

Abstract book



JOINT COMBAR WG MEETING
ANTHELMINTIC RESISTANCE IN RUMINANTS: FROM RESEARCH TO RECOMMENDATIONS

9-10 DECEMBER 2020 · ONLINE

  **COMBAR**

(LOCAL) ORGANISERS

4th Joint COMBAR WG meetings: “Anthelmintic Resistance in Ruminants: from Research to Recommendations”

Local organiser:

Dr. Smaro Sotiraki, HAO Demeter, Greece

With support from:

Dr. Johannes Charlier, Kreavet, Belgium

Dr. Hannah Rose-Vineer, University of Liverpool, UK



SCIENTIFIC COMMITTEE

4th Joint COMBAR WG meetings: “Anthelmintic Resistance in Ruminants: from Research to Recommendations”

Dr. Johannes Charlier, Kreavet

Dr. Smaro Sotiraki, Hellenic Agricultural Organization Demeter

Prof. dr. Laura Rinaldi, Università di Napoli Federico II

Prof. dr. Georg von Samson-Himmelstjerna, Freie Universität Berlin

Prof. dr. Edwin Claerebout, Ghent University

Dr. Dave Bartley, Moredun Research Institute

Prof. dr. Eric Morgan, Queen’s University of Belfast

Dr. Hervé Hoste, INRAE / Ecole Nationale Vétérinaire de Toulouse

PROGRAMME

4th Joint COMBAR WG meetings: “Anthelmintic Resistance in Ruminants: from Research to Recommendations”

Wednesday 09/12/2020

10:00-12:00	COMBAR 5 th Management Committee (MC) meeting	MC members only
Session 1	Improving diagnosis	Chair: Laura Rinaldi (UNINA)
14:00 - 14:10	Introduction	
14:10 - 14:40	Keynote: Monitoring anthelmintic resistance: from phenotypic assays to molecular markers.	Cédric Neveu (INRAE)
14:40 – 14:52	Survival of the fittest? Ecological fitness assessment in <i>Teladorsagia circumcincta</i> of known anthelmintic resistance status.	Kyra Hamilton (Teagasc)
14:52 – 15:04	Transgenically expressed <i>Haemonchus contortus</i> Cyp HCOI00827700 can modulate ivermectin susceptibility in <i>Caenorhabditis elegans</i> .	Natalie Jakobs (FUB)
15:04 – 15:16	Bulk tank milk <i>Ostertagia</i> ELISA as a tool for quantification of milk production losses in dairy herds : where do we stand?	Nadine Ravinet (Oniris)
15:16 – 15:25	One – minute poster presentations on droplet digital PCR for levamisole resistance screening, anthelmintic efficacy in Germany, the new Kubic FLOTAC microscope, <i>Dictyocaulus</i> bulk tank milk ELISA.	Presenters from SLU, FUB, UNINA and UGent
15:25 - 16:00	Moderated discussion with contributions from Georg von Samson Himmelstjerna (FUB), Smaro Sotiraki (HAO Demeter), Menno Holzhauser (Royal GD Deventer) and Eurion Thomas (FECPAK).	

Thursday 10/12/2020

Session 2	Socio-economics	Chair: Edwin Claerebout (UGent)
10:00 - 10:10	Introduction	
10:10 - 10:40	Keynote: OIE's activities on antiparastics resistance.	Maria Szabo (OIE)
10:40 – 10:52	Initial assessment of the economic burden of major parasitic helminth infections to the ruminant livestock industry in Europe.	Johannes Charlier (Kreavet)
10:52 – 11:04	Barriers and incentives for uptake of diagnostics for sustainable worm control by European dairy cattle farmers.	Fiona Vande Velde (UGent/NMBU)
11:04 – 11:16	Treatment against helminths in Norwegian sheep – a questionnaire-based survey.	Maiken Gravdal (NMBU)
11:16 - 11:25	One minute poster presentations on producer knowledge, effect of anthelmintic treatment on milk yield in sheep, ML resistance in Brazil.	Presenters from Universidade de Évora, University of León, Federal University of Parana
11:15 - 12:00	Moderated discussion with contributions from Dave Bartley (MRI), Lesley Stubbings (SCOPS), Erwin Wauters (ILVO) and Katarina Gustafsson (Farm and Animal Health, Sweden).	

Session 3	Sustainable control	Chair: Eric Morgan (QUB)
14:00 - 14:10	Introduction	
14:10 - 14:40	Keynote: Regulatory aspects of new anthelmintic veterinary medicines.	Barbara Cyrus (EMA)
14:40 – 14:52	<i>In vivo</i> anthelmintic efficacy of aqueous <i>Dryopteris filix-max</i> and <i>Punica granatum</i> macerates against gastrointestinal nematodes of sheep.	Fabio Castagna (University of Catanzaro)
14:52 – 15:04	A diluting strategy to reduce anthelminthic treatment: suckling dairy calf/nurse cow system and gastrointestinal infection during the first grazing season.	Christophe Chartier (INRAE/Oniris)
15:04 – 15:16	Efficacy of vaccination against <i>Teladorsagia circumcincta</i> in two native sheep in Gran Canaria is conditioned by age and breed.	Cynthia Machin (Universidad de Las Palmas de Gran Canaria)
15:16 – 15:25	One minute poster presentations on FAMACHA®, evaluation of seaweed, essential oils and nematode-killing bacteria for nematode control	Presenters from Instituto Nacional de Investigação Agrária e Veterinária Portugal, University of Copenhagen, University of Novi Sad, University of Ljubljana
15:25 - 16:00	Moderated discussion with contributions from Hervé Hoste (INRAE/ENV), Bruce Thompson (Irish dairy farmer), Athina Trachili (European Association of Veterinary Practitioners) and Thomas Geurden (Zoetis).	

INVITED PRESENTATIONS

4th Joint COMBAR WG meetings: "Anthelmintic Resistance in Ruminants: from Research to Recommendations"

Monitoring anthelmintic resistance: from phenotypic assays to molecular markers

Claude L. Charvet¹, Elise Courtot¹, Fabrice Guegnard¹, Abdallah Harmache¹, Guillaume Salle¹, Cedric Neveu¹

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The control of parasitic nematode infections in humans, livestock and companion animals is critically dependent on anthelmintic treatments. However, the indiscriminate use of anthelmintic drugs has inevitably led to the selection of resistant parasites. In this context, diagnosis tools are of particular interest to monitor the resistance spreading and refine strategies for the control of resistant parasites. In this presentation, recent advances in automated phenotypic assays performed on larval stages of the parasite will be discussed. In addition, based on the example of the imidazothiazoles/tetrahydropyrimidines anthelmintics, the identification and functional validation of molecular markers associated with resistance will be presented, opening a discussion about the advantages and limitations of the use of *C. elegans* as a model for parasitic species.

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OIE's activities on antiparasitic resistance

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Antiparasitic resistance (APR) is an important challenge across the world, including all World Organisation for Animal Health (OIE) Regions. This type of resistance poses a significant threat to animal health and welfare and can result in production losses in food-producing species, presenting a challenge for food security. There are currently no OIE guidelines or standards on antiparasitics.

The OIE first addressed this subject in the 4th and 5th cycles of their Training Seminars for the OIE National Focal Points for Veterinary Products. During these seminars, which were held worldwide, the OIE sought the views of its 182 Members on the potential need for OIE guideline(s) and standards on responsible and prudent use of antiparasitics. Standards and guidelines for antimicrobial agents already exist. The latter, entitled "Responsible and prudent use of antimicrobial agents in veterinary medicine", can be found in the OIE Terrestrial Animal Health Code, Chapter 6.9. and the Aquatic Animal Health Code; Chapter 6.2.

In 2019, the OIE Electronic Expert Group on Antiparasitic Resistance was set up as one of the outcomes of the Training Seminars for Focal Points for Veterinary Products. The Electronic Expert Group on Antiparasitic Resistance developed a simple user-friendly survey which was conducted in Asia, Africa, and the Middle East in 2020. The results will serve as inputs for a publication on Prudent use of antiparasitic agents to help control antiparasitic resistance. This could become the basis for OIE Standards for prudent and responsible use of antiparasitic if they are considered warranted.

¹Chargée for mission of veterinary products, OIE

Regulatory aspects of new veterinary anthelmintic medicines

Barbara Cyrus¹, Javier Pozo¹

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In the European Union (EU), a veterinary medicinal product (VMP) has to be authorised before being placed on the market, either by a national competent authority or by the European Commission (for a marketing authorisation valid throughout the EU). Dossier requirements are laid down in European legislation, i.e. the same dossier requirements apply throughout the EU. A new veterinary regulation, (EU) 2019/6, will apply from 2022.

For a new VMP, an applicant must provide data on the pharmaceutical quality, the safety (target animal, user, environment, and consumer, if applicable) and the efficacy of the VMP. If a pharmaceutical VMP is to be used in a food-producing species, a maximum residue limit (MRL) has to be established for the active substance. Once a marketing authorisation has been granted, a VMP will be subject to regular monitoring (pharmacovigilance) to ensure its safe and effective use; any post-authorisation change (e.g. new claim) also needs to be authorised.

Guidance on data and conduct of such tests have been developed by the EMA's Committee for Medicinal Products for Veterinary Use (CVMP), and by the International Cooperation on Harmonisation of Technical Requirements for Registration of VMPs (VICH). In addition, regulatory authorities may offer further support for novel product applications.

The majority of authorised anthelmintic VMPs are pharmaceutical products; for these VMPs it is mandatory to provide information about the potential for resistance development. No or limited regulatory experience is currently available for other types of anthelmintic VMPs, e.g. vaccines, other biologicals, or herbals.

¹ European Medicines Agency (EMA)

ORAL & POSTER PRESENTATIONS

4th Joint COMBAR WG meetings: "Anthelmintic Resistance in Ruminants: from Research to Recommendations"

Evaluation of the FAMACHA©-method for targeted selective anthelmintic treatment in Black and White varieties of Merino sheep in the Alentejo region, South of Portugal

*Ana Afonso*¹, *Maria Salomé Gonçalves*², *Tiago Perloiro*³, *Pedro Vieira*⁴, *Inês Sarraguça*², *Luís Madeira de Carvalho*^{L2}, *Pâmela Valente*², *Maria Alexandra Basso*², *Telmo Nunes*², *Catarina Oliveira*⁵, *Elsa Duarte*⁵, *Jacinto Gomes*^{1,2}, *Ana Cristina Ferreira*¹, *Luís Telo da Gama*², *Andreia J. Amaral*², *Helga Waap*^{1,2}

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Targeted selective treatment (TST) using the FAMACHA©-method was proposed as an alternative method for the management of GIN in small ruminants to slow down anthelmintic resistance. This study evaluated the accuracy of the FAMACHA©-method for TST in Black and White varieties of Merino sheep in the Alentejo region under field conditions. Paired fecal and blood samples were collected between September 2019 - September 2020 from 302 White Merino and 203 Black Merino sheep in 16 and 10 farms, respectively. Animals were scored for anemia by the FAMACHA-method and individual fecal egg counts (FEC) and packed cell volume (PCV) were determined. The presence of *Haemonchus* at farm level was assessed by L3 larvae identification. Two thresholds for anemia by the FAMACHA©-method were considered: scores ≥ 3 and ≥ 4 . Clinical anemia was defined as PCV $\leq 27\%$. Spearman's correlation test showed a significant correlation between FAMACHA scores and PCV in both breeds. Though PCV was significantly correlated with FEC, this was not observed for FAMACHA scores. Sensitivity for detecting anaemic animals was better considering scores ≥ 3 (79.1% for White Merino and 71.4% for Black Merino sheep), but specificity was low (52.1% and 32.3%, respectively). Overall sensitivity and specificity in detecting animals needing anthelmintic treatment (defined as FEC ≥ 500 EPG) was 57.1% and 40.8%, respectively. Within-herd prevalence of *Haemonchus* was 6.7-20% in 32% of farms but it was the less representative species. Based on these results, in the Alentejo region, where prevalence of *Haemonchus* is apparently low, the FAMACHA©-method may not be suitable for TST.

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Anthelmintic Fluorescent drugs, new probes to detect anthelmintic resistance in the nematode *Caenorhabditis elegans*

Mélanie Alberich¹, Jean-François Sutra¹, Marlène Lacroix¹, Anne Lespine¹

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Most grazing ruminants are infected by a variety of helminth parasites that have a massive impact on their health, welfare and their production. In that context, diseases caused by gastrointestinal nematode parasites are important animal health issue. The main mean to control such parasites relies on the use of anthelmintic drugs. However, intensive use of chemicals has selected drug-resistant parasite populations in many animal species. This includes resistance to the latest developed broad-spectrum macrocyclic lactones (MLs) such as ivermectin (IVM). There is now considerable evidence that ML resistance is a polygenic trait in nematodes, but the molecular mechanism(s) of ML resistance remains to be resolved. The problem is worsened by the lack of efficient tools to track resistance. In that context, we have developed a stable fluorescent probe of IVM, IVM-FLUO, and were able to visualize and quantify IVM into *Caenorhabditis elegans* using confocal imaging. We have compared the distribution and signal intensity of IVM-FLUO in susceptible and ML-resistant strains. For the first time, we could visualize the parental IVM in the whole worm. Signal quantification revealed a higher IVM amount in wild-type animals compared to the IVM-resistant worms, in line with a link between IVM concentration in the worm and IVM resistance status. Our fluorescent probe offers new perspectives to evaluate resistance status in parasite nematodes.

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

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Willows (*Salix*) are found worldwide and their secondary metabolites are used as a dietary supplement for animal feed. The objectives of the current study were to test the effects of irrigation type on secondary metabolite production and their potential to inhibit the nematode exsheathment process. Three willow ecotypes were cultivated and irrigated either using fresh tap water (FW) or treated wastewater (TWW) and we evaluated the effect of the plant extracts on nematodes using the larval exsheathment inhibition assay (LEIA). The TWW irrigation resulted in a doubling of biomass production compared with FW, probably due to the higher concentration of nutrients, particularly nitrogen, in treated wastewater compared with tap water. The type of irrigation water (TWW vs FW) didn't appear to have a significant effect on the quantity and quality of secondary metabolites, such as phenols, in the three willow ecotypes. The results showed that there is a difference in the chemical profile of glycoside phenols between willow ecotypes. We also found that biomass and secondary metabolites production greatly differed between ecotypes and was evident in the concentration of phenols and flavonoids accumulated by the plant leaves. These varying concentrations affected the biological activity and to inhibit the exsheathment process of larval L3 nematodes. The Keshon ecotype consistently resulted in more than 90% exsheathment inhibition when tested on the L3 larval stage, whereas the other ecotypes resulted in lower inhibition levels. A sub-fraction of ethyl acetate fraction obtained from Keshon showed maximum activity with more than 90% inhibition of the exsheathment of nematodes. The exsheathment inhibition potential of the phenolic compounds depends mainly on the willow ecotype tested.

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Droplet digital PCR screening for the Hco-acr-8b levamisole resistance marker in Swedish field populations of *H. contortus*

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Haemonchus contortus is one of the most prevalent and pathogenic parasitic nematode species in small ruminants. Infections with *H. contortus* and other parasitic gastrointestinal nematode species are controlled with anthelmintics. However, the development of resistance to drugs has become a major issue in livestock production. While the molecular detection of benzimidazole resistance in *H. contortus* is well developed, the molecular tools and protocols are far less advanced for the detection of levamisole resistance. Hco-acr-8 encodes a critical acetylcholine sensitive subunit that confers the levamisole-sensitivity to the receptor. A 63 bp deletion in the Hco-acr-8 gene was previously associated with levamisole resistance. Here, we report the development of a droplet digital PCR assay as a molecular tool to monitor this levamisole resistance marker in the field. Sequencing of single, adult worms recovered from Swedish sheep and reference isolates yielded 56 sequences, surrounding the region containing the aforementioned deletion. Primers and probes, developed based on the obtained sequencing data, were used to quantify the presence or absence of the “resistant” allele. We tested 60 individuals belonging to 6 different reference isolates (3 resistant, 3 susceptible) in conventional and (dd) PCR assays. Furthermore, *H. contortus* enriched larval culture samples, collected pre- and post-levamisole treatment on 8 sheep farms were tested to evaluate the proportion of the deletion in each. We did not find clear evidence linking the presence of this deletion to an increased survival and/or reduced levamisole efficacy among tested field populations from Sweden, suggesting that resistance to levamisole cannot only be explained by the presence of the 63bp deletion in the Hco-acr-8 gene.

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The effects of seaweed inclusion in feed on nematode burdens in pigs.

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Previous studies on extracts from Nordic seaweeds have shown promising effects against *Ascaris suum* L3 *in vitro*. Our current aim was thus to examine if this could translate into similar effects *in vivo*. Based on the *in vitro* trials the seaweed *Saccharina latissima* was used in a four week pilot feeding study with 3x3 pigs infected week one with 6000 *Oesophagostomum dentatum* L3 and week two with 2000 embryonated *A. suum* eggs. Three animals were thus either given a control feed, 5% (DM) lactobacilli-fermented *S. latissima* or 5% non-fermented *S. latissima* feed. There were no differences in worm burdens but an approx. 50% decrease in mean *O. dentatum* faecal egg counts was seen within the three weeks of infection in both seaweed groups compared to the control group. Based on the pilot a five week 2x2 study design of infected vs non-infected (6000 *O. dentatum* L3 week one and 2000 *A. suum* eggs week three) w/wo 8% fermented dried *S. latissima* dietary inclusion was set up (n=32). No significant differences were found in worm counts or *A. suum* liver spots. The study could not repeat the reduction in egg excretion seen in the pilot, although a 12% reduction in hatching percentages of cultured *O. dentatum* eggs due to *S. latissima* was observed (P>0.05). There were no substantial effect of diet on immune cells isolated from blood and lymph nodes. These results indicate short-term *S. latissima* inclusion does not affect parasite infections though long-term effects on parasite-host interactions cannot be excluded.

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Initial assessment of the economic burden of major parasitic helminth infections to the ruminant livestock industry in Europe

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We report a European wide assessment of the economic burden of gastrointestinal nematodes, *Fasciola hepatica* (common liver fluke) and *Dictyocaulus viviparus* (bovine lungworm) infections to the ruminant livestock industry. The economic impact of these parasitic helminth infections was estimated by a deterministic spreadsheet model as a function of the proportion of the ruminant population exposed to grazing, the infection frequency and intensity, the effect of the infection on animal productivity and mortality and anthelmintic treatment costs. In addition, we estimated the costs of anthelmintic resistant nematode infections and collected information on public research budgets addressing helminth infections in ruminant livestock. The epidemiologic and economic input data were collected from international databases and via expert opinion of the Working Group members of the European Cooperation in Science and Technology (COST) action COMbatting Anthelmintic Resistance in ruminants (COMBAR). In order to reflect the effects of uncertainty in the input data, low and high cost estimates were obtained by varying uncertain input data arbitrarily in both directions by 20%. The combined annual cost [low estimate-high estimate] of

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the three helminth infections in 18 participating countries was estimated at € 1.8 billion [€ 1.0 - 2.7 billion]. Eighty-one percent of this cost was due to lost production and 19% was attributed to treatment costs.

The cost of gastrointestinal nematode infections with resistance against macrocyclic lactones was estimated to be € 38 million [€ 11 – 87 million] annually. The annual estimated costs of helminth infections per sector were € 941 million [€ 488 – 1,442 million] in dairy cattle, € 423 million [€ 205 - 663 million] in beef cattle, € 151million [€ 90 - 213 million] in dairy sheep, € 206 million [€ 132 - 248 million] in meat sheep and € 86 million [€ 67 - 107 million] in dairy goats.

Important data gaps were present in all phases of the calculations which lead to large uncertainties around the estimates. Accessibility of more granular animal population datasets at EU level, deeper knowledge of the effects of infection on production, levels of infection and livestock grazing exposure across Europe would make the largest contribution to improved burden assessments. The known current public investment in research on helminth control was 0.15 % of the estimated annual costs for the considered parasitic diseases. Our data suggest that the costs of enzootic helminth infections which usually occur at high prevalence annually in ruminants, are similar or higher than reported costs of epizootic diseases. Our data can support decision making in research and policy to mitigate the negative impacts of helminth infections and anthelmintic resistance in Europe, and provide a baseline against which to measure future changes.

A diluting strategy to reduce anthelmintic treatment: suckling dairy calf/nurse cow system and gastrointestinal infection during the first grazing season.

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In dairy farms, new rearing practices of calves with nurse cows have been developed by organic farmers but still remain poorly documented regarding health implications. The objective was to assess the impact of rearing suckling calves with nurse cows on the risk of gastrointestinal nematode (GIN) infection in calves. Serum pepsinogen level and GIN egg excretion per gram of faeces (epg) were determined in 438 calves belonging to 38 groups from 30 farms in the western part of France at housing (October 2018 to January 2019). The maximum number of infective larval generations met by the animals (LG) in each pasture plot was modelled by Parasit'Sim expert system. The data were analysed using logistic univariate and multivariate regressions. Mean parasitological parameters per group were low. On average, the serum pepsinogen level was 1.1 units of tyrosine (U Tyr) and the GIN egg output was 130 epg. Pasture infectivity was above LG4 for 2/3 of the groups. These results suggest that rearing suckling dairy calves with nurse cows decreases the level of GIN infection in calves during the 1st grazing season compared to standard rearing of weaned heifers grazing alone. This can be explained by i) a cleansing effect of immune cows on pastures (eating larvae while excreting few eggs) and ii) a reduced intake of larvae/grass by suckling calves associated with a possible direct effect of milk on larvae.

¹ BIOEPAR, INRAE, Oniris, France

Current efficacy of anthelmintics in gastrointestinal parasitic nematodes of sheep and cattle in Brandenburg, Germany

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A worldwide increase of reports on lack of anthelmintic efficacy requests a comprehensive approach to monitor nematode prevalence and drug efficacy in livestock. This project aims to determine prevalence of parasitic nematodes and anthelmintic efficacy on fifteen dairy cattle and sheep farms in Brandenburg (northeastern Germany), using the faecal egg count reduction test. For sheep, first season grazing animals will be treated with ivermectin, moxidectin and fenbendazole while first- and second year grazing calves will be treated with ivermectin and fenbendazole (20 animals per group). The results will be evaluated by hierarchical statistical models using the R package eggCounts. Larvae will be collected from copro-cultures in order to conduct larval migration inhibition assays using the macrocyclic lactones ivermectin and moxidectin as well as the imidazolthiazole levamisole. Species composition of parasitic nematode populations will be explored using the nemabiome next-generation-sequencing approach. Presence of single-nucleotide polymorphisms in the isotype 1 β -tubulin gene associated with benzimidazole resistance will be quantified on species level using deep amplicon sequencing. A questionnaire analysis of the participating farms is being considered to estimate the awareness of anthelmintic resistance and to reveal management practices and routines in the field.

An additional questionnaire addressing farm animal veterinarians in Germany will elucidate difficulties in communication and transfer of scientific findings to practitioners.

We will present initial data of sheep farms with regard to faecal egg count reduction results for ivermectin, moxidectin and fenbendazole.

Funding statement: This study is co-funded by the Federal Office of Consumer Protection and Food Safety, Germany.

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² Federal Office of Consumer Protection and Food Safety (BVL), Berlin

Treatment against helminths in Norwegian sheep – a questionnaire-based survey.

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The objectives of this survey were to obtain information on the current situation regarding management of major helminth infections in Norwegian sheep flocks and to identify flocks potentially at high risk regarding development of anthelmintic resistance. The study focused especially on infections and possible production/clinical consequences related to *Haemonchus contortus* and *Fasciola hepatica*. A questionnaire was sent out in January 2020 to members of the Norwegian sheep recording system, representing farms in all counties of the country (total of 5968 members). The data consists of 1378 answers (23% response rate). The questionnaire was divided into three sections; general management; history of parasitic infections in the flock, and treatment against gastro-intestinal helminths. The last section included questions regarding the farmers' impression of the efficacy of anthelmintic treatments that they had used, especially in relation to *H. contortus* and *F. hepatica*. Answers indicated that 10% of the respondents suspected poor anthelmintic efficacy. Furthermore, 11% of respondents reported that gastro-intestinal parasites are an increasing problem in their flock. As this study is part of a 4-year PhD-project, selected flocks will be invited for further investigation; almost 65% of respondents were positive to participating in further investigations. More details/results will be presented and discussed at the meeting.

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Survival of the fittest? Ecological fitness assessment in *Teladorsagia circumcincta* of known anthelmintic resistance status.

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In recent years, levels of multi-drug resistant *Teladorsagia circumcincta* has increased. Documented evidence for reversion toward susceptibility has led to the hypothesis that resistance mutations result in an ecological fitness cost.

The fitness of four *T. circumcincta* isolates of known anthelmintic resistance status was assessed using *in vitro* assays: Isolates D and M were multi-drug resistant, whilst W and T were pan-susceptible. The *in vitro* assays were designed to assess larval length, egg hatch, ex-sheathment rate, egg to L3 development after cold-stress, and migration after heat-stress.

Isolate W was longer than all other isolates ($P < 0.005$). M was longer than D ($P = 0.005$). Isolates D and T had higher ex-sheathment than W at 24 hours incubation in artificial rumen fluid ($P < 0.01$). M had a higher egg hatch rate than W ($P = 0.03$). After storage at 4 °C all isolates exhibited decreased egg development; after 4 days exposure D and T had greater reduction than M ($P < 0.005$). All isolates displayed reduced migration ability after heat-stress; after 504 hours differences were observed between M and T ($P = 0.03$), and M and W ($P = 0.007$).

Although significant differences between isolates were observed for various fitness traits of *T. circumcincta*, reduced fitness was not definitively associated with multi-drug resistance. This suggests that variation in fitness parameters between isolates is large, making it difficult to determine whether resistance genotypes come with lower fitness. Future studies will focus on separating genotypes within isolates to overcome this issue.

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Transgenically expressed *Haemonchus contortus* Cyp HCOI00827700 can modulate ivermectin susceptibility in *Caenorhabditis elegans*

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Drug resistance has a major impact on the worldwide health system and became a widespread problem in helminthoses. Benzimidazoles and macrocyclic lactones (MLs) are the most used anthelmintic classes and revolutionized parasite control in livestock. However, anthelmintic resistance is now a global problem and particularly the highly pathogenic and most important ruminant parasite *Haemonchus contortus* is involved. Alterations at the drug target site as well as drug elimination by efflux proteins have already been associated as potential cause of resistance but drug metabolism via cytochrome P450 enzymes (CYPs) might also have a major impact either.

This study aimed to analyse the impact of the *H. contortus* CYP HCOI00827700 on the ivermectin (IVM) and moxidectin (MOX) susceptibility of *Caenorhabditis elegans*. Therefore, microinjection was used to generate a transgenic line expressing the candidate gene in *C. elegans* N2 background. After 24 h of incubation with either IVM or MOX, thrashing assays were performed to record dose-response curves. Data show that overexpression of the *H. contortus* CYP HCOI00827700 in the gut of *C. elegans* increased the EC50 value for IVM by approximately 4-fold. However, MOX had no significant effect on the EC50 value leading to the assumption, that MOX will be differently metabolized than IVM. This is the first time a direct effect of a CYP enzyme on ML susceptibility of a nematode has been demonstrated.

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Efficacy of vaccination against *Teladorsagia circumcincta* in two native sheep in Gran Canaria is conditioned by age and breed

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The resistance developed by gastrointestinal nematodes (GIN) to anthelmintics has led to the search for sustainable alternatives such as vaccines. Previously, a *Teladorsagia circumcincta* recombinant sub-unit prototype seemed to confer different levels of protection in Texel-cross sheep depending on their age. In the present study, the prototype vaccine was tested in two local breeds from the Canary Islands, Spain (Canaria Hair Breed and Canary Sheep), which present different susceptibility to GIN infection. Results from the immunization of both breeds at 3 and 6 months old were compared. Vaccination induced a reduction of several parasitological parameters in 3-month-old Canaria Hair Breed lambs (more resistant) and in 6-month-old Canaria Sheep lambs (more susceptible). The immunological mechanisms associated with this protection seem to be conditioned by the age of each breed and need to be elucidated in the future. Acknowledgements: This project received funding from European Union's Horizon 2020 Research and Innovation programme under the Grant Agreement No. 635408 (PARAGONE). Authors were funded by ACIISI, FULP, "La Caixa" Bank, "Universidad de Las Palmas de Gran Canaria" and "Cabildo Insular de Gran Canaria".

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Effect of anthelmintic treatment on milk yield in dairy flocks with different levels of infection by gastrointestinal nematodes

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The aim of this study was to determine the effect of anthelmintic treatment on milk yield in dairy flocks with different levels of infection by gastrointestinal nematodes (GIN).

Eight sheep flocks naturally infected by GIN, 4 with low level of eggs per gram (epg) before partum (LL) (< 150 epg) and 4 with high level of infection (HL) (> 400 epg), were included in this study. Faecal egg count reduction test (FECRT) was carried out in all flocks around 3-4 weeks before the partum. Two groups of 10 ewes each were constituted, the treated group administered with netobimin (Hapasil®), and the untreated group. Milk yield were measured individually and monthly in these 20 ewes at month 2, 3, 4 and 5 of lactation.

All flocks resulted susceptible to the netobimin treatment. Ewes belonging to LL flocks produced 125 kg more milk than ewes with high level of infection (55.4%). A significant and negative correlation was found between the mean epg before treatment and the mean milk yield per flock ($r = -0.860$; $p < 0.01$). In relation to the effect of anthelmintic treatment on milk yield, in LL flocks, the treated ewes produced 9.6% more milk than the untreated ewes, however, in HL flocks, treated ewes produced less milk than the untreated animals (-1.8%).

In conclusion, the treatment of flocks even with low level of infection can improve the milk yields. The lack of treatment effect in HL flocks could be due to the fact that ewes continued grazing on the same pastures after treatment, resulting in high levels of infection during lactation.

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Macrocyclic lactone resistance in helminths of cattle: blame it to the ticks

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Helminth control is performed in cattle two or three times a year, using mostly systemic macrocyclic lactone based-products. In Brazil, cattle raising is divided in *Bos indicus* (Red Angus, Brangus, Charolais, Hereford), found in the South and *B. taurus* (Nelore, Brahma, Gir, Guzera), distributed in the Central East and North of the country. Pure breed and their crosses are affected by multi-species parasite infections. The objective of this study was to evaluate the data from the literature regarding drug resistance in helminths of beef cattle (12 articles) and to correlate to parasite control practices based on a questionnaire to 32 farmers. The reports refer that there was a multi-drug (macrolactones, benzimidazols, levamisole), and multi-species (*Trichostrongylus* sp., *Haemonchus* spp., *Cooperia* spp., *Ostertagia* sp., *Dictyocaulus viviparus*) resistance. All farmers rotate the products after every treatment, the animals were treated monthly or biweekly, all treatments were based on visual evaluation (body condition score, tick counts), and 94% of the farmers treat all animals. All farmers use ivermectin, abamectin, doramectin or moxidectin in different concentrations in more than 80% of the time to control endo- and ectoparasites. Most of the studies (10/12) have reported nematode resistance to macrolactones (no refugia). The most important factor for parasite selection was the high level of drug exposure to cattle-tick (*Rhipicephalus microplus*) and horn-fly (*Haematobia irritans*) control. Horn-fly control is higher during the summer. Therefore, the selection of helminth populations in cattle is secondary to the suppressive use to control ticks and to a less extent to horn-flies.

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Bulk tank milk *Ostertagia* ELISA as a tool for quantification of milk production losses in dairy herds : where do we stand ?

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Based on the results obtained over the last 15 years, the aim of this presentation is to summarize current knowledge on the reliability of bulk tank milk (BTM) *Ostertagia* ELISA (expressed as ODR) as a guide for targeted treatment against gastrointestinal nematodes (GIN).

Preliminary work on this BTM ELISA showed significant statistical associations between high ODRs and reduced average milk production (MP). This BTM ODR was therefore seen as a promising tool to quantify MP losses. However, those statistical associations did not demonstrate a causal link between GIN infection and reduced MP. Further work assessed the relationship between this BTM ODR and the MP response after anthelmintic treatment. However, while some studies showed a trend towards a better MP response in high-ODR herds, others reported varying results. Additional results suggested to interpret cautiously this ODR taking into account the proportion of grazed grass in the diet and the Time of Effective Contact (TEC) with GIN of heifers. Furthermore, these studies were often based on a single ODR value measured at a given time, whereas the BTM ODR value can vary over time. In order to investigate whether combining several ODR values measured at different dates could allow to predict more reliably the MP response, 14 ways of taking multiple ODR values (with 4 thresholds) into account were tested. None of these combinations was shown to be effective in predicting the post-treatment MP response. The BTM *Ostertagia* ODR cannot alone reliably quantify MP losses due to GIN infection in dairy herds.

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Ovicidal potential of essential oils of *Juniperus communis* and *Achillea millefolium* to control gastrointestinal nematodes in sheep

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The worldwide increased difficulty to contrast gastrointestinal nematode (GIN) infections in sheep, due to growing anthelmintic resistance, has led to find alternative helminth control strategies. This study aimed to evaluate the *in vitro* ovicidal activity of essential oils (EOs) of *Juniperus communis* and *Achillea millefolium* (two chemotypes) against GINs of sheep. For this purpose, the nematode eggs were collected from naturally infected sheep by GINs in two farms located in Southern Italy. The egg hatch test (EHT) was performed at six different concentrations (50, 12.5, 3.125, 0.781, 0.195 and 0.049 mg/mL) for each EO and compared to the positive control (thiabendazole, 0.025 mg/mL) and the negative control (Tween 80 3%, v/v). In both farms, coprocultures revealed the presence of four genera of GINs: *Haemonchus*, *Trichostrongylus*, *Teladorsagia* and *Chabertia*. The inhibitory effect on GIN eggs' hatchability varied from 81% to 96.75% for *J. communis* EO. When using *A. millefolium* EO, the ovicidal activity against GIN was 46.5-99.5% (type 1) and 69.6-97.25% (type 2). Furthermore, the effect of tested EOs was very high at concentrations of 50 mg/mL (96.75%), 12.5 mg/mL (95.5%) and 3.125 mg/mL (94.75%) of *J. communis* EO, whilst for *A. millefolium* EO at concentrations of 50 mg/mL (99.5%), 12.5 mg/mL (98.0%) and 3.125 (95.25%) (type 1), and 50 mg/mL (97.25%) and 12.5 mg/mL (90.0%) (type 2), showing a similar effect ($p > 0.05$) to the positive control (98%). Therefore, the findings of this study showed the anthelmintic potential of the tested EOs and highlight the importance of ethnopharmacology to control GIN infection in sheep.

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Barriers and incentives for uptake of diagnostics for sustainable worm control by European dairy cattle farmers

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To mitigate emerging anthelmintic resistance in cattle, sustainable worm control strategies should be adopted, such as the use of diagnostic methods to take informed treatment decisions. To understand the factors affecting European farmers' intention to adopt diagnostic methods and to gauge for differences between European regions, a theoretical framework, based on theories in the field of behavioural psychology and health psychology, was validated through a questionnaire survey, carried out in dairy farms in 6 European countries. Despite insufficient data from 2 countries and several confounders prohibiting statistical comparisons between countries, three models were developed, which allowed to describe general trends and differences between regions. Data from Northern- (Norway) and Central-Europe (Austria and Germany) resulted in two similar models, which differed in 3 questions. Southern Europe's (Italy) data were more difficult to align with the conceptual model and several factors were deleted to fit the model. In all models, subjective norms, affected by a bandwagon bias, had the largest influence on farmers' adoption intention. Perception of anthelmintic resistance had no effect on adoption intention for Northern and Central, but to some extent for Southern Europe. In Northern and Central Europe, attitude towards anthelmintics had a negative effect on adoption intention, while attitude towards diagnostics had a positive effect. Both types of attitudes were affected by the default bias. Finally, by combining self-reported practices for Northern and Central Europe, a larger effect of descriptive norms on farmers' actual adoption of diagnostics was observed, compared to adoption intention and the default bias.

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Associations between *Dictyocaulus viviparus* bulk tank milk antibody levels and farmer-reported lungworm outbreaks, production losses and risk factors in grazing dairy cattle.

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Bulk tank milk (BTM) antibody levels against *Dictyocaulus viviparus* were used in two consecutive grazing seasons (2018 and 2019) to (1) investigate associations with farmer-reported lungworm outbreaks in dairy cows; (2) assess the impact of (sub)clinical lungworm infections on cow productivity and (3) identify risk factors for lungworm infections. A strong association was found between the occurrence of an outbreak and two consecutive positive BTM results (Odds ratio (OR) = 2.8 - 5.5), or a single positive BTM result in August (OR = 2.8 - 4.4) or October (OR = 1.8 - 3.7). In both years, there was a difference (NS) in the annual mean milk yield between farms with at least one BTM sample above the cut-off of 0.41 ODR and farms that stayed below this threshold (-0.17 and -0.70 kg milk/cow/day in 2018 and 2019, respectively). In 2019, this association was stronger when the cut-off was exceeded in at least two consecutive BTM samples (-1.74 kg milk/cow/day). BTM results were also significantly negatively associated with the closest milk production data during the two-weekly BTM sampling intervals in 2019. Purchase of new animals and the proportion of the first grazing season covered by anthelmintic treatments were positively associated with milk yield losses, while mowing of the pastures was negatively associated with reduced milk production. A positive BTM ODR could potentially have a predictive value for the occurrence of lungworm disease outbreaks and for lungworm induced production losses. Based on the risk factors, adjustments of the farm management could mitigate these losses.

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Influence of *Chryseobacterium nematophagum* on first stage larvae of *Haemonchus contortus* in micro plots and on naturally occurring free-living nematodes present on field-derived grass samples

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New solutions are needed to control of parasites, as roundworms are becoming increasingly resistant to anthelmintics and threatening livestock farming. Worm-killing bacteria, *Chryseobacterium nematophagum*, have been identified that rapidly destroy various species of parasitic ruminant worms grown *in vitro* on agar plates, but their effects on environmental free-living stages of parasitic nematodes are unknown.

The purpose of this study was to determine the effect of *C. nematophagum* bacteria on first-stage larvae of *Haemonchus contortus* (L1) in micro plots and on naturally occurring free-living nematodes present on grass samples. We have added bacteria *Chryseobacterium nematophagum* to grass grown in micro plots and L1 larvae *Haemonchus contortus*, and the same to mown grass samples with free-living nematodes.

Samples were incubated for 24, 42, or 72 hours, and then L1 was utilized using standard pasture larval counting techniques or the Baermann method. The number of L1 present in each sample was counted and their condition assessed.

Larvae obtained from micro plots exposed to *C. nematophagum* showed clear signs of damage within 24 hours. Larvae obtained from soil appear to show the effect of the dose of *C. nematophagum* used, with lesions occurring earlier in nematodes with higher doses of bacteria, whereas no clear dose effect was observed in grass samples. The number of free-living stages of parasitic nematodes obtained from grass samples exposed to *C. nematophagum* was decreased.

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Parasite epidemiology and sustainable management in Northern semi-arid climatic zones

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The prevalence of gastrointestinal nematodes (GIN) in northern semi-arid climatic zones is not as well understood as the other climatic regions which hinders the development of sustainable grazing management. This presentation introduces two studies on GIN epidemiology in western Canada and the results of these studies were interpreted into empirical support on sustainable grazing strategies.

For both studies, environmental samples (e.g. grass and fecal samples) were collected at certain time points followed by larval enumeration. Detailed meteorological data at farm level were recorded by solar powered weather stations and nemabiome metabarcoding was used to determine the GIN species composition. The first study examined the overwintering capability of bovine trichostrongylid third stage larvae (L3) on western Canadian pastures. Environmental samples were collected from the same pasture before and after winter. L3 overwintering was further investigated using parasite-free tracer calves. The results showed relatively efficient overwintering of *O. ostertagi* and *C. oncophora* L3 and their further establishment of spring infection in grazing animals. The second study investigated the epidemiology of bovine GIN throughout the grazing season in nine independent pastures. We found on most pastures, L3 could not be recovered until 6th week after fecal deposition and L3 count peaked at the 9th week after fecal deposition.

These two studies, in combination, cover the epidemiology of GIN for the whole calendar year. The results can be used as regional-level basic advice before any farm-specific management. Therefore, two empirical grazing strategies were developed according to different number of available grazing pastures.

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Survey on the knowledge and practices of sheep producers' associations towards the control of helminth infections

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Sheep production has been an ancestral tradition in Portugal and currently sheep population is about 2.2 million in approximately 52,000 farms, mainly for meat production. Gastrointestinal nematode infections are a constraint in meat and dairy herds and cause a decrease in animal health, productivity and farm profitability. In order to assess current deworming practices and improve our knowledge on anthelmintic resistance (AHR), a questionnaire was sent to 105 Livestock Producers' Organizations (LPO). This questionnaire included 15 questions concerning: 1) general information about sheep herds (such as size, breeds and geographical location); 2) deworming practices (including deworming criteria, anthelmintics usage and dosage and pasture management); 3) general knowledge about the development of AHR. This questionnaire was carried out by telephone and information from 21 LPO from 5 NUTSII regions covering 25,524 farms was collected. Deworming was performed once a year in most farms (61.9%) and Ivermectin was the substance most frequently used (71.4% of herds). Dosage calculation *per* animal is made by estimating the average weight through visual observation in two thirds of the farms. Pasture rotation was mentioned as the most common control method for helminth infections. All veterinary practitioners surveyed said to be aware of anthelmintic resistance and 42.9% already doubted under some circumstances of anthelmintics efficacy. More than half never had recommended a Fecal Egg Count Reduction Test. These results allowed a preliminary assessment on the knowledge, attitudes and practices of small ruminant veterinarians concerning anthelmintic resistance.

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The prevalence of anthelmintic resistance on Lithuanian sheep farms

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Introduction: Anthelmintic resistance (AR) of gastrointestinal nematodes (GIN) of sheep is the main problem worldwide, including Lithuania, where the number of sheep was increasing the last 15 years. The intensive and incorrect use of anthelmintics led to the development of AR. The aim of the study was to evaluate the prevalence of AR on Lithuanian sheep farms by using in vitro tests: egg hatch test (EHT) and micro-agar larval development tests (MALDT).

Materials and methods: During 2019-2020 the study was carried out in 35 sheep farms from all over Lithuania territory. In vitro tests were used to detect AR. Data were analysed using a threshold discriminating concentration of 0.1 µg/ml-1 for thiabendazole in EHT and 0,04 µg/ml-1 for thiabendazole, 21.6 ng/ml-1 for ivermectin-aglycone, 2 µg/ml-1 for levamisole in MALDT.

Results: The resistance to thiabendazole by using EHT was indicated in 31 farms (88.6%), while MALDT - 12 (34.3%). Resistance to ivermectin-aglycone was indicated in 14 farms (40.0%). Resistance to levamisole was not indicated in Lithuanian sheep farms. *Trichostrongylus* spp. were the most prevalent GIN, which developed in the highest drugs concentrations.

Discussion and conclusion: AR of GIN is common in Lithuanian sheep farms. Based on EHT the most prevalent is resistance to thiabendazole whereas according to MALDT - ivermectin. These anthelmintics are the most commonly used in sheep farms in Lithuania.

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***In vivo* anthelmintic efficacy of aqueous *Dryopteris filix-max* and *Punica granatum* macerates against gastrointestinal nematodes of sheep**

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Gastrointestinal nematodes (GINs) are amongst the most important ruminant production-limiting pathogens with negative impact also on animal welfare and health, aggravated by increasing anthelmintic resistance (AR). Emergence of AR requires alternative remedies for helminth control, mainly from plants. The present study aimed to evaluate the *in vivo* anthelmintic activity of aqueous macerates based on *Dryopteris filix-max* plant (AE1), fruit and peel of *Punica granatum* (AE2) against GINs in sheep. This study was conducted in a farm located in the Calabria region (southern Italy). A total of 45 sheep naturally infected by GINs were selected and divided into 3 groups of 15 animals each: TG1 group and TG2 group were treated orally with 50 ml of AE1 and AE2, respectively; CG group was untreated. Individual faecal samples were collected rectally on the day of treatment (D0) and after 7, 14 and 21 days (D7, D14 and D21) to evaluate Faecal Egg Count Reductions (FECR) using the FLOTAC techniques. The formula used to evaluate the anthelmintic efficacy was $FECR = 100 \times (1 - [FEC-TG/FEC-CG])$. The results of FECR (%) for each treated group at the different days were: **TG1-** D7 (2.7%), D14 (10.3%), D21 (3.1%); **TG2-** D7 (56.2 %); D14 (54.1%); D21 (45.7%). The *in vivo* results showed an anthelmintic ineffectiveness of *Dryopteris filix-max* and a low anthelmintic efficacy of *Punica granatum*. Despite this, the use of aqueous macerates based on fruit and peel of *Punica granatum* with FECR values above 50% could be an alternative or complementary to drugs for GIN control in sheep, thus reducing the use of drugs to an acceptable threshold.

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Anthelmintic resistance of horse strongyle nematodes to ivermectin and pyrantel in Lithuania

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With intensive use of anthelmintic drugs over the past decades, anthelmintic resistance (AR) in horse nematodes is becoming a growing issue in many countries. However, little information is available 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 AR on horse farms in Lithuania. The study was conducted in 25 stables. On each farm a faecal egg count reduction test (FECRT) was performed after deworming tests with ivermectin (IVM), (Bimectin 0,2 mg/kg, Ireland) and pyrantel (PYR), (Embotape 19 mg/kg, Ireland).

This study showed that strongyle infections were highly prevalent in horses residing in stud farms in Lithuania, with high infection intensities according to an over dispersed distribution pattern. Of all tested subjects (n=707) 93% exceeded the value of 200 strongyle EPG, which is considered the cut-off limit with in the new equine strongyle control strategy based on targeted selective treatment. The efficacy of IVM was comparatively high: 98.8% of 250 horses had a zero egg count 14 days after treatment. Resistance to PYR was present on five stables (20% of all tested herds). Egg count had a significant ($p<0.01$) difference between treatment and control groups.

This *in vivo* study showed that horse farms in Lithuania already had problems with AR, with resistance to pyrantel. Our findings should guide the implementation of more sustainable management of strongyles infestation among horses in Lithuania.

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The Path of Anthelmintic Control Face to the New CAP 2020-2027

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The European Parliament following the Treaty of Lisbon finally approve the new Common Agricultural Policy (CAP) based on the proposed Multiannual Financial Framework (MFF) for the period 2021-2027. Therefore, the final negotiations can now be started with the Council of the European Union (EU) to reach a final understanding new CAP. The present CAP agreements until reached reinforce the main principle of subsidiarity among the Member States (MS) of European Union (EU) and, on the other hand, they reducing the main general CAP policies and MFF 2021-2027 due the Brexit and correspondent reducing of expenses to financing the European budget. In addition, in line with the new MFF architecture for a more flexible and agile financing framework, food chain measures will be integrated in other budget priorities such as research, innovation and digital policies (Horizon Europe, Digital Europe). Those instruments, as well as the European Social Fund Plus (ESF+), will also cover health-related actions, such as measures against antimicrobial resistance, in the context of the 'One Health' approach. Moreover, food safety, animal welfare, fight against food waste or sustainable use of pesticides are part of the new Common Agricultural Policy objectives.

Nowadays the antihelminth control due the resistance phenomena require an increase in costs to fight this problem among the MS.

The main aim of the paper is analyzing the main challenges of combat the antihelminth due the restrictions of European budget. The methodology is based on a systematic literature review. The main results outline confirms the need of increasing research and strong network efficiency as occurs with COMBAR.

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Investigation on triclabendazole resistance of *Fasciola hepatica* in German sheep flocks

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The trematode *Fasciola hepatica* leads to severe production losses in livestock when not treated effectively, especially in sheep. Triclabendazole has been a frequently used drug in the last decades due to its unique efficacy range against all fluke stages. During recent years, there have been numerous cases of confirmed resistance worldwide. However, there is a lack of published data about the susceptibility of *F. hepatica* to flukicides in Germany.

The project objective is to evaluate the efficacy of triclabendazole against *F. hepatica* in German sheep flocks. We will investigate if it is still effective to treat fasciolosis successfully or if there is no reduction of egg shedding and copro-antigen-levels in the field as reported in other countries.

Individual faecal samples will be collected from 15 sheep farms in Northern Germany to evaluate the faecal egg count reduction using the sedimentation method and to determine the copro-antigen-levels with the commercial BIO K 201-Antigen-ELISA-kit before and 14 days after triclabendazole treatment. Additionally, an egg hatch assay is planned to investigate the ovicidal effect of albendazole on *F. hepatica* eggs collected from these flocks.

There is a need to identify resistant markers of *F. hepatica* to triclabendazole as the resistance mechanisms are still unclear. We will search for putative resistance markers in surviving *F. hepatica* after triclabendazole treatment by next generation sequencing, to identify genomic regions associated with triclabendazole resistance.

Funding statement: This study is co-funded by the Federal Office of Consumer Protection and Food Safety, Germany.

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The kubic flotac microscope: a new tool for faecal egg count of gastrointestinal nematodes

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The Kubic FLOTAC Microscope (KFM) is a compact, low-cost and portable digital microscope designed to analyse faecal specimens prepared with the Mini-FLOTAC/FLOTAC in both field and laboratory settings. The KFM combines the high sensitivity, accuracy and precision of the Mini-FLOTAC/FLOTAC techniques with a reliable system to capture and analyse pictures. In this study we performed a first validation of the KFM on faecal egg count (FEC) of gastrointestinal nematodes (GINs) in cattle. Thirty faecal samples were collected from cattle experimentally infected by GIN and analysed at the Faculty of Veterinary Medicine, Ghent University, Belgium, using the Mini-FLOTAC technique. Then, a comparison was performed between a traditional optical microscope (OM) and the KFM. The results showed a substantial agreement (CCC = 0.999) with a low discrepancy (-0.425 ± 7.370) between the GIN counts obtained. Moreover, captured images by KFM showed a quality comparable with the view provided by the OM. This is very important for the real-time identification, quantification of parasitic structures and for the Artificial Intelligence software development, that is actually in progress. Moreover, these pictures can be transferred via internet to other laboratories for a remote diagnosis as expected by Tele-Parasitology. Therefore, the KFM is a promising automated system for an accurate and efficient FEC to improve parasite diagnosis and to assist a new generation of operators in veterinary and public health.

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In vitro* anthelmintic activity of *Kyllinga odorata* Valh (Cyperaceae) using the Larval Exsheathment Inhibition Assay (LEIA) and Eggs Hatch Assay (EHA) on *Haemonchus contortus* and *Trichostrongylus colubriformis

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The increasing development of resistance to anthelmintics (AH) has prompted the search for alternatives to control gastrointestinal nematodes (GINs). The evaluation of plants used in traditional medicine is one of these options. *K. odorata* was mentioned in a survey in Paraguay as AH in humans and animals among ethnobotanical and ethnoveterinary criteria. The aims of the current study were: 1. To evaluate the AH activity of methanolic extracts of *K. odorata* against *H. contortus* (Hc) and *T. colubriformis* (Tc) *in vitro* LEIA and EHA assays were used, 2. To determine the role of tannins/polyphenols in the AH effects, the inhibitor polyvinylpolypyrrolidone (PVPP) was used. The concentration required to inhibit 50% (EC50) for both assays were calculated. The EC50 values for LEIA were 565.06 µg/ml (Hc), and 610.61 µg/ml (Tc) and for EHA 176.99 µg/ml (Hc) and 143.82 µg/ml (Tc). Incubation with PVPP for both EHA and LEIA has produced a partial restauration towards control values in the inhibitory response for the 2 GIN species. These *in vitro* results confirmed the potential use of *K. odorata* as an alternative to control GINs. The effects were dose dependent in both species. The results in PVPP also suggest that tannins are not the sole class of metabolites responsible for the AH effects, which open new investigations to determine the role of other metabolites. As far as we know, it is the first work to evaluate the anthelmintic action of this plant, widely used in traditional medicine in South America.

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Sustainable agriculture: Use of FAMACHA method in Santa Ines sheep in the Semi-arid region of Brazil

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Sheep farming is of great importance in generating income to smallholders in semi-arid areas worldwide. Gastrointestinal helminths cause important damage to the animals reflecting in poor welfare conditions. The objective of this work was to evaluate the FAMACHA method (FMC) in a herd of 60 Santa Inês from Paraíba, Brazil, and compare the data to previous production years. In order to determine anemic sheep, the FMC chart was used biweekly for 12 months. Blood and fecal samples were collected monthly to determine packed cell volume (PCV), and parasite fecal egg count (FEC), respectively. The animals were divided into: dry, pregnant and lactating ewes, lambs and rams. *Haemonchus* was the predominant helminth genus (94.7%) during the year. There were up to 97% of success on the FMC interpretation with 90 to 100% efficacy in the treatment decision. Among the 1356 FMC evaluations, in only 40 (3%) animals were required to be dewormed, being 27 during the rainy season and 13 during the long dry season. A statistically significant correlation ($P < 0.01$) was observed between FEC and PCV for the dry, lactating, and pregnant categories. The data reports a 94% reduction in deworming with a greater need for anthelmintic use in lambs and lactating ewes ($P < 0.05$). Our data validates the use of FMC in Santa Inês sheep, which is considered a local resilient breed in the semi-arid region of Brazil. Therefore, we recommend that selective evaluations should be directed during the rainy season to prevent clinical problems.

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Experimental haemonchosis in lambs: effect of medicinal herbs on histopathology

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The purpose of my STSM at Warsaw University of Life Science was to evaluate the effect of medicinal plants on abomasum tissue, and mucosal immunity of *Haemonchus contortus* infected lambs. During my stay, I learned how to prepare samples for histopathological analysis and how to evaluate histopathological changes of tissue. Abomasum tissues were obtained in the experiment in Košice, in which twenty-four lambs were divided into four experimental groups: infected animals (UNS), infected animals supplemented with *Artemisia absinthium* (ART), infected animals supplemented with *Malva sylvestris* (MAL), and infected animals supplemented with a mix of *A. absinthium* and *M. sylvestris* (ARTMAL). Lambs were infected with 5000 L3 larvae of *H. contortus*. The plants were mixed daily with the oats during the experiment. All animals were humanely killed after 75 days post-infection, and histological dissection was performed. Histopathological changes were detected in the mucosal membrane of the abomasum, with the presence of inflammatory cells infiltrates in MAL, ART, and ARTMAL. Increase mucus production was observed in the ARTMAL. The number of mast cells was significantly higher in UNS and ART than MAL ($P < 0.01$ and $P < 0.05$). Plasma cell numbers were higher in ARTMAL than MAL ($P < 0.05$). Abomasal tissue regenerated more frequently in ARTMAL than the other experimental groups. Treatment with the medicinal plants helped trigger a local immune response in tissues. Dietary supplementation with medicinal plants may increase the resistance of lambs to infection with *H. contortus*. The STSM was supported by COST COMBAR Action number CA16230.

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Biochemical profiling of digestive proteolytic enzymes from *Haemonchus contortus*

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Haemonchus contortus is one of the most pathogenic gastrointestinal (GI) nematodes of ruminants worldwide. Severe infections caused by this parasite negatively affect animal health and welfare with result in economic impact on grazing livestock productivity. The control of GI nematode infections in livestock is traditionally based on the use of anthelmintic drugs. However, anthelmintic resistance in GI nematodes has evolved within the past decades as a result of frequent and inappropriate use of anthelmintics. Thus, alternative approaches to control GI nematode infection are needed. Both, the fourth-stage larvae (L4) and adults of *H. contortus*, are known for their blood-feeding activities. Host blood protein digestion is an essential process for parasite growth, development, and reproduction. This study is focused on the proteolytic digestive system of L4s as a promising target for the development of novel parasite control strategies. We performed activity profiling of major proteolytic enzymes in the L4s extract using selective peptide substrates and inhibitors. A network of proteases with different specificities was identified, which efficiently degrades ingested blood proteins. Such an approach may help to identify targets for novel anthelmintics based on protease inhibitors.

Acknowledgements: This research is supported by the Ministry of Education, Youth and Sport of the Czech Republic (INTER-ACTION LTAUSA19109).

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The use of High Resolution Melting analysis for reliable differentiation of trichostrongylid nematodes *Haemonchus contortus* and *Ashworthius sidemi*

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Infection of ruminants by helminth parasites, especially gastrointestinal nematodes, has worldwide a considerable economic and social impact. From this perspective, correct taxonomical identification is a key prerequisite of initiation of appropriate measures. However, differentiation of some nematode species according to morphological features is complicated, e.g. in the case of important haematophagous abomasal parasites, *Haemonchus contortus* and *Ashworthius sidemi*.

In this study, we employed qPCR followed by High Resolution Melting (HRM) analysis as an advanced molecular method. This approach enables to detect sequence alterations by increasing temperature. Differences in melting profiles can be visualised as the fluorescence of saturating dye that is gradually disassociated from the dsDNA amplicons. As an output, a difference plot representing the most transparent expression of specific matrix curves were used.

The aim of this study was to develop a fast and usable qPCR-HRM assay, which uses polymorphisms in internal transcribed spacer 1 (ITS-1) to distinguish both nematodes without the necessity of additional confirmation by electrophoretic separation and/or sequencing.

Based on the specific melting curves, we identified a total of 45 specimens of adult nematodes of *H. contortus* and *A. sidemi* that came from a wide range of domestic and wild ruminant hosts that occur in the Czech Republic. We also confirmed that qPCR-HRM analysis is applicable for determination of the infective larval stages of the nematodes, which promises a significant improvement in intravital diagnostics.

Acknowledgements: This research is supported by Ministry of Education, Youth and Sport of the Czech Republic (LTC19018) and EU COST Action (CA16230).

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Analysis of blood parameters in sheep provided spores of parasiticide fungi

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With the aim to establish the possible influence of giving frequently spores of parasiticide fungi on the health of grazing sheep, blood samples were collected by venipuncture over a two-years period and analyzed by means of a coulter-counter. Between 2018 and 2019, two groups of twelve sheep under a grazing management in Guipuzcoa (Sakona Farm, Azpeitia, Basque Country, Spain) were provided twice a week a dosage of 10^6 spores of both *Mucor circinelloides* and *Duddingtonia flagrans* / ovine. At the beginning of each season (Autumn), anthelmintic treatment was administered to all sheep.

Blood analyses showed normal (physiological) counts of leukocytes during the study, with values higher than normal for the percentages of lymphocytes. Physiological values were recorded for lymphocytes, granulocytes and monocytes during the intake of fungal spores. The examination of red blood parameters revealed the absence of abnormal values for the counts of red cells, hemoglobin, hematocrit, mean corpuscular volume, mean hemoglobin concentration and mean corpuscular hemoglobin concentration, and counts out of normal were seldom observed. Based on the lack of significant alterations on the blood parameters in sheep grazing and receiving spores of parasiticide fungi, it is concluded this is a healthy and innocuous procedure.

Partially supported by the research projects RYC-2016-21407 (Ministry of Economy and Competitiveness, Spain) and ED431F 2018/ 03 (Consellería de Cultura, Educación e Ordenación Universitaria, Xunta de Galicia, Spain).

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