



FINAL COMBAR CONFERENCE
Combating anthelmintic resistance
in ruminants: options for the future

ATHENS 2022

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Combating anthelmintic resistance in ruminants

COMBAR (CA16230) (www.combar-ca.eu) is a COST Action (23 June 2017-18 March 2022) that aimed to advance research on the prevention of anthelmintic resistance in helminth parasites of ruminants in Europe and disseminate current knowledge among all relevant stakeholders.

COMBAR focuses on integrated approaches to reduce the reliance on anthelmintics as the only option for parasite control. Thus, researchers in the fields of diagnostics, targeted (selective) treatment (TT/TST) approaches, vaccines, anti-parasitic forages and decision support were brought together to develop more holistic and complementary control approaches. A particular focus was to start using economic and sociologic methodologies, novel to the field of parasitology to understand economic aspects and barriers to uptake of novel approaches by farmers. Over **200 scientists from 39 countries** participated in the activities.

Here, we present the program of our **Final Conference** where we aim to announce our so far achievements. We hope COMBAR will stay alive after March 2022 and evolve to a bigger research initiative pursuing the achievement of sustainable helminth control, promoting good practices and the optimal use of the control options that are available today.



Making
parasite control
simple and
sustainable



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Keynote presentations

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Juan Felipe de Jesus Torres Acosta

Preparing future veterinarians to implement integrated gastrointestinal nematode control in small ruminant farms

Profile

Veterinarian from the University of Yucatán, México.

He is a Professor in the same University where he is teaching veterinary medicine since 1989.

Has worked with sheep and goats farmers under tropical conditions to investigate the anthelmintic resistance situation in different regions of México, with the collaboration of many research groups.

Has contributed to define the conditions required to implement different gastrointestinal nematode control methods in small ruminant farms.

Is currently investigating the sustainable integration of different GIN control methods.

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The present work identifies those elements required for the training of future Veterinarians that wish to implement protocols of integrated gastrointestinal nematode control in ruminant herds. It is proposed to carry out an international training course for Veterinarians with 5 components.

COMPONENT 1: Understanding on the epidemiology and pathogenesis of all relevant GIN in each region, with clear emphasis on the effects of environmental conditions of different ecosystems throughout the year, seasonality of parasitism, and the characteristics of parasitized ruminants in terms of productivity or their resilience/resistance against GIN.

COMPONENT 2: Specialized techniques for the clinical diagnosis of animals infected with GIN, including ante-mortem and post-mortem helminthological techniques (including molecular) to identify and quantify stages of eggs, larvae (1, 2, 3, 4 and 5) and adults of different species. These methodologies can be used to identify resilient or resistant animals.

COMPONENT 3: Sustainable use of conventional anthelmintics (AH), including selective (ST) and targeted selective (TST) schemes appropriate for the economic and technological conditions of farmers.

COMPONENT 4: Methodologies for the diagnosis/identification of AH susceptible/resistant GIN populations (field and in vitro tests, and molecular).

Finally, **COMPONENT 5:** Strategies/techniques to implement one or more GIN alternative control methodologies. The complexity of this program will require the collaboration of international experts providing their inputs in a virtual platform accessible to Veterinary schools worldwide. A national or international certification could be feasible. Farmers' feedback on the appropriateness of contents and proposed protocols is mandatory.



Iliopoulos Constantine & Irimi Theodorakopoulou

The drivers of Change in Animal Farming Management Decisions: A systems - based approach

Profile

Constantine Iliopoulos, PhD

Dr Iliopoulos serves as Director of, and Senior Researcher at, AGRERI, Greece, and a Visiting Research Fellow at IAMO in Germany. His research program focuses on the transformation of systems and awareness-based collective action, with an emphasis in food and natural resource systems. He has over 27+ years of professional experience in 30+ countries.

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Dr. Irimi Theodorakopoulou

Dr. Theodorakopoulou is an expert on rural innovation management and supply chain economics and organization and a facilitator for participatory processes in organizations and communities. Social labs, platforms, dialogue and in-depth interviews, story-telling, solution focus workshops, are some of the methods she uses to bring together people from across a system and help them work on solutions to their problems.

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Animal farms are parts of wider Supply Chain Systems (SCS). We view SCSs as living systems characterized by complexity and constant emergence of new constellations, interactions, processes, and outcomes designed to address societal needs.

Actor engagement is a crucial process in order to ensure that any animal disease control strategy is tailored to the needs of the very people whose needs it is supposed to serve. It also guarantees that a strategy is adopted by a crucial mass of actors and thus has a high potential to result in systemic social change. The practices actors employ redefine the relationship among them, their perspectives, and their network routines. Examples of such practices include creating common cognitive frames, rules, norms, and physical infrastructures.

All the above will build a "container" for the group that is going through a change process together. This means that all stakeholders should leave their own institutional bubbles and realize the degree of connectedness and interdependency of the system. Theory U provides the theoretical framework and "technology" to guide stakeholders at all levels and in all systems through the process of letting go of old thinking and behavioral patterns and to co-design with research teams, groups and organizations future possibilities for animal farming systems.

An important element in theory U is the role, function, and capacities of scientists as social leaders. Successful leadership depends not only on the leaders' knowledge and action but also on several leadership capacities and the degree of attention and the quality of intention the leader brings in any given situation.



Dimitrios G. Karpouzas

The presence of veterinary drugs in agricultural soils and their interactions with the soil microbiota: toxicity, microbial degradation and bioaugmentation approaches to avert environmental pollution.

Profile

Dimitrios G. Karpouzas is a Professor of Environmental Microbiology and Biotechnology, in the Department of Biochemistry and Biotechnology, University of Thessaly and currently serves as a Head of Department.

His main research focus is on the interactions of agrochemicals (pesticides, nitrification inhibitors) and veterinary drugs with soil microorganisms.

His group tries to shed light into the mechanisms involved in either the microbial degradation of these compounds or their potential toxicity on the soil microbiota.

He has authored more than 120 articles in scientific journal and coordinated several EU-funded projects (FP7-IAPP-MCSA; MCSA-IF-H2020, MSCA-ITN-EID-H2020 etc).

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Veterinary drugs constitute a major mean to control microbial and parasite infestations of productive animals. Upon their administration in animals, veterinary antibiotics and anthelmintics are only partially metabolized in animal tissues with 40-90% of the administered dose found in animal excreta.

These are either stockpiled in ambient conditions or treated via anaerobic digestion or composting before applied as manures in agricultural soils. Despite the application of these intermediate treatments, manures contain residual amounts of veterinary antibiotics and anthelmintics ($\mu\text{g}/\text{kg}$ up to mg/kg level) which end up in agricultural soils. From there they could be either degraded or transferred to other environmental compartments or taken up by plants where they could enter the food chain challenging human health. In addition to the above exposure routes, anthelmintics could end up in grassland soils through direct deposition of the excreta of grazing animals, although this route leads to lower exposure levels.

Soil microorganisms seem to interact with antibiotics and anthelmintics, once in soil, with the outcome of this interaction being either detrimental, appearing as inhibition of key soil microbial groups and associated ecosystem services, or beneficial, taking the form of a growth-linked microbial degradation. Our aim was to disentangle the complex interactions between veterinary drugs and soil microorganisms.

We will (i) present data on the degradation of anthelmintic compounds by the soil microbiota (ii) show examples of microorganisms able to degrade veterinary drugs like benzimidazole anthelmintic compounds and (iv) present applications of these microorganisms in the bioremediation of feces and soils.



Erwin Wauters & Helena C. de Carvalho Ferreira

DISARM - a Thematic Network on Disseminating Innovative Solutions for Antibiotic Resistance Management: achievements and reflections

Profile

Erwin Wauters is senior researcher at the Social Sciences Unit of the Institute for Agricultural and Fisheries Research (ILVO), Belgium. He has a Bachelor in Bioscience Engineering, a Master (2002) and PhD (2010) in Agricultural Economics, all from the Katholieke Universiteit Leuven.

His research focuses on understanding the economic, institutional, and social drivers that shape our farming and food systems, and how to create a more enabling environment that fosters more sustainable farming and food systems.

Currently he coordinates a Horizon 2020 Thematic Network on Disseminating Innovative Solutions for Antimicrobial Resistance Management (DISARM) and is in the core group of 2 Horizon 2020 projects on AMU and AMR.

He is board member of the Flemish Society for Veterinary Epidemiology and Economics (VEE) and board member of the International Society of Economic and Social Sciences on Animal Health (ISESSAH).

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To contribute to fighting antimicrobial resistance (AMR), a more responsible use - including a substantial reduction - of antimicrobial use (AMU) in livestock is needed. The EU's Farm to Fork strategy has made this very explicit by aspiring a 50% reduction in the aggregate use in the EU by 2030.

As part of its support for initiatives that can further reduce AMU and curb AMR, the EC funded, since 2019, a Thematic Network in its Horizon 2020 research & innovation funding program.

Thematic networks (TNs) are multi-actor projects which collect existing knowledge and best practices on a given theme to make it available in easily understandable formats for end users such as farmers, foresters, advisers and others. TNs follows the ideas of 'interactive innovation', the idea that innovations are not developed and spread top-down only, but also bottom-up in collaborative modes of innovation.

Close before its finalization, this paper presents the key assumptions and ideas that form the backbone of the project's architecture and activities and reflects on its achievements and challenges. It provides lessons learned for future TNs, for instance regarding anthelmintic resistance.

DISARM had 3 main core parts in its design: creating and facilitating national and international networking possibilities; collecting a database which reflects the current state of the art regarding existing best practices to reduce the need for AMU; demonstrating an approach to efficiently deliver existing knowledge to the individual farm(er). As the networking project was under some degree of COVID-19 restrictions for more than 2/3 of its time, substantial challenges appeared, but also digital alternatives for networking and dissemination were explored.

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Oral presentations

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Occurrence of benzimidazole resistant *Haemonchus contortus* in Norwegian sheep, detected by droplet digital PCR

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The aim of this study was to investigate the occurrence of BZ-resistant *Haemonchus contortus* in Norwegian sheep flocks. Screening was performed based on detection of one of the resistance-conferring mutations in the β tubulin isotype 1 gene. Fecal samples were collected in 2021 from flocks in the northern (n=3), coastal (n=5), and inland (n=22) areas of Norway. Flocks were recruited from the Norwegian sheep recording system, using data previously obtained by a questionnaire. In total, 25 flocks were randomly selected from the dataset. Additionally, five flocks were enrolled due to suspicion of AR. Individual faecal samples were collected from 10 randomly selected ewes (spring) and 10 lambs (autumn). From each flock samples, 4X5 (lambs and ewes pooled separately) faeces, were pooled and cultured for L3 development. After harvest of larvae with the Baermann method, DNA was extracted and then analyzed using droplet digital PCR. The assay was performed using primer/probe sets targeting the F200Y (TAC) mutation. The mutation was found in 20 of the total 30 flocks (67%). In positive flocks, median allele frequencies were 100% in both age groups, with a range of 23-100 and 82-100% in lambs and ewes, respectively. The mutation was only found in flocks from the coastal (n=5) or inland (n=15) areas (including the AR-suspected flocks). However, so far, few flocks have been investigated from northern Norway. Samples collection from additional flocks in this region will therefore continue in 2022.



Genome-wide analysis of the response to ivermectin treatment by a Swedish field population of *Haemonchus contortus*

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Haemonchus contortus is a pathogenic nematode of small ruminants which contributes to significant losses in the animal production sector. Despite decades of research, little is known about the mechanism(s) driving ivermectin resistance let alone about the putative targets for ivermectin resistance diagnosis in this species. Here we describe a genome-wide approach to detect evidence of selection by ivermectin treatment in a field population of *H. contortus* from Sweden, using parasites sampled from the same animals before and seven days after ivermectin exposure followed by whole-genome sequencing. Despite an 89% reduction in parasites recovered after treatment measured by the fecal egg count reduction test, the surviving population was highly genetically similar to the population before treatment, suggesting that resistance has likely evolved over time and that resistance alleles are present on diverse haplotypes. Pairwise gene and SNP frequency comparisons indicated the highest degree of differentiation was found at the end of chromosome 4, whereas most striking difference in nucleotide diversity was observed in a region on chromosome 5 previously reported to harbor a major quantitative trait locus involved in ivermectin resistance. These data provide novel insight into the genome-wide effect of ivermectin selection in a field population and confirm the importance of the previously established locus in the development of resistance to ivermectin. Thus, genome-wide association studies are likely to yield novel, high quality data leading towards the understanding of ivermectin resistance and specific changes in the genomes of parasites which can be used to create diagnostic assays to detect resistance.



High endoparasitic burden in cattle grazed on alpine pastures in Austria

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Climate change and the development of anthelmintic resistance are factors demanding for a constant monitoring of parasite infections in grazed cattle. In our study, we examined the dynamic of parasite infections in Tyrolian dairy farms with transhumance over one grazing season. Samples from 326 cattle (first grazing season cattle and dairy cows) from ten farms were examined at three timepoints: before driving the animals up to alpine pastures in spring, during the alpine grazing season in summer and after their return to the lowlands in autumn. Faecal samples were collected and examined by Mini-FLOTAC, sedimentation and baermanisation. Trichostrongyloid-positive samples were further processed for larval differentiation. Milk samples were examined by *Ostertagia ostertagi* ELISA and *Fasciola hepatica* ELISA. In summer, young stock showed trichostrongyloid egg excretion with a mean of 114 EpG and a max of 1500 EpG. Although *Cooperia* was the predominant trichostrongyloid overall, in young cattle the proportion of *Ostertagia* was up to 60%. The *Ostertagia*-ELISA of bulk milk samples was highest in autumn with a mean ODR of 0.8, ranging from 0.7-1.1. On six farms, *F. hepatica* could be detected by sedimentation and ELISA (mean ODR 0.6), with high burdens on two of these farms (ODR 0.8 each), and reported treatment failure. *Dictyocaulus* was found on two farms. These results show a considerable endoparasite burden, indicating the need for evaluation and optimization of applied control strategies and the monitoring of flukicidal treatment to gain insight into weak points of parasite management in this special way of livestock production.

This study was partly sponsored by Boehringer Ingelheim RCV GmbH & Co KG, Vienna, Austria.



Improving burden estimation of gastro-intestinal strongyles to target whole-group treatments and to preserve anthelmintic efficacy in small ruminants in Italy

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A precise diagnosis is crucial for more sustainable target treatments (TT) and to prevent the spread of anthelmintic resistance. A mathematical formula was recently proposed to calculate the sample size needed for a sufficiently precise determination of parasite burden in a group of animals, in the framework of a farm-tailored monitoring approach (Maurizio et al., 2021, Vet. Sci. 8, 69). This approach was implemented in Italy in 13 goat and 19 sheep farms. In each farm, the number of treatments/year was recorded and individual faecal samples were collected (3 months post-treatment minimum) and analyzed by McMaster technique. The mean FEC and its variability (95%CI) were compared with treatment practices. Assuming 500EPG as treatment threshold, farms were divided in three categories: catA) mean and upper CI limit < 500EPG; catB) CI including 500EPG; catC) mean and lower CI limit > 500EPG. Out of 17 catA farms, treatments were regularly performed (1 or 2 times/year) in 13 farms (4/7 for goat and 9/10 for sheep), highlighting that a likely unnecessary use of drugs is widespread. Regular treatments were reported also in all catC (3/3) and in most catB (11/12) farms. CatB farms would especially benefit from a careful interpretation of FEC mean and CI, when considering TT. A relatively wide CI (high parasitic aggregation) could also suggest when treating only the few animals with high burden is preferable (2 catB farms in our study). Genera identification represents a further evolution of this approach.



Essential oil as natural anthelmintic for combating sheep gastrointestinal nematodes

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Treatment of parasitic gastroenteritis usually based on regular usage of broad-spectrum anthelmintics that has been compromised due to development of multi-drug resistance parasite populations. The anthelmintic activities of bioactive phyto-compounds, such as essential oils and their main components, can provide an alternative natural, safe and more sustainable approach. The anthelmintic effects of five pure essential oil constituents (geraniol, geranyl acetate, eugenol, methyl iso-eugenol, and linalool) were tested, using larval motility assay, on the third-stage larvae (L3s) of *Haemonchus contortus*. The geraniol, eugenol and linalool showed a strong inhibitory efficacy against larval motility of *H. contortus* comparable to positive control (20 mg/mL levamisole) within 24 hr ($p < 0.05$). The three oils also induced a considerable structural damage to L3s. Results of treatments were validated by quantitative fluorometric microplate-based assays using Sytox green, propidium iodide and C12-resazurin, which successfully discriminated live/dead larvae. The cytotoxicity of components on Madin-Darby Canine Kidney cells was evaluated using sulforho-damine-B (SRB) assay and no significant cytotoxic effect at concentrations $< 1\%$. These results indicate that testing essential oils components may help to find new potential anthelmintic compounds, while at the same time reducing the reliance on synthetic anthelmintics. More comprehensive studies are required to assess the bioavailability of the tested oils for post ruminal delivery by encapsulation in natural biodegradable polymeric nanocarrier to enhance the thermal stability of the EOs, protect their phenolic contents, reach sustained release profile, improve their anthelmintic activities.



Detection, confirmation and characterisation of fenbendazole resistance in *Ostertagia ostertagia* in clinically affected grazing dairy calves in Great Britain

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Following the first reports of anthelmintic resistance (AR) in small ruminants, a surveillance system for detection of new and emerging AR cases was created in Great Britain. This system is based on initial detection of suspected lack of anthelmintic efficacy by Veterinary Investigation Centres through postmortem examinations, worm counts, faecal egg count reduction tests and/or on-farm investigation. Thanks to this multidisciplinary approach, suspected reduced fenbendazole efficacy was identified in *Ostertagia ostertagia* in a Welsh dairy herd. Diarrhoea, malaise and weight loss were noted in post-weaned calves. Abomasal nodules, *O. ostertagia* worms and eggs were detected at postmortem examination, despite prior fenbendazole ruminal bolus administration. The same pasture and anthelmintic had been used for first season grazing calves for several years. The sensitivity of the *O. ostertagia* isolates to benzimidazoles was investigated by controlled efficacy test, conducted in conjunction with sequencing of the β -tubulin isotype-1 gene of larvae pre- and post-fenbendazole administration. Fenbendazole resistance in *O. ostertagia* was confirmed with total treatment failure (0% efficacy) in reducing worm burden. Sequence analysis of the β -tubulin isotype-1 gene from infective larvae from control and treated groups was performed. The three commonest single nucleotide polymorphisms (SNPs) associated with benzimidazole resistance were examined. The predominant AR-associated SNPs were F200Y (78% control and 79% treated groups) and F167Y (remaining genotypes; no 198 SNPs were identified) emphasising the importance of these SNPs in clinical disease in this isolate. This investigation highlights the importance of a clinical, pathological and parasitological approach for the surveillance of AR in livestock.



Increasing importance of anthelmintic resistance in European livestock: creation and meta-analysis of an open database

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Anthelmintic Resistance (AR) in parasites is now widespread throughout Europe, although there are still gaps in our knowledge in some regions and countries. A better understanding of the extent of AR in Europe is needed to develop and advocate more sustainable parasite control approaches. A database of European published and unpublished AR research on gastrointestinal nematodes (GIN) and liver fluke (*Fasciola hepatica*) was collated by members of the European COST Action "COMBAR" (Combatting Anthelmintic Resistance in Ruminants). 197 publications on AR in GIN were available for analysis, representing 535 studies in 22 countries and spanning the period 1980-2020. Estimated regional (country) prevalence was highly heterogeneous, ranging between 0% and 100% depending on livestock sector and anthelmintic class, and generally increased with increasing research effort in a country. In the few countries with adequate longitudinal data, there was a tendency towards increasing AR over time for all anthelmintic classes in GIN. Suspected AR in *F. hepatica* was reported in 21 studies spanning 6 countries. For GIN and particularly *F. hepatica*, there was a bias towards preferential sampling of individual farms with suspected AR, and research effort was biased towards Western Europe and particularly the United Kingdom. Ongoing capture of future results in the live database, efforts to avoid bias in farm recruitment, more accurate tests for AR, and stronger appreciation of the importance of AR among the agricultural industry and policy makers, will support more sophisticated analyses of factors contributing to AR and effective strategies to slow its spread.



Sustainable gastrointestinal parasite control in dairy cattle: educational tools for veterinarians and farmers

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The objective of this communication is to present educational tools that have been co-developed by experts and Ceva on sustainable gastrointestinal parasite control. Indeed, the control of helminth infections in ruminants is still primarily based on the routine use of anthelmintics but this approach is more and more unsustainable as resistance to anthelmintics is widespread and increasing. The current therapeutic arsenal must be preserved as much as possible. Selective anthelmintic treatment is considered a key element of this new paradigm to reduce the development of resistance but to be embraced by the farmer community and veterinarians, evidence-based criteria along with appropriate educational tools should be available. Ceva has recently collaborated with J. Vercruyse (Ghent University) and Nadine Ravinet (INRAE, Oniris, Nantes) to explain the urgency of this approach and to clarify the possible ways to operate the change in dairy cattle. These discussions led to the creation of two distinct documents, that cover several aspects of the sustainable control of GIN parasites in dairy cattle: the prevalence of anthelmintic resistance in Europe, its assessment in the field, the principles of refugia, the challenges and necessity associated to this approach, the impact of gastrointestinal nematode infections on milk yield and the criteria for selective treatment. A final third document was created in a comic strip format with the idea of an open conversation on parasites between a veterinarian and a farmer. These documents are freely available in printed or digital format upon request.



Eprinomectin-resistant *Haemonchus contortus* strains in French dairy sheep farms: can we balance pastoral traditions and control of gastro-intestinal nematodes?

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In French ovine dairy farms, eprinomectin is increasingly used during lactation as it is the only anthelmintic with a zero-day milk withdrawal period, and consequently to the increase in milk withdrawal periods for benzimidazole products that occurred in 2014. In France, eprinomectin is commercially available in sheep as a pour-on dosed at 1 mg/kg or as an injectable solution dosed at 0.2 mg/kg. This overreliance on one molecule in the 2 main sheep cheese regions of France - namely the Roquefort area and the Pyrénées Atlantiques département - where sheep grazing is culturally deeply imbedded and mandatory to comply with European specifications, brings deep concern about the rise of resistant isolates. Fecal Egg Count Reduction Tests conducted in dairy sheep farms of these areas detected eprinomectin-resistant isolates of *Haemonchus contortus* in 21/27 farms explored in this study (Pyrénées Atlantiques) and 1/6 farms (Roquefort area). Eprinomectin concentration was individually measured in treated ewes 2 and 5 days after treatment in 9 farms, hence confirming implication of resistant isolates in the FECRT results in 8 farms, and demonstrating on one farm that the observed efficacy default was due to underexposure of the worms to the drug when the pour-on formula was used. These results plead once more for implementation of Targeted Selective Treatment (TST) using an adapted form of eprinomectin for the control of sheep nematodes, the feasibility of which is currently evaluated by the multi-partner ANTHERIN program led by the Veterinary School of Toulouse (ENVT) as well as the French Agronomical Institution (INRAE).



Delineating the species-specific impact of four anthelmintic classes on gastrointestinal nematodes on 52 sheep farms in the U.K.

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Sheep host a diversity of gastrointestinal nematode (GIN) parasites with seasonally shifting co-infection patterns. While multiple GINs can be present, species differ drastically in their impacts on sheep health. As such, determining the presence of different species is critical to informing control strategies. Species level diagnostics also improve anthelmintic drug efficacy testing, pinpointing resistant species. While the current diagnostic of choice, faecal egg counting (FEC), cannot determine individual species, FECs are used to determine anthelmintic drug efficacy by quantifying eggs pre- and post-treatment. With no species identification, it is impossible to determine what species an anthelmintic is clearing, and what species are resistant. To overcome this issue, individual sheep farms were split into four treatment groups: benzimidazole, levamisole, ivermectin, and moxidectin. Pooled pre-treatment and 15 individual post-treatment FECs were compared to a rapid and simple larval multi-plex PCR screen targeting 8 GIN species. FECs revealed failure in all four drug groups against strongyle GINs on at least 1 farm. Benzimidazole treatment largely failed to reach 90% egg clearance, with the majority of species surviving treatment (average reduction = 1.65 species). Other anthelmintics were more effective at clearing eggs but none removed all species; with *Teladorsagia circumcincta*, *Trichostrongylus colubriformis*, and *Trichostrongylus vitrinus* surviving in all drug groups. Worryingly, *Haemonchus contortus* was identified in 27% of pre-treatment farm pooled faecal samples and persisted on some farms post bezimidazole, lavemisole, and ivermectin treatment. This method highlights the need for species-specific identification of GINs as well as the efficacy of anthelmintics.



Geographical and temporal diversity of parasite prevalence revealed by computer modelling

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Gastrointestinal nematodes (GIN) are amongst the most important pathogens of grazing ruminants worldwide, resulting in negative impacts on cattle health and production. The dynamics of infection are driven in large part by impacts of climate and weather on free-living parasite stages on pasture, and computer models have been developed to predict infective larval abundance and guide management strategies. New version of a modern population dynamic model, GLOWORM-FL, was recently developed and validated with nemabiome data permitting species-specific estimates for *Ostertagia ostertagi* and *Cooperia oncophora* in mixed natural populations. This model has been applied to explore differences in seasonal and temporal transmission patterns with climatic data obtained from weather stations of different regions. When combining five years data (2015-2019) from a variety of regions in Canada and Europe, model simulation shows a consistent seasonal pattern in L3 development potential on temperate pastures, which across regions tends to produce the highest L3 abundance during a 6-week pasture grazing period starting in July. However, a considerably distinct pasture L3 dynamic was revealed with model simulation using climatic data of 2020 and 2021 from the same region (Belgium), both in magnitude and seasonality. These results are important to develop evidence-based strategies for parasite control under increasing pressure from climate change and drug resistance.



Sheep deworming strategies and AR in Sweden - insights from a national survey and wildlife nemabiome analysis

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A questionnaire was sent to Swedish sheep farmers to investigate their use of anthelmintic drugs and problems associated with gastrointestinal nematode infections (GIN), especially *Haemonchus contortus* (Hc). 45% of the respondents reported anthelmintic use. Of these, most only drenched ewes once per year, using ivermectin or albendazole. In most cases, treatment was done after fecal examination, but routine drenching was common, especially among conventional farms. Problems with Hc primarily perceived by farmers doing routine drenching, sometimes with uncalibrated dosing guns, and veterinarian are an important source of advice for GIN infections. Most of the farms grazed their animals on a combination of natural pastures and arable land, changing paddocks to reduce parasite pressure, thus reduce the need for anthelmintic drugs. On pastures, roe deer are frequently observed throughout the country, and fallow deer and red deer in the southern part. A common question commented in the survey was whether these deer can transmit parasites to the sheep, especially Hc. It is known that Roe deer can spread Hc to domestic hosts. Therefore, we set out to investigate this under Swedish condition. We gathered samples from 250 deer and a handful moufflon and analyzed their nemabiome using ITS2 barcoding. We found Hc sequences in <10 samples, but other species found in sheep were common, such as *Chabertia ovina*, *Teladorsagia circumcincta*, *Trichostrongylus vitrinus* and *Oesophagostomum venulosum*. In conclusion, we have identified factors contributing to AR, such as use of uncalibrated dosing guns, while nemabiome analysis show low Hc prevalence.



Validation of the image-based FECPAKG² system for counting sheep nematode eggs with a sensitivity of 5 epg.

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Helminth control in ruminants heavily relies on anthelmintics. Consequently, anthelmintic resistance in parasites is widespread throughout Europe threatening the future of the pasture-based livestock industry. Faecal egg count reduction tests (FECRT) are the recommended field-based methodology used to estimate anthelmintic efficacy. A pre- and post-treatment faecal egg count is performed, and the calculated percentage reduction gives an indication of treatment success. Updated WAAVP and COMBAR FECRT guidelines recommend using 'raw eggs counted' instead of epg, with a minimum of 200 eggs counted in the pre-treatment FECs to ensure a reliable indication of treatment efficacy. As such, FEC methods with lower multiplication factors are preferable to ensure the target of >200 eggs is met. FECPAKG² is a remote location, image-based system used to enumerate helminth eggs in faeces. The automated image capture function of the FECPAKG² enables efficient record keeping, and ensures the transparency and assurance of results. Additionally, the digitised FECPAKG² system removes the requirement for the end user (e.g., farmer) to have specialist knowledge of helminth egg identification. The objective of this experiment was to modify the standard FECPAKG² protocol to reduce the detection sensitivity from 35 epg to 5 epg. The precision of the new FECPAKG² 5 epg method was evaluated against another standard FEC technique, using sheep faecal samples collected from UK farms. The new FECPAKG² 5 epg method has a lower egg detection sensitivity making it more suitable for the new FECRT guidelines and for processing post-treatment samples that are expected to have lower numbers of eggs.



A novel benzimidazole derivative shows in vivo anthelmintic activity against *Haemonchus contortus* in gerbils and sheep

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The objective of the current study was to assess the toxicity and the in vivo anthelmintic potential of three novel synthetic compounds identified in a previous study, one benzimidazole (BZD) and two diamine derivatives (DD1 and Dd2). Acute toxicity assays were carried out on mice while in vivo efficacy of compounds was assessed on both gerbils and sheep experimentally infected with *Haemonchus contortus*. For the acute toxicity in mice, all compounds were administered at a dose of 250 mg/kg body weight; no animal died and no macroscopic or microscopic lesions were observed. Gerbils infected with 1,000 *H. contortus* L3 were orally treated with 200 mg/kg or 10 mg/kg of each compound at day 10 post-infection and slaughtered 3 days later. DD1 was the most effective compound in terms of L4 reduction (16.5%). However, regarding pre-adult reduction, BZD showed efficacies of 95.63% and 30.64% at 200 and 10 mg/kg, respectively. Then BZD was tested at 120 mg/kg in infected sheep with 250 L3 per kg body weight at day 28 post-infection. The BZD was able to reduce 99.13% of the number of eggs in faeces at day 7 post treatment and 95.45% of worms after the slaughter of all animal. Sheep did not show any clinical signs of toxicity after BZD administration during the whole study. No macroscopic or microscopic lesions were found either. In the next studies, BZD will be tested at lower doses and against albendazole-resistant strains.



STAR-IDAZ International Research Consortium: Global coordination of animal disease research

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STAR-IDAZ International Research Consortium (IRC), an international network of R&D programme owners/managers and international organisations from around 50 countries, was established to coordinate research funding by addressing identified research needs, sharing results and delivering new and improved animal health strategies for priority animal health diseases/issues. Under the IRC, 28 partners from 19 countries moved to a higher level of commitment by committing a minimum of \$US 10 million to research on IRC priorities over a five-year period, so there is a dedicated five-year budget of approximately \$2.5 billion to contribute to IRC objectives. The five IRC Regional Networks encourage regional coordination and cooperation, by bringing programme owners together to work with network partners, explore opportunities for sharing resources and to identify international funding opportunities. They also report regional needs and emerging issues to the Executive and Scientific Committees. The IRC is supported by the EU-funded secretariat (SIRCAH). To achieve IRC goals, Working Groups have been established on priority topics, to conduct gap analyses to identify and prioritise research and knowledge gaps, which are organized into research roadmaps. Details of current and planned research projects are mapped onto these roadmaps to determine the extent to which gaps are being addressed. This forms the basis of funding recommendations made to the IRC partners to encourage more focused and coordinated research on the priority gaps.



In vitro and in vivo anthelmintic efficacy of essential oil of *Satureja montana* (L.) against gastrointestinal nematodes of sheep

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The development of anthelmintic resistance in gastrointestinal nematodes (GINs) requires the search for alternative strategies. The aim of this study was to examine in vitro and in vivo anthelmintic efficacy of essential oil (EO) of *Satureja montana* (L.) against GIN of sheep, as well as toxic effects in sheep, in order to evaluate the possibility of its use in the veterinary practice. In vitro Egg Hatch Test (EHT) was conducted at eight different concentration (50, 12.5, 3.125, 0.781, 0.195, 0.049, 0.025 and 0.0125 mg/mL) of EO. In addition, for the in vivo Faecal Egg Count Reduction Test (FECRT), *S. montana* EO was administered orally to sheep of two different farms in southern Italy at the mean dose of 150 mg/kg. The chemical composition of EO was determined by GC-MS. In the EHT, *S. montana* EO showed ovicidal potential against sheep GINs with inhibition of egg hatchability that varied from 17.3-83.0% depending on the used concentration. The EO also showed in vivo anthelmintic potential with the total reduction of FEC of 15.7% and 33.0% at days 7 and 14 after treatment, respectively. No toxic effects were observed during clinical evaluation, blood count and liver function tests of sheep. The main represented compounds of EO were p-cymene (42.8%), carvacrol (28.1%) and γ -terpinene (14.6%). The obtained results suggest that *S. montana* EO may be used in the sustainable future management of GIN infections in sheep as a complementary and sustainable method to reduce the use of chemicals and to counteract anthelmintic resistance.



Investigation of eprinomectin resistance in gastrointestinal strongyles on a dairy goat farm in Northern Serbia

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The use of eprinomectin (EPR) to control gastrointestinal strongyles in dairy ruminants has economic benefits considering its zero milk withdrawal time. In summer of 2019 at the goat farm in the village of Male Pijace (Vojvodina province, Serbia), where resistance to ivermectin (IVM) was recently confirmed, the use of pour-on EPR at 1 mg/kg was recommended for strongylid treatment over fully effective albendazole in order to avoid milk loss expenses. Simultaneously, a faecal egg count reduction test (FECRT) performed in nine goats using McMaster technique (analytical sensitivity=25 epg) to evaluate its efficacy showed 83% of reduction of GIN eggs. In order to further investigate EPR failure, FECRT was repeated in January 2020 in 18 goats using Mini FLOTAC for more sensitive parasitological diagnosis (5 epg). Percentage of egg reduction and 95% Confidence Intervals were calculated using "egg-Counts 2-3" on shiny web interface <http://shiny.math.uzh.ch/user/furrer/shinyas/shiny-eggCounts/>, and the results were interpreted according to the guidelines of the World Association for Advancement in Veterinary Parasitology. The efficacy of EPR pour-on against goat gastrointestinal strongyles was 65% (95% CIs: 64-67), so the AR was confirmed. At the individual level, the anthelmintic was effective (percentage of egg reduction more than 95%) in only four out of 18 tested goats. These results have confirmed EPR resistance gastrointestinal nematodes of dairy goats in Serbia. As EPR was never used on the goat farm before these trials, it is possible that cross-resistance with long using IVM has occurred, which warrants closer examination.



Plant supplementation combined with Targeted Selective Treatment in goat farmers from Botswana: a survival analysis approach

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This study aimed to determine the effect of plant supplementation combined with Targeted Selective Treatment (TST) in poor resource goat farmers from Botswana. Twenty goat farmers were selected and randomly split into PTST (N=390 goats) and DTST (N=366 goats). Firstly, monthly five-point checks were used to assign a health status to goats. Goats displaying body condition score (BCS)=1.5, FAMACHA=3, dag score (DAS)=3 were considered borderline status. Secondly, animals showing BCS ≤ 1 , FAMACHA ≥ 4 , DAS ≥ 4 or submandibular oedema were dewormed with Levamisole + Rafoxanide at a dose of 12mg/kg and classified as sick goats. Health status was considered FAMACHA ≤ 2 , BCS ≥ 2 , DAS ≤ 2 , and absence of oedema. Finally, animals in the PTST with Borderline and Sick status were offered ~200 g fresh basis of *Terminalia sericea* or *Viscum rotundifolium*, compared to DTST goats without supplementation. Kaplan-Meier analyses were implemented to compare the time (days) until goats require a drug intervention (final day). Lastly, it was compared the proportion of goats in each status between PTST and DTST. A higher survival probability without drug intervention was recorded on the PTST vs DTST after 489 days of the study ($P < 0.05$). Survival analysis compared the number of plant interventions between 0, 1, 2, 3 or > 4 times. Furthermore, 73% of goats supplemented > 4 times lasted 382 days without drug intervention ($P < 0.05$). Plant supplementation reduced 15% of drug intervention and improved by 6% the recovery rate compared with goats in the DTST ($P < 0.05$).



The veterinarians' perspective regarding pasture parasites in Norwegian sheep and cattle, a questionnaire study

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Aim: To investigate veterinarians' perception of the impact of pasture parasites (PP) on Norwegian sheep and cattle and their current management strategies. **Method:** A net-based pre-tested questionnaire was distributed to veterinarians that worked in farm animal practice in September 2021. Questions concerned demographics, impact, diagnostics, advice and anthelmintic resistance regarding *Eimeria* spp., Gastrointestinal nematodes (GIN) and *Fasciola hepatica*. STATA were used for the descriptive analysis. **Result:** The response rate was 282/945 (30%). In sheep flocks, 189/236 (80%) and 130/231 (56%) thought *Eimeria* spp. and GIN, respectively, had a medium/large clinical importance, while *F. hepatica* was ranked as having non/small clinical importance by most; 181/233 (77%). In dairy cattle 140/236 (58%) considered pasture parasites as clinically important, while 78/220 (35%) thought so in beef cattle. Half (125/236, 53%) suspected that GIN and *Eimeria* spp. had a negative impact on production in dairy cattle. Disease history and pasture management were the most important factors when deciding to prescribe anthelmintics for both species. Furthermore, diagnostics were used seldom/never by 153/237 (65%) and 196/235 (83%) in sheep - and cattle respectively. In sheep 65/233 (28%) had suspected reduced effect of anthelmintics, while 11/232 (5%) suspected this in cattle. **Conclusion:** Most Norwegian veterinarians consider the impact of *Eimeria* spp. and GIN clinically important in sheep- and dairy cattle herds. However, diagnostic tools were used rarely, especially in cattle where the actual parasite status must be considered as unknown. This study emphasized the need to further investigate the prevalence of PPs in Norwegian cattle.



Exposure to *Ostertagia ostertagi* in Austrian dairy cattle: Preliminary results

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Monitoring parasite exposure by detection of helminth-specific antibodies (Ab) in bulk tank milk (BTM) enable targeted parasite control strategies in dairy herds. Here, we used this approach to evaluate the exposure of dairy cattle to *Ostertagia ostertagi* in Austria. BTM samples were collected between October-November 2018 in farms from Upper Austria (n=742) and Tyrol (n=499). BTM were analysed with the SVANOVIR *O. ostertagi*-Ab ELISA. Samples were classified as positive (ODR>0.6), negative (ODR<0.3) or inconclusive (ODR 0.3-0.6). Information on average milk yield/cow, access to pasture and farming system (organic/conventional) was obtained. From all farms, 50% of the BTM samples were positive (ODR>0.6), 39% were inconclusive and 11% were negative. A mean ODR (25th-75th percentile) of 0.59 (0.42-0.75) was detected across all farms, with significant differences between states (Upper Austria mean ODR = 0.50 vs. Tyrol mean ODR = 0.73; p<0.0001) and among farms with or without access to pasture (mean ODR with grazing vs. without grazing: 0.70 vs. 0.48; p<0.001). A significant negative correlation was detected between higher ODR and milk yield, with *Ostertagia*-positive farms producing 780 L milk/cow less compared with negative farms (r=-0.21; p<0.0001). Linear regression confirmed that grazing and organic farms had a significant effect on increased ODR, while ODR had a significant effect on reduced milk yield. The exposure of Austrian dairy cattle to *O. ostertagi* warrants further monitoring of parasite exposure and the implementation of sustainable helminth control approaches adapted to the local production systems. This study was financed by Boehringer Ingelheim-Austria.

Poster presentations



Changes in the microbiome composition in Churra sheep with a resistant phenotype to *Teladorsagia circumcincta* infection

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Gastrointestinal nematodes (GIN) are a major threat to health and welfare in small ruminants. *Teladorsagia circumcincta* is a parasite that inhabits the abomasum of sheep, especially in temperate regions, causing important economic losses. Given that *T. circumcincta* and microbiome share the same niche, interactions between them and the host are expected. Although it is known that within a sheep breed there are animals that are more resistant than others to infection by this GIN, it is not known if the microbiome influences the phenotype of these animals. Under this condition, 12 sheep were classified according to their cumulative Faecal Egg Count at the end of an experimental infection, 6 as resistant phenotype (RG) and 6 as susceptible phenotype (SG) to *T. circumcincta* infection. Then, all sheep were experimentally infected with 70.000 L3 of *T. circumcincta* and at day 7 days post-infection were humanly slaughtered to collect gastric mucosa tissue and gastric content from abomasum. Microbiome DNA from gastric mucosa and gastric content was extracted to Illumina sequencing and bioinformatic analysis. Our results showed that diversity and structure remained similar between the two groups. However, sheep with the resistant phenotype showed a higher number of bacteria butyrate-fermenting species as *Clostridium sensu stricto*-1 (abundance in RG: 1.29% and in SG: 0.069%; $p = 0.05$) in gastric content and also of *Serratia* sp in gastric mucosa (abundance in RG: 0.12% and in SG: 0.041%; $p = 0.07$). These data may suggest that the composition of the microbiome may be an influential factor in host response to *T. circumcincta* infection.

Local differential gene expression response in animals successfully protected by a recombinant vaccine against *Teladorsagia circumcincta*

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Teladorsagia circumcincta is one of the most important gastrointestinal nematodes in temperate areas for its prevalence and economic consequences. It has been traditionally controlled by strategic drenching, although increased resistance is promoting the identification of alternative or complementary control measures. Vaccination would be a very attractive option. Recently, a successful recombinant vaccine prototype that conferred protection in sheep to *T. circumcincta* has been developed. However, great individual variability in the response to this immunogen has been also recorded, hampering its commercialization. In this work we have compared the gene expression in the abomasum of two groups of sheep -one vaccinated and one control- experimentally infected with *T. circumcincta*. Successfully vaccinated animals showed several genes upregulated, most of them were associated with local immune response (mast cell sensitization, antigen recognition, mucus production/composition, immunoglobulin and cytokine production, etc.). Data obtained here may be useful for improving the understanding of the protective mechanisms of these immunogens and for reducing the variability in the response to the vaccine as well.



Occurrence and anthelmintic resistance of *Fasciola hepatica* in German sheep flocks

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Fasciola hepatica leads to severe production losses in livestock when not treated effectively. During recent years, there have been numerous cases of confirmed flukicide resistance worldwide. However, there is a lack of published data about the susceptibility of *F. hepatica* in Germany. The project's objective was to identify *F. hepatica* infections in German sheep flocks by coproscopical/serological examinations and to evaluate the efficacy of triclabendazole or albendazole. In 2020-2022, 1463 faecal samples from 69 farms were coproscopically examined (Flukefinder® method) for *F. hepatica* eggs. The efficacy of triclabendazole was tested on 10 farms and albendazole on one farm (3-53 sheep/farm). Individual faecal samples were collected before and 14 days after treatment to evaluate the faecal egg count (FEC) reduction using the Flukefinder® and to determine the coproantigen reduction with the BIO-X 201-antigen-ELISA-kit. The frequency of *F. hepatica* findings at individual sheep and farm level was 15.4% and 29% respectively, however the individual FEC were low (often <10 eggs per gram) on most farms. Resistance to triclabendazole was observed on one farm. On this farm, numerous sheep from the study population died due to acute fasciolosis and even the double dose of triclabendazole had no effect while treatment with closantel showed 95% FEC reduction and a negative cELISA result. On a dairy sheep farm, albendazole was found to be effective. In conclusion we hypothesise that the dry summers in 2019 and 2020 led to low infection pressure. Resistance to triclabendazole does occur but is not widespread so far. Funding statement: This study is co-funded by the Federal Office of Consumer Protection and Food Safety (BVL), Germany.



Effect of medicinal plants and organic selenium on immune responses in lambs with haemonchosis

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Haemonchus contortus is one of the most pathogenic gastrointestinal nematodes (GIN) of small ruminants. One of the solutions to reduce GIN infections is the improvement of host immune responses through nutritional manipulation. The objective of this study was to evaluate the effect of medicinal plants (HERB) and selenium (SE) on the immune responses of *H. contortus* infected lambs. Thirty-three lambs were infected with 5000 L3 larvae of *H. contortus*, re-infected two times on days 49 and 77, and subsequently divided into three experimental groups: Control (C), HERB and SE. During the experiment, blood samples were regularly taken to determine immunological parameters. Animals were slaughtered after 119 days, and the abomasum of each lamb was dissected for histopathology. Treatment with HERB and SE significantly affected the number of total leukocytes, neutrophils, and monocytes, indicating stronger immune responses. There were no significant differences between serum and mucosal antibodies between experimental groups. The mean length of adult female worms was C > HERB > SE (2.1, 2.08 and 2.03 cm, respectively), probably caused by a stronger local immune response in SE group. HERB group showed strong local inflammation in the abomasum and the infiltration of immune cells, but higher numbers of eosinophils and plasma cells in the mucosa was in SE. In conclusion, HERB and SE lambs could elicit stronger immune responses to *H. contortus* infection than infected animals. This study was supported by funds from the Slovak Research and Development Agency (APVV 18-0131) and SAS Programme for PhD students' grants (DoktoGrant APP0029).



In vitro evaluation of novel benzimidazole derivatives against *Fasciola hepatica*

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Fasciolosis is an important parasitic disease affecting significantly the ruminant production by means of reducing the growth, conversion rate, milk production, quality and quantity of meat and reproduction. The triclabendazole and albendazole resistance phenomenon is already present in many countries. Because of the absence of novel drugs against fluke infections, it is necessary to test and compare the efficacy of new compounds with potential anthelmintic activity against *Fasciola spp.* The aim of this study was to test a total of 53 compounds, benzimidazole derivatives, against *Fasciola hepatica* eggs collected directly from gall bladder of naturally infected cattle, susceptible to albendazole. All compounds were tested with the in vitro test "Egg Hatch Test" (EHT), including albendazole at a dose of 0.5 µM. To determine the ovicidal activity of these compounds, they were tested at an initial concentration of 50 µM, and those whose activity was greater than 90% were evaluated again at a concentration of 10 µM. Then, compounds with an efficacy greater than 80% at 10 µM were tested at 5 µM using eggs from albendazole-susceptible and albendazole-resistant strains. Of the 53 compounds evaluated, 11 showed ovicidal activity between 91.2 and 100% at 50 µM and 3 of them between 83.7 and 89.9 at 10 µM. Finally, 2 benzimidazole derivatives showed an ovicidal activity of 71.2% and 89.2% at a concentration of 5 µM in an albendazole-susceptible strain.



Anthelmintic resistance of horse strongyle nematodes to fenbendazole in Lithuania

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Background: with intensive use of anthelmintic drugs in recent decades, anthelmintic resistance (AR) in horse nematodes is becoming a growing issue in many countries. However, there is little available information about the parasites, treatment practices or AR in the horse population in Lithuania. The aim of this study was to assess the current situation of fenbendazole AR on horse farms in Lithuania. **Results:** the study was conducted in six stables and only the horses with a strongyle faecal egg count (FEC) of ≥ 200 eggs per gram were selected. One hundred and twenty-one faecal samples were examined with McMaster technique Faecal egg count reduction tests (FECRT) were performed on the 89 horses that met the inclusion criteria. Resistance to fenbendazole (FBZ) was found in three stables (50% of all tested herds). The FEC showed a significant ($P < 0.01$) difference between the treatment and control groups. Only cyathostomin larvae were detected in larval cultures derived from strongyle-positive faecal samples collected 14 days after treatment of a test group with FBZ. **Conclusions:** this in vivo study showed that resistance to FBZ is prevalent on horse farms in Lithuania. These findings should guide the implementation of more sustainable management of strongyle infections in horses in Lithuania.



Prevalence of anthelmintic resistance in strongyle nematode populations on sheep and cattle farms in north-eastern Germany

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Anthelmintic resistance is rapidly emerging in Europe in strongyles infecting sheep and cattle. Since few reports are available for Germany, this study focused on monitoring the prevalence and level of resistance of strongyle nematodes in Brandenburg and surrounding federal states using the faecal egg count reduction test on 12 sheep and 9 cattle farms. Grazing animals younger than 24 months were divided into different treatment groups on farm level. Sheep were split into fenbendazole, ivermectin and moxidectin and cattle into fenbendazole and eprinomectin treatment groups. Drug efficacy was calculated using the R package eggCounts in a hierarchical statistical approach using paired data before and after treatment. Sufficient eggs of *Nematodirus* spp. were found on two farms to calculate a separate egg count reduction for this genus. Furthermore, larvae were isolated from faecal samples and frozen for species composition analysis and to detect single-nucleotide-polymorphisms in the β -tubulin isotype 1 gene associated with benzimidazole-resistance using next-generation-sequencing. On sheep farms, resistance against fenbendazole and ivermectin was widespread and identified on each 6 of 8 farms. Moxidectin resistance was only observed on 2 of 12 farms. Two of the farms showed resistance against all three anthelmintics tested. On cattle farms only one single farm lacked efficacy of eprinomectin. In conclusion, anthelmintic resistance including multi-drug resistance is widespread in sheep flocks in northeast Germany while cattle are less affected.

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Study of anthelmintic resistance in the south of Spain: Preliminary results

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Parasitosis caused by gastrointestinal nematodes (GIN) are frequent in small ruminants. Traditionally, its control is based in the use of anthelmintics drugs in a non-strategic way, causing the appearance of resistance phenomena to different formulations, with considerable economic losses affecting livestock production. Thus, the aim of this study was to evaluate the resistance of GIN against moxidectin and febendazole. For the design of the experience, 15 different farms were sampled, where 30 animals were selected and divided equally into three groups: control, lactone-treated and febendazole-treated. Faecal samples were collected at day zero (before treatment) and 15 days post-treatment. Coprological analysis and quantification of parasite burden were carried out using McMaster technique and the results expressed in eggs per gram of faeces (EPG). To determine resistance, the FECRT was applied. The results showed no resistance in 53% of the farms, 26% resistance against febendazole, 6% resistance against moxidectin, 20% inconclusive results against moxidectin and finally 6% inconclusive results against febendazol. This is the first study about anthelmintic resistance in the south of Spain although more studies should be carried out in order increase the knowledge about the situation in our farms.



Essential oil of *Mentha pulegium* induces anthelmintic effects and reduces parasite-associated oxidative stress in rodent model

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Following the previous findings reported by the present authors on the anthelmintic effect of hydro-ethanolic extract of *Mentha pulegium*, the volatile constituents of *M. pulegium* are now assessed in the present study by exploring its anthelmintic and its antioxidant properties using *in vitro* and *in vivo* assays. Egg hatch assay (EHA) and adult worm's motility assays (AWMA) were used to assess the *in vitro* activity against *Haemonchus contortus*. The *in vivo* anthelmintic potential was evaluated in mice infected with *Heligmosomoides polygyrus* using faecal egg count reduction (FECR) and total worm count reduction (TWCR). *M. pulegium* EO demonstrated 100% inhibition in the EHA at 200 µg/mL (IC₅₀=56.36 µg/mL). In the AWM assay, EO achieved total worms paralysis 6 h after treatment exposure. This nematicidal effect was associated to morphological damages observed in the cuticular's worm using environmental scanning electron microscopy (ESEM). At 400 mg/kg, *M. pulegium* oil showed 75.66% of FECR and 80.23% of TWCR. The antioxidant potential of this plant was also monitored by several *in vitro* assays: total antioxidant capacity was 205.22 mg GAE/g DW, DPPH quenching effect was IC₅₀=140 µg/mL, ABTS activity IC₅₀=155 µg/mL and FRAP effect of 660 µg/mL. Regarding the *in vivo* assay, *M. pulegium* EO demonstrated a protective effect against oxidative stress by increasing the activity of the endogenous antioxidants (SOD, CAT and GPx) during *H. polygyrus* infection.



Current efficacy of anthelmintics and treatment against gastrointestinal nematodes of sheep in Lithuania

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Intensive use of anthelmintics to control helminth infections has led to anthelmintic resistance (AR), which has become an important issue in Lithuania. The aim of the study was to assess the GIN control practices used by Lithuanian sheep farmers and to evaluate the prevalence of AR to ivermectin (IVM), benzimidazoles (BZ), levamisole (LEV). During 2019-2020 all members of the Lithuanian Sheep Breeders Association were surveyed. The questionnaire was completed by 52 members (26,9% response rate). Sheep's were treated on average 1,52 times per year. Only 5,8% of sheep breeders regularly monitor GIN infection by fecal egg count test. IVM was the most frequently used anthelmintic - 63,5%. *In vitro* micro-agar larval development test was used to evaluate the prevalence of AR in 38 randomly selected farm. Resistance to IVM was indicated in 47,8 % farm. Moderate levels of IVM resistance were detected in 66,7 % farm, 16,7 % - mild and 16,7 % severe levels of AR. BZ resistance was indicated in 39,5%. Moderate level had 53,3 %, 26,7 % - severe and 20,0% mild levels of BZ resistance. AR to LEV was not detected. 16 farms were free from AR, while in 7 farms multidrug resistance was detected. Farms, which kept Lithuanian local breeds or non-Lithuanian breeds was significantly positive lined to BZ resistance. IVM using was not significant with the presence of IVM resistance in farms. The AR levels are currently low and required an effective anthelmintic treatment of GIN control strategy and better understanding of AR spread in Lithuania.



Parasitic infections in dairy cows and assessment of their carcass at the abattoir

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Gastrointestinal nematode (GIN) infections and fasciolosis affect the wellbeing, productivity and reproductivity of cows. These parasitic infections can affect a significant profitable part of the carcass and offal, being declared as unfit for human consumption. This study was conducted in S^o Miguel Island, Azores archipelago, Portugal. The dairy sector is of major importance to the regional production and economy. The objective of this study was to assess in 120 cows the prevalence of GIN, the number of rejected livers associated with *Fasciola* infection and assessment of the carcass's classification at the abattoir. The slaughtered cows came from natural pasture-based dairy herd, where they remained throughout the year. The adult females were Holstein dairy aptitudes that had already given birth and were designated as 'category D' at the abattoir. The cows weighed between 600 and 700 kg and were sent to slaughter for meat market when reproductive and dairy performance was no longer profitable. Faecal samples were collected and analyzed individually by a modified McMaster technique for egg count. According to the faecal egg count, 27% of the cows were infected with GI nematodes (50-350 eggs per gram). During veterinary inspection, 84% of the carcass were approved for consumption and the liver rejection rate reached 97%. According to the SEUROP carcass classification system, 50,8% approved carcass corresponded to the classification "O" (Fair) and 35% "P" (Poor). Regarding the factors affecting weight, reproductive efficiency and feed costs, parasitic diseases play an important role in the quantity and quality of the slaughtered cows.



Usefulness of ground cereal soaked with a mixture of chlamydospores of parasitological fungi for developing sustainable strategies for helminth control in sheep

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Sixteen first-season pasturing ewe lambs were monitored between September and May. Analysis of feces showed eggs of strongyles, identified by means of coprocultures as *Trichostrongylus* (71%), *Teladorsagia* (63%), *Nematodirus* (29%), *Chabertia* (25%) and *Oesophagostomum* (15%). Accordingly, they were administered albendazole, and then sorted into two groups. Lambs in group G-S were given thrice a week, milled cereal soaked with a liquid medium containing a blend of chlamydospores of *Mucor circinelloides* and *Duddingtonia flagrans* (individual dosage of 106 of each fungus); those in group G-C were maintained as controls and did not receive the chlamydospores. Thirteen days after deworming, the anthelmintic efficacy was 95.6% in G-S and 96.6% in G-C. After the deworming, in G-C the EPG numbers increased and counts higher than 300 EPG recorded three months later, and then rose until values around 600 EPG. In G-S, the EPG counts reached levels below 300 EPG throughout the study. The EPG numbers halved at the third month in G-C, and counts close to the starting of the study were attained at the end. In G-S, a significant reduction was observed during the trial, (57% at the end). All lambs of G-C passed eggs of strongyles by two months after deworming, whereas 75% in G-S did it between November and March. This formulation offers a very helpful procedure for developing strategies of biological control of strongyles affecting pasturing animals. Partially supported by PID2020-120208RB-I00 (Ministry of Economy and Competitiveness, Spain; FEDER) and ED431B 2021/07 (Xunta de Galicia, Spain).



Gastrointestinal Nematode Prevalence and Fecal Egg Counts in Merino White and Merino Black lambs in Portugal

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Merino White (MW) and Merino Black (MB) sheep are Portuguese autochthonous breeds originating from the Alentejo region, characterized by their high rusticity, adaptation to edaphoclimatic conditions, and natural resistance to diseases. The aim of the study was to determine the prevalence of natural gastrointestinal nematode (GIN) infections and variance of fecal egg loads in lambs of the two breeds sampled in different farms across the Alentejo region. A total of 1022 sheep aged between 4 and 12 months were sampled between September 2019 and December 2021 in 35 farms. Individual fecal egg counts were determined using the Mini-Flotac technique. Differences in worm egg burdens between breeds were assessed using a mixed logistic regression model with farms as a random factor. Ninety three percent of lambs were infected with one or more GIN genera as follows: 92% strongylids (95% CI: 90,2 - 93,5%), 17,9% Nematodirus (95% CI: 15,7-20,4%), 16,1% Strongyloides (95% CI: 14-18,5%) and 10,5% Trichuris (10,5%; 95% CI: 8,7-12,5%). The median egg loads in MW and MB lambs were 270 EPG (IQR:80-660) and 200 EPG (IQR: 90-390), respectively. Although egg counts were generally lower in MB sheep, the differences observed were not statistically significant and did not allow to conclude about a higher resistance to gastrointestinal parasitism compared to MW sheep. With regard to deworming needs, fecal egg counts showed that anthelmintics would be necessary only in a small proportion of lambs, stressing out the relevance of targeted selective treatment as a more sustainable approach for worm management. Acknowledgements: this study was funded by Project MERINOparasite PTDC/CVT-CVT-28798/2017



COMBAR COST-EU, a mobile application proposal

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Diagnosis and treatment options/strategies for helminth parasitic diseases are not standardized globally, or even across Europe, due to factors such as epidemiology, resources availability, socioeconomics and the lack of adequate information. Good communication of options can help to overcome some of these problems. In this sense, it is proposed to develop a mobile application that allows the tailoring, and sharing, of knowledge related to the various diagnoses and treatments in a format appropriate for all stakeholders (e.g. farmers, veterinarians, universities, researchers, laboratories, industries). The proposal aims to present the theoretical functionalities for the creation of a mobile application, the application should have a sequence of options that must be grounded in theoretical requirements. First of all, there is a necessity to have inputs standardized by the application (e.g. country/region, applicable legislation, animal typology, disease characteristics, symptoms) that should be entered into the application. The aim is to provide a range of tailored options to the end-user. Second, given the user's choices, the application can offer information and guidelines (including veterinarians available, laboratories, diagnosis, treatments, stores, among others). It is highlighted that the main gain may be the collection of information, whenever the user allows it. Finally, considering that most farmers use the language of their country, it is crucial to have the application in different European languages.



Effect of sainfoin (*Onobrychis viciifolia*) pellets on ruminal fermentation, microbiome and hematological parameters in lambs experimentally infected with *Haemonchus contortus*

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Presence of plant secondary metabolites in feed substrates can affect processes of ruminal fermentation in ruminants infected by gastrointestinal nematodes. A present work analyzed the ruminal fermentation parameters, microbiome and hematological profile of lambs experimentally infected with a gastrointestinal nematode *Haemonchus contortus* and fed sainfoin pellets (SFPs; 600 g DM/day/animal) for 14 day period. Twenty four lambs were divided into two groups of twelve animals each on day 30 after infection, when all parasites had matured to the adult stage: control animals fed MH (control, MH, 600 g DM/d/animal) and animals fed sainfoin pellets (SFPs, 600 g DM/d/animal). Both groups also fed commercial concentrate (300 g DM/d/animal). In ruminal contents, fermentation parameters, and microbiome in vitro and in vivo were determined using molecular and microscopic techniques. Analysis of ruminal contents in the SFP group showed smaller populations of total Archaea ($p < 0.001$), Methanomicrobiales ($p = 0.009$) and decreased methane production in vitro ($p = 0.046$) and in vivo ($p = 0.030$) compared to the control group. The relative abundance of genus *Butyrivibrio fibrisolvens* quantified by real-time PCR was more present in the lambs with the SFP diet ($p = 0.05$). The number of red blood cells of the lambs was affected by haemonchosis ($p < 0.001$). Finally, the consumption of SFPs by GIN-infected lambs may affect ruminal methanogens and subsequently decrease methane emission without undesirable changes in the ruminal microbiome. The study was supported by funds from the Slovak Research and Development Agency (APVV 18-0131) and DoktoGrant (APP0162).



In vitro anthelmintic efficacy of natural extracts against gastrointestinal nematodes of sheep

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Resistance to anthelmintic drugs in gastrointestinal nematodes (GINs) of sheep is of high concern for livestock production worldwide. Therapeutic properties of plants may be used in ethnoveterinary practices against GIN infection and would provide successful alternative remedies to synthetic anthelmintic drugs. The aim of this study was to determine the in vitro anthelmintic activity of aqueous, ethanolic and hydroethanolic extracts from 10 medicinal plants (*Borago officinalis*, *Malva sylvestris*, *Matricaria inodora*, *Mentha suaveolens*, *Plantago lanceolata*, *Potentilla reptans*, *Rosmarinus officinalis*, *Rumex acetosa*, *Thymus serpyllum* and *Thymus vulgaris*), native to southern Italy. For this purpose, the Egg Hatch Test (EHT) was used to estimate the in vitro anthelmintic efficacy of the plant extracts using GIN eggs from sheep naturally infected by *Teladorsagia circumcincta*, *Trichostrongylus colubriformis* and *Haemonchus contortus*. Each extract was analyzed in three replicates and tested at decreasing concentrations from 50.00 mg/mL for the aqueous extract, 1.024 mg/mL for the ethanolic extract and 7.776 mg/mL for the hydroalcoholic extract. Thiabendazole and deionized water were used as positive and negative controls, respectively. The results indicated that *B. officinalis*, *M. sylvestris*, *M. inodora* and *M. suaveolens* extracts caused a high inhibition of egg hatching within 48 hours of exposure, showing efficacy ($\geq 91\%$) at the first two higher concentrations. Further in vivo studies are needed to evaluate the anthelmintic potential of these natural extracts against GINs of sheep.



Effect of irrigation water source on secondary metabolites in *Salix acmophylla* and their potential to impair exsheathment of gastro-intestinal nematodes

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Willows (*Salix*), which are found worldwide, have secondary metabolites that are valuable as dietary supplements for animal feed. The objectives of the current study were to test the effects of irrigation source on the production of secondary metabolites and their potential to inhibit nematode exsheathment. Three willow ecotypes (Kishon, Meah Shearim, and Golan) were irrigated with fresh tap water (FW) or treated wastewater (TWW). TWW irrigation resulted in a doubling of biomass production compared with FW, probably due to the presence of more nutrients, particularly nitrogen. The type of irrigation water (TWW vs FW) did not affect the quantity and quality of secondary metabolites, such as phenols, in all three willow ecotypes, although there was a difference in the chemical profile of glycoside phenols between willow ecotypes. Biomass and secondary metabolite production differed greatly between ecotypes, which was evident in the differing concentrations of phenols and flavonoids. These varying concentrations in turn affected the inhibition of the exsheathment of larval L3 nematodes. The ethanol extract of the Kishon ecotype consistently resulted in more than 90% exsheathment inhibition when tested on the L3 larval stage, whereas extracts from Meah Shearim and Golan resulted in lesser inhibition. A sub-fraction of the ethyl acetate fraction obtained from Kishon had maximum activity with more than 90% exsheathment inhibition.



Impact of dimethylsulphoxide (dmsO) on the development of gastrointestinal larvae in the larval development test

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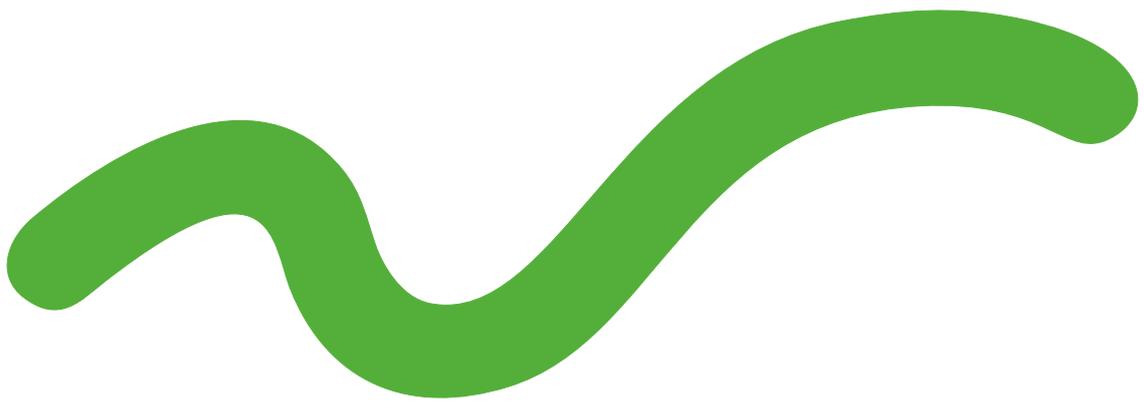
Introduction: Parasitic infections, especially those caused by gastrointestinal nematodes, are one of the main causes of economic losses in goat farming worldwide. Their control is mainly based on the use of anthelmintics. The widespread use of these products has led to the emergence of drug-resistant parasite strains. One of the available tests for the detection of drug resistance in parasites is the in vitro larval development test (LDT). Dimethyl sulfoxide (DMSO) is used in the larval development test as a drug solvent and has the potential to influence the development of the larvae and the results obtained.

Materials and methods: The eggs of gastrointestinal nematodes were isolated from faecal samples collected from 2 naturally infected goats. The LDT was performed according to standard procedure. Instead of increasing concentrations of anthelmintics, only DMSO was used in the following concentrations: 0% (distilled water - control), 0.6%, 1.3%, 2.6%, 5.2%, 10.4% and 20.8%. The number of developed larvae was counted and calculated % development at each concentrations. The results are presented below in the table as the arithmetic mean \pm SD and min-max in parentheses and plotted on the graph as the arithmetic mean and 95% confidence intervals:

DMSO concentration	n	Percentage development [%]	Dunnett's test p-value of the comparison with water control (0% DMSO)
Control (water)	30	95.5 ± 3.1 (87.5 - 100)	-
0.6%	30	97.0 ± 2.6 (89.2 - 100)	0.859
1.3%	30	92.8 ± 4.4 (81.0 - 100)	0.342
2.6%	30	76.3 ± 8.8 (50.0 - 91.8)	<0.001
5.2%	30	27.6 ± 10.4 (10.8 - 50.0)	<0.001
10.4%	30	5.7 ± 6.6 (0 - 33.7)	<0.001
20.8%	30	0 ± 0.2 (0 - 1.2)	<0.001

Results and discussion: The results of the study showed that the percentage reduction in the development of larvae at the concentration of 1.3% DMSO was not significantly lower than the percentage of development of the larvae in the control sample (distilled water; 0% DMSO). The use of DMSO concentration above 1.3% in the larvae development test may inhibit the development of the larvae, and thus the test results.

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