

2nd COMBAR Working Groups Meeting

Anthelmintic Resistance: Past, Present and Future

26-27th September, León, Spain

Hotel Real Colegiata de San Isidoro



**universidad
de león**



2nd COMBAR Working Groups meeting, 26-27th September, León 2018, Spain

Hotel Real Colegiata de San Isidoro

PROGRAMME

COMBAR Management Committee meeting

25th September

17:30	19:30	Management Committee Meeting
19:30	20:30	Guided Tour of the <i>Museum of San Isidoro</i> Meeting point: Hotel Real Colegiata de San Isidoro. Plaza de Santo Martino, 5 24003 León.
21:00	23:00	Dinner, Local Leonese Cuisine. Restaurante Ezequiel. Calle Ancha, 20, 24003 León.

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PROGRAMME

COMBAR Working Groups meeting "Anthelmintic resistance: past, present and future"

26th September

8:30 9:00 Registration

9:00 9:10 Welcome, Dr. María Martínez-Valladares

9:10 9:50 **Plenary Session, Dr. Gerald Coles**

Anthelmintic resistance, past, present and future.

9:50 10:05 **Introduction Working Group 1. Dr. Laura Rinaldi and Dr. Georg von Samson-Himmelstjerna**

10:05 10:35 **Guest Speaker, Dr. John Gilleard**

Application of sequencing technologies for the detection and investigation of anthelmintic resistance.

10:35 11:05 **Oral Presentations**

Dr. J. Höglund. Droplet Digital PCR - a novel diagnostic tool in nematodes of veterinary interest.

Dr. M. Kašný. From genes to diagnostics of fasciolid flukes.

11:05 11:35 Coffee Break and Poster session

11:35 12:05 **Guest Speaker, Dr. Laura Peachey**

Novel options for worm control; exploiting helminth-microbiota interactions in gastrointestinal helminth infections of livestock.

12:05 13:20 **Oral Presentations**

Dr. B. Hinney. High frequency of benzimidazole resistance alleles in *Haemonchus contortus* and *Teladorsagia circumcincta* from sheep flocks in Austria

Dr. P. Halvarsson. Meta-community analysis reveal new insights to ovine parasite diversity.

Dr. M. Martínez-Valladares. Evaluation of the Egg Hatch Assay as diagnostic method to determine the resistance in gastrointestinal nematode of sheep.

Dr. V. Marián. Does in vitro egg hatch test predict the clinical failure of benzimidazole treatment?.

Dr. I. Stojanov. The number of endoparasite eggs in different parts of the sheep intestinal tract.

13:20 13:35 **General Discussion, Working Group 1**

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13:35 14:45 Lunch

14:45 15:00 **Introduction Working Group 3. Dr. Eric Morgan and Dr. Hervé Hoste**

15:00 15:30 **Guest Speaker, Dr. Carlos Lanusse**

Some pharmacological issues contributing to extend the lifespan of traditional and novel anthelmintic drugs.

15:30 16:15 **Oral Presentations**

Dr. C. Canton. Nematocidal drug combinations: a valid strategy for parasite control in cattle?.

Dr. N. Ravinet. Targeted-selective treatment of dairy cows against gastrointestinal nematodes: a stepwise decision making strategy

Dr. S. Sotiraki. Efficacy of tannin rich feed against multi resistant *Haemonchus contortus* and interactions with ivermectin subcutaneous treatment in sheep on farm conditions.

16:15 16:45 Coffee Break and Poster session

16:45 17:15 **Guest Speaker, Dr. Jan Felipe Torres Acosta**

Are we ready to enter a post-anthelmintic era?!

17:15 18:15 **Oral Presentations**

M. Vilá Pena. Fungal spores to develop the integrated control of horse strongyles.

Dr. J. F. González. The Canaria Hair Breed sheep: a resistant breed to gastrointestinal nematodes.

Dr. M. Kesik-Brodacka. Immune response of rats, calves and lambs vaccinated with various forms of *F. hepatica* cysteine proteinase when challenged with *F. hepatica* metacercariae.

Dr. T. V. A. Hansen. Functional expression of *Trichuris suis* acr-16 in *Xenopus laevis* oocytes – a pharmacological screening tool for the anthelmintic effect of synthetic and natural compounds on nicotinic acetylcholine receptor in parasitic nematodes.

18:15 18:30 **General Discussion, Working Group 3**

19:30 21:00 Guided Tour of the Old Town.

Meeting point: Hotel Real Colegiata de San Isidoro. Plaza de Santo Martino, 5 24003 León.

21:00 23:30 Dinner at the Hotel Real Colegiata de San Isidoro.

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COMBAR Working Groups meeting "Anthelmintic resistance: past, present and future"

27th September

9:00 9:15 **Introduction Working Group 2. Dr. Edwin Claerebout and Dr. Fiona Kenyon**

9:15 9:45 **Guest Speaker, Dr. Jan Van Wyk**

We 're all here because we 're not all there!.

9:45 10:15 **Guest Speaker, Dr. Andy Greer**

Targeted Selective Treatments: Lessons learnt and future considerations.

10:15 11:00 **Oral Presentations**

Dr. C. Milne. Understanding the resistance of farmers.

Dr. B. Sekovska. Farmers and veterinary practitioners focus group experience regarding vector borne disease outbreak in Macedonia.

Dr. O.M. Dourado Martins. Contribution of Social Marketing to change the behaviour of veterinarians and farmers in worm control.

11:00 11:30 Coffee Break and Poster session

11:30 12:00 **Guest Speaker, Dr. Rens van Dobbenburg**

EPRUMA view on responsible use of anthelmintics.

12:00 13:00 **Oral Presentations**

Dr. J. Ježek. Experiences with questionnaire survey and faecal sampling in small ruminant flocks.

M. De Alcubierre. Monitoring the efficacy of anthelmintics in ruminants in southern Italy.

Dr. H. Hertzberg. Impact of transfer of goats to alpine pastures on the dynamics of infections with gastrointestinal nematodes.

Dr. T. Letra Mateus. Practices on helminth control in organic cattle from northern Portugal and Spain

13:00 13:15 **General Discussion, Working Group 2**

13:15 13:30 Conclusion of the Conference and Take Home Messages, Dr. Johannes Charlier

13:30 14:30 Lunch

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COMMUNICATIONS

Guest Speakers - Oral Communications

Presenter	Communication
Gerald Coles	Anthelmintic resistance, past, present and future.
John Gilleard	Application of sequencing technologies for the detection and investigation of anthelmintic resistance.
Laura Peachey	Novel options for worm control; exploiting helminth-microbiota interactions in gastrointestinal helminth infections of livestock.
Carlos Lanusse	Some pharmacological issues contributing to extend the lifespan of traditional and novel anthelmintic drugs.
Jan Felipe Torres Acosta	Are we ready to enter a post-anthelmintic era?!
Jan Van Wyk	We 're all here because we 're not all there!.
Andy Greer	Targeted Selective Treatments: Lessons learnt and future considerations.
Rens van Dobbenburg	EPRUMA view on responsible use of anthelmintics.

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COMMUNICATIONS

Oral Communications

Working Group	Presenter	Communication
1	J. Höglund	Droplet Digital PCR - a novel diagnostic tool in nematodes of veterinary interest.
1	M. Kašný	From genes to diagnostics of fasciolid flukes.
1	B. Hinney	High frequency of benzimidazole resistance alleles in <i>Haemonchus contortus</i> and <i>Teladorsagia circumcincta</i> from sheep flocks in Austria
1	P. Halvarsson	Meta-community analysis reveal new insights to ovine parasite diversity.
1	M. Martinez-Valladares	Evaluation of the Egg Hatch Assay as diagnostic method to determine the resistance in gastrointestinal nematode of sheep.
1	V. Marián	Does in vitro egg hatch test predict the clinical failure of benzimidazole treatment?.
1	I. Stojanov	The number of endoparasite eggs in different parts of the sheep intestinal tract.
2	C. Milne	Understanding the resistance of farmers.
2	B. Sekovska	Farmers and veterinary practitioners focus group experience regarding vector borne disease outbreak in Macedonia.
2	O.M. Dourado Martins	Contribution of Social Marketing to change the behaviour of veterinarians and farmers in worm control.
2	J. Ježek	Experiences with questionnaire survey and faecal sampling in small ruminant flocks.
2	M. De Alcubierra	Monitoring the efficacy of anthelmintics in ruminants in southern Italy.
2	H. Hertzberg	Impact of transfer of goats to alpine pastures on the dynamics of infections with gastrointestinal nematodes.
2	T. Letra Mateus	Practices on helminth control in organic cattle from northern Portugal and Spain.
3	C. Canton	Nematocidal drug combinations: a valid strategy for parasite control in cattle?.
3	N. Ravinet	Targeted-selective treatment of dairy cows against gastrointestinal nematodes: a stepwise decision making strategy.

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3	S. Sotiraki	Efficacy of tannin rich feed against multi resistant <i>Haemonchus contortus</i> and interactions with ivermectin subcutaneous treatment in sheep on farm conditions.
3	M. Vilá Pena	Fungal spores to develop the integrated control of horse strongyles.
3	J. F. González	The Canaria Hair Breed sheep: a resistant breed to gastrointestinal nematodes.
3	M. Kesik-Brodacka	Immune response of rats, calves and lambs vaccinated with various forms of <i>F. hepatica</i> cysteine proteinase when challenged with <i>F. hepatica</i> metacercariae.
3	T. V. A. Hansen	Functional expression of <i>Trichuris suis</i> acr-16 in <i>Xenopus laevis</i> oocytes – a pharmacological screening tool for the anthelmintic effect of synthetic and natural compounds on nicotinic acetylcholine receptor in parasitic nematodes.

COMMUNICATIONS

Poster Communications

Working Group	ID	Presenter	Communication
1	1	A. Amadesi	Mini-FLOTAC automated system for helminth egg counts in ruminants
1	2	M. Macrelli	First detection of ivermectin resistance in <i>Oesophagostomum dentatum</i> in pigs.
1	3	K. Alzbeta	A model transmission of gastrointestinal nematode <i>Haemonchus contortus</i> between domestic and wild ruminants under field conditions
1	4	M. Mickiewicz	Evaluation of levamisole effectiveness in two goat herds with previously detected resistance of gastrointestinal nematodes to ivermectin and benzimidazoles.
1	5	E. Devaney	The BUG Consortium- Using the <i>Haemonchus contortus</i> genome to identify mechanisms of anthelmintic resistance.
2	6	T. Letra Mateus	Who cares about helminths of backyard pigs in Portugal?.
2	7	T. Letra Mateus	Preliminary data suggest resistance to anthelmintics in sheep in northern Portugal.
2	8	M. Dos Santos	Towards Helminth Sustainable Control in Grazing Ruminants in Europe.
2	9	Z. Ruano	Practices on helminth control in an endangered autochthonous Portuguese sheep breed.
2	10	A.M. Duque de Araujo Munhoz	Seroprevalence of <i>Fasciola hepatica</i> in dairy cattle and management practices that may influence the onset of the disease on São Miguel Island, Azores – Portugal.
2	11	S. Petkevičius	The present status of anthelmintic resistance on sheep farms in Lithuania
3	12	H. Akkari	In vitro evidence that the pastoral <i>Artemisia campestris</i> species exerts an anthelmintic effect on <i>Haemonchus contortus</i> from sheep.
3	13	E. Valderas-García	Anthelmintic activity of amino alcohols against eggs and larvae of the ovine nematode <i>Teladorsagia circumcincta</i> .
3	14	E. Valderas-García	In vitro effect of benzaldehydes and phthalazinones against different eggs and larvae of <i>Teladorsagia circumcincta</i> .
3	15	E. Valderas-García	New synthetic benzimidazole with potential anthelmintic activity.
3	16	V. Castilla	Differences in the immune response between adult sheep belonging to Churra breed.

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3	17	H. Azaizeh	Seasonal variation in the effects of polyphenolic extracts of <i>Salix</i> sp (Willow) on the exsheathment of gastrointestinal nematode larvae.
3	18	S. Stuen	Toltrazuril resistance in a field isolate of ovine <i>Eimeria</i> spp.
3	19	J. Vadlejch	To feed or to develop? Evaluation of anthelmintic properties of six plant extracts using two in vitro tests.
3	20	C.F. Cazapal-Monteiro	How to reduce the risk of infection by <i>Trichuris</i> spp. in zoological parks? the answer is spreading spores of parasiticide fungi.
3	21	J. N. Hernández	Recombinant <i>Teladorsagia circumcincta</i> vaccine in native sheep breeds from the Canary Islands.
3	22	J.M. Molina Caballero	Local Immune Responses of Goats Immunized with Thiol-Proteinases Enriched Fractions from E/S products of <i>Teladorsagia circumcincta</i> Adult Worms
3	23	M.S. Arias Vázquez	Biological control as a solution against parasites affecting captive wild ruminants.
3	24	M. Voinot Meissner	Biological control of helminths affecting sheep in the Basque country.
3	25	J. Voller	High-throughput screening of anthelmintic drugs using <i>Caenorhabditis elegans</i> .
3	26	C. Chartier	Assessment of the potential of mid-season targeted selective anthelmintic treatment based on flexible weight gain threshold for nematode infection control in first-grazing season dairy calves.
3	27	B.M. Blomstrand	In vitro anthelmintic effects of bark extracts from <i>Picea abies</i> and <i>Pinus sylvestris</i> against sheep nematodes.
3	28	J. F. Torres-Acosta	In vivo nutraceutical evaluation of <i>Gymnopodium floribundum</i> leaf meal against <i>Haemonchus contortus</i> in lambs.
3	29	P.G. González Pech	Can we observe differences in feeding behavior between kids without browsing experience and goats with browsing experience on heterogeneous vegetation?.
3	30	S. Katerina	Antiparasitic activity of tannin-rich Mediterranean plants against sheep gastrointestinal nematodes: in-vitro studies.

Guest Speakers

Oral Communication

Anthelmintic Resistance, Past Present and Future

Coles G.

Ubley Biologics, P.O.Box 170, Ubley, Bristol BS40 6JA, UK

Past. Anthelmintic resistance has been made much more serious by the recommendations of veterinary parasitologists who encouraged dose and move to clean pasture and in Australia dosing during drought. Government officials who allowed farmers to buy and use anthelmintics without diagnosis has led to overdosing. Using ivermectin/moxidectin to control sheep scab has led to over use of one product type. Most farmers have failed to apply quarantine dosing. However, wild roe deer have been found with benzimidazole resistant *Haemonchus* and rabbits and deer can transmit *Fasciola* ensuring the spread of resistance.

Present. Diagnosis. Arrow Labs (UK) have image recognition software that can count nematode eggs and provide some speciation and a novel machine for preparing eggs for counting. Sociology. We need more information on how to persuade farmers to adopt recommendations to slow the development and spread of resistance. It is essential that all farms that have liver fluke know whether triclabendazole resistance is present or serious loss of animals can occur. If closantel resistance also develops, farms where acute fluke occurs may have to stop keeping sheep.

Future. Overcoming anthelmintic resistance: A) 'Farmers eye' a no cost method of practising TST? B) Breeding of resistant or resilient sheep and cattle. C) Modified natural products. D) Possible use of ozone in solution? E) Novel vaccines. Some bacterial vaccines are oligosaccharide based and nematodes are coated in a glycocalyx. So why not oligosaccharide vaccines? Patents suggest a liver fluke vaccine can be made from oligosaccharides.

Guest Speakers

Oral Communication

**Application of “Next-Gen” Sequencing Technologies for the Detection
and Investigation of Anthelmintic Resistance**

Gilleard J. S., Avramenko R., Redman E., Wit J., Queiroz C., Workentine M., Levy M.

Faculty of Veterinary Medicine, University of Calgary

There is a need for scalable and accurate diagnostic tools to detect the emergence of anthelmintic resistance particularly in its early stages. The so-called Next-Generation sequencing technologies have multiple applications in these areas including whole genome sequencing as a tool to help identify drug resistance mutations in parasite genomes to use as molecular diagnostic markers. However, the focus of this talk will be the application of deep amplicon sequencing approaches to detect and study the molecular epidemiology of anthelmintic resistance mutations in parasitic nematode populations. Over the last few years, we have developed an approach known as Nemabiome sequencing which targets regions of the rDNA cistron to provide tools to determine the relative quantities of different livestock parasite species eggs or larvae present in fecal samples. This is a powerful surveillance and diagnostic tool that can be used in many different ways including as an adjunct to the fecal egg count reduction test. We have also developed deep amplicons sequencing to detect and measure the frequency of anthelmintic resistance mutations in sheep and cattle GIN nematode populations. This approach provides an accurate measure of resistance allele frequencies and can reliably detect resistance alleles down to a frequency of 0.1%, making it particularly valuable for screening mutations at the early stages of resistance. The power of these approaches, using a number different examples, and future plans for further development will be discussed.

Guest Speakers

Oral Communication

**Novel options for worm control; exploiting helminth-microbiota interactions
in gastrointestinal helminth infections of livestock**

Peachey L.^{1,2}

¹Bristol Vet School, University of Bristol, Langford, UK

²Department of Veterinary Medicine, University of Cambridge, Cambridge, UK

A growing body of evidence supports the existence of a complex network of interactions occurring between gastrointestinal (GI) helminth parasites and the gut commensal bacteria. In particular, ongoing work from our group and others, suggests that acute helminth infection impacts negatively on GI microbial metabolism, and that certain microbial taxa may be responsible for promoting increased worm burdens. These findings point towards two potential approaches for microbiota-modulatory dietary intervention; 1) to counteract the negative impact of infection on GI microbial metabolism through supplementation with targeted prebiotics, thus improving host health and production parameters; and 2) supplementation with probiotics which improve host immune responses to GI helminth infection. Parallel to this, dietary interventions in the form of bioactive forages are being explored as adjuncts to sustainable parasite control; however, despite the promise they have shown, their widespread inclusion into parasite control regimens is complicated by discrepancies in efficacy between studies and the narrow therapeutic index of some of the active compounds. An improved understanding the mechanism/s of action of each bioactive forage would facilitate their optimisation. One potential mechanism is that bioactive forages may exert their effect, at least in part, through alterations to the GI microbiota and microbial metabolism. Indeed, there is evidence that bioactive feeds containing high levels of fructan may exert some anthelmintic effect via a prebiotic effect on the host GI microbiota. For the majority of bioactives forages, associations with the GI microbiota have not been explored, hence this is an important area for further investigation.

Guest Speakers

Oral Communication

Some pharmacological issues contributing to extend the lifespan of traditional and novel anthelmintic drugs

Lanusse C.

Med Vet., Dr Cs Vet., Ph.D., Dip ECVPT

*Laboratorio de Farmacología, Centro de investigación Veterinaria de Tandil (CIVETAN),
CONICET-CICPBA-Universidad Nacional del Centro, Tandil, Argentina*

The use of pharmacology-based information is critical to achieve sustainable parasite control in ruminants. The inadequate use of anthelmintics has led to therapeutic failures and to the dramatic widespread development of parasite resistance, which has been spreading in prevalence and severity. The accumulated scientific knowledge on the pharmacology of anthelmintics has been relevant to design strategies for parasite control in livestock. Relevant scientific work supporting the main strategies to optimize anthelmintic therapy in ruminants under the current drug resistance scenario, is now available. Different approaches to enhance parasite exposure, the combination of drugs from different chemical families and the use of phytochemicals, have been proposed as valid strategies to delay the development of anthelmintic resistance. The need for further integrated pharmaco-parasitological knowledge to extend the lifespan of both traditional and novel anthelmintic compounds, and to progress in the identification of complementary/alternative measures of parasite control in livestock animals, will be emphasized in this presentation.

Guest Speakers

Oral Communication

Are we ready to enter a post – anthelmintic era?

Torres-Acosta J. F. J.

^bFacultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán. Km 15,5 Carretera Mérida-Xmatkuil 97315 Mérida, Yucatán, México.

Ready or not, many sheep and goat farms in Latin America host gastrointestinal nematodes (GIN) with multiple anthelmintic resistance (AR) against two or more drug classes and some farms are already in the post-anthelmintic era. Our research on targeted selective treatment against GIN has shown that large proportions of tropical goats and hair sheep host low GIN burdens. We are investigating whether this is due to low pasture infectivity or the phenotypic expression of parasite resistance. Our work on nutritional supplementation confirmed that growing animals improve their resilience and resistance against GIN, and recent surveys showed that adult hair sheep with good body condition score (BCS) are in no need of AH treatment during the whole year even with frequent reproductive activity, provided that good BCS is maintained. We confirmed that some plants from the tropical forests affect the biological cycle of GIN populations inside their hosts, helping to endure parasites, and we are investigating plants that could be considered nutraceutical. The *Haemonchus contortus* of sheep and goats can be controlled with copper oxide wire particles, with a persistent effect of > 28 days, and the Barvervax[©] vaccine showed high efficacy (>90% EPG reduction). In conclusion, we have tools and protocols that could be implemented to face partial or total AH failure of multiple drug classes. The building blocks of a sustainable GIN control are present, but the construction still require trained staff (vets) with knowledge and ability to be the architects and engineers building solid strategies for each farm.

Guest Speakers

Oral Communication

We 're all here because we 're not all there!

van Wyk J. A.

Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria

This presentation laments the lack of progress in placing modern technology functionally in the hands of farmers, especially the resource-poor (R-P), for instance for largely automated helminth management. Relatively soon after the advent of electronics it was construed in a periodical that the only limitation to the application of electronics was that of the human mind, thereafter soon supported in 1957 by technology such as telemetrical monitoring of vital signs of dogs in orbit, on Sputnik 2. Of special import subsequently, was the cellphone, developed over the 1970's to the present wide range of models used, for instance, by an estimated 80% of R-P farmers in Sub-Saharan Africa. Furthermore, fully thirteen years ago it was suggested in an international electronic conference that all the technological aids required for developing automated mathematical models/ systems for enabling particularly R-P farmers to commence with commercialisation, were already on hand. Seven years later a so-called "blueprint" (concept) for an automated system followed, but had similarly little impact. So the question posed here is not what can be done to address this prerequisite for helping such desperately needy people to advance, but rather how and when the process can be kickstarted in earnest, despite the hurdles under remote rural conditions. In other words, what have we done/are we doing today, with this powerful hand we've been dealt by fellow scientists. The suggestion is that gap reviews are urgently required going forward, regarding: (i) development of appropriate system(s); and (ii) potential problems regarding uptake by R-P farmers.

Guest Speakers

Oral Communication

Targeted Selective Treatments: Lessons learnt and future considerations

Greer A. W.

Faculty of Agriculture and Life Sciences, Lincoln University, Christchurch, New Zealand
Andy.Greer@lincoln.ac.nz

There are numerous examples of targeted selective anthelmintic treatment (TST) regimes. Although each has its limitations, in young growing animals utilising information on animal performance, namely growth, has been commonly investigated. In evaluating the suitability of liveweight gain based TSTs there have been a number of lessons which have been learnt. Lesson 1: Not all animals require treatment at all times, with the timing and frequency of treatments to individuals showing considerable variability within and between mobs. Lesson 2: Parasitological measures (primarily faecal egg counts) are frequently poorly correlated with animal performance, reflecting either resilience or inadequacy of these measures to predict those which are suffering from parasitism. Lesson 3: Setting appropriate liveweight gain targets can be a challenge, particularly when dealing with variation in expected performance between animals, mobs and seasons. Lesson 4: There is still room for improvement and refinement, particularly when utilising relatively crude indicators which can be influenced by factors other than parasites and in light of the recent availability of advanced grazing sensors. Lesson 5: The potential benefits of a TST regime extends beyond simply providing refugia, particularly in the context of utilizing information on the number of treatments received to assist with genetic selection and providing information marketing based on the responsible use of chemicals in food producing animals. While it is anticipated that many more lessons remain to be learned, consideration of these five key lessons can assist in developing appropriate TST regimes in growing livestock.



Guest Speakers

Oral Communication

**EPRUMA best practice framework
on responsible use of anthelmintics in food-producing animals**

van Dobbenburgh R.

European Platform for the Responsible Use of Medicines in Animals (EPRUMA)

EPRUMA – the European Platform for Responsible Use of Medicines in Animals – is a multi-stakeholder platform working on a coordinated and integrated approach with regard to best practices and use of medicines in animals. It brings together European and national associations of professions and industries involved in animal health. Veterinarians, farmers and agri-cooperatives, pharmacists, manufacturers of animal medicines, diagnostics and feed work together on identifying best practices that contribute and promote responsible use of medicines.

EPRUMA aims at raising awareness and showing to the outer world we do care about this responsibility. Today responsible use often reflects to the use of antibiotics and AMR, but there is more than that.

Last year EPRUMA focused on the use of anthelmintics in livestock. Another area where we have to take responsibility in order to fight resistance and keep efficacy of available products. EPRUMA best practice framework on responsible use of anthelmintics in food-producing animals is the result of this work that is about to be launched and promoted.

Working Group 1. Improving Diagnosis

Oral Communication

Droplet Digital PCR - A Novel Diagnostic Tool in Nematodes of Veterinary Interest

Elmahalawy S., Baltrusis P., Halvarsson P., Höglund J.

*Swedish University of Agricultural Sciences, Department of Biomedical Sciences and Veterinary Public Health,
Section for Parasitology, P.O. Box 7036, Uppsala, Sweden*

Droplet Digital PCR (ddPCR) is an automated third generation PCR technology based on microfluidics, which could be a useful complement in the arsenal of laboratories dealing with routine veterinary diagnostics. Unlike similar probe-based diagnostic assays such as qPCR, in ddPCR the sample template is fractionated into thousands water-oil emulsion droplets before amplification occurs in each individual droplet. The massive sample partitioning is a key aspect of the ddPCR and provides an absolute quantification of target DNA copies without the need for running standard curves. In this presentation we will outline some recently developed ddPCR based diagnostic applications that have been developed and validated in our laboratory. We have used this technology for speciation of key gastrointestinal (GI) nematodes of sheep. We also developed two ddPCR primer-probe assays for estimating allele frequencies of single nucleotide polymorphisms (SNP) in different candidate genes linked to anthelmintic resistance (AR). According to our results, ddPCR is capable of quantifying both universal and genus specific ITS2 copies of both eggs and larvae in all three major GI including *Haemonchus*, *Teladorsagia* and *Trichostrongylus*. We have also demonstrated that this technology is a powerful tool for the detection and absolute quantification of certain SNPs in *Haemonchus contortus*. This has been carried out both with the *dyf7* gene purported to occur in relation to ML resistance, and for the transversion after selection to benzimidazoles in the 200th codon (TTC→TAC) in the β -tubulin isotype 1 gene. It can be concluded that ddPCR is a very useful technology for quantitative speciation of GI nematodes in complex samples containing multiple species. On the other hand, the value of the AR-resistant markers and especially *dyf7* can be questioned as we showed that the allele frequencies were unrelated to the level of ML-resistance.

Working Group 1. Improving Diagnosis

Oral Communication

From Genes to Diagnostics of Fasciolid Flukes

Ježková M.¹, Leontovyč R.¹, Marek M.², Kašný M.^{1,3}

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³Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

Current serodiagnostics of flukes and number of other parasites is predominantly based on mix of specific antigens from excretory-secretory products (ESPs). Due to high heterogeneity of ESPs - serological markers, their standardized isolation, purification and subsequent reproducibility of results could be problematic. Therefore, the recombinant forms of particular antigenic ESPs proteins are often adopted in commercial serodiagnostic kits; the previous identification of proper protein/antigen typically requires sophisticated and expensive instrumentation, such as e.g. mass spectrometer.

Due to the recent fast development of robust sequencing technologies the huge genomic, transcriptomic and proteomic data were generated and they are available in public databases. This data represents an important source of information which can be transformed by bioinformatics tools into the sets possessing the novel quality.

In our work we used transcriptomic/proteomic data for *in silico* predictions of the antigenic epitopes corresponding to antigenic protein molecules potentially presented in ESPs of fasciolid fluke *F. magna*, close relative species to *F. hepatica*. Nine of predicted epitopes were combined and three multi-antigenic fusion protein molecules designed, produced and tested by ELISA using the sera from infected/not-infected animals.

Acknowledgement: The research was financially supported by the Masaryk University (MUNI/A/1362/2016) and Charles University in Prague (PROGRES Q43, UNCE/SCI/012-204072/2018 and SVV260432/2018). M.K. is a member of COMBAR COST-action project (CA16230).

Working Group 1. Improving Diagnosis

Oral Communication

High Frequency of Benzimidazole Resistance Alleles in *Haemonchus contortus* and *Teladorsagia circumcincta* from Sheep Flocks in Austria

Hinney B.¹, Schoiswohl J.², Melville L.³, Wille-Piazzai W.¹,
Bauer K.⁴, Joachim A.¹, J. Skuce P.³, Krametter-Frötscher R.²

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Establishing a baseline, followed by regular monitoring of the spread of AR is necessary as it allows farmers and veterinarians to make informed decisions about parasite management. The diagnosis of single nucleotide polymorphisms (SNPs) for the detection of AR of Benzimidazoles (BZ) has a high sensitivity for the detection of resistance alleles (RA).

To monitor the efficacy of anthelmintics, faecal samples from 243 sheep out of 14 flocks in Austria were examined by the McMaster-method. Positive samples were pooled for larval cultures. Out of these larval cultures *Haemonchus contortus*- and *Teladorsagia circumcincta*-larvae were examined by pyrosequencing for the detection of RA frequencies. To detect the efficacy of moxidectin (MOX) an egg count reduction test (EZRT) was performed.

Frequencies of RA against BZ in codon 200 of the β -tubulin isotype-1 gene ranged from 87% to 100% for *H. contortus* and <5% to 66% for *T. circumcincta*. Hence a high level of AR against BZ can be assumed for these nematodes. The efficacy of MOX against trichostrongylids detected by EZRT was 93% so that AR was suspected here. In all other farms efficacy was >95%.

In this study a very high frequency of RA against BZ was diagnosed in pooled samples of *H. contortus* and *T. circumcincta*. Also resistance against MOX was suspected. These results show that a close monitoring of anthelmintic efficacy and the development of new control strategies to sustainably control trichostrongylid infection in sheep in Austria is urgently required.

Working Group 1. Improving Diagnosis

Oral Communication

Meta-community Analysis Reveal New Insights to Ovine Parasite Diversity

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Accurate determination of gastrointestinal nematodes (GIN) are a cornerstone to successful treatment and control of parasitic infections. With the advent of Next Generation Sequencing it is today possible to sequence whole metabiomes and their correlated nemabiomes. The strength of this approach is the simultaneous identification of all GI nematodes and their abundance in a host using universal primers. Using ITS2 universal primers for identifying stronglylidae nematodes, we have sequenced samples from Swedish sheep farms on the PacBio sequencing platform. Samples have been collected both before and after treatment with anthelmintic drugs to investigate changes in species composition and also on a temporal scale.

DNA was extracted from fecal larval cultures. Our result show that *Haemonchus*, *Teladorsagia* and *Trichostrongylus* are the three most common parasites and that we also detect low frequencies of other ovine GIN. A bit surprising, we identified GINs sequences matching *Cyathostomum*, *Coronocyclus* and *Cylicostephanus* (identity 80%), which are genera that have not previously been found in sheep samples. In some samples, this was the most frequently occurring GIN. This is an important finding as it has implications on animal welfare and on treatment with anthelmintic drugs

Working Group 1. Improving Diagnosis

Oral Communication

Evaluation of the Egg Hatch Assay as Diagnostic Method to Determine the Resistance in Gastrointestinal Nematode of Sheep

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Egg hatch assay (EHA) is a widely used in vitro method to detect benzimidazole resistance in gastrointestinal nematodes although when it is used under field conditions present a high inter-sampling variation. Due to this fact we analyzed the possible factors affecting the presence and level of resistance including those ones related with isolates (genera composition), parasite (genetic markers related with resistance: SNPs at codons 200 and 198 of the β -tubulin isotype-1 gene), host (faecal egg count), environment (sampling season) and laboratory procedure (storage condition of faecal samples). For that, the levels of anthelmintic resistance to thiabendazole (TBZ), measured as hatching ratio (Hdd) at different doses, including the discriminant dose of 0.1 $\mu\text{g/ml}$, were determined in naturally infected sheep flocks. According to different general linear models, the Hdd was negatively related to the number of days in anaerobic-refrigerated storage conditions of samples, before doing the EHA, and positively to the interactions between the relative abundance of *Trichostrongylus* spp. and *Haemonchus* spp with the resistant allele frequency at SNP200 and SNPS200 and 198, respectively. In a different model, the presence of *Haemonchus* spp. explained 5.69% of the resistance found in naturally infected sheep. On the other hand, the sensitivity and specificity of the EHA was determined using different doses of TBZ as discriminant dose and the percentage of resistant SNPs as gold standard; the highest values were shown with a dose of 0.2 $\mu\text{g/ml}$ of TBZ.

Working Group 1. Improving Diagnosis

Oral Communication

Does *In vitro* Egg Hatch Test Predict the Clinical Failure of Benzimidazole Treatment?

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The objective of the study was to compare *in vivo* the faecal egg count reduction (FECR) test, the *in vitro* Egg Hatch Test and the molecular determination of the beta-tubulin isotype 1 codon 200 BZ-resistance associated allele frequencies in *H. contortus* stages obtained from infected goats. In this study 54 goat kids were each infected with mixture of 2500 third stage larvae of resistant (benzimidazole) and susceptible *H. contortus* isolates. Composite infection doses consisted of 10, 20, 30, 40, 60 and 80 % of the respective resistant isolate. Faecal samples for *in vitro* test were collected on separate days. The results of the *in vivo* FECR test showed that albendazole treatment reduced infections by 91.3, 78.0, 63.3, 48.4, 36.5 and 41.4 %, respectively at the above listed infection groups. The results of ED₅₀ values in the *in vitro* EHA varied from 0.09±0.01 to 15.63±12.10 µg/ml TBZ. The results of PyrosequencingTM method revealed that the frequency of the susceptible (TTC) and resistant (TAC) beta-tubulin codon 200 alleles of *H. contortus* in larvae derived from different groups of goats were closely related with the respective composition of larvae which have been used during infection. The results of the *in vitro* tests show solid base for an estimation of clinical resistance *in vivo*. This was also confirmed by comparing the FECRT data with hatching percentages in EHT in 22 goat farms in Slovakia with natural mixed infections of GI parasites.

Working Group 1. Improving Diagnosis

Oral Communication

The Number of Endoparasite Eggs in Different Parts of the Sheep Intestinal Tract

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The presence of different parasites (*Nematodes*) in domestic and wild animals may lead to health and economic issues. The nematode control in animals represents the first step in the fight against their unwanted impact. The choice of appropriate anthelmintic will depend on the type of pre-used preparations and time applications as well as the development of resistance to anthelmintic. The question that arises is whether an equal number of parasites can be found in each part of the intestinal tract and is it possible to determine the number of adult's parasites in the host according to the number of the established nematode eggs? In our research we determined the number of endoparasite eggs in the caecum and the colon in dead sheep. We examined the samples of feces taken from the caecum and colon of three dead sheep by the method of flotation and sedimentation, and the total number of eggs was determined by the McMaster egg counting technique. The goal of our work was to determine whether there are differences in the number of eggs in different parts (caecum and colon), as well as whether there is a difference in the presence of the found species of parasites? The nematode eggs of *Nematodirus filicolicis*, *Chabertia ovina*, *Trichostrongylus sp.* were found in the caecum samples. The mean value of eggs was 800/grams of the feces. In the colonic samples, *Chabertia Ovina*, *Trichostrongylus sp.* were found and the mean value of the number of eggs was 300/grams.

Key words: nematode, eggs, sheep

Working Group 1. Improving Diagnosis

Poster Communication

Mini-FLOTAC Automated System for Helminth Egg Counts in Ruminants

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Gastrointestinal nematode (GIN) helminths are amongst the most important production-limiting pathogens of grazing ruminants. Diagnosis of these parasites is traditionally based on the detection of eggs in faecal samples by copromicroscopic techniques that are still time-consuming, require technical expertise and laboratory equipment and impractical to use in the field (pen-side). Mini-FLOTAC is a validated technique for faecal egg counts (FEC) and faecal egg count reduction test (FECRT) of GIN and several parasites in livestock, other animals and humans. Mini-FLOTAC has achieved a good level of Technologies Readiness level (TRL), also thanks to more than 40 scientific publications on International Journals having a high citation index. Even if the Mini-FLOTAC technique is considered accurate and precise, results are prone to issues such as operator dependency, method variability, equipment requirements, and time commitment. The aims of this study was to design and test a prototype of the Mini-FLOTAC automated system that allows a rapid laboratory workup and can be used directly on livestock farms for automated FEC/FECR including image software analysis. The use on farm of this simple, automated system will allow a rapid assessment of anthelmintic resistance in large and small ruminants to assist the new generation of veterinarians and farmers.

Working Group 1. Improving Diagnosis

Poster Communication

First Detection of Ivermectin Resistance in *Oesophagostomum Dentatum* in Pigs

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Efficacy of ivermectin (IVM) against *Oesophagostomum* species worms has been investigated to determine whether reduced efficacy on a mixed indoor and outdoor breeder-finisher pig farm in England reflected true IVM resistance under controlled experimental conditions. On days 0 and 40 of the trial, twenty helminth-naïve pigs were infected *per os* with 5000 *Oesophagostomum* L3 obtained from the farm under investigation. The pigs were allocated to treatment or control groups (n =10 per group). Treatment group pigs received IVM by sub-cutaneous injection as per manufacturer's instructions on day 44. Control group animals were not treated. On day 50 all pigs were euthanased to assess the worm burdens. Resistance to IVM was confirmed in *Oesophagostomum dentatum* based on the results of a faecal egg count reduction test (FECRT) and a controlled efficacy test (CET). Efficacy based on mean reduction in faecal egg count of IVM-treated pigs compared to untreated control pigs was 91%. Mean reduction in IVM-treated pig worm burdens was 35% against an adult worm population and 94% against an L4 population. The detection for the first time of IVM resistance in *Oesophagostomum* species worms in UK pigs is particularly important considering the global anthelmintic situation where resistance to pyrantel, levamisole and benzimidazoles in *Oesophagostomum* species worms in pigs have already been previously reported. The results provide an opportunity to discuss the wider issue of anthelmintic usage and efficacy on pig farms and highlight the need for wider surveillance for the occurrence of nematode resistance in pigs.

Working Group 1. Improving Diagnosis

Poster Communication

A model transmission of gastrointestinal nematode *Haemonchus contortus* between domestic and wild ruminants under field conditions

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The rapid spread of anthelmintic resistance has raised the question of the role of wildlife ruminants in transmission of resistant parasites to domestic sheep and goats. This study was conducted to confirm possible transmission of gastrointestinal blood-sucking nematode *Haemonchus contortus* between sheep and roe deer, sharing the same pastures, under field conditions. Two lambs were experimentally infected with 2500 third stage larvae (L₃) of resistant strain MHco4 of *Haemonchus contortus*. 28 days after infection, both lambs with high worm egg count were moved to a clean pasture, where no animals have grazed before. After 6 weeks two roe deer were introduced to the same pasture. Faecal samples of roe deer were collected and EPG were determined on day 21, 28, 35, 42 and 49. Maximum intensity of infection in roe deer was documented on day 35 (800 EPG, 4400 EPG). *In vitro* egg hatch test (EHT) and larval development test (LDT) were used for determine ED₅₀ and LD₅₀ for thiabendazole (TBZ) and ivermectin aglycone (IVM) in roe deer samples. Values of ED₅₀ in EHT were 0.111±0.004 - 0.115±0.008 µg/ml TBZ. In LDT LD₅₀ values for TBZ were 0.021±0.002 - 0.031±0.001µg/ml and for IVM 46.670 ± 5.508 ng.ml⁻¹. Results obtained from *in vitro* tests and L₃ larvae from coprocultures confirmed the direct transmission of multidrug resistant *H. contortus* from sheep to roe deer under field conditions.

The study was supported by Grant Agency VEGA No. 2/0120/16 and Slovak Research and Development Agency Project No. 14-0169.

Working Group 1. Improving Diagnosis

Poster Communication

Evaluation of Levamisole Effectiveness in Two Goat Herds with Previously Detected Resistance of Gastrointestinal Nematodes to Ivermectin and Benzimidazoles

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Introduction: Very little is known on the anthelmintic resistance (AR) in gastrointestinal nematodes of sheep and goats population in Poland and only resistance to benzimidazoles has so far been detected.

Materials and methods: The study was conducted in two dairy goat herds (A and B), where one year before resistance of gastrointestinal nematodes to benzimidazoles and macrocyclic lactones had been confirmed. Only levamisole (LEV) was then effective – percentage reduction for LEV in Faecal Egg Count Reduction Test (FECRT) was 100% (95% CI: 98%, 100%) and 98% (95% CI: 80%, 100%), respectively. In each herd, FECRT using LEV was performed on 20 goats. Moreover, Larval Development Test (LDT) for *in vitro* detection of AR to LEV was performed. Larval cultures were prepared for each group and larvae stage L3 were identified at the species level using morphological criteria.

Results and discussion: Percentage reduction for LEV in FECRT decreased compared to the situation from before a year – it was 95% (95% CI: 75%, 99%) and 87% (95% CI: 73%, 93%) in herd A and B, respectively. LD₅₀ values from LDT were 23.1 µg/ml and 1.2 µg/ml, respectively. Main species present after the treatment was *Haemonchus contortus* in both herds together with *Trichostrongylus colubriformis* in herd B. FECRT performed after one year of the extensive use of LEV showed a decline in LEV efficacy in both herds, with suspected resistance in herd A and the presence of resistance in herd B.

Working Group 1. Improving Diagnosis

Poster Communication

The BUG Consortium- Using the *Haemonchus contortus* Genome to Identify Mechanisms of Anthelmintic Resistance

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The BUG project (Building Upon the Genome) is a UK-based project aiming to develop tools, including diagnostics, for the better control of anthelmintic resistant nematode infections of sheep. We are using the genome of the parasitic nematode *Haemonchus contortus* to identify markers of anthelmintic resistance. So far, we have constructed a genetic cross between a drug sensitive (MHco3, ISE) and a drug resistant isolate (MHco18, UGA04) of *H. contortus*. L3 of the F2 generation of the MHco3xMHco18 cross were used to infect sheep, which were then treated with a standard dose of either ivermectin (IVM), benzimidazole (BZ) or levamisole (LEV). Pre and post-treatment L3 were cultured from faecal samples and sequenced with the aim of identifying regions of the genome under selection by each class of drug. Fst analysis demonstrates clear signals of selection for each drug. BZ and LEV, where good candidates already exist for drug resistance loci, acted as proof of principle and known loci (β -tubulin and *acr-8*, respectively) were identified. However other minor loci were also apparent. For IVM, where resistance is thought to be multi-genic, several loci were identified, which are under further investigation. In addition, we are collecting field populations of both *H. contortus* and *Teladorsagia circumcincta* pre- and post-drug treatment for sequencing, in order to confirm results with the laboratory isolates. In the longer term we hope these studies will result in novel markers for monitoring drug resistance in the field. This project is funded by the BBSRC.

Working Group 2. Understanding the Socio-economic Aspects

Oral Communication

Understanding the Resistance of Farmers

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Anthelmintic resistance (AR) is necessitating a new era of innovation in gastrointestinal nematode control. An array of alternative strategies and tools are being promoted and developed for farmers with the expectation that they will rapidly adopt them. Behavioural studies however have found that many are continuing to use control measures that are favourable to AR development. This is perceived by some as a failure on the part of farmers and can seem to make little sense. Yet as explained in this paper both of these impressions are incorrect when the problem of GIN control is viewed from the farmer perspective and in light of social science understandings of innovation adoption. It will focus on a socio-economic framework that can bring structure to and insights about barriers known to be restricting farmer behaviour change. A number of other factors that may also be influential are also revealed highlight important gaps in our knowledge about the human factors that may be contributing to the development of AR. In addition, reasons why GIN control measures that are risky for AR can remain the farmer's choice despite interventions to aid behavioural change are discussed. These understandings of the choices farmers make about anthelmintics and other GIN control measures provide an insight on the potential future pathways of AR and how they may be directed to achieve the greatest possible benefits from scientific research.

Working Group 2. Understanding the Socio-economic Aspects

Oral Communication

Farmers and Veterinary Practitioners Focus Group Experience Regarding Vector Borne Disease Outbreak in R. Macedonia

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Introduction:

Vector-borne diseases (VBD) are of increasing global Public Health interest given ongoing changes in the environment, global trade and worldwide travel. Improved concepts of adequate risk management together with meaningful risk communication strategies are badly needed to enhance risk governance.

Methods:

The findings are based on focus group research as a method to analyse risk perception patterns, coping strategies, and media usage. The core theme of the focus group is to elucidate perceptions, coping strategies, information expectations and information behaviours regarding VBD in particular. Moreover, the influence of social interactions between the participants in regard to all these mentioned themes is an additional dimension which can be clarified with this method.

Results:

Three focus groups in total with about 8-10 attendees each were conducted in endemic areas of the country. The setting of the focus group were followed certain socio-demographic variables that are known to influence the risk perception, information behaviour, coping strategies and knowledge: We included different sex different age groups and different spatial characteristics though all of them in endemic regions). At the beginning of the three focus groups, participants were asked to write down or present their first associations of mice or tick transmitted diseases. To facilitate and stimulate discussion between the participants, we provided a collage composed of newspaper headlines from the respective local newspaper. The headlines referred to news reporting on previous VBD outbreaks in this region, including some photographs also. The analysis of this first associative

phase in regard to risk perception patterns is done by using the visualization technique called 'word cloud' (or tag cloud). The presence of VBD - the situation in the R. Macedonia is evident.

Conclusion:

Conducted focus groups gave a clear picture that there is no knowledge about VBD among population in the rural area, and also among some of the participants in the occupational risk group. VBD was mostly known among the oldest participants in the occupational risk group. However, it is positive that participant from all groups have some basic information that ticks can transfer diseases, although most of them from the rural area thought that these diseases cannot be very dangerous. In general, risk perception for the VBD in the rural area is on a very low level.

Keywords: focus groups, VBD, risk strategies

Working Group 2. Understanding the Socio-economic Aspects

Oral Communication

Contribution of Social Marketing to Change the Behaviour of Veterinarians and Farmers in Worm Control

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Human behaviour is cognitive and has a value of its own (Fishbein & Ajzen, 1975). It is dependent of external factors (Lee et al., 2005), which interfere in many different ways, making it difficult to define a single model to describe and explain all of the behaviours (Ajzen, 1991). Although intention is often assumed to be the best predictor of behaviour (Fraser et al., 2010), and many predictive models are based on cognitive belief systems (Ajzen, 2011), the ‘intention-behaviour gap’ should be considered when studying factors influencing behaviour.

Conscious decision-making can be divided in three types of behaviour (Mills et al., 2017), initiated by different motivations: i) compulsory behaviour based on regulation (Jansen et al., 2012); ii) incentive-driven behaviour based on economic rationality and iii) voluntary behaviour driven by socio-psychological factors. Efforts to influence farmer’s or veterinarian’s behaviour should take into account these different levels. This article presents the basic assumptions of Social Marketing to inform, to educate and to persuade (Kotler & Lee, 2007) the veterinarians and farmers to adopt sustainable worm control practices. Since educative campaigns have better results if the behaviour is volunteered, cognitive and emotional benefits could be the way to achieve success (Peattie & Peattie, 2009). For many marketing authors, before promoting the individual change it is necessary to overpass structural barriers (Wymer, 2011). Donovan (2011) argued about the influence of legislation, protection and education, and Wallack (1994) defended the necessity to involve the main decision-makers to change the behaviour.

Working Group 2. Understanding the Socio-economic Aspects

Oral Communication

Experiences with Questionnaire Survey and Faecal Sampling in Small Ruminant Flocks

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In years 2015-16, we conducted a study on parasites and parasite control practices in small ruminants flocks. Our experiences with the sampling and cooperation of breeders in the survey will be presented.

Questionnaires about rearing and parasite control practices were sent to breeders. Those who participated in the survey had the opportunity to send faecal samples of their animals to a free parasitological examination before and after dehelminthisation. With the questionnaire, they received an instruction for faecal sampling and number of samples to be submitted, which was based also on the number of animals.

The questionnaires were completed by 225 sheep and goat breeders. 172 breeders sent faecal samples before the treatment. Approximately half of breeders (84) sent faecal samples also after treatment and only 67 performed sampling according to the instructions. Parasitological examination revealed differences between samples from the same flock, both in terms of the parasite species and the number of eggs per gram of faeces.

A good response of breeders to fill in the questionnaire was noted, while the response to sending the samples was worse, in particular after the treatment. Response of breeders could reflect a lack of knowledge about this problem or that they attribute too little importance to it. The proposed way of sampling was appropriate, as it showed that with more samples from the same herd, we got better information about the parasitological situation in the flock, and on the other hand, it would be still time and cost acceptable for breeders.

Working Group 2. Understanding the Socio-economic Aspects

Oral Communication

Monitoring the Efficacy of Anthelmintics in Ruminants in Southern Italy

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Anthelmintic resistance (AR) of gastrointestinal nematodes (GINs) represents today a very important threat in ruminant production systems, resulting in huge economic losses all over the world. The spread of AR is due to the last decade misuse of drugs for the control of GIN infections in ruminants, leading to the development of new parasite populations which are resistant to various classes of anthelmintics. A high prevalence of AR is reported in almost all European countries, with some exceptions: in Italy occurrence of AR is very rare so far. However, even in areas where AR is not yet a problem, monitoring the drug-susceptibility of GIN populations in large and small ruminants must be a high priority because early detection of AR is crucial to avoid production losses. Therefore, from 2016 to 2018 field studies were conducted in two regions (Campania and Basilicata) of southern Italy. Faecal Egg Count Reduction Tests (FECRTs) were run on a total of 41 sheep farms and 10 cattle farms to estimate anthelmintic efficacy of the following anthelmintics administered orally: ivermectin, netobimin, moxidectin and albendazole. In these studies Mini-FLOTAC technique on pooled samples was used for FEC analysis showing a good correlation with averages of individual FECs. Very high efficacy was obtained with all anthelmintics tested: from 97.5 to 100% in sheep and 100% in cattle farms. The present findings confirm that AR is rare in cattle and sheep in southern Italy and moreover support the idea that Mediterranean climate combined to the farm management, i.e. reduced number of treatments per year and animal seasonal movement, have a predominant role in preventing the development of resistance.

Keywords: ruminants, anthelmintic resistance, pooled samples, Italy

Working Group 2. Understanding the Socio-economic Aspects

Oral Communication

Impact of Transfer of Goats to Alpine Pastures on the Dynamics of Infections with Gastrointestinal Nematodes

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Whereas the effect of alpine grazing on infections with gastrointestinal nematodes (GIN) is well documented in cattle, little is known about the respective situation in small ruminants. Therefore, the development of GIN infections was studied in dairy goats on two swiss alpine pastures on altitudes between 1400 - 1880 m a.s.l. (Farm I) and 1860 – 2000 m a.s.l. (Farm II) respectively during one season. Individual faecal egg counts (FEC) as well as clinical examination including FAMACHA-analysis were performed at three occasions following the goal that the study should influence the herd management as little as possible. As there were no requirements to treat the goats anthelmintically prior to turnout on the alpine pastures, GIN populations obtained on the home pastures remained in the goats during transfer to the alpine pastures. At this stage the mean GIN FEC ranged between 600 and 1000 epg. During the alpine grazing period a marked reduction of the mean FEC by 48 and 52% resp. was observed. Furthermore, the partial FEC of *Haemonchus contortus* was reduced by 86% in both herds, whereas the FAMACHA-scores recovered during the same period. Clinical parasitic gastroenteritis was not observed during the entire season in both herds. Recommendations for anthelmintic treatment on the basis of FEC and/or clinical status had to be given only after 5 and 9% of the examinations resp. and we assume that the treatment which were actually done by the owners were much less. The results indicate that grazing goats on alpine pastures may reduce the risk for GIN infections.

Working Group 2. Understanding the Socio-economic Aspects

Oral Communication

Practices on Helminth Control in Organic Cattle from Northern Portugal and Spain

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Organic farms with cattle production are growing in the north of Portugal and Spain but little is known about their prevalence on intestinal helminths and practices on their control. The aim of this survey was to contribute to this knowledge. For this purpose, we constructed and administrate a questionnaire for farmers and collect pool faecal samples from 20 different farms in the northern Portugal and Spain, in autumn and again in spring. Farmers were mostly more than 45 years old and attended school only during 6 years. Most of the farms had only cattle, between 11 and 50 animals each one, and cattle had contact with other animals namely wild ones. All the farms were located in mountain regions and in Portugal the breeds were autochthonous. The base of the feeding was pasture. Half of the farmers affirmed to practice deworm twice a year. From the 20 farms, helminths (strongyles, *Moniezia benedeni* and *Capillaria* spp.) were found in 18 in autumn and in 17 in spring. Besides the low diversity found, the burden was very low: 50 eggs per gram was the highest burden found. Deworming did not influence the presence and burden of parasites. These results emphasize the need to evaluate the parasite burden before deworming, in order to reduce the risk of developing resistance to anthelmintic, especially in animals of autochthonous breeds and well adapted to the ecosystem they inhabit.

Working Group 2. Understanding the Socio-economic Aspects

Poster Communication

Who cares about helminths of backyard pigs in Portugal?

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The Bísaro pig is an autochthonous portuguese breed, whose biological and cultural heritage has for centuries been an ally of the rural world, playing a very important role in human nutrition, namely through the sausages associated with some regions of northern Portugal. Helminth infections are common in domestic pigs of all production regimes and throughout the world, as well as their negative influence on the health status and weight gain of animals, but little is known about backyard pigs. The aim of this study was to assess the gastrointestinal parasite burden and diversity in backyard pigs from Portugal. For this purpose, we collected and analysed (using Willis and McMaster techniques) 462 faecal samples from backyard pigs from the north and centre of Portugal. The overall prevalence was 87%. Eight different eggs/oocysts were identified: strongyles (76%), *Eimeria* spp. (42%), *Cystoisospora* spp. (26%), *Ascaris suum* (13%), *Balantidium coli* (13%), *Trichuris suis* (2%), *Metastrongylus* spp. (0.4%) and *Hymenolepis diminuta* (0.4%). Severe (EPG>2000) strongyle infections (n=130) and *Ascaris suum* infections (n=13) occurred and deworming significantly influence their presence or absence. These results suggest the need to raise awareness among pig owners (that many times are not professional farmers and require to be educated concerning animal and public health, as well as food safety) to the importance of deworming in a one health concept – to prevent environmental contamination and animal and human infection.

Working Group 2. Understanding the Socio-economic Aspects

Poster Communication

Preliminar Data Suggest Resistance to Anthelmintics in Sheep in Northern Portugal

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Sheep and goat production are an important factor for the survival and sustainability of the mainland in the north of Portugal, a region affected by human aging and abandonment of land by new generations migrating to urban areas on the coast or abroad, mainly to other European Countries. Since Portugal integrated the European Union in 1986, the promotion of Organizations of livestock producers (OPPs) have promoted actions to support the eradication of zoonoses in livestock species (Brucellosis and Tuberculosis) and, husbandry measures to increase yield production (animals reared in extensive system are affected by a diversity of gastrointestinal parasites, which may cause an enormous impact on its health and productivity, so farmers have been lead to the use deworm compounds in their animals on a seasonality basis annual or biannually) and, net income, which is including de-worming against gastrointestinal nematodes (GIN). In the present work, we evaluated the number of OPGs in sheep and goats, and we started the evaluation of resistance to antiparasitic compounds (FECRT). From the results obtained so far, we can observe that OPG sheep are excreting (below 100 OPG) does not justify deworming actions and, although the regularity of deworming is an annual deworming, our results already show the presence of resistance of parasites, principally to benzimidazoles. Different approaches should be established in order to contribute for a rational use of pharmaceutical compounds as well as to the genetic diversity of GIN that may re-establish the susceptibility of parasites to antiparasitic drugs.

Working Group 2. Understanding the Socio-economic Aspects

Poster Communication

Towards Helminth Sustainable Control in Grazing Ruminants in Europe

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Infections with parasitic helminths (IPH) have a huge economic, social, environmental and political negative impacts in global ruminant livestock industry in Europe in particular and across the world in general. The increasing prevalence of anthelmintic resistance means that current control programmes are in economic terms costly and unsustainable in the long term. So, the sustainable control of IPH requires a holistic approach and requires detailed knowledge of these various impacts and sub-indices of the sustainability of the IPH. Hence, the main aim of this work is to analyse the scientific state of the art of the IPH at a sustainable level whereas the sub-indices of the IPH sustainability. This will be essential to the continued research at IPH sustainability to promote at European level the productivity and profitability of livestock farming and its contribution to regional and global food security at European and world level. Thus, we did a revision of the scientific contributes in the sustainability sub-index of IPH at European and world level (based on SCOPUS Q1 and Q2 references and WoS) and we used Google Trends data as a quantitative methodology, namely, multivariate analysis and econometric models in order to nowcast and forecast new insights about the importance, the role and the new trends about IPH in Europe. The results show an interesting development and an increasing trend in IPH search terms as a *proxi* of IPH development in research and interest in Europe.

Keywords: Infections with parasitic helminths; sustainable control; Europe; Google Trends; ruminants

Working Group 2. Understanding the Socio-economic Aspects

Poster Communication

Practices on Helminth Control in an Endangered Autochthonous Portuguese Sheep Breed

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Helminth infections can be threatening to an autochthonous sheep breed at extinction risk like Churra Galega Mirandesa. The aim of this study was to assess practices on helminth control and the diversity and burden of helminths in these sheep flocks. Coprological analysis (flotation, natural sedimentation and McMaster method) and a questionnaire were applied. A total of 512 faecal samples from 49 flocks were collected. Helminths eggs were identified in 100% of the flocks, such as strongyles, *Nematodirus* spp., *Skrjabinema* spp., *Moniezia expansa*, *Moniezia benedeni*, *Trichuris* spp., *Capillaria* spp., *Dicrocoelium* spp. and *Fasciola hepatica*. Strongyles eggs were the most prevalent and with the higher burden (mean: 415.5; range: 50-6250). Forty nine questionnaires were recovered. Most farms (48.9%) have less than 130 sheep and 95.9% have other cohabiting animals: dogs (93.9%) and donkeys (24.5%). The vast majority of flocks (81.6%) graze on communitary pastures. Most farmers (75.5%) report deworming. The non-deworming only influenced the prevalence of *Nematodirus* spp. ($P < 0.05$). The prevalence of *Trichuris* spp. ($P < 0.05$) increased when animals grazed in the early morning and late in the day. The prevalence of *Nematodirus* spp. ($P < 0.05$) and *Dicrocoelium* spp. ($P < 0.01$) increased, when animals grazed during all the day.

The month of sampling was significantly associated with the occurrence of *Moniezia benedeni* ($P < 0.05$) and *Dicrocoelium* spp. ($P < 0.01$). The risk of occurrence of *M. benedeni* and *Dicrocoelium* spp. is 5.9 and 3.9 times higher, respectively, in October. Awareness about the human behaviour and practices is needed to implement well adapted measures for helminth control.

Working Group 2. Understanding the Socio-economic Aspects

Poster Communication

Seroprevalence of *Fasciola Hepatica* in Dairy Cattle and Management Practices That May Influence the Onset of The Disease on São Miguel Island, Azores – Portugal

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Between January and July 2016, a study was carried out in São Miguel Island (SMI), Azores in order to know the prevalence of *Fasciola hepatica* and the management techniques that could contribute to its control. There are 1.424 dairy farms with a total of 51.590 cows, which are mostly Holstein-Friesian breed. The seroprevalence of *F. hepatica* was confirmed by the ELISA technique in 46.7% of the animals and is present in 74.5% of the farms. According to a slaughterhouse study (Barbosa, 2017), 39% of these farms were located in low prevalence areas, 28% in medium prevalence and 33% in high prevalence areas. According a survey in 149 farms on management practices, parasite control was performed in 88% and the remaining 12% did not performed any treatment. The treatments with efficacy against *F. hepatica* was carried out in 62% of the farms, 35% had no efficacy and 3% were unknown. In 83% of the farms the animals did not have access to the areas where snails could serve as intermediate hosts were present and 17% of the farms the animals grazed freely. The practice to clean the drinking water daily was observed in 5% of the farms, 13% every 15 days, 15% every 30 days, 9% every 60 days, 16% every 90 days, 12% every 180 days and 11% annually. This study contributed to know the main management practices that may influence the onset of fasciolosis in dairy herds on SMI, Azores.

Working Group 2. Understanding the Socio-economic Aspects

Poster Communication

The Present Status of Anthelmintic Resistance on Sheep Farms in Lithuania

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The aim of the performed study was to determine the prevalence of anthelmintic resistance (AR) in parasitic nematodes on sheep farms in Lithuania. The study was made from May to November in 2017. Thirteen sheep farms in total were enrolled in this study from various regions of Lithuania. Farms were chosen to have their antihelmintic treatment for at least 10 weeks prior the beginning of study. Both, local (Lithuanian Black Head) and imported (mixed) sheep breeds were involved in the study. To estimate AR - larval development test (LDT) was used. Data was analysed using a threshold discriminating concentration of 21.6 ng/ml⁻¹ for ivermectin-aglycone, 2 µg/ml⁻¹ for levamisole and 0.04 µg/ml⁻¹ for thiabendazole.. The *in vitro* survey showed the anthelmintic resistance to macrocyclic lactones in 11 sheep farms (84.6%), to levamisole in 1 farm (7.7%) and to benzimidazoles in 2 farms (15.2%). The study showed that the main genus of anthelmintic resistant gastrointestinal nematode identified in sheep farms were *Teladorsagia* (P<0.05). The results of *in vitro* survey showed that sheep farms have increased problem with AR to macrocyclic lactones with higher using ivermectins.

Working Group 3. Innovative, Sustainable Control Methods

Oral Communication

Nematocidal Drug Combinations: a Valid Strategy for Parasite Control in Cattle?

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In an attempt to manage anthelmintic resistance, nematocidal drug combinations could be considered as a valid strategy to delay resistance development, contributing to optimize control of resistant parasite populations. The rationale behind using combinations is based on the fact that individual worms may have a lower degree of resistance to a multiple component formulation (each chemical with a different mode of action/resistance) compared to that observed when a single anthelmintic molecule is used. A pharmaco-parasitological assessment of different nematocidal combinations was performed at different cattle commercial farms in Argentina. Ivermectin(IVM)-ricobendazole(RBZ), IVM-levamisole(LEV) and RBZ-LEV combinations were assessed. The observed pharmacokinetic data demonstrated that the co-administration of two anthelmintics did not modify the plasma pharmacokinetic behaviour of either drug in cattle. The combinations were the only treatments achieving 100% clinical efficacy, even when a highly IVM-resistant *Haemonchus* spp. isolate was present. Overall, after combined treatments a therapeutic additive effect was observed under all susceptible/resistance scenarios. However, the efficacy of IVM-RBZ against nematodes resistant to IVM and RBZ was greater than an additive effect. Achieving the highest possible efficacy is a powerful argument for using combinations; since fewer resistant parasites will survive treatment, the diluting effect with susceptible unselected parasites in refugia will be greater, and thus the development of resistance may be slowed. Additionally, the weight gain was significantly higher for the combination IVM-RBZ in calves naturally infected with resistant nematodes. Overall, anthelmintic combinations could be useful to control gastrointestinal nematodes in cattle farms. However, any treatment decision should be based on a previous diagnosis and rational use of combinations according to the situation of each individual farm.

Working Group 3. Innovative, Sustainable Control Methods

Oral Communication

Targeted-Selective Treatment of Dairy Cows against Gastrointestinal Nematodes: a Stepwise Decision Making Strategy

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In dairy cows, the milk production (MP) response to anthelmintic treatment (AT) is variable, and the decision to treat still depends on how much risk averse farmers or vets are.

The objective of this study was to develop a stepwise decision making strategy based on nested criteria associated with an increasing probability of MP response.

A randomized controlled trial was conducted at housing in 123 grazing dairy herds (injectable eprinomectin). Monthly individual test-day milk yields were obtained. Linear mixed models were used to assess the effect of AT on MP. In a first step, all herds were included in the analysis. The criterion associated with the highest MP response was identified. In a second step, only herds meeting the first criterion were included in order to identify the criterion associated with the highest MP response in this subset. This was repeated a third time on herds meeting the first 2 criteria.

When all the herds were taken into account, the effect of AT was significant but low (+0.3 kg/cow/day). The first discriminating criterion (step 1) was identified as the % of grazed grass in the diet (no MP gain in low-pasturing herds versus +1 kg/cow/day in moderate/high-pasturing herds). Among moderate/high-pasturing herds, the second discriminating criterion (step 2) was the TEC (+0.6 kg/cow/day in high-TEC herds versus +1.4 kg/cow/day in low-TEC herds). Finally, among moderate/high-pasturing AND low-TEC herds, the third discriminating criteria (step 3) was the bulk tank milk *Ostertagia* ODR (+0.2 versus +1.8 kg/cow/day when ODR < 0.9 and ≥ 0.9, respectively).



The 3 criteria identified, when applied sequentially, are associated with increasing expected MP gains and are useful for farmers and vets with different attitudes regarding risk.

Working Group 3. Innovative, Sustainable Control Methods

Oral Communication

Efficacy Of Tannin Rich Feed Against Multi Resistant *Haemonchus contortus* and Interactions with Ivermectin Subcutaneous Treatment in Sheep on Farm Conditions.

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The objectives of this study were to investigate the anthelmintic activity of condensed tannin(CT) feed resources against resistant parasite nematodes *in vivo* in sheep and the possible interactions between them and ivermectin treatment(IVM). This experiment included 36 lambs comparing standard (non-tannin) with 2 types of tannin-rich diets and receiving or not IVM treatment. Two-month-old lambs were experimentally infected (Do) with 3,000 L3 larvae of *H. contortus* (white river multi-resistant strain). The CT rich diets used were based on either sainfoin pellets or carob-pod meal both incorporated in isonitrogenous, isoenergetic balanced rations (at DAY-24). The animals were randomly allocated into 6 groups of 6 animals each as follows: Group-1 (receiving sainfoin pellets-no treatment); Group-2 (receiving sainfoin pellets + IVM); Group-3 (receiving carob-pod meal-no treatment) Group-4 (receiving carob-pod meal + IVM); Group-5 (receiving lucerne-no treatment); Group-6 (receiving lucerne + IVM). The IVM was administered SC at the dose rate of 0.2 g/kg (DAY36). Faecal samples were collected from D18 and on weekly basis onwards and blood samples were collected on D36 (To, 5h 10h), D37, D38, D40, D43, D46, D51, D56, D62. Faecal egg counts were performed and after the animals were sacrificed (DAY-64) worm burdens and female fertility was calculated.

Results showed that after IVM subcutaneous injection, the drug concentration profiles were similar in lambs fed lucerne, carob or sainfoin. Under these conditions, tannin-rich feed did not impact the drug kinetics. However, interactions between tannins and drug may occurred since a lower antiparasitic activity of ivermectin measured in animals fed with sainfoin pellets compared to animals fed with carob-



pod meal. Such observations do impose caution when both tannin-rich diet and drug-based treatments are combined. Further studies are required to clarify the mechanisms that support such interactions.

Working Group 3. Innovative, Sustainable Control Methods

Oral Communication

Fungal Spores to Develop the Integrated Control of Horse Strongyles

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Control of strongyles is aimed to the elimination of the stages affecting horses. Despite the high efficiency of chemical dewormers, horses become infected promptly, due to the presence of infective stages (third-stage larvae) in the soil. The current study was conducted on adult horses maintained under a continuous grazing system. At the beginning, all horses passed counts of strongyles higher than 300 eggs per gram of feces (EPG), thus a treatment comprising ivermectin was administered; efficacy was established by estimating the reduction of EPG (FECR) and the egg-reappearance period (ERP). With the objective to prevent the development of the eggs of strongyles in the feces, spores of the nematophagous fungi *Duddingtonia flagrans* and *Mucor circinelloides* were cultured in edible gelatins. One group containing five horses received (twice a week) the gelatins, and the other group remained without spores as control. The FECR values were of 99-100% fourteen days after the deworming, and the ERP was 2.5 months. Gelatins were well taken by the horses and none refused to take them. Horses receiving the fungal spores attained values of strongyle egg-output lower than 300 EPG. However, the counts were higher than 600 EPG in the control group. This assay seems to demonstrate that edible gelatins containing spores of parasiticide fungi appears very useful to develop a sustainable strategy in the integrated control of parasites of horses.

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Working Group 3. Innovative, Sustainable Control Methods

Oral Communication

The Canaria Hair Breed Sheep: a Resistant Breed To Gastrointestinal Nematodes

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Gastrointestinal nematodes (GINs) are one of the main constraints in small ruminant production worldwide. Traditionally, they have been controlled by regular anthelmintic treatment. However, rising drug resistance makes this control method non-sustainable. One of the most attractive alternatives is the selection of resistant animals. In the last decade, our group have identified several mechanisms that could explain the greater resistance of the Canaria Hair Breed than the Canaria Sheep to one of the most pathogenic GINs, *Haemonchus contortus*. This protection targets the adult stage instead of the larva stage (L3) that has been described in other commercial breeds. In this presentation, we will summarise some of the results obtained in our studies and the potential role of gammadelta T cells, eosinophils, IgA, and galectins in mediating protection. We will also discuss the utility of this knowledge to identify new biomarkers of resistance (genetic and/or phenotypic) and/or new pharmacological or immunological therapeutic targets.

Working Group 3. Innovative, Sustainable Control Methods

Oral Communication

Immune Response of Rats, Calves and Lambs Vaccinated with Various Forms of *F. Hepatica* Cysteine Proteinase when Challenged with *F. Hepatica* Metacercariae.

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It has been found that cysteine proteinases of *F. hepatica* are very important candidates for a vaccine antigen because of their role in fluke biology and in the host-parasite relationship. In our experiments we found that vector carrying cDNA encoding for a cysteine proteinase of *F. hepatica* when administered intramuscularly or when given intranasally induced a high level (61-75%) of protection against subsequent infection with *F. Hepatica* metacercariae in rats.

In another attempt we used a recombinant cysteine proteinase (CPFhW) expressed in *Escherichia coli* for enteral vaccination of rats. The vaccination induced a 78-80% protection against challenge with fluke metacercariae. We also evaluated the protectivity of CPFhW in the mucosal vaccination of calves and lambs. Female calves vaccinated intranasally with recombinant CPFhW showed 54.2% protection against the subsequent challenge. Flukes which developed in vaccinated calves showed a reduction of reproductive potential. Vaccinated male Corriedale lambs showed 56.5% of protection against the subsequent challenge.

We also used CPFhW produced in a transgenic-plant-based system in oral vaccination of rats. To avoid inducing tolerance and to maximise the immune response induced by oral immunisation, we used the hepatitis B virus core protein (HBcAg) as a carrier for CPFhW. The vaccinated animals showed 65.4% protection.

Working Group 3. Innovative, Sustainable Control Methods

Oral Communication

Functional Expression of *Trichuris suis* acr-16 in *Xenopus laevis* oocytes – a Pharmacological Screening Tool for The Anthelmintic Effect of Synthetic and Natural Compounds on Nicotinic Acetylcholine Receptor in Parasitic Nematodes

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Background and purpose:

Anthelmintic resistance and/or decreased susceptibility to anthelmintics is a major global problem in parasitic nematodes of livestock. Therefore, there is an urgent need to understand the molecular mechanisms of existing drugs. *Trichuris* is an interesting nematode genus due to its broad host spectrum and its low susceptibility to several anthelmintic drug classes. However, the cholinergic agonists oxantel has a high efficacy against *Trichuris* spp. Despite this knowledge, the exact molecular target of oxantel within *Trichuris* is still unknown. In *Ascaris suum*, oxantel has a low effect on the nicotinic Ach receptor (nAChR) acr-16. We therefore hypothesized that the sensitivity of *T. suis* acr-16 to oxantel is different from *A. suum* acr-16.

Methodology:

The sensitivity of *T. suis* acr-16 was evaluated using PCR, molecular cloning, functional expression of *T. suis* acr-16 cRNA in *Xenopus laevis* oocytes and two-electrode voltage clamp electrophysiology.

Results:

In electrophysiological experiments, oxantel produced a high and robust current response of *T. suis* acr-16, whereas pyrantel activated acr-16 moderately. The cholinergic anthelmintic compounds morantel and levamisole did not activate the receptor significantly. Other nAChR agonists, including epibathidine, nicotine, cytosine, dimethylpiperazine and 3-bromocytisine resulted in minor responses.

Conclusion and perspective:

T. suis acr-16 is more sensitive to oxantel than *A. suum* acr-16. *T. suis* acr-16 may not be the only target of oxantel, but the functional expression of this nAChR can prove a valuable screening tool to evaluate the effect of new synthetic or natural compounds against this genus.

Working Group 3. Innovative, Sustainable Control Methods

Poster Communication

***In vitro* Evidence That the Pastoral *Artemisia Campestris* Species Exerts an Anthelmintic Effect on *Haemonchus Contortus* from Sheep**

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Occurrence of anthelmintic resistant strains of helminthes is increasing. The aim of this study was to evaluate the *in vitro* anthelmintic activity of *Artemisia campestris* in comparison to albendazole against *Haemonchus contortus* of sheep. In this respect, *in vitro* anthelmintic activities of crude aqueous and crude ethanolic extracts of aerial parts of *A. campestris* were investigated on eggs and adults of *Haemonchus contortus*. Chemical analyses revealed that overall profile of both extracts samples were dominated by flavonoids among them quercetin and apigenin derivatives were the most abundant phenolics constituents. Both extract types completely inhibited egg hatching at a concentration close to 2 mg/ml. Lethal concentration 50% of *A. campestris* ethanolic and aqueous extracts were 0.83 and 1.00 mg/ml respectively ($p < 0.05$). The ethanolic extract showed better *in vitro* activity against adult parasites than the aqueous extract in terms of the paralysis and/or death of the worms at different hours post treatment. Dose dependent activity was also observed for both extract. After 8 and 24 h of exposure, the ethanolic extract induced 91.3 and 100% mortality at the highest tested concentration respectively, while the aqueous extract induced 3.22 and 70.96% at the same concentration respectively.

To our knowledge, these results depict for the first time that *A. campestris* possesses *in vitro* anti-*Haemonchus contortus* properties.

Working Group 3. Innovative, Sustainable Control Methods

Poster Communication

Anthelmintic activity of amino alcohols against eggs and larvae of the ovine nematode *Teladorsagia circumcincta*

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The importance of gastrointestinal nematodes in sheep is due to the huge economic losses as a result of decreased production and increased healthcare cost. The aim of this study was to test the potential anthelmintic activity of 20 synthetic molecules belonging to the amino alcohols family, with demonstrated activity against parasites such as *Echinococcus granulosus* and *Trypanosoma cruzi*. The molecules were tested against different phases of the gastrointestinal nematode *Teladorsagia circumcincta* using the in vitro Egg Hatch Assay (EHA) at a single concentration of 50 µM. After an incubating period of 48 hours, the ovicidal and larvicidal activity was calculated by the percentage of inhibited eggs and dead larvae, respectively. In those molecules that showed ovicidal activities higher than 80%, the concentration required to inhibit the 50% of the activity (IC₅₀) and the cytotoxicity were calculated to determine the selective index (SI). With those molecules with high larvicidal activity (>90%), the Larval Feeding Inhibition Assay (LFIA) was performed with the aim to identify a possible mechanism of action based on laryngeal paralysis. Out of all molecules tested, 9 showed larvicidal activity higher than 95% at 50 µM and 4 an egg hatching inhibition higher than 80%. One molecule showed a promising SI of 20.93, 10.11, 5.92 and 9.52 when cytotoxicity was tested in mammalian Vero cells, mouse splenocytes cells (Balb/c strain), mouse macrophages (Balb/c strain), and human CaCo-2 cells, respectively. LFIA showed that one molecule inhibited the larvae food ingestion, showing a similar mechanism of action as imidazothiazoles or macrocyclic lactones. Study funded by AGL2016-79813-C2-1/2-R.

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Poster Communication

In vitro* Effect of Benzalphthalides and Phtalazinones against Different Eggs and Larvae of *Teladorsagia Circumcincta

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Gastrointestinal nematode infections cause direct and indirect losses of great economic impact in ruminants by decreasing productions yields and increasing health care cost. The objective in this study is to test the anthelmintic activity of 41 molecules against eggs and larvae of the ovine nematode *Teladorsagia circumcincta*. The molecules are included into 2 families of drugs: benzalphthalides and phtalazinones, with previous activity demonstrated against protozoan parasites such us *Cryptosporidium parvum*. All molecules were tested by means of the Egg Hatch Assay (EHA) using an initial concentration of 50 uM. After an incubating period of 48 hours, the ovicidal and larvicidal activity was calculated by the percentage of inhibited eggs and dead larvae, respectively. In those molecules with activities higher than 90%, the concentration required to inhibit the 50% of their activity (IC₅₀) and the cytotoxicity were calculated to determine the selective index (SI). The first screening of the molecules at 50 uM showed that only 4 compounds had ovicidal activity, 2 of them had an activity higher than 90% (molecule A and B), and the other two, ranging between 50-90%. The ovicidal and larvicidal activity in molecule A were 98% and 4%, respectively, and in molecule B were 99% and 11%, respectively. Both molecules showed SI higher than 7 when the cytotoxicity was tested in HepG2, RAW or CaCo-2 cell lines, indicating that they could be good candidates for further studies to determine their *in vivo* activity. The only compounds with good potential belong to the benzalphthalides family. Study funded by AGL2016-79813-C2-1/2-R.

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Poster Communication

New Synthetic Benzimidazole With Potential Anthelmintic Activity

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Gastrointestinal nematodes cause direct and indirect losses of great economic impact in sheep. The excessive preventive use of the drugs of choice, as well as their incorrect administration has led to the development of anthelmintic resistances worldwide, increasing the costs of production and reducing the efficiency of the system and the quality of the products. Thus, the aim of this study was to test the anthelmintic activity of a series of newly synthesized molecules belonging to the benzimidazole family. A total of 16 new molecules were tested to determine their activity against *Teladorsagia circumcincta* eggs and larvae. Initially all molecules were tested at a final concentration of 50 μ M. After an incubating period of 48 hours, the ovicidal and larvicidal activity was calculated by the percentage of inhibited eggs and dead larvae, respectively. In those molecules in which ovicidal or larvicidal activity was higher than 85% at 50 μ M, the concentration required to inhibit the 50% of their activity (IC₅₀) as well as their cytotoxicity were calculated to determine the selective index (SI). Initially 5 molecules showed ovicidal activities higher than 99% at 50 μ M and one of them also showed a larvicidal activity higher than 90%. All these molecules had a SI higher than 1 when the cytotoxicity was measured in mouse macrophages (Balb/c strain), human CaCo-2 cells or human HepG2 cells. Consequently, the next step would be to test these molecules with benzimidazole resistant strains of *T. circumcincta*. Study funded by AGL2016-79813-C2-1/2-R.

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Poster Communication

Differences in the Immune Response between Adult Sheep Belonging to Churra Breed

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The infection by the gastrointestinal nematode (GIN) *Teladorsagia circumcincta* is one of the most common diseases in grazing sheep. Within the Spanish Churra breed, sheep can show different level of GIN infection according to their faecal egg count (FEC). In the present study, the mechanisms by which Churra breed sheep can present the susceptible or resistant phenotype to the *T. circumcincta* infection were studied. A total of 12 adult sheep were selected from a 119 commercial sheep flock, 6 resistant and 6 susceptible according to their FEC, and then were infected with third stage larvae (L3) of *T. circumcincta*. During the experimental infection the title of IgA was always higher in the resistant group than in the susceptible group although without significant differences. At day 21 days post-infection, a slight negative correlation was found between IgA and their cumulative FEC ($r = -0.516$; $p = 0.08$). Afterward, sheep were dewormed, experimentally infected again with L3 of *T. circumcincta* and slaughtered at 7 days post infection. At slaughtered, IgA title in serum and gastric mucus was higher in the resistant group although slight significant differences were only shown in serum samples ($p = 0.1$). Histological and immunohistochemistry analysis of abomasum tissue also showed that the number of CD4+ lymphocytes, $\gamma\delta$ T+/WC1 lymphocytes, eosinophils and globule leukocytes was higher in the resistant group; however these differences with the susceptible group were slight significant only in the eosinophils ($p = 0.1$). Slight positive correlation were shown between $\gamma\delta$ T+/WC1 lymphocytes and eosinophils ($r = 0.41$; $p = 0.1$) but also between globule leukocytes and CD4+ T cells ($r = 0.49$; $p = 0.09$). In conclusion, although the immune response was higher in the resistant group, the differences between them were not significant or were slight in some of the immune parameters analysed.

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Poster Communication

Seasonal Variation in the Effects of Polyphenolic Extracts of *Salix* sp (Willow) on the Exsheathment of Gastrointestinal Nematode Larvae

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Willow trees (genus *Salix*, family Salicaceae) are found worldwide where the trees range in height from 0.2 to 20 meters, depending on species and habitat, and are deciduous. Willow has been proposed as a fodder for both small and large ruminants. The leaves and fine stems of willow are rich in nutritive value for grazing ruminants. Bioactive ingredients from plants can be both seasonal and species-dependent and we hypothesized that this may cause variability in anthelmintic bioactivity. We tested this hypothesis by using the larval exsheathment inhibition assay. Total polyphenol, flavonoid and salicylic acid content were evaluated from ten local ecotypes of *Salix*, using dry foliage biomass that was collected during spring and summer. The 70% ethanolic extract yield ranged between 18-33%. Polyphenol content of extracts as quebracho equivalents ranged between 8-20% for spring and 23-33% for summer. Flavonoid content varied between 16-48%. Salicylic acid content ranged between 5-14%. Crude polyphenol extracts from spring harvests of four ecotypes resulted in more than 80% exsheathment inhibition when applied to L3 larvae. With summer harvests, however, only two ecotypes resulted in more than 80% exsheathment inhibition. Consumption of *Salix* foliage by ruminants could lead to an overall decrease in the intestinal load of health-sapping nematodes, however, seasonal variation in anthelmintic activity should be taken into account when plants are integrated into anthelmintic strategies. Using HPLC-PDA showed that the chemical constituent of the different ecotypes is composed of a similar mixture of flavonoid and phenolic acid derivatives.

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Poster Communication

Toltrazuril resistance in a field isolate of ovine *Eimeria* spp.

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Coccidiosis due to *Eimeria* spp. infections in lambs causes increased mortality and substantial production losses, and anticoccidials are important for control of the infection. Anticoccidial resistance (ACR) has been reported in poultry and swine, in addition to anecdotal reports of reduced anticoccidial efficacy in lambs. Due to the substantial differences between nematodes and coccidia, current WAAVP methods for assessing anthelmintic efficacy are not suitable for such evaluations. The present objectives were to investigate methods to evaluate anticoccidial efficacy in ovine *Eimeria* spp. and the possible occurrence of anticoccidial resistance. Based on these studies, we described the first evidence-based report of reduced toltrazuril efficacy in ovine *Eimeria* spp. in Norwegian sheep farms using a newly developed faecal oocyst count reduction test (FOCRT). To verify this reduced efficacy, a control efficacy test (CET) were performed, which confirmed toltrazuril resistance in ovine *Eimeria* spp., including the highly pathogenic species *E. ovinoidalis*. This verification also supports the use of FOCRT as an appropriate tool for field evaluation of anticoccidial efficacy. To our knowledge this is the first report of ACR in ovine isolates of *Eimeria* spp. Confirmation of resistance in ovine *Eimeria* spp. increases the urgency of identifying alternative options for treatment and control. Due to limited anticoccidial treatment alternatives, these findings may have important implications for the sheep industry, particularly in Northern Europe.

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Poster Communication

To Feed or To Develop? Evaluation of Anthelmintic Properties of Six Plant Extracts Using Two *in vitro* Tests

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Gastrointestinal nematode infections are traditionally controlled by anthelmintic drugs. Nevertheless, the anthelmintic resistance evolved as a result of long-term relying on chemical drugs as the only strategy to control gastrointestinal nematodes. Currently, parasites resistant to available anthelmintics represent a significant problem in many parts of the world. Anthelmintic properties of different plant species are intensively studied as an alternative approach to chemical control. Several *in vitro* assays have been applied to explore larvicidal effects of plant extracts; however, sensitivity and reliability of all these tests has yet to be investigated. In this survey the efficacy of *Althaea officinalis*, *Foeniculum vulgare*, *Hyssopus officinalis*, *Chamomilla recutita*, *Inula helenium* and *Malva sylvestris* methanolic extracts against *Haemonchus contortus* larvae were evaluated using Larval development assay (LDA) and Larval feeding inhibition assay (LFIA). Applying the majority of plant extracts within the above-mentioned *in vitro* tests LDA produced higher median lethal dose (LD₅₀) than LFIA and, simultaneously, these LD₅₀ values differed significantly ($P < 0.01$) in individual extracts. Only LD₅₀ values of two extracts produced by *in vitro* tests correlated with each other; i.e. 236 µg/ml in LDA and 128 µg/ml in LFIA applying *A. officinalis*, and furthermore, 53 µg/ml in LDA and 61 µg/ml in LFIA using *M. sylvestris*. Based on the obtained results it was difficult to evaluate the real anthelmintic effect of the majority of tested plant extracts against *H. contortus* larvae. Reliability of LFIA should be further investigated.

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Poster Communication

How To Reduce the Risk of Infection by *Trichuris* spp. in Zoological Parks? The Answer Is Spreading Spores of Parasiticide Fungi

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Infection by helminths *Trichuris* spp. is common among animals without dependence of their management. As occurs with domestic species under continuous pasturing, animal species captive in zoological parks are at important risk of infection due to the eggs passed in their feces can survive viable for long periods. Despite the administration of successful anthelmintics, animals become infected soon. For the purpose to decrease the risk of infection by lessening the viability of eggs of *Trichuris* spp., spores of *Mucor circinelloides* and *Trichoderma atroviride* were sprayed directly onto fecal pats from dromedaries. The fungal effect was ascertained by measuring their viability and development rate. Viable eggs without cellular division (zygote) were classified as non-developed, and as developed when containing a morula, blastula, gastrula or larva inside. Percentages of non-viable eggs around 50% were obtained thirty days after the addition of spores. Fifty percent of the viable eggs were classified as developed in the pats receiving spores, by 66% in the controls. The presence of eggs containing a L1 was near to 10% in the fungal-treated pats, by 33% in those without spores. These results point that spreading of spores of parasiticide fungi directly onto soil (or feces) offers a very useful tool to limit both the viability and the development rate of eggs of *Trichuris* spp., which results in a significant reduction of the risk of infection among wild animals captive in zoological parks.

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Poster Communication

Recombinant *Teladorsagia circumcincta* Vaccine in Native Sheep Breeds from The Canary Islands

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Teladorsagia circumcincta is one of the main parasites that compromise sheep production in temperate areas. It has been controlled by drugs, however, the worm has developed resistance and new alternative control methods are needed. Several options have been considered, such as biological control, targeted selective treatment, vaccines, genetic resistance etc. Although several of these have been considered very promising, nowadays, it is believed that their integrated use will improve on their individual effectiveness. In this work, we present the data obtained after the administration of a recombinant vaccine prototype against *T. circumcincta* in lambs younger than 6 months in two native sheep breeds from the Canary Islands with differences in their relative susceptibility to gastrointestinal nematodes: the Canaria Hair Breed (resistant) and the Canaria Sheep (susceptible). The vaccine conferred protection only in the lambs of the resistant breed, with reductions in worm burden and parasite egg excretion of about 70%. These data confirm the relevance of integrating several control methods in the search for alternatives to classical chemoprophylactic control methods in order to apply them in farms.

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Poster Communication

Local Immune Responses of Goats Immunized with Thiol-Proteinases Enriched Fractions from E/S products of *Teladorsagia circumcincta* Adult Worms

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Several studies have shown the possibility of inducing protective immune responses to gastric nematodes using native antigens from both larvae 3 and adult worms (somatic antigens or E/S products). Some of the antigens investigated include thiol-proteinases, enzymes that seem to play a very important role in the survival of parasites within the host. The immunization using these antigens has given promising results in experimental trials against *H. contortus* in both sheep and goats. However, no information is available on the use of these immunogens against other gastric nematodes of small ruminants such as *T. circumcincta*.

In this trial, the immunoprotection induced in goats by thiol-proteinases enriched fractions (obtained from E/S products of *T. circumcincta* adult worm) has been evaluated at local level after a homologous challenge. The results showed that the immunization provides a partial protection against the parasite, which translated into a reduction (42,2 %) in the cumulative fecal egg counts (FEC) throughout the study. However, at the end of the experiment, no significant differences in adult worm burden was observed.

These parasitological findings were associated with an increase of eosinophils and globule leukocytes in the abomasal mucosa as well as the levels of specific antibodies (IgG and IgA) in the mucus of immunized goats. The immunohistochemical analysis on abomasal mucosa revealed that MHC-II+ cells, CD4⁺ and IgA⁺ lymphocytes counts were increased in immunized goats when compared to the challenge control group.

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Poster Communication

Biological Control as a Solution against Parasites Affecting Captive Wild Ruminants

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Strongyles and ascarids are parasites frequently detected among captive wild animals, because of their direct biological cycle is improved by maintaining them ever in the same parcels. With the aim to find a beneficial strategy for contributing to the integrated control of these parasites, the usefulness of using spores of two parasiticide fungi, *Duddingtonia flagrans* and *Mucor circinelloides*, to reduce the risk of infection was checked. Two assays were conducted. First, feces collected from yaks from the zoological park “Quinta dos Plátanos” (Abrantes, Portugal) were sprayed spores of the aforementioned fungi. An in vivo probe was carried out on ruminants (goats, bison and reindeer) from the zoological park “Marcelle Natureza” (Lugo, Spain), by incorporating fungal spores into nutritional pellets. The efficiency of the fungi *D. flagrans* and *M. circinelloides* was evaluated by measuring the number of eggs per gram of feces (EPG). For that purpose, monthly fecal samples of different animal species were taken. In the in vitro assay, viability of eggs of *Toxocara vitulorum* reduced by half in the presence of fungal spores. Ruminants provided pellets containing spores maintained values of gastrointestinal egg-output around 100-150 EPG. It is concluded that the presence of nematophagous fungi such as *D. flagrans* and *M. circinelloides* in the feces of ruminants infected by gastrointestinal parasites would turn out to be very profitable to limit the risk of infection among individuals captive in zoological parks decreasing the need and frequency of conventional treatments.

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Biological Control of Helminths Affecting Sheep in the Basque Country

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Sheep in the Basque Country are mainly reared under an extensive grazing system, which increases the risk of some parasitic diseases. Analysis of feces obtained from sheep in different farms belonging to the regions of Azpeitia and Aretxabaleta (Guipúzcoa, N Spain) demonstrated the infection by helminths affected more than 50% of all sheep. The highest prevalences were obtained among gastrointestinal nematodes (50-100%), followed by trematodes (13-18%) and bronchopulmonary nematodes (3-9%). In order to avoid that helminths could attain the infective stages in the soil, spores of parasitocidal fungi (*Mucor circinelloides* and *Duddingtonia flagrans*) were sprayed onto different coprocultures. Next step consisted of analyzing the more appropriate way to ensure the presence of spores in the feces. With this aim, spores were cultured in different media and added to commercial feeding composed by grain. A significant reduction in the viability of helminths in the coprocultures added fungal spores was recorded. These results show that the presence of spores in feces offers a very effective solution to prevent the development of the infective stages of certain parasites, limiting the risk of infection and thus the need to apply treatments too frequently. The most suitable formulation for giving the spores to the sheep involved their culturing in edible gelatin, lyophilization and subsequent grinding. Finally, this product was mixed with the feeding prior to be provided to the sheep.

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Poster Communication

High-Throughput Screening Of Anthelmintic Drugs Using *Caenorhabditis Elegans*

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We employ HighRes Biosciences robotic platform for ultra-high-throughput screening of small molecules with the goal to identify candidate drugs. Recently, assays for screening of anthelmintic activity using *C. elegans* as a model nematode have been optimized. The assays are based either on fluorescence measurement (chitinase assay, quantification of the decrease of the GFP labeled bacterial food) or automated microscopy followed by image analysis (shape and movement). The therapeutic index of the compounds is estimated by testing of toxic effect in primary human cell cultures using MTS assay. Assays for in vitro evaluation of absorption (PAMPA and CaCo2, MDR-MDCK permeability assays) and metabolism (stability in plasma, microsomal stability) are available. Screening of a pilot sample (N~2000) of our diverse chemical library (N> 110,000) revealed several hits.

We are looking for collaboration with laboratories that could test the activity of our compounds in parasitic helminths or infection models. We also offer an expertise in image analysis of helminths.

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Poster Communication

Assessment of the Potential of Mid-Season Targeted Selective Anthelmintic Treatment Based on Flexible Weight Gain Threshold for Nematode Infection Control in First-Grazing Season Dairy Calves

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The suitability of a single mid-season targeted selective treatment (TST) for gastrointestinal nematode control, based on flexible average daily weight gain (ADWG) thresholds, was investigated in 23 groups of first grazing season calves 3-4 months after turnout (levamisole 7.5 mg/kg). In each group, animals were randomly allocated into 2 sub-groups: one sub-group where all calves were treated (whole treatment, WT) and the other sub-group where a TST was applied on the animals showing an individual pre-treatment ADWG inferior to the corresponding mean pre-treatment ADWG in the WT sub-group. At housing, blood anti-Ostertagia antibody and pepsinogen levels and breech soiling score were assessed at individual level. In TST sub-groups, the ADWG thresholds for treatment ranged from 338 to 941 g/day and the percentage of treated animals from 28 to 75 %. Pre and post-treatment ADWG as well as parasitological and clinical parameters measured at housing were similar between WT and TST sub-groups. In the 6 highest exposed groups (pepsinogen level

≥2.5 U TYR), the average effect of treatment on post-treatment ADWG was the highest and estimated up to 14 kg for 4 months of grazing. In contrast, in 6 other groups showing the lowest exposure, no effect of treatment was seen suggesting an absence of production losses when the level of infection is low. This study demonstrated that a mid-season TST strategy for first grazing season calves based on the use of flexible thresholds of AWG allowed similar growth compared to WT strategy while keeping a nematode population in refugia.

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***In vitro* anthelmintic effects of bark extracts from *Picea abies* and *Pinus sylvestris* against sheep nematodes**

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Introduction:

Gastrointestinal nematodes (GIN) in sheep are a common cause of reduced animal welfare and performance, and thus, high economical losses. The emerging drug resistance in GIN prompts for new methods in combating infections. Several studies have indicated an anti-parasitic effect of tannin-rich plants when included in the diet.

Objective:

To assess the potential *in vitro* anthelmintic efficacy of tannin-rich extracts from bark of common Norwegian trees (spruce (*Picea abies*) and pine (*Pinus sylvestris*)) against GIN of sheep.

Materials and methods:

Bark from spruce and pine was milled in a hammer mill (5 mm sieve), freeze-dried and ground to < 1 mm. For extraction, acetone, methanol or water was used, and subsequently the extracts were freeze-dried. Eggs of GIN from faeces of naturally infected sheep (>90% *Teladorsagia circumcincta*) were isolated by flotation and used within 1 hour. L3 were obtained after culturing eggs for 10 days at 20°C, using Baermann technique for L3 extraction. Two *in vitro* methods were applied: egg hatch assay (EHA) and larval motility assay with real time cell analyser (LMA-RTCA). EHA: 300 µl extract (five concentrations in two-fold dilutions from 1000 to 62.5 µg/ml) in 1% dimethyl sulfoxide and 150 eggs were added to each well in 96 well

plates (n=3). Eggs and larvae were counted after 48 hours incubation at room temperature, and the egg hatch percentage (EH%) was calculated. LMA-RTCA: 3000 L3 in PBS were added to 300 μ l wells in three 16 well resistor plates, and the Cell Index (CI; electronic impedance) was measured for 24 hours. Subsequently, extracts were added, followed by a 24 hours CI measuring period. CI was converted to Motility Index (MI), calculated as the standard deviation over 800 data points of the CI difference from the rolling average over 20 data points. 100% motility was calculated as a mean MI of the untreated wells, 0% motility as a mean MI of the dead L3. Tests were run in triplicate.

Results:

EHA showed that water and methanol extracts of spruce practically had no effect (EH% above 90). The pine extracts had a high efficacy independent of extraction method, with the acetone extract giving the highest egg hatch inhibition (EH% <10). In the LMA-RTCA both pine and spruce extracts caused a dose-dependent motility reduction: the water extracts showed a motility reduction only at the highest concentrations, whereas the acetone and methanol extracts reduced the L3 motility to varying degrees at all concentrations.

Conclusions:

The pine extracts demonstrated promising anti-parasitic activity in both the EHA and LMA-RTCA. Pine water extract need to be retested at higher concentrations to obtain conclusive results.

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Poster Communication

***In vivo* Nutraceutical Evaluation of *Gymnopodium floribundum* Leaf Meal against *Haemonchus contortus* in Lambs**

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This study evaluated the nutraceutical value of graded levels of *Gymnopodium floribundum* (GF) leaves meal on total diet intake, digestibility and its effect on *Haemonchus contortus* burden in hair-sheep lambs. Thirty animals (17.8 ± 3.44 kg BW) were infected with 6 000 L₃ of *H. contortus*. On day 28 post-infection, animals were assigned to groups with homogeneous infection (n = 6): T1 (20% GF), T2 (30% GF) or T3 (40% GF), T4 (0% GF) and T5 (worm free + 0% GF). All diets were isoproteic and isoenergetic. Intake, dry matter digestibility (DMD) and organic matter digestibility (OMD) were measured, as well as faecal excretion of eggs per gram (EPG) and total faecal egg count (TFEC) of *H. contortus*. At day 38 post-infection, animals were humanely slaughtered to recover and count adult parasites, assess length and fecundity of female worms. Inclusion of GF did not affect intake or growth of lambs, but high levels of GF (T2 and T3) reduced DMD and OMD ($P < 0.05$). The EPG and TFEC for T1 and T3 were lower than for T4 ($P < 0.05$). Meanwhile, the number of adult female worms was lowest in T3 ($P < 0.05$). Fecundity and length of female worms were similar between groups ($P > 0.05$). In conclusion, the highest GF inclusion level (T3) showed a good nutraceutical value as it did not reduce production or intake of lambs and reduced the EPG of lambs associated with fewer adult female worms. Funding CONACYT, Mexico (CB-2013-1/221041).

Working Group 3. Innovative, Sustainable Control Methods

Poster Communication

Can We Observe Differences in Feeding Behavior between Kids without Browsing Experience and Goats with Browsing Experience on Heterogeneous Vegetation?

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Feeding behavior was compared between kids without browsing experience and adult goats with browsing experience on 2.2 ha of tropical heterogeneous vegetation on Mérida, México. Twelve adult goats (35 ± 5 kg LW) naturally infected with gastrointestinal nematodes (GIN) and ten tracers kids (19 ± 3 kg LW) born from goats of a different flock, and raised free of GIN browsed at the same site four hours daily during ten weeks covering the rainy season. Kids were introduced by pairs for three weeks every fortnight. After exposition traces were maintained without browsing 28 days and humanly slaughtered to recover GIN worm burdens. The dry matter intake (DMI) was estimated with the continuous bite monitoring method. The proportion to the DMI of shrubs, dicot herbaceous, vines and grasses but also of strata (low < 25 cm, medium 25-50 cm, high >50 cm of height) consumed by kids and goats was compared with Chi-squared test. Kids and goats obtained 62.8% and 57% of their DMI from grass respectively, 35.3% and 40.4% from shrubs, 1.6% and 2.15 from dicot herbaceous, and 0.3% and 0.6% from vines; also a 66.8% and 58.2% of their DMI from the low strata respectively, a 18.7% and 17.3% from mid strata, and 14.6% and 24.5% from high strata. No significant differences ($p < 0.05$) was found on the proportion of life forms or strata consumed by kids and goats. No correlation was found between worm burden and the proportion of life form or strata consumed. Funding came from CONACYT, México.

Keywords: natural GIN infection, heterogenous vegetation, plant strata and life's forms, small ruminants

Working Group 3. Innovative, Sustainable Control Methods

Poster Communication

Antiparasitic Activity of Tannin-Rich Mediterranean Plants against Sheep Gastrointestinal Nematodes: *In-vitro* Studies

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The potential anthelmintic (AH) effect of 31 tannin-rich plants (*Arbutus adrachnae*, *A. unedo*, *Quercus coccifera*, *Amorpha fruticosa*, *Coronilla varia*, *Olea europea*, *Robinia pseudoacacia*, *Lotus corniculatus*, *Erica arborea*, *E. manipuliflora*, *Punica granatum*, *Onobrychis peloponnesiaca*, *On. alba-laconica*, *Opuntia ficus-indica*, *Pyrus spinosa*, *Lupinus albus*, *L. angustifolius*, *Rosa canina*, *Rubus sanctus*, *Prunus webbii*, *Pr. mahaleb*, *Potentilla recta*, *P. pedata*, *P. speciosa*, *P. hiyraldiana*, *Sorbus umbellata*, *Aremonia agrimonoides*, *Geum urbanum*, *Alchemilla bulgarica*, *A. xanthochlora*, *Fragaria vesca*) was investigated in an *in-vitro* study. Precisely, the extracts of above plants were tested against third stage larvae of *Haemonchus contortus* and *Trichostrongylus colubriformis* by using the Larval Exsheathment Inhibition Assay at different concentrations (1200/600/300/150 µg/ml in PBS). Results showed high and dose-dependent AH effects against both parasites for *A. adrachnae*, *On. peloponnesiaca*, *P. granatum*, *P. spinosa* and *P. hiyraldiana*, while *Q. coccifera*, *C. varia*, *O. europea* pulp and *A. agrimonoides* showed AH effect only at the highest concentration. The extracts of *R. pseudoacacia*, *On. alba-laconica*, *A. fruticosa*, *A. unedo*, *E. arborea*, proved to be more effective against *H. contortus*, while *E. manipuliflora* was the single extract proved to be more effective against *Tr. colubriformis*. Finally, *R. canina*, *P. webbii*, *P. recta*, *P. pedata*, *A. xanthochlora*, *P. speciose*, *F. vesca* were effective only against *Tr. colubriformis* at the highest concentration, in contrast to *A. bulgarica* which was efficient only against *H. contortus*. Overall, the study confirms that tannin-containing resources show a potential to alter the biology of nematodes representing an option for their sustainable control. However, the variability in the *in-vitro* effects suggests a strong need for further research and *in-vivo* studies.