and 39 mV). The maximum encapsulation efficiency was 74.2% (evaluated by filtration in a molecular cutting centrifuge tube and subsequently quantified by chromatography). Stability tests were carried out, noting around 10% degradation of the active ingredient in the nanostructured formulation after 21 days of synthesis. We can conclude that the metronidazole molecule is stable in aqueous media after encapsulation and shows promise for evaluation in the treatment of bovine trichomoniasis, promoting the rational use of antimicrobials.

Determination of doramectin protocols using computational modeling for the treatment of *Rhipicephalus microplus* in cattle.

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The control of ectoparasites in cattle farming is essential to ensure productivity and animal health. Macrocyclic lactones, such as doramectin, are widely used due to their low toxicity, broad spectrum of action, and biological activity at low concentrations. However, optimizing do-

sing regimens is crucial to maximize therapeutic effects, improve efficacy, and minimize the selection of resistant parasites. In this context, pharmacokinetic/pharmacodynamic (PK/PD) modeling emerges as a strategic tool for optimizing therapeutic protocols. The objective of this study was to develop a PK/PD model for doramectin 3.5% protocols against *Rhipicephalus microplus* in cattle. For this purpose, previously published PK and PD data were used. Plasma concentrations and efficacy against ticks were estimated for three dosing regimens: a single dose (700 µg/kg on day 0), two doses (350 µg/kg on days 0 and 14), and four doses (175 µg/kg on days 0, 14, 28, and 42) of doramectin 3.5% (via subcutaneous route) over 90 days, using Monolix and Simulix Suite 2020R1 software. The estimated pharmacokinetic parameters included latency time, absorption rate constant, clearance, and central compartment volume. The pharmacodynamic model presented a maximum effect (*Emax* = 100%), the concentration that induces 50% of *Emax* (*EC50* = 4.88 µg/mL), and a Hill coefficient (*gamma* = 1.29). The analysis demonstrated that dose optimization led to a 75% increase in the residual efficacy period without increasing the environmental load. The results indicate that the developed PK/PD model is a valuable tool for optimizing doramectin therapy, allowing dose adjustments that ensure treatment efficacy. Additionally, the pharmacokinetic model proved to be effective in predicting doramectin 3.5% doses in cattle, highlighting its potential in assisting the development of more precise therapeutic protocols. Therefore, computational modeling presents itself as an ethical and viable approach, reducing the need for animal experimentation, aligning with the principles of the 3Rs (Reduction, Refinement, and Replacement), while also contributing to cost reduction and the promotion of animal welfare.

Striking the Epigenetic Core: A Golden Bullet Against Apicomplexan Development

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The complex life cycles of Apicomplexan parasites—such as *Plasmodium*, *Toxoplasma*, and *Cryptosporidium*—involve multiple differentiation stages that require tightly regulated gene expression. Emerging evidence highlights the central role of chromatin structure and epigenetic modifications in orchestrating these developmental transitions. Despite their importance, chromatin remodeling mechanisms remain poorly characterized across the phylum. Here, we investigate the function of an ISWI-related

ATPase, exemplified by PfSnf2L in *Plasmodium falciparum*, which actively repositions nucleosomes and is essential for both asexual proliferation and sexual differentiation. This ATPase regulates stage-specific gene expression by precisely coordinating nucleosome positioning at developmental gene promoters. The distinctive features of ISWI-family remodelers in Apicomplexans enabled us to identify a small-molecule inhibitor that selectively disrupts chromatin dynamics and blocks parasite transmission. These findings suggest that chromatin remodelers are conserved yet mechanistically specialized across Apicomplexans and represent promising targets for next-generation antiparasitic therapies. We are currently identifying similar inhibitors for other apicomplexans, such as Toxoplasma gondii, Cryptosporidium spp, and diverse coccidia.

Parasites of companion animals & exotic pets

Underdiagnosis of *Lynxacarus radovskyi* infestation in cats from São Luís, Maranhão: evidence of a silent clinical profile

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Lynxacarus radovskyi is an ectoparasite that primarily affects cats and often presents with discrete or absent clinical signs, favoring underdiagnosis. Identification of mite requires the use of methods such as microscopy of hairs obtained through trichotomy, since it is located on the hair shaft. Therefore, the present study aimed to report the high prevalence of domestic cats naturally infested by *L. radovskyi* in São Luís (Maranhão, Brazil), emphasizing the frequency of asymptomatic cases and the subclinical nature of the infestation. Cats from different regions of the city were subjected to the avulsion of approximately 50 hairs from three pre-determined areas (scapular region, lateral aspect of the hind limb, and perianal region). Samples were stored in containers with 70% alcohol and analyzed in the laboratory. Animals were randomly selected, and their clinical and dermatological signs were assessed. Results were categorized according to the presence of the parasite and clinical manifestation. A total of 80 cats were examined, of which 78.7% tested positive for *L. radovskyi*. Among the infested animals, 12.7% exhibited clinical signs such as pruritus, self-induced alopecia, or excoriations, while the remaining 87.3% showed no visible alterations. These findings highlight the high prevalence of *L. radovskyi* infestation in domestic cats and the potential compromise of animal welfare, particularly when infestations manifest dermatological signs. This underscores the need for clinical suspicion along with the performance of diagnostic tests for mites, especially in environments housing multiple animals. Furthermore, the subclinical nature

of infestation contributes to its persistence in the environment and hinders effective parasite control.

Prevalence of feline leishmaniasis through molecular testing in the Semiarid Region of Paraíba, Brazil

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Leishmaniasis is a zoonosis caused by protozoa of the genus *Leishmania spp*., with *Leishmania infantum* being the species that most affects animals worldwide. Domestic cats can become infected and serve as sources of infection. However, the epidemiology of the disease in the feline species is not yet well understood. Therefore, the aim of this study was to determine the prevalence of *L. infantum* in blood samples from domestic cats (*Felis catus*) using molecular tests in the semiarid region of Paraíba, Brazil. A total of 350 whole blood samples were collected from cats in random neighborhoods of Sousa, Paraíba. The samples were extracted using the Purelink Genomic DNA Mini Kit (Invitrogen®) and stored at -20°C until analysis. To detect the target gene, a specific primer for the *L. infantum* kDNA gene was used, which amplifies a 120 bp fragment. PCR amplification was carried out using the following program: denaturation at 86 °C for 5 minutes, followed by 33 cycles (denaturation at 95 °C for 30 seconds, annealing at 52 °C for 30 seconds, and extension at 72 °C for 1 minute), with a final elongation at 72 °C for 10 minutes. Amplified products were analyzed by electrophoresis on a 1.5% agarose gel. The prevalence was 0.5% (2/350) positive samples. The results suggest a low occurrence of *L. infantum* infection in cats in the semiarid region of Paraíba, indicating that, despite the positive detections, felines likely play a secondary role in the epidemiological chain of visceral leishmaniasis in the region. However, it is important to note that the use of peripheral blood as a sample for PCR may have lower diagnostic sensitivity, which could have contributed to the low detection rate observed. Nevertheless, the recorded positivity reinforces the importance of monitoring the feline species as a potential host in endemic areas.

Prevalence of Ectoparasites and Vector-Borne Diseases in Domiciled Dogs in Angra dos Reis, Rio de Janeiro, Brazil (2023-2024)

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In the southeast of Brazil, dogs are often parasitized by ectoparasites. Ticks are the main

vectors of pathogens for animals, but there are other ectoparasites as fleas and mosquitoes that can transmitted pathogens. The main hemoparasitosis are caused by vector-borne pathogens and are the main cause of morbidity in dogs. The aim of this study was to determine the prevalence of ectoparasites and some vector-borne diseases in domiciled dogs in Angra dos Reis, RJ, Brazil, during the period from December 2023 to December 2024. Samples of blood and ectoparasites were collected from 111 dogs with no recent history of parasiticide use, according to their owner. The animals were included during the consultation at the veterinary clinic. Whole blood samples were used to perform tests for the detection of *Dirofilaria immitis* antigen, and antibodies to *Anaplasma phagocytophilum*, *Anaplasma platys*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Ehrlichia ewingii* (SNAP 4Dx Plus Test - IDEXX) and for detection of *Leishmania donovani* or *Leishmania infantum* antibodies (SANP Leishmania- IDEXX). The ectoparasites collected were identified under stereomicroscopy (Ethics Committee number 0106862022). Of the 111 dogs evaluated, 10.81% had infestations by fleas (*Ctenocephalides felis felis*), 3.6% had infestations by ticks from the *Amblyomma cajennense* Complex and the *Rhipicephalus sanguineus* Complex, 2.7% had mixed infestation by fleas and ticks and 82.88% did not have infestations by ectoparasites. The blood tests detected 6.31% of dogs with *Anaplasma* spp. antibodies, 33.33% with *Erlichia* spp. antibodies, 0% with *B. burgdoferi* antibodies, 9% with *D. immitis* antigen, 0% with *Leishmania* spp. antibodies and 51.53% of the dogs were negative in all tests. Although the prevalence of ectoparasites found was low, approximately half of the dogs had already been exposed to vector-borne pathogens.

Molecular detection of Trypanosoma cruzi and Trypanosoma evansi infection in dogs of Paraguay.

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Canine trypanosomosis is a vector-borne disease caused by protozoa of the genus *Trypanosoma* (Kinetoplastida: Trypanosomatidae). Trypanosomes such as *T. evansi*, *T. cruzi*, *T. caninum*, and *T. rangeli*, have been reported to infect dogs. These species are widespread in South America and are endemic in most countries in the region. Chagas disease is a widespread infection in Paraguay and is an endemic zoonosis caused by *Trypanosoma cruzi*, which has been detected in several mammalian reservoirs, including dogs. Surra is an animal trypanosomosis caused by *T. evansi*, which is known to infect a wide variety of mammalian hosts. It has been reported that *T. evansi* infection in dogs is detected by molecular techniques in Paraguay, and their clinical signs observed are weight loss, dermatitis, hair loss, mouth and skin ulcers, enlarged lymph nodes, onychogryphosis, arthritis, and conjunctivitis. These clinical signs are also observed in *Leishmania infantum* infection, which is highly prevalent in dogs in this country. This study aimed to determine the

presence of *T. cruzi* and *T. evansi* by a cross-sectional molecular epidemiological survey for canine trypanosomosis. Two hundred forty-six samples of canine whole blood were collected from different animal hospitals that sent their samples to a veterinary private laboratory (CEDIVEP). Eight departments constitute Paraguay, and 218/246 of the samples were from the Central department, which is the most populated department in the country. Data on sex and age were obtained. No notable findings on blood smears nor special information (clinical signs) about health conditions were obtained. A complete cell count (CBC) was performed (Huma-Count 80Ts, Germany). Thirty µL of blood samples were preserved using an FTA card (Qiagen, Hillden, Germany). DNA extraction was performed following the manufacturer´s protocols. The PCR reactions were performed using S35/S36 primers and TBR primers for *T.* cruzi and *T. evansi*, respectively. A 10 µL reaction mixture containing 1 µL of DNA samples, 5 µL of 2× MightyAmp buffer Ver. 3 (Takara Bio Inc., Shiga, Japan), 0.3 µM of each forward and reverse primer, 1 µL of 10× Additive for High Specificity (Takara Bio Inc.), 0.2 µL of MightyAmp DNA polymerase Ver.3 (Takara Bio inc.), and 2.2 µL of double distilled water was prepared for PCR for *T. evansi*. PCR cycling conditions were as follows: initial pre-denaturation step at 98 °C for 2 min, followed by 40 cycles of amplification with denaturation at 98 °C for 10 s, annealing at 62 °C for 15 s, and extension at 68 °C for 10 s following manufacture's protocol. A 10 µL reaction mixture containing 1 µL of DNA samples, 2 µL of 5× Phusion® HF reaction buffer (New England BioLabs Japan Inc., Tokyo, Japan), 0.8 µL of 250 µM dNTPs, 0.5 µM of each forward and reverse primer, 0.1 µL of Phusion® DNA polymerase (New England BioLabs), and 5.1 µL of double distilled water was prepared for PCR for *T. cruzi*. PCR cycling conditions were as follows: initial pre-denaturation step at 98 °C for 1 min; followed by 40 cycles of amplification with denaturation at 98 °C for 10 s, annealing at 70 °C for 10 s, and extension at 72 °C for 10 s following manufacture's protocol. After agarose gel electrophoresis, the amplicons were visualized using WSE-5400 Printgraph Classic UV transilluminator (Atto Corporation, Tokyo, Japan). Positive control and double-distilled water were used as a negative control. Risk factor analysis by X2 test or Fisher's exact test, were performed to investigate the association of kinetoplast parasite infection among department, sex, and age. Association between prevalence of kinetoplast parasites in dog in Paraguay (*T. evansi, T. cruzi)* and explanatory values in the study were statistically analyzed by Multivariable logistic regression. Comparison of blood parameters among infection status were analyzed by Kruskal-Wallis test or one-way ANOVA. The prevalence of *T. evansi* and *T. cruzi* using TBR1/2, S35/36 was 14.63% (36/246) and 20.33% (50/246), respectively. In addition, the prevalence of mixed infection with *T. evansi* and *T. cruzi*, detected using both TBR1/2 and S35/36 was 4.47% (11/246). Positive samples for multiple infection of *T. evansi* and *T. cruzi* were detected in six of eight departments of the country. All of them are distributed in the eastern region. No data were available for 16 of 246 samples. In Asunción, the main city of the country (population density: 4,000/km2), we found positive samples at the highest percentage, 12.78% (17/133) and 17.29% (23/133) for *T. evansi* and *T. cruzi*, respectively. There was no association of kinetoplastid infection among department, sex, and age by the statistical analysis, and no significant difference in blood parameters among infection status were observed. Canine trypanosomosis is prevalent in dogs from Paraguay.

T. cruzi has a higher prevalence than *T. evansi*, and both species are detected in different departments from the eastern region of the country. Further studies will be necessary to understand the dynamics of the host, vectors, and environment regarding the urbanized geographical situation of the dogs, including the zoonotic potential as reservoirs of these pathogens.

Epidemiological study of parasites and vector-borne diseases in dogs and cats from curitibanos, santa catarina, brazil

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Parasitic and vector-borne diseases are common in dogs and cats, posing a risk to animal and human health due to their zoonotic potential. To evaluate the occurrence and prevalence of internal and external parasites in dogs and cats in the city of Curitibanos, Santa Catarina state, Brazil. This work was part of a multicenter research in Brazil and was sponsored by Boehringer Ingelheim Animal Health, having been approved under CEUA/UFSC 9454210922. The study occurred at the UFSC Veterinary School Clinic, in Curitibanos. Dogs and cats with access to the outside environment and without recent use of antiparasitic drugs were included between February and November 2024. A physical examination, blood collection for rapid tests (IDEXX) and copro parasitological examination were performed for all animals, and skin scraping or ectoparasite collection have been made when necessary. Cats were tested for FIV/FeLV, and dogs for 4Dx Plus and Leishmaniasis. A total of 158 animals (72 cats and 86 dogs) participated in the study. Among the felines, 9% were positive for FeLV and 2.77% for FIV+FeLV. Of the 78 dogs tested, only 5.8% were positive (3.5% for *Erlichia canis* and 2.3% for *Dirofilaria immitis)*. There were no Leishmaniasis positive samples. Dermatological complaints were reported in 30.5% of cats and 34.9% of dogs, with identification of *Ctenocephalides* spp., *Demodex* spp., *Rhipicephalus sanguineus* and *Dermatobia hominis*. Of the fecal samples from cats, 50% were positive, with emphasis on *Giardia* spp. (38.5%) and *Ancylostoma* spp. (28.8%). Among the dogs, 33.8% were positive, with prevalence of *Ancylostoma* spp. and *Giardia* spp. (both with 34.78% of prevalence). These results indicate a high prevalence of parasites and vector agents, highlighting the importance of early diagnosis, continuous prevention and the role of veterinarians in public health.

Molecular detection and phylogenetic perspective of *Hepatozoon canis* in domestic dogs from ecological transition zones in Southeastern Brazil

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Hepatozoonosis is an infection caused by protozoa of the genus *Hepatozoon*, affecting both

domestic and wild carnivores, typically presenting with nonspecific or asymptomatic clinical signs. In dogs in Brazil, *Hepatozoon canis* is the main species involved, and transmission is primarily associated with the ingestion of infected arthropods, such as *Rhipicephalus sanguineus* (sensu lato) and *Amblyomma ovale* ticks. This study aimed to investigate the circulation of *Hepatozoon* spp. in dogs from the municipality of Itabirito, Minas Gerais, Brazil, covering urban, peri-urban, and rural areas. Whole blood samples in EDTA were collected from 100 domiciled dogs, and molecular screening was conducted via PCR targeting the 18S rRNA gene, followed by sequencing of 17 nucleotide sequences. Evolutionary history was inferred using the Maximum Likelihood method and the Tamura 3-parameter model for specific identification, using the MEGA11 software. Seven samples (7/100) tested positive for *Hepatozoon* spp., with phylogenetic analysis indicating 100% identity to *H. canis* isolates previously detected in wild carnivores in Brazil, such as *Cerdocyon thous* (crab-eating fox), *Lycalopex gymnocercus* (Pampas fox), and felids; in addition to high similarity with isolates from an Iberian wolf (*Canis lupus signatus*) in Spain and a red fox (*Vulpes vulpes*) in Italy. These findings suggest that *H. canis* has a broad host range and that wild and domestic canids may share vectors in Brazil, while different vectors may be involved in the transmission of the same pathogen in other parts of the world. The results of this study contribute to a better understanding of *Hepatozoon canis* host diversity and transmission ecology, reinforcing the importance of integrated approaches for monitoring hemoparasites in transition areas between natural and anthropogenic environments.

Seroprevalence of *Toxoplasma Gondii* in dogs in the state of São Paulo

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Toxoplasma gondii may cause neurological, ocular, and cutaneous diseases in dogs. This study investigated anti-T. *gondii* antibodies in dogs treated at the FCAV/Unesp Veterinary Hospital, upstate São Paulo State. From August 2023 to December 2024, 78 dogs with access to the street were evaluated, 58.8% females and 41% males, aging from 2 months to 17 years old. Geographical location and previous raw meat intake were recorded. Serum samples were evaluated by IFAT with commercial kits (Imunoteste *T. gondii* Imunodot®). Fisher's exact test was used to associate the test result with sex and consumption of raw meat. Most of the dogs (80.7%; 63/78) were from Jaboticabal city. 26.9% (21/78) of the tested animals were positive. The most frequent antibody titer was 1:80 (15.38%). Raw meat ingestion was related by 10.2% (08/78) of the dog owners, but 6.4% (05/78) could not

inform as the animals were recently adopted. There was no association between raw meat consumption and host sex with the infection status (both P>0.05). *T. gondii* seroprevalence in Brazilian dogs ranges between 10.5% and 43.9%. In this study, the seroprevalence was similar to other studies in Brazil using the same diagnostic test. Some dogs in the study were recently adopted, which may explain their seropositivity for *T. gondii*, since the risk factors associated include access to the street, contact with cats, the presence of rodents at home and, especially, the diet these animals receive. In this study, there were no significant differences between the group that consumed raw meat and the group that did not. However, the possibility of an association between the seropositivity of animals and the consumption of raw meat is not ruled out due to the low n of animals exposed to this risk factor. None of the seropositive dogs showed clinical signs compatible with *T. gondii* infection. Our study demonstrated the circulation of *T. gondii* in dogs from the study area in frequency similar to other Brazilian regions.

Vertical transmission of *Leishmania infantum* in dog from a non-endemic region

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Canine leishmaniasis (CanL) is a zoonotic disease caused by sand fly-transmitted *Leishmania infantum* parasites and is endemic in several tropical and subtropical countries. In Europe, CanL is endemic in areas of the Mediterranean basin. Vertical transmission of *L. infantum* in dogs has been confirmed. In areas where vector transmission is limited or absent, the transplacental route accounts for *L. infantum* transmission and perpetuation within canine populations. The aim of this study is to report an autochthonous case of CanL in São Miguel Island (Azores Archipelago, Portugal) in a 5-year-old female Beagle dog who was born on the island and had never left it. At the age of 4 years, the dog was admitted to a veterinary clinic in São Miguel with an exfoliative dermatitis covering the chest. Later, in January 2024, she delivered a litter of three puppies. During pregnancy and lactation, no clinical signs were registered on physical examination. However, a relapse of the exfoliative dermatitis, with a generalized presentation, was diagnosed 4 months after birth. In April 2024, an elective ovariohysterectomy was performed. In August 2024, the dog was found seropositive for *Leishmania* by an IDEXX Snap test. In September 2024, the female parent, litter of puppies, and male parent were tested for antibodies to *Leishmania* (ELISA) and for kDNA (PCR). Positive results were obtained for the mother, while puppies and father were ELISA and PCR negative. This case highlights the possible vertical transmission of *Leishmania* in a non-endemic area and in the absence of vectors. International relocation of dogs from CanL-endemic to non-endemic regions contributes to the spread of the parasite. Veterinarians' awareness is essential for effective control of this disease. In conclusion, vertical transmission of *L. infantum* in dogs is an established phenomenon that may play an important role in the epidemiology of CanL, particularly in areas where vectorial transmission is limited or absent.

Genetic diversity and epidemiology of Cryptosporidium in dogs and cats in Northern Paraná, Brazil.

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In Paraná, *Cryptosporidium* species have been identified in ruminants, captive wild birds, and primates, but no studies have addressed the genetic diversity of *Cryptosporidium* in dogs and cats. In this study, 104 fecal samples from dogs and 61 from cats were collected from urban and rural areas across eleven municipalities in northern Paraná. *Cryptosporidium* presence was investigated using the Sheather technique and Nested PCR, with diagnostic sensitivity and specificity calculated. Positive samples underwent genetic sequencing. Risk factors such as age, sex, habitat, and others were analyzed. No oocysts were detected using Sheather; however, *Cryptosporidium* was identified in 1.82% (3/165) of samples by Nested PCR, all from dogs. The occurrence in dogs was 2.9% (3/104), 3.6% (2/55) in urban dogs and 2% (1/49) in rural dogs. Positive urban dogs were from Cornélio Procópio and Itambaracá, and the rural dog from Siqueira Campos. All positive dogs were under 1.5 years old, and one had diarrhea. The diagnostic sensitivity of the Sheather technique was 0%, with specificity at 98.2% (95% CI: 94.8–99.6%), confirming its negative predictive value (100%). Genetic sequencing identified *C. andersoni* and *C. parvum* (99% similarity) in urban dogs, but species could not be determined in the rural dog. The *C. andersoni* sample came from a dog with frequent contact with rural environments and livestock. Among risk factors, young animals (up to 1 year) were significantly more susceptible (Odds Ratio = 47.44) compared to adults. This study concludes that *Cryptosporidium* is present in the region. Although its clinical relevance appears limited due to its low occurrence, the detection of a zoonotic species underscores its epidemiological importance. While *C. andersoni* may represent mechanical transmission, further research on host specificity is warranted.

Efficacy of a single monthly dose of an oral formulation of sarolaner, moxidectin, and pyrantel (Simparica Trio®) against transmission of *Dipylidium caninum* by *Ctenocephalides felis* fleas

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Two laboratory studies were conducted to confirm the efficacy of Simparica Trio® in preventing Dipylidium caninum infection in dogs for one month through killing of the flea intermediate host, *Ctenocephalides felis*. These studies were compliant with local regulatory, animal welfare, and legal requirements. Two groups of 10 dogs were used in each study. On Day 0, dogs were administered either placebo or Simparica Trio at the minimum dose of 1.2 mg/kg sarolaner, 24 µg/kg moxidectin and 5 mg/kg pyrantel. One placebo dog was excluded from

study due to incomplete dosing. Dogs were infested with 200 (±5) unfed, D. caninum-infected fleas on Days 0 (a er treatment), 7, 14, 21, and 30. Live flea counts were conducted on Day 33 (72±2 hours after Day 30 infestation). Following euthanasia on Day 58, necropsies were performed to enumerate *D. caninum* scoleces from the gastrointestinal tracts. Placebo-treated dogs maintained flea infestations of 5-159 fleas throughout the duration of eachstudy, accounting for at least 70% of control dogs having ≥ 50 C. felis fleas on Day 33. Scolex counts of 1-100 were present at the me of necropsy in placebo--treated dogs with at least 88.9% of dogs having ≥ 2 *D. caninum* scoleces. Two dogs in Study 1 (1 placebo and 1 Simparica Trio-treated) were found to have proglotids at necropsy without scoleces and were therefore each counted as having a single scolex. Significantly lower mean flea counts were recorded for Simparica Trio--treated dogs compared to placebo-treated dogs ($P \leq 0.0007$) and efficacy based on arithmetic mean flea counts was 100% for both studies on Day 33. Scolex counts in Simparica Trio-treated dogs were also significantly decreased compared to placebo-treated dogs ($P \leq 0.0033$) and the efficacy of Simparica Trio, based on least squares mean scolex counts, was 92.1% (Study 1) and 100% (Study 2).

Case report of canine leishmaniasis with discordant laboratory results: relevance of clinical staging

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Canine leishmaniasis is an infectious, zoonotic, and incurable disease caused by protozoa of the genus Leishmania spp., transmitted by phlebotomine sandflies. Accurate diagnosis is essential, as it guides therapeutic decisions and impacts both animal and public health. Considering the diagnostic complexity and the risks associated with unnecessary treatment, this report aims to describe a clinical case that underscores the importance of careful interpretation of laboratory tests and clinical staging of the disease. In July 2024, a mixed-breed dog, one year old and weighing 10 kg, was referred for veterinary care following a positive quantitative PCR result for Leishmania spp. in a blood sample (cycle threshold – 31.49), and negative results for Anaplasma sp., Babesia sp., and Ehrlichia sp. These tests were requested due to previous episodes of vomiting and diarrhea. On physical examination, the animal was alert, responsive, and showed normal physiological parameters. Biochemical testing revealed total protein of 7.1 g/dL, albumin 3.8 g/dL, globulin 3.3 g/dL, creatinine 0.9 mg/dL, urea 31 mg/dL, and ALT 45 U/L. The complete blood count showed leukocytosis due to lymphocytosis (18,900 leukocytes/mm^3; 5,278 lymphocytes/mm^3), and urinalysis revealed proteinuria (++) associated with the presence of spermatozoa (+++). Serology by ELISA and IFAT (RIFI) was non-reactive, and quantitative PCR using blood and bone marrow samples was also negative. In view of the discrepancy between the results of direct and indirect tests, treatment was not initiated, and the patient was kept under monitoring. A new serological evaluation (ELISA and IFAT), performed in March 2025, remained

nonreactive. The patient continues to be clinically stable and with no laboratory abnormalities. This report highlights the importance of clinical staging and integrated interpretation of diagnostic tests, preventing premature diagnoses and unnecessary treatments in suspected cases of leishmaniasis.

Survey of major parasitic diseases in dogs and cats attended at a veterinary teaching hospital in Porto Alegre, Rio Grande do Sul, Brazil

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Parasitic diseases are common in the clinical routine of dogs and cats, affecting both animal and public health and causing economic losses. Understanding the main parasitic infections in a region is essential for prevention strategies, education, and veterinary improvement, and the development of new antiparasitic treatments. To identify and quantify parasitic diseases in dogs and cats treated at a Veterinary Teaching Hospital in Porto Alegre, RS, Brazil. A prospective study conducted between September 2023 and November 2024. The study included dogs and cats seen in the clinical routine of the Veterinary Clinics Hospital at UFRGS, without anthelmintic use in the past two months or ectoparasiticides in the past month. The animals underwent examinations for ectoparasites (inspection, skin scraping, cerumen parasitology), endoparasites (Mini-FLOTAC), and ELISA SNAP tests (*SNAP 4DX Plus*, *SNAP Leishmania*, and *SNAP Feline Triple*). A total of 147 animals were analyzed: 83 dogs (38 spayed and 12 intact females, 16 neutered and 17 intact males) and 64 cats (20 spayed and 9 intact females, 23 neutered and 12 intact males). In the *SNAP 4DX Plus* test, dogs tested positive for *Ehrlichia spp.* (n=5), *Anaplasma spp.* (n=5, one coinfected), and *Leishmania spp.* (n=6). In cats, 4 had FIV and 7 had FeLV, with one co-infection. Coproparasitological tests detected *Toxocara spp.* (9 cats, 1 dog), *Ancylostoma spp.* (9 dogs, 1 cat), *Cystoisospora spp.* (1 cat), along with isolated cases of *Trichuris spp.*, *Neospora spp.*, and *Haemonchus spp.* Ectoparasites identified included *Ctenocephalides felis felis* (6 dogs, 5 cats), *Ctenocephalides canis* (6 dogs, 1 cat), and *Rhipicephalus sanguineus* (4 dogs). Notably, five dogs were infected with *Ehrlichia spp.*, an agent considered uncommon in southern Brazil, and seven animals had *Ctenocephalides canis*, a flea species considered infrequent in the country.

Occurrence of *Anaplasma platys* and *Ehrlichia* spp. in healthy dogs in Londrina, Paraná, Brazil

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Ehrlichia spp. and *Anaplasma platys* are gram-negative, obligate intracellular bacteria transmitted mainly by *Rhipicephalus sanguineus* ticks. *Ehrlichia* spp. infects monocytes, lymphocytes and macrophages, and may remain hidden in target organs such as lymph nodes, spleen and liver. *Anaplasma platys* infects platelets. As the same vector transmits them, it is not uncommon for a patient to present both agents. Diagnosis is based on associating history, clinical signs and compatible laboratory changes with agent identification. The study aimed to identify dogs with *A. platys* and/or *Ehrlichia* spp. Without clinical signs through molecular and serological testing. Sixty dogs brought for blood donation at HV-UEL from January to December 2024 were selected. These animals underwent history taking, physical examination, complete blood count, serum biochemistry, IDEXX® SNAP 4Dx Plus Test and PCR for the dsb gene (409 bp) and 16S rRNA gene (504 bp). The SNAP 4Dx test detected 1.6% (1/60) dogs positive for *Anaplasma* spp. and 13.3% (8/60) for *Ehrlichia* spp. By PCR, 3.3% (2/60) were detected for both *Anaplasma platys* and *Ehrlichia* spp. Additionally, one dog was positive on SNAP 4Dx for both diseases. However, there were no patients who tested PCR positive for both. Dogs may test positive for *Ehrlichia* spp. and *Anaplasma platys* without showing any clinical signs.

Filarids in dogs of Uruguay. What do we know so far?

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Filarids are parasites that live inside several tissues and use arthropods as vectors. Among filarid species, *Dirofilaria immitis* and *Dirofilaria repens* are well-documented zoonotic agents. In Latin America, *D. immitis* has a wide distribution but it has never been reported in Uruguay. In 2023, the first confirmation of an imported case of *D. immitis* infection in a dog brought from Argentina was reported. In 2024, a second case of *D. immitis* infection was informed in a Brazilian dog brought to Uruguay a few months before. These cases triggered a survey to determine whether this parasite could be transmitted to local dogs and establish an autochthonous circulation. The aim of this study was to detect microfilariae in dogs from Uruguay and to perform their molecular characterization. Dog blood with EDTA was submitted by private veterinary clinics from several locations in the country and analyzed using the Knott test in our laboratory. Microfilarial-positive samples were subjected to DNA extraction, PCR targeting a fragment of the 16S RNA gene, and sent for sequencing. A total of 1050 dog samples were analyzed by Knott test with 10 samples being microfilarial-positive. Sequencing results revealed that all the positive samples were *A.*

reconditum. This study highlights the importance of including *A. reconditum* in the differential diagnosis of canine filariasis. Although only two imported cases of *D. immitis* have been reported to date, the detection of *A. reconditum* in multiple regions indicates that this parasite is already circulating locally.

Cystoisospora spp. infections and other intestinal parasitoses in dogs and cats with diarrhea in Italy and Greece

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The occurrence and clinical impact of *Cystoisospora* spp. were evaluated in <2-year-old dogs (n. 117) and cats (n. 118) with diarrhea. Individual fecal samples were subjected to flotation and sucrose concentration; fecal and clinical scores were calculated. Significant associations between clinical signs' severity and presence of coccidial and/or other parasitic infections were assessed *via* Binomial logistic regression and Fisher's exact test. *Cystoisospora canis* and *Cystoisospora ohioensis*-complex oocysts were found in 8 (6.8%) and 4 dogs (3.4%); 1 (0.8%) was coinfected by both; *Cystoisospora felis* and *Cystoisospora rivolta* oocysts were shed by 34 (28.8%) and 7 (5.9%) cats; 1 shed both (0.8%). Other intestinal parasites, i.e. *Giardia*, hookworms, roundworms, *Trichuris vulpis*, cestodes, were also detected. All coccidia-infected dogs had very soft feces. Watery/hemorrhagic diarrhea occurred in 4/21 (19%) and 2/5 (40%) cats with *C. felis* and *C. rivolta*-only infection. Overall, 2/3 (66.7%) and 1/3 (33.3%) *C. canis*-only infected dogs had mild and moderate disease. Mild disease was recorded in 2 *C. ohioenesis*--only infected dogs; severe illness occurred in the dog with mixed infection. Very mild, mild, and moderate disease occurred in 4/21 (19%), 13/21 (61.9%), and 4/21 (19%) cats infected by *C. felis* only. Among cats with *C. rivolta*-only infection, 2/5 (40%) had mild disease; the other 3 had very mild, moderate and severe disease. Significant associations were found between positivity to coccidia in dogs (lower) vs cats (higher) (p<.0001), *C. canis* infection/reduced attitude (p=0.021), hookworm infection/very soft feces (p=0.007) (dogs); hookworm infection and vomitus (p=0.04), dehydration (p=0.009), watery/hemorrhagic diarrhea (p=0.007) (cats) (Fisher's exact test). These results indicate that (i) young cats with diarrhea may be more frequently infected with *Cystoisospora* than dogs (p= <.0001), (ii) *C. canis* and *C. rivolta* may be more pathogenic than other species in dogs and cats.

Dipteran larvae parasitizing wild animals in Sorocaba's city, SP, Brazil

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Blowflies from the families Calliphoridae, Muscidae, and Cuterebridae are widely distributed, with species classified into over 150 genera. Adults and larvae are important in medical and veterinary fields, causing economic losses in livestock due to severe cutaneous, subcutaneous, and muscular lesions. Calliphorids aid forensic science in postmortem interval estimation and serve as debriding agents in wound treatment. This study aimed to taxonomically identify dipteran larvae in myiasis lesions of wild animals rescued from free-ranging environments or kept at Sorocaba Zoo. From 2021 to 2024, larvae from wild hosts with myiasis were collected, preserved in 70° GL ethanol, and sent to Fluminense Federal University (UFF) for taxonomic diagnosis. Samples from nine wild species were examined: *Pteronura brasiliensis, Cerdocyon thous, Didelphis albiventris, Didelphis aurita, Alouatta clamitans, Dasyprocta azarae, Mazama gouazoubira, Cervus elaphus*, and *Lama glama*. The genus *Cochliomyia* was most prevalent, found in 88.9% (8/9) of samples from *P. brasiliensis, C. thous, A. clamitans, D. azarae*, and *M. gouazoubira*. The species *Cochliomyia hominivorax* was confirmed in 44.5% (4/9) of *D. albiventris, A. clamitans, D. azarae*, and *C. elaphus*. Additionally, *Chrysomya* was found in *A. clamitans*, *Muscidae* in *D. aurita*, and *Dermatobia hominis* in *L. glama*, all with 11.1% (1/9). Myiasis in wild animals is frequent, especially in the rainy season, revealing a complex ecological dynamic where dipterans impact wildlife health and indicate environmental changes. However, data on larval identification as etiological agents in such lesions are scarce. This study contributes to the taxonomic identification of parasitic fly larvae in wild animals and encourages collection and identification efforts in both live animals and carcasses. Understanding these interactions is essential for comprehending ecosystem dynamics involving these dipterans.

Parasitological findings in wild and exotic artiodactyls in Brazil: five years of surveillance

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Wild and exotic artiodactyls are subject to infection by several parasites, which can impact

their health and management. This study aimed to describe the parasitological findings in different species of artiodactyls analyzed by the Laboratory of Diagnostic Support in Parasitic Diseases of the Fluminense Federal University (LADDP-UFF). To this end, from March 2018 to November 2023, LADDP-UFF carried out the parasitological diagnosis of 82 samples from Barbary Sheep (*Ammotragus lervia*), Cervicapra (*Cervicapra antelope*), Pantanal Deer (*Blastocerus dichotomus*), Buffalo (*Bubalus bubalis*), Dromedary (*Camelus dromedarius*), Red Deer (*Cervus elaphus*), Red Deer (*Mazama gouazoubira*), Alpaca (*Vicugna pacos*) and Llama (*Lama glama*). Of these samples, 76 were fecal masses and one specimen of adult endoparasites and five ectoparasites. The samples were from Sorocaba, SP, Rio de Janeiro, RJ and Nova Friburgo, RJ. The prevalence of positive samples for at least one parasite species was 68.3% (56/82). Of these, 53.6% (30/56) were positive for monoinfections and 46.4% (26/56) for multi-infection. Samples of *L. glama* with a prevalence of 46.4% (26/56) and *V. pacos* with 21.4% (12/56) stand out. Of the positive samples for endoparasites, 64.3% (36/56) were for *Haemonchus* sp., 34% (19/56) for *Trichostrongylus* sp., 16% (9/56) for strongylids, 14.3% (8/56) for *Eimeria* sp., 3.6% (2/56) for *Moniezia* sp., 1.8% (1/56) for *Toxocara vitulorum* and *Taenia omissa*. Of the ectoparasite samples diagnosed, the prevalence was 5.3% (3/56) for *Cochliomyia* sp., 1.8% (1/56) for *Ctenocephalides felis* and *Rhopalopsyllus* sp. The results reveal a high prevalence of parasitic infections, with emphasis on gastrointestinal helminths in South American camelids. These findings reinforce the importance of continuous parasitological surveillance in animals of interest for conservation, research or production, aiming at animal health and welfare.

Flea-infested dogs (Insecta: Siphonaptera) and their risk factors in peri-urban areas of the Cajamarca district, Peru

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Fleas are hematophagous ectoparasites of endothermic animals, with medical and veterinary importance due to allergic reactions and their role as pathogen vectors. In Peru, fleas are widespread; however, formal data from Andean regions remains limited. This study reports the prevalence of flea infestation in dogs and associated risk factors in the Cajamarca district, northern Peru (2750 m a.s.l.). One hundred and two randomly selected households with dogs were visited, and structured owner surveys were conducted in six peri-urban clusters. Between one and ten fleas per infested dog were collected for morphological identification. The prevalence of infestation was 89.22 ± 6.02% (91/102). Among the 689 fleas collected, *Ctenocephalides felis* was the most prevalent (66.47 ± 3.53%), followed by *C. canis* (31.35 ± 3.46%) and *Pulex irritans* (2.18 ± 1.09%). Coinfestation with all three species occurred in four cases, and dual-species infestations were recorded in 49 cases. *C. felis* was significantly associated with dog age and housing material, whereas *C. canis* was linked to resting place ($p < 0.05$). In contrast, sex, breed, cohabitation with other ani-

mals, presence of stray dogs, and synanthropic rodents showed no significant associations ($p > 0.05$). Logistic regression indicated that dogs older than 8 years were 97.10% less likely to be infested with *C. felis* than puppies (≤1 year) (*OR* = 0.029; 95% CI = 0.003–0.310, $p = 0.003$). Dogs in concrete/brick homes were 93.90% less likely to be infested with *C. felis* than those in earthen homes (*OR* = 0.061; 95% CI = 0.006–0.605, $p = 0.017$). Finally, dogs that spent most of the day outdoors had a 3.33- fold higher likelihood of *C. canis* infestation than those indoors (95% CI = 1.065–10.433, $p = 0.000$). These findings highlight the high flea prevalence and may inform improved prevention and control strategies in Cajamarca.

First autochthonous outbreak of *Leishmania infantum* in domestic dogs from Guanacaste, Costa Rica

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Leishmania infantum is a protozoan parasite responsible for visceral leishmaniasis, affecting millions globally. Dogs act as key environmental reservoirs and are considered sentinels of infection. In Costa Rica, only three cases had been reported in humans or animals—none in the past 25 years. Objective: To describe and analyze four cases of canine *L. infantum* infection in Guanacaste, Costa Rica, and examine DNA sequences from the detected parasites. We evaluated clinical signs, treatments, and disease progression in each case. Dogs were screened using serological and molecular tools. When available, skin scrapings, spleen biopsies, and lymph node aspirates were collected. Samples were stained and analyzed by PCR, targeting the *hsp70*, kinetoplastid DNA, and *ITS1* loci. Phylogenetic and haplotype analyses were performed to determine the origin of the strains. The first case was identified in 2023 and involved cutaneous, hematological, and renal abnormalities. Infection with *L. infantum* was confirmed using morphological, serological, and molecular techniques. This prompted additional screening in the community, leading to the identification of three asymptomatic dogs. In 2025, another dog presented with blood abnormalities, and a splenectomy revealed multiple intracellular amastigotes in macrophages and multifocal granulomatous nodules. DNA sequencing from all cases confirmed the presence of *L. infantum*, and clustering with strains from Spain, Morocco, and Italy rather than those from South America. This suggests the infections were likely imported from the Old World. This is the first confirmed autochthonous outbreak of *L. infantum* in Costa Rican dogs. The detection of new cases two years after the initial ones indicates possible establishment of the parasite's life cycle locally—raising concerns for both canine and human public health.

Molecular characterization of *Spirometra* isolates across the United States of America

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Spirometra (Cestoda: Diphyllobothriidea) is a genus of zoonotic cestodes with an ambiguous species–level taxonomy. Previously, *Spirometra mansonoides* was considered the only species present in North America. However, recent molecular data revealed the presence of at least three distinct species in the United States of America (USA): *Spirometra* sp. 2 and 3, and *Spirometra mansoni*. In this study, we aimed to elucidate the diversity and potential host associations of *Spirometra* species among companion animals in the USA. We examined 302 samples from at least 13 host species, including mammals, amphibians, and reptiles. Sample types included eggs isolated from feces (n=222), adult specimens (n=71), and plerocercoids (n=9) from 18 different states and 2 territories across the USA. Extracted genomic DNA was subjected to PCR targeting a fragment of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene. Generated sequences (n=136) were included in a comprehensive phylogenetic analysis. *Spirometra mansoni* was detected in domestic cats (n=76), dogs (n=12), a White's tree frog (n=1), a Cuban knight anole (n=1), a green iguana (n=1), and a serval (n=1) across 15 states and Puerto Rico. *Spirometra* sp. 2 was found only in dogs (n=3) from Florida, and *Spirometra* sp. 3 was found only in cats (n=41) from 17 states. The findings of all three genetic lineages in carnivorous definitive hosts indicate patent infections. All plerocercoid samples were consistent with *S. mansoni*. The results confirm that at least three distinct *Spirometra* species are present and established in companion animals such as dogs and cats and likely are using various native and exotic species as paratenic hosts within the USA.

Coproparasitological findings in mammal from the uberlândia municipal zoo – Minas Gerais, Brazil

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The study aimed to evaluate the parasitological profile of mammals at the Uberlândia Municipal Zoo – Minas Gerais, Brazil. 178 samples were analyzed between July 2020 and February 2024 using spontaneous fluctuation and sedimentation methods. 34 samples (19.1%) were positive for endoparasites. Tayassu pecari had the highest number of positive samples (n=9/20; 45%) and Mazama gouazoubira had the highest percentage of parasitism (n=4/6; 66.7%).

The most frequent findings were Hookworm eggs (n=7/37; 18.91%), Strongylid eggs (n=6/37; 16.21%) and Balantidium spp. cysts. The majority of positive samples occurred in the summer. It is concluded that mammals at the Uberlândia Municipal Zoo had a relatively low prevalence of endoparasitism and the importance of parasitological monitoring of animals in captivity is highlighted in order to develop management protocols that are efficient for controlling parasitic infections.

Molecular prevalence of *Enterocytozoon bieneusi* and *Encephalitozoon cuniculi* in cats

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Microsporidia are emerging, opportunistic fungi that infect a diverse population of vertebrates and invertebrates. Domestic cats can be a source of infection for humans, and the average worldwide prevalence of *Enterocytozoon bieneusi* is 8.1%, but other species of microsporidia are neglected. *E. bieneusi* has also been detected in many animals such as cats, dogs, cattle, goats, horses, and primates. In Brazil, clinical and epidemiological data are lacking. The aim of this study was to analyze the occurrence of the zoonotic microsporidian *Encephalitozoon intestinalis, Encephalitozoon cuniculi* and *Enterocytozoon bieneusi* in the feces of domestic cats. Fecal samples from domestic cats with or without diarrhea (n=30) were subjected to DNA extraction with the QIAamp Fast DNA Stool Mini Kit. Amplification by PCR was performed with generic primers and the product generated from this reaction was subjected to nested PCR with specific primers. Of the 30 fecal specimens analyzed, 4 (13.3%) samples were positive for microsporidia. Of the 4 positive samples, 3 (75%) were diagnosed as *E. bieneusi* and one (25%) was positive for *E. cuniculi*. The 3 samples positive for *E. bieneusi* belonged to cats aged 1 to 3 years, one was a domestic cat and 2 were rescued from a shelter, and all were associated with intermittent diarrhea. The only sample positive for *E. cuniculi* belonged to a 5-year-old cat with no history of diarrhea. We can conclude that the prevalence was 13.3%, higher than that observed in other countries, and the presence of *E. bieneusi* was associated with diarrhea, and the pathogen should be included in the etiological list for the diagnosis of chronic diarrhea.

Coccidiosis in a flock of exotic birds in the municipality of Santa Bárbara – Bahia

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Exotic birds are increasingly being kept as companion animals, which requires attention to the sanitary risks associated with captive breeding. Among the relevant infectious agents, coccidia stand out as enteric protozoa that can com-

promise the clinical condition and productive performance of birds. This study aimed to describe an outbreak of coccidiosis in an ornamental flock located in the municipality of Santa Bárbara, Bahia, consisting of one pair of each of the following species: Ring Neck (*Psittacula krameri*), Kakariki (*Cyanoramphus novaezelandiae*), Plum-headed parakeet (*Psittacula cyanocephala*), and Rosella (*Platycercus eximius*). The animals showed diarrhea, cachexia, opaque and fragile feathers, presence of lice, pale mucous membranes, dry skin, and evident signs of dehydration. Fresh fecal samples were collected from the floor, which had been previously covered with plastic to prevent environmental contamination. The samples were placed in sterile containers and submitted for laboratory analysis using the Willis-Mollay technique, which revealed the presence of unsporulated oocysts compatible with coccidia. The therapeutic protocol consisted of the administration of Clopidol diluted in water by oral route and the application of Toltrazuril 5% for four consecutive days, also diluted in water, with a three-day interval between medications. After 30 days, resolution of clinical signs was observed, with an average weight gain of 7 g and restoration of plumage. New fecal samples were collected and, upon coproparasitological examination, tested negative. This case highlights the importance of continuous sanitary monitoring in exotic bird breeding facilities, aiming at endoparasite control and the maintenance of animal welfare.

Coproparasitological analysis of red-footed tortoises (*Chelonoidis carbonarius*) admitted to a wildlife screening center in the state of Bahia

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Tortoises are reptiles of the order Testudines, with *Chelonoidis carbonarius* (red-footed tortoise) and *Chelonoidis denticulata* (yellow-footed tortoise) being the most common species in Brazil. These animals are frequently kept illegally as pets, which represents a serious threat to One Health, as wildlife trafficking facilitates the circulation of infectious agents between animals and humans, increasing the risk of outbreaks and compromising the sanitary balance among species. In this context, this study aimed to conduct a coproparasitological analysis of red-footed tortoises admitted to the Wildlife Screening Center (CETAS) in Cruz das Almas, Bahia. Fecal samples were collected from 21 individuals between September and December 2024 and processed using simple flotation and spontaneous sedimentation techniques. All samples (100%) tested positive for at least one type of parasite. Strongylid eggs were identified in 57.14% (12/21) of the samples, coccidian oocysts in 19.05% (4/21), and protozoan cysts in 52.38% (11/21). Protozoan trophozoites were observed in 19.05% (4/21), and helminth larvae were found in 47.62% (10/21) of the samples. The results highlight the importance of performing coproparasitological examinations on tortoises rescued by screening centers to ensure appropriate therapeutic intervention, protect

local fauna, and prevent risks to public and environmental health.

Efficacy of NexGard® Plus against naturally acquired *Otodectes cynotis* ear mites in dogs

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Otodectes cynotis is a parasitic mite of cats and dogs of considerable veterinary importance. The mites live in the external ear canal of the host, being the most common aetiological agent of otitis externa in pets. Therefore, using an effective miticidal is important to treat dogs against *O. cynotis* infestations. The study provided efficacy data of NexGard® Plus (Boehringer Ingelheim), a combination of afoxolaner, moxidectin and pyrantel, against naturally acquired *O. cynotis* infestations in dogs. It was a parallel group designed, blinded, randomised, negative controlled, efficacy study. It was conducted in a single kennel using three groups (A, B and C) of eight dogs, each individually caged: A) untreated negative controls, B) treated with NexGard® Plus as a single oral dose on Day 0 and C) treated with NexGard® Plus as an oral dose once on Days 0 and 30. On Days 0, 14, 30, 42 and 56, clinical and otoscopic examinations to evaluate the presence of live mites and debris/cerumen were performed. At the end of the trial (D56) ear flushing to collect and count the mites was additionally performed. A written informed consent from the dog owner and the approval by the Ethics Committee of the Aristotle University of Thessaloniki were obtained. The level of significance was set at $p<0.05$. The mean numbers of *O. cynotis* mites at the end of the trial for dogs in groups A, B and C were 58.8±35.2, 0 and 0.3±0.7, respectively. The numbers of mites at the end of the trial differed significantly between the untreated control and treated animals, while no significant difference was noted between the dogs treated once or twice. The assessment of the ear canal with the otoscopic examination showed a significant improvement in the treated dogs. The efficacy was calculated to be practically 100% for either one or two treatments. It was concluded that NexGard® Plus offers a highly effective and safe option for *O. cynotis* control in dogs.

Parasiticides in UK pets: the impact of prevention on pet health and veterinary costs

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Parasites of dogs and cats pose a significant One Health burden due to the close interaction and strong emotional bonds between pets and humans. Prevention practices rely on the routine administration of parasiticides, but the efficacy paradox and societal concerns on environmental impact of parasiticides are challenging the need for preventative parasiticide treatments. This study aims to develop a novel understanding of the impact of preventative parasiticide use on prevented cases and owner costs in the UK. Data on prevalence, parasiticide use, treatment compliance, veterinary costs were combined to make a quantitative assessment via a compartmental model. Analysis focused on roundworms, tapeworms, fleas and ticks, sourcing information from scientific literature, market reports, and

regulatory data. Two scenarios were evaluated: scenario A representing the current coverage of parasiticide treatment, and Scenario B representing optimal treatment coverage with all pets receiving parasiticide treatment according to ESCCAP guidelines. The analysis found that current parasite control levels (scenario A) in the UK likely prevent up to 5.5 million cases annually, including approximately 852,600 cases of *Toxocara* spp., 556,700 tapeworm infections, 23,100 *Angiostrongylus vasorum* infections, 1.95 million flea infestations, and 2.09 million tick infestations. Under Scenario B, the number of cases prevented would rise by ~80% to 9.9 million. Economically, current parasite control saves UK households up to £334 million annually, including £6.8 million from *Toxocara* spp., £5.6 million from *A. vasorum*, and £320 million from flea prevention. Enhanced regular treatments could increase household savings by ~143% to £812 million. Regular parasite prevention significantly reduces the number of parasite cases and associated economic burdens on UK households. Increasing consistent use of parasiticides could further enhance pet health and provide substantial financial benefits, highlighting the importance of improved parasite control strategies in companion animals. These data can be further used in cost-benefit assessments and informing public and animal health policies.

Exploring the genetic diversity of *Ehrlichia canis* lineages (16S-rDNA) from Brazil in a worldwide perspective

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Ehrlichia canis is a tick-borne pathogen transmitted by *Rhipicephalus sanguineus* s.l., relevant to veterinary and human health. Despite its global spread, recent studies on the genetic variability of *E. canis* are lacking, with most research focusing on phylogenetic analysis using the 16S-rDNA marker. Thus, the presente study aimed to perform molecular characterization of *E. canis* populations in dogs from southeastern Brazil and to explore the haplotypic relationships of 16S-rDNA sequences from samples obtained from hosts across five continents. Blood samples were collected from two dogs, one from Juiz de Fora and another from Viçosa, Minas Gerais, Brazil, both confirmed positive for *E. canis*. DNA extraction followed the QIAGEN DNeasy Blood & Tissue kit protocol. After quantification with a Nanodrop 2000 spectrophotometer, samples were standardized to 60 ng/µL using QIAGEN Tris-EDTA (TE) buffer. The 16S rDNA of *E. canis* was amplified using specific primers, and sequenced at the University of São Paulo, resulting in two new sequences. These sequences were grouped into a dataset containing 58 sequences of *E. canis* and 5 of *E. chaffeensis* (outgroup), with over 1,200 base pairs and locality records. The dataset was aligned using MAFFT v.7.0 and edited in GBlocks. A haplotype network was built via PopART's Median Joining method. Haplotype analysis revealed that sequence from Juiz de Fora, belong to the most widely distributed haplotype. The sequence from Viçosa shares a haplotype with a lineage from São Paulo. A total of 24 haplotypes were identified from various locations, with Asia being the most represented

continent, highlighting the coexistence of global and regional lineages. The analysis suggests that host shifts may be linked to speciation and genomic evolution. To better understand this genetic variation, more samples and other markers for species delimitation are needed.

Seroepidemiology and spatial distribution of Toxoplasma gondii in dogs from Ourinhos, São Paulo, Brazil

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Toxoplasma gondii is an intracellular coccidian protozoan that can infect all warm-blooded animals, including humans. The parasite has a worldwide distribution, and it is estimated that around 30% of the human population is infected, which makes toxoplasmosis one of the significant public health problems in the world. Dogs can play a crucial role in the epidemiology of toxoplasmosis by acting as mechanical vectors and sentinels for environmental contamination. Thus, the present study aimed to evaluate the seroprevalence and spatial distribution of *T. gondii* in dogs from Ourinhos, São Paulo state, Brazil. Blood samples from 602 dogs from different areas of Ourinhos municipality were collected during the rabies vaccination campaign. Information about the age, gender, breed of the dogs, and residential address of each tutor was collected to evaluate risk factors. The indirect fluorescent antibody test (IFAT) was performed to evaluate the presence of antibodies against the parasite, while the Kernel intensity estimator was used for spatial analysis. In total, 23.7% (143/602) of samples were considered positive, with titers ranging from 16 to 4,096. No significant association between gender, age, and breed were observed. The distribution map showed that seronegative and seropositive dogs were distributed across the municipality; however, the kernel map showed a high intensity of positive animals in the peripheral neighborhoods of the municipality. In conclusion, this study indicates that dogs from Ourinhos, Brazil, are exposed to *T. gondii*, and peripheral neighborhoods probably have greater environmental contamination due to the low socioeconomic levels. These results can support the development of public health to minimize the occurrence of *T. gondii* in dogs and other animals, including humans.

First Molecular Evidence of Vertical Transmission of *Ehrlichia canis* in Naturally Infected Female Dogs in Brazil

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Ehrlichia canis (Rickettsiales: Anaplasmataceae) is an obligate intracellular bacterium that infects mononuclear cells and is transmitted by the brown dog tick, *Rhipicephalus sanguineus* sensu lato: *Rhipicephalus linnaei*. Since its identification in dogs in Brazil in 1973, the bacterium has been detected in various hosts, and its occurrence in humans in Venezuela and Costa Rica highlights its zoonotic importance. In dogs, *E. canis* infection causes Canine Ehrlichiosis (EC), characterized by clinical signs that vary in intensity across the acute, subclinical, and chronic stages. The main hematological findings are thrombocytopenia and anemia, and diagnosis is made through blood smears, molecular methods, and serological tests. Vertical transmission of the bacterium has been suggested in Brazil, but not yet proven, and the mechanisms of this transmission route require further studies.This research aimed to investigate the occurrence of vertical transmission of *E. canis* in naturally infected female dogs in Belo Horizonte, Minas Gerais, Brazil. Whole blood samples in EDTA were collected from 51 female dogs before cesarean delivery, and after the birth, 22 stillborn puppies, 51 placentas, and 40 blood samples from the live-born puppies were analyzed. Molecular screening was performed by PCR targeting the *16S rRNA* gene, followed by sequencing of 23 nucleotide sequences. The analysis revealed that 27.45% of the blood samples from bitches, 5.88% of the placentas, 9.09% of the organs from stillborns and 2.5% of the blood from 3-day-old neonates were positive for *Ehrlichia* spp., and of the positive samples, 35.71%, 33.33%, 100% and 100% respectively were positive for the species *E. canis*. Thus, vertical Three of the positive samples were sent for genetic sequencing, and the results showed 100% identity with other previously characterized *E. canis* sequences from domestic dogs in Brazil, Japan, India, and Turkey, corroborating its classification within the same phylogenetic clade. The results emphasize the need for further research into the vertical transmission of hemopathogens, considering that this transmission route plays a crucial role in the maintenance and dissemination of these agents.

Molecular Evidence of Transplacental Transmission of Hemoplasmas from Blood and Placental Samples of Naturally Infected Canines in Minas Gerais, Brazil

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Hemotropic micoplasmas are uncultivable, cell wall-deficient bacteria that parasitize erythrocytes in vertebrate hosts, including dogs. Human infections have been reported in both China and Brazil, underscoring the parasite's zoonotic potential and its significance within the One Health concept. The present study aimed to evaluate the vertical transmission of hemoplasmas by detecting the DNA the pathogen in whole blood, placental remnants, and pooled organ samples from stillborn puppies of three naturally infected female dogs in Belo Horizonte, Minas Gerais, Brazil. The deliveries were performed via cesarean section in a sterile hospital environment, and the stillborn puppies, along with their placentas, were collected for

analysis. Blood samples from the three female dogs were collected via jugular vein puncture using sterile vacuum tubes containing an anticoagulant during preoperative examinations. DNA was extracted from the collected samples using the Promega® kit, followed by Polymerase Chain Reaction (PCR) to detect the *Mycoplasma* sp. genus. Molecular analysis of the 16S rRNA gene of *Mycoplasma* sp., conducted on the three whole blood samples from the female dogs, placental remnants, and pooled organ samples from the stillborn puppies, revealed 100% positivity in all tests. These findings confirm the occurrence of vertical and transplacental transmission of the pathogen, phenomenon can be explained by blood leakage at the maternal-fetal interface, followed by the phagocytosis of infected cells or extracellular agents, either free-floating or temporarily extracellular, resulting in the infection of new cells through trophoblasts located in the marginal hematophagous zone. The findings of this study highlight the critical role of this route in pathogen dissemination and the need for further studies on its epidemiological significance and transmission dynamics.

One Health and welfare

Public health risk associated with zoonotic canine parasites in Barros Blancos, Canelones, Uruguay

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Barros Blancos, located in southern Canelones (Uruguay), has experienced marked socio-territorial transformations, including the emergence of informal settlements and recent commercial and urban revitalization. These processes have shaped a fragmented, unequal setting where urban and rural areas coexist—often lacking basic sanitation and affected by free-roaming dogs. These factors led to a case of visceral larva migrans in a child, highlighting the need for this study to assess the presence of Toxocara canis and other zoonotic endoparasites. The aim of this study is to provide updated information on the prevalence of zoonotic canine parasites in Barros Blancos. Dog feces were collected from public spaces and households.For biosafety, the feces were inactivated at –80°C for at least 72 h and then thawed at 4°C for 24 h. Willis's flotation technique was carried out using a sucrose-salt solution with a specific gravity of 1.33, and samples were analyzed under a microscope. Parasitic elements were identified using the morphological keys. Data analysis was performed using Quantitative Parasitology 3.0 software, and 95% confidence intervals (CIs) were calculated using Sterne's exact method. Out of 200 samples analyzed, endoparasites were detected in 63% of the samples (95% CI: 56.01- 69.53%). The identified parasites included ancylostomids (36.5%), Trichuris vulpis (29.5%), Toxocara canis (14.0%), Eucoleus spp. (11.5%), Cystoisospora spp. (5.5%), Toxascaris leonina (1%), and Dipylidium caninum (1%). At least one zoonotic taxon was detected in 49.5%

of the samples. The results demonstrate a high prevalence of zoonotic parasites in the canine population of Barros Blancos posing a significant public health risk, particularly within this socioeconomic context. Immediate actions of public health measures are essential to safeguard community well-being and equity.

Owners' attitudes and practices in flea prevention for domestic dogs and their association with infestation in Cajamarca, Peru

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Fleas are the most common ectoparasites in dogs worldwide. Their presence is often linked to various owner-related practices and attitudes that can vary by region. This study aimed to identify dog owners' attitudes and practices regarding flea prevention and control and to analyze their association with dog infestation, highlighting factors that may contribute to the persistence of parasitism. One hundred two domestic dog owners in the Cajamarca district of Peru were surveyed, and their pets were examined for fleas. The prevalence of flea infestation was 89.22 ± 6.02%. Only 49 owners (48.05%) reported deworming their pets, using sprays (4 [8.16%]), spot-on formulations (11 [22.45%]), chewable tablets (6 [12.24%]), talc-like powders (22 [44.90%]), or medicated shampoos (6 [12.24%]). However, flea positivity (FP) was not significantly associated with these practices (p = 0.083). Additionally, 72 owners (70.59%) bathed their dogs more than three times a year (FP: p = 0.117), 42 (41.18%) washed their pet's bedding more than twice a year (FP: p = 0.238), and 63 (61.76%) noticed free-roaming fleas in their homes (FP: p = 0.002). Most owners (97 [95.10%]) had between one and four dogs, while only five (4.90%) owned up to eight dogs (FP: p = 0.425). Moreover, 83 (81.37%) of dogs went outside daily to streets or parks (FP: p = 0.008), and only 26 (25.49%) were accompanied by a family member (FP: p = 0.013). One person (0.98%) reported always sleeping with their pet (FP: p = 0.011), while only nine (8.82%) were aware that fleas transmit diseases (FP: p = 0.022). Furthermore, 76 owners (74.51%) reported experiencing flea bites (FP: p = 0.381), and the greatest number of owners had only completed basic education (35 [34.31%]) (FP: p = 0.014). In conclusion, flea infestation in Cajamarca is associated with various owner-related attitudes and practices.

Special topic – Dirofilaria

Prevalence of *Dirofilaria immitis*, *Dipylidium caninum* and *Taenia* spp. in populations of cats from shelters and research colonies, in two endemic regions of Eastern Australia

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There is a paucity of available prevalence data for key parasite species and genera infecting cats in Australia. The present study aimed to determine the prevalence of key parasites in-

fecting cats in Australia, namely the feline heartworm (*Dirofilaria immitis*), *Dipylidium caninum* and *Taenia* spp. Prevalence of these parasites was assessed in cat populations in five separate locations of eastern Australia and within two geographic regions – humid subtropical and oceanic. A total of 141 cats were enrolled in this study. Out of these cats, 91 were tested for *D. immitis* by feline heartworm Antigen/Antibody testing, 93 samples were tested for *Di. caninum* and for *Taenia* spp. by faecal floatation and a subset of 48 cat samples were tested for *Di. caninum* and *Taenia* spp. by PCR performed on peri-anal swabs. Test results were negative for the two species with a single positive result observed for *Taenia* spp. Point estimates of prevalence were therefore 0% for feline heartworm and *Di.* caninum and 1.1% for *Taenia* spp. Associated 95% confidence intervals around the point estimate were 0 - 4.1% for feline heartworm, 0 – 3.9% for *Di. caninum* and 0 – 5.8 % for *Taenia* spp. It should be noted that estimates were calculated using nominal values for test sensitivity and specificity, however the overall prevalence of all three parasite species in these geographic regions is likely to be minimal based on data from this study.

Evaluation of preventive efficacy against *Dirofilaria immitis* in dogs monthly treated with oral NexGard Spectra®

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Canine heartworm disease is a zoonosis caused by the nematode *D. immitis*. It is a parasitic disease that affects the cardiovascular system of dogs and cats. Vectored by *culicidae* mosquitoes, the infection occurs when parasite larvae enter the animal's skin and develop into adulthood, lodging in the pulmonary arteries and, in advanced cases, in the heart. The disease can lead to heart failure, respiratory distress and even death. Due to the severity of the infection, prevention is essential for the well-being of dogs. The objective of the study was to evaluate the preventive efficacy for heartworm infection (*D. immitis*) of NexGard Spectra®. The study was approved by CEUA from Universidade Federal do Rio de Janeiro. The study was performed in different households in the city of Angra dos Reis-RJ, Brazil. A total of 104 dogs were included, 49 animals allocated in Group 1 (Milbemax™ C) and 55 animals in Group 2 (NexGard Spectra®). The study included 64 female dogs and 40 male dogs, aged from 4 to 168 months and weighing between 2.1 and 48.5 kg. From day -7 to day -1 the dogs were subjected to physical examination, weighing, blood collection for microfilaria and heartworm antigen tests (SNAP® 4Dx tests). On days 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300 and 330 the dogs were examined, weighed and treated with the dose according to the respective label, and general health observation was performed after treatment. Blood collection for microfilaria and heartworm antigen tests were performed from day 60 onwards in bi-monthly intervals. Throughout the 360 days of evaluation, no animal of the two groups did show positive results, neither for circulating *Dirofilaria* antigens nor for microfilariae. Based on the study results Nex-

Gard Spectra® was effective in preventing the establishment of *D. immitis* infections.

Prevalence and risk factors of *Dirofilaria immitis* in dogs in a previously non-endemic area on the southern coast of Bahia, Brazil

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Canine heartworm disease caused by *Dirofilaria immitis* is one the most clinically relevant parasitic conditions in dogs. Nevertheless, epidemiological data is scarce from many regions worldwide. We conducted an epidemiological survey of *D. immitis* in dogs from Ilhéus, a previously non-endemic area in Bahia, Brazil, from January to December 2024. Dogs were randomly selected from across various regions (coastal, inland, rural, and urban areas), using QGIS software to define the sampling points. A total of 482 dogs, domiciled or semi-domiciled, over one year of age and living in the area for at least one year, had blood sampled. A questionnaire was administered to the owners to collect patient information, epidemiological data, and risk factors related to transmission. Diagnosis was performed using the modified Knott's test and the SNAP® 4Dx® antigen detection test (IDEXX Laboratories, Inc.). To assess possible associations with positive cases, bivariate and multivariate regression analyses were performed (statistical significance - $p < 0.05$). A prevalence of 16.2% was identified (78/482; 95% CI: 12.9%–19.5%). Risk factor analysis revealed that dogs allowed to roam free 100% of the time had a 3.72-fold increased risk of infection (33/107; prevalence = 30.8%; $p = 0.001$), while those spending half of their time outdoors also showed an increased risk (12/59; prevalence = 20.3%; OR = 2.45; $p = 0.043$). The presence of mosquito breeding sites within the household was also significantly associated with infection (OR = 2.85; $p = 0.001$), compared to those without standing water sources (prevalence = 8.7%; 20/229). Other variables such as sex, age, coat type, use of repellent collars, length of residence in the municipality, and area of residence were not significantly associated with infection. This study identifies Ilhéus as an endemic area with a substantial level of *D. immitis* infection, reinforcing the need for ongoing epidemiological surveillance and preventive strategies.

Prevalence and risk factors associated with *Dirofilaria immitis* infection in dogs from Mexico including serum pre-heat treatment for the dissociation of immunocomplexes.

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Dirofilaria immitis is a nematode found worldwide and is capable of infecting several animal species, including dogs. In Mexico, *D. immitis* in dogs has been detected in 22 states with a wide range of prevalence. The objective of this research was to determine the prevalence of *D. immitis* in dogs from four agroecological zones and 11 localities in Mexico, the risk factors associated, and the impact of heat treatment for immune-complex dissociation. Blood samples of 535 dogs from 4 ecological zones and 11 localities in Mexico were processed with ELISA (IDEXX SNAP 4Dx® Plus) with non-treated--serum samples (NTS) and pre-heat-treated sera for immune-complexes dissociation (pre-ICD). Thick blood smear tests were also performed. The prevalence of *D. immitis* in each locality was estimated. The results of *D. immitis* with pre-ICD were analyzed using $\chi 2$ to identify the associated factors and the variables with $p<0.2$ were analyzed using a logistic regression. Positive cases of *D. immitis* were different ($p<0.05$) when the ELISA test was used with NTS or pre-ICD sera. Thick smear tests were also significantly less sensitive. Observed infection rates were 15.5%, 22.6%, and 7.2%, respectively. Eighty-three dogs were positive by ELISA using both NTS and pre-ICD, and 38 dogs (7.1%) were positive only with pre-heat-treated sera. The higher prevalence was found in Ciudad del Carmen (48.3%), Tampico (48.3%), and Puerto Morelos (64%). The risk factors associated with *D. immitis* infection were the ecological zone (warm subhumid with summer rains: AW_1, OR 2.8, $p=0.003$ and AW_0, OR 20.4, $p=0.003$) and age of the animals (dogs >6 years, OR 2.8, $p<0.001$). In Mexico, dogs from four ecological zones are highly infected by *D. immitis*, the associated risk factors were the ecological zone and age of the animals. Pre-ICD allowed to detect 7.1% of false negative ELISA antigen tests based on non-treated.

Demystifying treatment of *Dirofilaria immitis* infection with doxycycline alone

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Canine heartworm (HTW) infections are frequent in Brazil and is spreading its territory. Since many asymptomatic infections are detected by the presence of microfilariae (mff) on routine CBC or by the movement beneath the buffy coat in a microhematocrit, unfamiliar with *D. immitis* veterinarians struggle with misinformation provided on internet. Melarsomine is unavailable in the Brazilian market and many veterinarians believe macrocyclic lactones can cause adverse reactions when administered to HTW dogs, leading them to the treatment with doxycycline (Doxy) as the sole treatment. Although it has been published that Doxy alone reduces mff but it does not eliminate the infection, based on empirical media reports, many Brazilian veterinarians choose Doxy alone. The intimacy veterinarians developed with Doxy due to its extensive use for treatment of ehrlichiosis and other infections may explain that choice. Aiming to understand and to show if Doxy alone eliminates HTW infection, 9 HTW antigen (Snap 4DX) positive dogs presenting over 300 mff/mL on Knott's test were included. All dogs received Doxy for 21 days (10mg/kg BID). On day 0 microfilaremia ranged from 2,163 to 30,425 ($\bar{x}$=14,448±11,381mff/

mL) on day+21 (+3days) it ranged from 5,392 to 29,913 ($\bar{x}$=14,152±9,040mff/mL) and on day+90 (+5days) after the last Doxy dose, 6 dogs were still microfilaremic, ranging from 13 to 138 mff/mL ($\bar{x}$=58±46mff/mL). The fact that microfilaremia drops below the threshold for casual detection on CBC, microhematocrit or fresh blood smear after Doxy administration may mislead veterinarians to the conclusion that the dog cleared all the HTWs. Therefore, even in Brazil, where melarsomine is not registered, veterinarians must be aware that Doxy is part of the best treatment a dog may receive, but it must be associated with other drugs such as macrocyclic lactones.

Detection and seroprevalence of *Dirofilaria* spp. in internationally transported dogs from endemic regions

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Dirofilaria spp. are parasitic filarial nematodes endemic globally that infect dogs and can cause human dirofilariasis. Traveling dogs can act as carriers, facilitating the spread of *Dirofilaria* spp. and other zoonotic parasites to new regions; however, the risk associated with importation from endemic areas is unclear. Our study aimed to assess whether dogs traveling from Central and South America are infected with *Dirofilaria* spp. Canine serum samples (n = 6,556) were obtained from 34 countries between September 2022 and December 2023 from the Kansas State Veterinary Diagnostic Laboratory. Samples were grouped by country and pooled, with pool sizes determined using EPITOOLS based on reported prevalence. We validated the DiroCHEK® ELISA (Zoetis) for pooled testing up to 100 samples using positive control serum (TRS Labs). Pooled samples were tested for *D. immitis* and cross-reactive nematodes, both with and without heat treatment to dissociate immune complexes. ELISA reactions were quantified via spectrophotometry. We determined the ELISA-cutoff for different spectrophotometric wavelengths (490, 620, or 650 nm) with receiver operating characteristic (ROC) curves. At 650 nm, the area under the curve (AUC) was 1.00. The optimal cutoff was 0.155, providing 100% sensitivity and specificity. Of 649 total pools, 50 heat-treated and 11 unheated pools tested positive. Individual samples from positive pools were retested. A subset (n = 23) of pools that tested positive had no positive individuals upon follow-up testing. Country-specific prevalence estimates ranged from 0 - 18%. These findings provide evidence that traveling dogs are carrying *D. immitis* and cross-reactive nematodes across the globe, increasing the risk of introduction to new areas or new parasite genetics to endemic areas. Our results highlight the need for re-evaluating current U.S. and global canine importation laws to mitigate the spread of *Dirofilaria* spp. and other zoonotic nematodes.

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