



Abstract book JOINT COMBAR – ACSRPC MEETING

Anthelmintic resistance in ruminants: Who cares?

August 27-29th, 2019
Het Pand, Ghent, Belgium



MID-TERM MEETING

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

**Mid-term meeting of COST Action COMBAR,
August 27-29th 2019 in Ghent, Belgium**

After 2 successful joint Working Group meetings in Warsaw and León in 2018, this year COMBAR lands in the historical centre of Ghent (Belgium) for a meeting with specialists from Europe, Near-Neighbour Countries and beyond to discuss their work and innovations in the field of sustainable helminth control. It will be the Action's mid-term meeting and with the central theme "Anthelmintic resistance in ruminants: Who cares?", we aim to investigate the "who and how" of a shift towards sustainable management practices in ruminant parasite control. The meeting is held jointly with [the American Consortium for Small Ruminant Parasite Control](http://www.acsrpc.org) (ACSRPC) and is expecting around 100 participants from different parts of the world. Further information can be found on the meeting's website: www.combar-acsrpc2019.org



**Joint COMBAR – ACSRPC meeting,
Ghent, 27-29 August 2019**
www.combar-acsrpc2019.org

Anthelmintic resistance in ruminants: Who cares?

Preconference workshop public speaking
Invited speakers
WG meetings
Social programme

COMBAR

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IN SCIENCE & TECHNOLOGY



Parasitic worms are important production-limiting diseases of grazing ruminants and are mainly controlled through grazing management practices and the strategic use of anthelmintics. Today, anthelmintic resistance (AR) has become a global threat for effective parasite control and rentable livestock farming. The [COST Action COMBAR](http://www.combar-acsrpc2019.org) aims at



coordinating research at the European level to find solutions for the AR problem. The action works in a structure of 3 working groups: (1) Diagnosis; (2) Socio-economic aspects and (3) Integrated control.

SPONSORS

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

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LOCAL ORGANISERS

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Dr. Johannes Charlier, Kreavet

Prof. dr. Edwin Claerebout, Ghent University

Prof. dr. Jozef Vercruysse, Ghent University



PROGRAMME

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Tuesday 27/08

17:00-19:00	COMBAR 4th Management Committee (MC) meeting	MC members only
20:00-...	Social dinner	

Wednesday 28/08

08:15-09:00	Registration	
09:00-09:15	Welcome address	Edwin <u>Claerebout</u>
09:15-09:25	Quo vadis <u>COMBAR</u> ?	Johannes Charlier
09:25-09:50	Session 1: Diagnosing (the impact of) AR	Chairs: Laura Rinaldi, <u>Jozef Vercruvse</u> Jarkko Niemi
09:50-11:00	Keynote: Understanding economics of AMR and incentives for prevention Oral presentations (8 min + 2 min discussion) <ul style="list-style-type: none"> - Worm control on organic farms in the EU: Management and the farmers' point of view - Investigating farmer's perceptions of <u>Nematodirus battus</u> infection in grazing sheep and current helminth control practices in the UK - Gastrointestinal nematodes as cause of death in sheep and goats in Switzerland - The presence of a new SNP at codon 198 of the beta tubulin gene is associated with benzimidazole resistance in <u>Teladorsagia circumcincta</u> - Sheep, <u>strongyles</u> and sequencing for sustainability: investigating ivermectin resistance on UK farms - Illustrating the application of next generation amplicon sequencing to investigate anthelmintic drug resistance in North American beef cattle 	Steffen Werne Lynsey Melville Hubertus Hertzberg María Martínez-Valladares Jennifer McIntyre John Gilleard
11:00-11:30	Coffee break/Poster session	
11:30-11:55	Session 2: Sustainable parasite control	Chair: Smaro Sotiraki Gustavo Sabatini
11:55-13:00	Keynote: Sustainable ruminant parasite control: an industry view Oral presentations (8 min + 2 min discussion) <ul style="list-style-type: none"> - Weather and the development of anthelmintic resistant <u>Haemonchus contortus</u> - Is pasture management at fault for anthelmintic resistance in small ruminants? - Successful implementation of TST for sustainable integrated internal parasite management - Sustainable control of gastrointestinal nematodes in Gran <u>Canaria</u> island (Spain). Is it possible? - Ellagitannins as potential natural <u>anthelmintics</u> - ACSRPC web site provides information to a growing audience 	Hannah R. Vineer James P. Muir Gareth Bath Jorge Francisco González Maarit Karonen Susan <u>Schoenian</u>
13:00-14:00	Lunch/Poster session	
14:00-15:30	Joint COMBAR-ACSRPC session: Enhancing transatlantic collaboration in helminth research <ul style="list-style-type: none"> - Current status of small ruminant parasite control in the US and Canada - The BUG Consortium: unravelling the complexities of AR - New guidelines for the faecal egg count reduction test 	Chair: James Miller Anne <u>Zajak</u> Eileen Devaney Ray Kaplan
15:30-16:00	Coffee break/Poster session	

PROGRAMME

Joint COMBAR- ACSRPC meeting: “Anthelmintic Resistance in ruminants: Who cares?”

16:00-17:00	Session 4: Short term scientific missions Oral presentations (8 min + 2 discussion) - Potent <i>in vitro</i> anthelmintic effects of the seaweed <i>Saccharina latissima</i> against <i>Teladorsagia circumcincta</i> - Mini-FLOTAC and DNA-based technology for detection of helminths, protozoa and microbiome in large ruminants - Towards the development of the Mini-FLOTAC Automated System - <i>In vitro</i> techniques to detect anthelmintic resistance of parasites of domestic animals - Genotyping of <i>Haemonchus contortus</i> L1 larvae in the Egg Hatch Test via droplet digital PCR - Resistance to Benzimidazoles identification in sheep parasite <i>Haemonchus contortus</i> via Pyrosequencing and droplet digital PCR	Chair: María Martínez-Valladares Charlotte Smith Bonde Morgoglione Maria Elena Alessandra Amadesi Marcin Mickiewicz Michaela Komáromyová Paulius Baltrusis
Evening	Guided tour through historical Ghent and Walking dinner in Saint-Vincent Chapel.	

Thursday 29/08

08:30-09:00	Registration	
09:00-09:50	Session 5: Preserving drug efficacy Keynote: <i>WormBoss</i> —effective and sustainable Australian sheep worm control strategies Keynote: Monitoring drug efficacy and anthelmintic resistance in human STH: an update from <i>Starworms</i>	Chair: Georg von Samson-Himmelstjerna Deborah Maxwell Bruno Levecke
09:50-10:00	Organisation of break-out groups per COMBAR WG	
10:00-11:20	WG discussions: collaboration, progress, deliverables	WG leaders
11:20-11:50	Coffee break/Poster session	
11:50-12:30	Reporting of WG discussions	WG leaders (15 min per WG)
12:30-12:45	Wrap-up and close of meeting	
12:45-14:00	Lunch	

SCIENTIFIC COMMITTEE

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Dr. Johannes Charlier

Dr. Smaro Sotiraki

Prof. dr. Laura Rinaldi

Prof. dr. Georg von Samson-Himmelstjerna

Prof. dr. Edwin Claerebout

Dr. Dave Bartley

Prof. dr. Eric Morgan

Dr. Hervé Hoste

Prof. dr. Tom Terrill

OVERVIEW INVITED PRESENTATIONS

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

	Presenter	Titel
1	E. Devaney	The BUG Consortium: unravelling the complexities of anthelmintic resistance
2	R. M. Kaplan	Further progress on developing new World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for the Faecal Egg Count Reduction Test
3	B. Levecke	Monitoring drug efficacy and anthelmintic resistance in human soil-transmitted helminths: an update from the Starworms project
4	D. Maxwell	WormBoss-effective and sustainable Australian sheep worm control strategies
5	J. K. Niemi	Understanding economics of AMR and incentives for prevention
6	G. Sabatini	Sustainable Ruminant Parasite Control: an industry view
7	A. Zajac	Current status of small ruminant parasite control in the U.S. and Canada

INVITED PRESENTATIONS

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

The BUG Consortium: unravelling the complexities of anthelmintic resistance

Eileen Devaney¹, Roz Laing¹, Kirsty Maitland¹, Barbara Mable¹, Collette Britton¹, Jennifer McIntyre¹, Louise Matthews¹, Jamie Prentice¹, Dave Bartley², Alison Morrison², Neil Sargison³, Umer Chaudhry³, Eric Morgan⁴, Hannah Vineer⁵, Katie Bull⁶, Cath Milne⁷, Matthew Berriman⁸, James Cotton⁸, Stephen Doyle⁸

¹University of Glasgow; ²Moredun Research Institute; ³University of Edinburgh; ⁴Queen's University Belfast; ⁵University of Liverpool; ⁶University of Bristol; ⁷Scotland's Rural College; ⁸Wellcome Sanger Institute

The BUG Consortium is a multi-centre BBSRC-funded project aiming to use the resources of the *Haemonchus contortus* genome to investigate anthelmintic resistance in parasitic nematodes. Our long-term aim is to identify markers of resistance and to use these to optimise worm control strategies on farm. Much of our effort has focused on the use of a genetic cross between a susceptible (MHco3, ISE) and a triple resistant isolate (MHco18, UGA2004 - resistant to ivermectin, IVM; benzimidazole, BZ; levamisole, LEV) of *H. contortus*. Pooled whole genome sequencing of 200 L3 collected pre- and post-treatment of the genetic cross F2 in vivo with each of the drugs confirmed the known mechanisms of BZ and LEV resistance and the likely complexity of IVM resistance. A locus on Chromosome V was shown to be under IVM selection in the cross and a range of additional experiments have been undertaken to improve the resolution of this region. Studies from other laboratories indicate that the region under selection is conserved in isolates from other geographical locations. As findings emerge from the experimental data, mathematical models are being developed to capture the spread of resistance under different genetic architectures, and to examine the role of sheep movement in the spread of resistance. Through a BBSRC International Partnership Award we were able to join forces with colleagues at the University of Liverpool, UK and the University of Georgia, Athens, USA to further our research on co-infections and resistance. This has led to two additional grant applications and further collaboration that underpins a Fellowship application.

INVITED PRESENTATIONS

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Further Progress on Developing New World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for the Faecal Egg Count Reduction Test

Ray M. Kaplan¹, Bruno Levecke², Paul R. Torgerson³, Robert J. Dobson⁴, Stig M. Thamsborg⁵, Matt Denwood⁵, Martin K. Nielsen⁶, Jozef Vercruyse²

¹Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Georgia, USA

²Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium

³Section of Epidemiology, Vetsuisse Faculty, University of Zürich, Switzerland

⁴School of Veterinary and Life Sciences, Murdoch University, Australia

⁵Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

⁶Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, Kentucky, USA

In recent years there have been many new insights into the fecal egg count reduction test (FECRT) regarding optimal experimental design, and the analysis and interpretation of data. Additionally, there are important host-specific and parasite-specific differences that require protocol modifications to address these distinctions. These issues highlight the necessity for developing new World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines that improve both the general methodology and standardization of the FECRT. Extensive data simulations and analyses identified many factors that can affect the outcome and interpretation of a FECRT. Proper consideration of these factors made it obvious that a simple protocol would be unlikely to yield consistently accurate results. However, there is a strong demand among the veterinary parasitology community for a simplified guideline that will facilitate the performance of FECRT at the individual farm level. To address these conflicting demands, we have developed a guideline that has several components. In the first part, we address the major issues relevant to experimental design and make a series of general recommendations. We then provide two separate guidelines for the performance of the FECRT, (1) a more rigorous version that is intended for use in scientifically-based studies (e.g. in studies intended for publication or registration of new drugs), and (2) a simpler version that has fewer experimental and analytical demands, and is intended for use by veterinarians and livestock owners.

INVITED PRESENTATIONS

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Monitoring Drug Efficacy and Anthelmintic Resistance in Human Soil-Transmitted Helminths: An Update From the Starworms Project

Bruno Levecke, Piet Cools, Johnny Vlaminck & Jozef Vercruysse

Laboratory of Parasitology, Faculty of Veterinary Medicine, Ghent University

Infections with soil-transmitted helminths (STHs) pose an important threat to public health in subtropical and tropical countries. Global estimates indicate that more than 1.4 billion people are infected by STHs and that they are responsible for an estimated 3.3 million disability-adjusted life years. Current means to control morbidity caused by soil-transmitted helminthiasis is the blanket administration of drugs to populations that suffer the most from disease. However, the success of these large-scale deworming programs is threatened by the emergence of anthelmintic resistance (AR), as sustained in veterinary medicine. Although there is urgent necessity for thoroughly designed surveillance systems that allow for the detection of any changes in drug efficacy arising through the evolution of AR in these helminthes, these systems are currently lacking. As a response to this the Bill & Melinda Gates Foundation has brought together a group of scientists with the ultimate aim to strengthen the monitoring of drug efficacy and the emergence of AR in STH control programs, which resulted in the Starworms (Stop Anthelmintic Resistant Worms project). During the meeting an update will be given on Starworms project, highlighting the opportunities and challenges to set-up a global surveillance system.

INVITED PRESENTATIONS

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

WormBoss-effective and sustainable Australian sheep worm control strategies

Deborah Maxwell

ParaBoss, The University of New England, deb@paraboss.com.au

Australia's 70 million sheep live in 8 broad climatic worm regions, so a one-size-fits-all program of worm control is not possible. WormBoss is the national authority for ruminant worm control, crossing state and organizational boundaries to provide a unified, proven, practical and profitable program endorsed by industry and stakeholders.

The eight regional programs are built on common strategies and contain a cohesive set of anthelmintic resistance management principles that are adapted to regional conditions. Similar programs have been developed for Australia's smaller national population of goats, and will soon be released for cattle.

While drench resistance testing is strongly promoted, Faecal Egg Count Reduction Tests have limited uptake due to cost, complexity, difficulty obtaining small volumes of drenches, and, above all, because most people don't believe they have a drench resistance problem, despite recognizing it as an industry issue. DrenchChecks - pre- and post-drench worm egg counts - are used more often, but not widely enough.

WormBoss promotes breeding for worm resistance (Sheep Genetics calculates Worm Egg Count and Dag breeding values), preparing low worm-risk pastures and targeted treatment to reduce drench use. Regular testing to confirm drench need is encouraged, with effective (98%), short-acting, combination anthelmintic products the cornerstone of wise drench choice.

The recent commercial releases of Barbervax® (*Haemonchus contortus* vaccine) and Bioworma® (*Duddingtonia flagrans* concentrate) provide two novel approaches to reduce the need for drenches.

WormBoss, with its sister programs: FlyBoss, LiceBoss and TickBoss, form ParaBoss, a suite of extension, communication and training resources for ruminant parasite management.

INVITED PRESENTATIONS

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Understanding economics of AMR and incentives for prevention

Jarkko K. Niemi

Natural Resources Institute Finland (Luke), Finland

Antimicrobial resistance (AMR) is globally one of the most important societal challenges and economics plays an important role in combatting antimicrobial resistance (AMR). This presentation will analyze economics of AMR both analytically and by using an empirical case study with relevance to antiparasitic field. It will elaborate economics of disease prevention versus treating the disease in ways which increase the risk of AMR.

First, economic impacts of disease and AMR at the farm and at the sector level will be elaborated. Next, an optimization model, which accounts for profit-maximising behavior of farmer, to analyze antimicrobial use will be presented. The analysis will focus on optimizing disease prevention measures. It will address the issue from both farm perspective and from societal perspective to account for both private and public costs, where the later can be important in externalities. The model will address farmer's incentives to put effort on disease prevention measures and how changes in incentives can influence on one hand costs caused by the disease, and on the other hand, willingness to comply with societally preferred practices. Finally, analogies between AMR and anthelmintic resistance will be discussed.

INVITED PRESENTATIONS

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Sustainable Ruminant Parasite Control: an Industry View

Gustavo Sabatini

Boehringer-Ingelheim Animal Health

With regards to ruminant parasite control, sustainability is based on three pillars: delay of selection of parasiticide resistance, environmental impact management, and optimization of quality protein production. In this sense, cattle producers, academics and the pharmaceutical industry have key roles to play. Producers should pursue sustainability by implementing best practices for parasite control. This means treating the right animals, with the right parasiticide, at the right dose, at the appropriate time. The academic community is essential in this process. There is high expectation on the generation of practically applicable scientific information about how to control parasites sustainably and effectively with the tools available today. The pharmaceutical industry is likewise important in continuing to direct investments toward the development of efficacious parasite control solutions that are safe to the environment and food consumer. If every stakeholder plays their role accordingly, it will be possible to extend the lifetime of current antiparasitics with negligible harm to the environment and, at the same time, to develop the solutions demanded for a sustainable and effective parasite control program in the future. In this way, the forthcoming increased demand for animal protein production will be manageable.

INVITED PRESENTATIONS

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Current status of small ruminant parasite control in the U.S. and Canada

Anne Zajac

Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg VA

There are approximately 6 million sheep and 2.5 million goats in the US and Canada. Historically, many of the largest sheep operations were found in arid western states, which limited the impact of parasitism on production. Sheep numbers have been falling in these areas, however, with growing interest in small ruminants and increased numbers of small flocks and herds in eastern states where gastrointestinal nematodes (GIN) flourish. Many owners identify parasite control as their most serious disease issue. Throughout the eastern and midwestern U.S. and into Canada, control of *Haemonchus contortus* is challenging and responsible for substantial losses in production and health. In the US, macrocyclic lactones, benzimidazoles and levamisole are approved for use in sheep with fewer approved products for goats. Resistance to these products is widespread and, although no combination products are available in the U.S., simultaneous treatment with 2 or more drugs is becoming commonplace. In Canada, closantel and abamectin/derquantel are also available as prescription only products. Because of the severity of anthelmintic resistance, many producers understand the value of refugia based control programs and the FAMACHA® system has been widely promoted. Copper oxide wire particles are also being used increasingly, especially in goats. In addition to other management practices, interest in parasite resistant animals has grown with increased use of breeds considered more parasite resistant, such as Katahdin sheep and Kiko goats. In addition, an Estimated Breeding Value for fecal egg count is now offered by the U.S. National Sheep Improvement Program.

OVERVIEW ORAL PRESENTATIONS

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

WG	Presenter	Titel
1	WG1 J. Gilleard	Illustrating the application of next generation amplicon sequencing to investigate anthelmintic drug resistance in North American Beef cattle
2	WG1 H. Hertzberg	Gastrointestinal nematodes as cause of death in sheep and goats in Switzerland
3	WG1 M. Martínez-Valladares ¹	The presence of a new SNP at codon 198 of the beta tubulin gene is associated with benzimidazole resistance in <i>Teladorsagia circumcincta</i>
4	WG1 J. McIntyre	Sheep, strongyles and sequencing for sustainability: investigating ivermectin resistance on UK farms
5	WG2 L. Melville	Investigating farmer's perceptions of <i>Nematodirus battus</i> infection in grazing sheep and current helminth control practices in the UK
6	WG2 S. Schoenian	ACSRPC web site provides information to a growing audience
7	WG2 S. Werne	Worm control on organic farms in the EU: Management and the farmers' point of view
8	WG3 G. Bath	Successful implementation of TST for sustainable integrated internal parasite management
9	WG3 J.F. Gonzalez	Sustainable control of gastrointestinal nematodes in Gran Canaria island (Spain). Is it possible?
10	WG3 M. Karonen	Ellagitannins as potential natural anthelmintics
11	WG3 J. P. Muir	Is Pasture Management at Fault for Anthelmintic Resistance in Small Ruminants?
12	WG3 H. R. Vineer	Weather and the development of anthelmintic resistant <i>Haemonchus contortus</i>

ORAL PRESENTATIONS working group 1

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Illustrating the application of next generation amplicon sequencing to investigate anthelmintic drug resistance in North American Beef cattle

John Gilleard¹, Russell Avramenko¹, Elizabeth Redman¹, Eranga De Seram², Roy Lewis³, Fabienne Uehlinger²

¹Faculty of Veterinary Medicine, Host-Parasite Interactions group. University of Calgary, Calgary,

²Western College of Veterinary Medicine, University of Saskatchewan, ³Merck Animal Health

We provide several examples to illustrate the value of molecular diagnostics, using next generation sequencing, to investigate anthelmintic resistance in north American beef cattle. We first surveyed ivermectin pour-on efficacy in first season grazing calves in 50 herds across Canada. There was low treatment effectiveness in many herds, and ITS-2 rDNA nemabiome sequencing revealed *Cooperia oncophora* and *Cooperia punctata* as the species surviving ivermectin treatments. We confirmed ivermectin resistance in several species of gastrointestinal nematodes in western Canadian beef cattle using a fecal egg count reduction test: 234 auction market-derived steer calves were randomly assigned to three groups (each with replicates of 6 pens of 13 calves). Group 1 treated with injectable ivermectin (IVM), Group 2 with injectable IVM plus oral fenbendazole and Group 3 untreated. FECs were performed on fecal samples collected pre- and 14 days post-treatment, and ITS-2 rDNA nemabiome sequencing applied to harvested L3. IVM produced an 82.5% reduction (95% CI, 67.8-90.5) in FEC whereas IVM /FB combined produced a 100% reduction. The integrated FECRT and nemabiome sequencing data revealed the presence of ivermectin resistant *Cooperia oncophora*, *Cooperia punctata* and *Haemonchus placei*. We have also applied isotype-1 B-tubulin deep amplicon sequencing to screen 70 beef herds across Canada and southern USA for benzimidazole resistance mutations. The codon 200 mutation was present at low levels in *T. axei*, *Ostertagia ostertagi* and *Cooperia spp.* in multiple herds suggesting the early emergence of resistance that would be undetectable by FECRT or in vitro bioassays.

ORAL PRESENTATIONS working group 1

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Gastrointestinal nematodes as cause of death in sheep and goats in Switzerland

Raila Ruprecht^{1,2}, Monika Hilbe², Walter Basso³, Hubertus Hertzberg¹

Institute of Parasitology¹, Institute of Veterinary Pathology², University of Zurich, Institute of Parasitology³, University of Berne, Switzerland

Routine post-mortem examination at the Vetsuisse Faculty in Zurich revealed that gastrointestinal helminths have represented a significant cause of death in sheep and goats during the last decade. Aiming to quantify these observations, a retrospective analysis was undertaken. The post-mortem examination protocol always included opening of selected parts (all segments of the small and large intestine) of the gastrointestinal tract and in the case of suspected parasitism an examination of the total abomasal and intestinal content and/or a faecal examination were done. Between 2012 and 2018 a total of 717 sheep and 461 goats were necropsied. In 342 sheep (48%) and 221 goats (46%) the presence of parasites was recorded in the post-mortem protocols. Based on all protocols, parasites were determined as cause of death in 18% and 20% of sheep and goats respectively, with gastrointestinal helminths (focus *Haemonchus contortus*) accounting for most of these cases. Similar observations were made in a parallel project at the Vetsuisse Faculty in Berne, underlining the high significance of gastrointestinal helminths on small ruminant welfare and productivity. There are no statistics on the rate of annual death cases of small ruminants in Switzerland, but it is evident that only a small minority of these will undergo post-mortem investigation. The practicing veterinarian often diagnoses haemonchosis on the basis of pale mucous membranes and edema without submission to a pathology unit so that the death rate due to this parasite will actually be much higher than calculated within this study.

ORAL PRESENTATIONS working group 1

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

The presence of a new SNP at codon 198 of the beta tubulin gene is associated with benzimidazole resistance in *Teladorsagia circumcincta*

María Martínez-Valladares^{1,2}, Elora Valderas García^{1,2}, Verónica Castilla Gómez De Agüero^{1,2}, Philip Skuce³, Alison Morrison³, Javier Gandasegui⁴, Camino González Lanza², Francisco A. Rojo Vázquez^{1,2}

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²Instituto de Ganadería de Montaña (CSIC-Universidad de León), Grulleros 24346, León, Spain; Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de León, Campus de Vegazana s/n, León 24071, Spain.

³Moredun Research Institute, Pentlands Science Park, Edinburgh, UK.

⁴Instituto de Salud Global de Barcelona (ISGlobal), Barcelona, Spain.

In the present study we analyzed the level of resistance to benzimidazoles by the detection of Single Nucleotide Polymorphisms (SNPs) at codons 198 (E198A) and 200 (F200Y) of the beta tubulin gene in the gastrointestinal nematode, *Teladorsagia circumcincta*, present in field samples. The molecular identification of this species was confirmed before treatment with albendazole in 6 flocks, amongst other species, but also as the only species surviving treatment. The result of the Faecal Egg Count Reduction Test in these flock ranged between 17-88%, and the resistance was confirmed by the Egg Hatch Assay as well. By pyrosequencing, neither of the two SNPs was present before or after treatment. Therefore, with the aim of confirming these results, a gene fragment of 390bp was sequenced including SNPs at codon 167 (F167Y), 198 and 200 in a pool of L1 recovered before and after treatment in these 6 resistant flocks. Again, in all samples, the three resistance SNPs were absent but a different amino acid was identified at codon 198 in all samples collected after treatment, namely, a leucine (CTA). In the pool of larvae collected before treatment, 5 samples were homozygous G/C and T/A at the first and second base of the codon 198, respectively, leading to the possibility of two amino acids at codon 198, glutamic acid (GAA) or leucine (CTA). In the remaining flock, glutamic acid (GAA) was present before treatment. Further analysis is being performed in individual larvae and other different isolates. All results will be shown at the next COMBAR meeting.

Funding: RTA2013-00064-C02-02, RYC-2015-18368.

ORAL PRESENTATIONS working group 1

Joint COMBAR- ACSRPC meeting: “Anthelmintic Resistance in ruminants: Who cares?”

Sheep, strongyles and sequencing for sustainability: investigating ivermectin resistance on UK farms

McIntyre J^{1a}, Maitland K¹, McGoldrick J¹, Doyle S³, Cotton J³, Holroyd N³, Morgan E⁴, Vineer H⁵, Bull K⁶, Bartley D², Morrison A², Hamer K¹, Sargison N⁷, Laing R¹, Devaney E¹

¹ University of Glasgow, Glasgow, UK [a\(j.mcintyre.1@research.gla.ac.uk\)](mailto:j.mcintyre.1@research.gla.ac.uk); ² Moredun Research Institute, Edinburgh, UK; ³ Wellcome Sanger Institute, Hinxton, UK; ⁴ Queen’s University Belfast, Belfast, UK; ⁵ University of Liverpool, Liverpool, UK; ⁶ University of Bristol, Bristol, UK; ⁷ Royal (Dick) School of Veterinary Studies, Edinburgh, UK

Parasitic gastroenteritis is a major production limiting disease of small ruminants worldwide. The abomasal nematode *Teladorsagia circumcincta* is the primary pathogen on most sheep farms in the UK during the summer months, when lambs are in their primary growth phase. Expertly adapted to temperate climates, its small ruminant hosts and capable of surviving year round, *T. circumcincta* is challenging to control, particularly with the rapid development of resistance to all widely used anthelmintic classes. Ivermectin is the most commonly used anthelmintic in the UK but resistance is highly prevalent; recent studies have demonstrated rising numbers of farms with detectable ivermectin resistance by faecal egg count reduction test (FECRT), a concern for sustainable control of parasites in the future. There are many limitations to the FECRT, and such limitations, combined with a highly complicated disease, make understanding the effect of different management practices on the development of anthelmintic resistance difficult. Molecular tests are needed for sensitive diagnosis of resistance, but such tests require knowledge of the regions of the genome conferring resistance. In this study we undertook genome-wide sequencing of two UK farm populations of *T. circumcincta* pre- and post-ivermectin treatment using next generation sequencing techniques (ddRAD-Seq and Pool-Seq) to identify regions of the genome under selection. Despite the fragmented nature of the *T. circumcincta* draft genome assembly, we found a single large locus to be under ivermectin selection, in addition to many smaller loci. Though many selected loci appear farm specific, several are under selection on both farms.

ORAL PRESENTATIONS working group 2

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Investigating farmer's perceptions of *Nematodirus battus* infection in grazing sheep and current helminth control practices in the UK

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Following reports of benzimidazole resistant *Nematodirus battus* and its changing epidemiology in the UK it was decided to investigate farmers' perceptions surrounding this pathogenic and economically important roundworm. Questionnaires were developed in a couple of formats (hard-copy and web-based), piloted and distributed to the farming community via direct email, social media, veterinarians and agricultural bodies. The survey consisted of 42 questions, divided into four sections; i) farm demographics, ii) farmer perceptions of *N. battus* and specific control practices, iii) grazing management and iv) general anthelmintic usage and quarantine. Perception questions were designed to gather information on respondent's observations of *N. battus* on their farm, severity of disease, which months signs of *N. battus* were typically observed and their opinion of if/how the disease had changed over the preceding five years. A total of 197 completed questionnaires were collected and analysed using descriptive statistics and GLMM. Management practices and perceptions of disease severity varied regionally, as did the uptake of 'best-practice' approaches. Respondents in the north perceived more severe disease, restricted to spring/summer whilst a large proportion of farms in the south reported varied timing of infection and greater uptake of evidence-based anthelmintic administration (including faecal egg counting, online risk maps). The differences in disease perception, management decisions and practices reported could be a result of the recent fluctuations in *N. battus* epidemiology or changing sensitivities to benzimidazole in this species. Understanding management practices and decision making in farmers will inform the development of more usable and sustainable control options.

ORAL PRESENTATIONS working group 2

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

ACSRPC web site provides information to a growing audience

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The American Consortium for Small Ruminant Parasite Control (ACSRPC) was organized in 2003 in response to the widespread emergence of anthelmintic-resistant worms. A web site (acsrpc.org) was established in 2004 to provide up-to-date information to stakeholders. The web site (wormx.info) was upgraded in 2012 and is now hosted by the University of Maryland. According to Google Analytics, the web site received 134,109 page views (110,254 unique) during the past year (May 1, 2018 – April 30, 2019); +7.76% compared to the previous 12 months. There were 44,993 users; +11.3%. While the majority of users were from the US (82.22%), the web site was accessed by 159 countries during the past year. Non-US countries with the most users were Canada (3.01%), Australia (2.32%), UK (1.55%), and South Africa (1.04%). The average site visit lasted 114 s. Almost half (49.1%) used a mobile device to access the web site; 44.1% from a desktop. While more users are accessing the web site from mobile devices, those using desktops and tablets visit more pages and stay longer. Search engines were responsible for over half of the web site traffic (51.3%), with direct traffic (22.8%), social media (18.2%), and referrals (7.7%) accounting for the balance. Last year, the most viewed pages were the home page (18.49%), a page with links to information about dewormers (6.71%), and an article about other causes of anemia and bottle jaw (4.82%). The web site for the ACSRPC serves (since 2004) as an important resource for a growing audience.

ORAL PRESENTATIONS working group 2

Joint COMBAR- ACSRPC meeting: “Anthelmintic Resistance in ruminants: Who cares?”

Worm control on organic farms in the EU: Management and the farmers’ point of view

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A structured survey was developed to determine farmers’ disposition towards recently developed refugia-based concepts. The focus was on individual drenching, possible increased workload or costs and reduced production that might come with innovations. Furthermore, the survey aimed at assessing the status-quo of gastrointestinal nematode (GIN) control on organic small ruminant farms in 5 European countries. In total 441 surveys of organic certified small ruminant farms were evaluated, of which were 129 dairy goat and 320 meat sheep surveys. Amongst other things, the survey covered applied alternatives such as feeding bioactive forage, increased protein supply, phytotherapy, homeopathy and culling of nematode-susceptible animals. The number of drenches per animal per year in dairy goats varied from 1.3 (CH) to 1.6 (FR), in sheep from 0.8 (LT) to 1.5 (CH) and in lambs from 0.9 (LT) to 1.6 (CH). Applied individual drenching of lambs was relatively low from 2% (LT) to 33% (UK). Sixteen percent (NL) to 36% (CH) of the farmers drenched their dairy goats individually. Eight percent (UK) to 49% (LT) did not drench their lambs at all. Seven percent (CH) to 21% (FR) of the organic farmers did not apply any anthelmintic to their dairy goats. Between 10% (LT) and 40% (NL) of the farmers used faecal sampling to monitor GIN, whereas performance parameters (weight gain or milk yield) were moderately used from 19% (CH) to 41% (LT). The use of phytotherapy varied considerably between livestock species and country from 13% (LT) – 69% (CH). The majority of farmers rather or fully agreed that anthelmintic resistance will worsen in future (51% – 92%). When asked about performance losses that may be caused by the introduction of alternative methods, the agreement was more variable with 7% in NL to 93% in LT. Our data suggest that innovations might be accepted despite higher labor input or costs. As some organic farmers make already use of phenotypic traits like production loss, it should not be a big step towards a “Targeted Selective Treatment” approach for farmers that are open minded towards innovations.

ORAL PRESENTATIONS working group 3

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Successful implementation of TST for sustainable integrated internal parasite management

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The success of parasite research can be measured by the extent of its implementation by farmers as beneficiaries. The paper describes the origins of the concept of Targeted Selective Treatment (TST) for ovine haemonchosis that evolved in the 1980s and its refinement as the FAMACHA© System in the 1990s, followed by launches, first in South Africa and then internationally in 2000. The system has undergone many modifications and improvements over time and these will be listed and discussed. International testing, with increasing acceptance and implementation, has followed wherever haemonchosis is a problem. Its role in slowing Anthelmintic Resistance has been demonstrated and the system is now used widely in the world. Distribution and training agreements have been concluded in many countries and these will be highlighted. The reasons for the worldwide success of the FAMACHA© System are listed:

it is simple, cheap, quick, reliable and robust on-farm and in the hands of farmers. These are essential ingredients for the successful implementation of research-proven parasite management systems. Subsequently, the principle of practical TST has been extended to other major parasites of sheep and goats in the form of the Five Point Check©, using similar principles. The approaches found useful or necessary to get farmer and industry acceptance of measures for sustainable parasite control will be listed and briefly outlined. These should prove useful when further and future ways of management are to be promoted.

ORAL PRESENTATIONS working group 3

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Sustainable control of gastrointestinal nematodes in Gran Canaria island (Spain). Is it possible?

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Gastrointestinal nematodes (GIN) are one of the main problems in the ruminant production Worldwide. Control have relied on strategic drenching. However, increasing drug resistance has prompted the search of alternative control methods. Gran Canaria is called the "tiny continent", because, in spite is small size, several climatic areas are presented there. The South-East is very dry and the North-West is more humid and these different landscapes also affect the ruminant species exploit (goat vs sheep) and main parasitological problems. GIN are a productive constraint in the humid area. Local breeds of sheep are relatively resistant to GIN and previous works showed that, at least in part, this resistance is immunomediante. Lambs of some of these breeds can respond to immunogens at earlier age than commercial breed lambs. In this work we will discuss potential alternatives for GIN control in this island, combining the breed resistance and the singular orography of the island, in order to develop a sustainable model of GIN control with great value both locally and as a model for similar epidemiological environments.

ORAL PRESENTATIONS working group 3

Joint COMBAR- ACSRPC meeting: “Anthelmintic Resistance in ruminants: Who cares?”

Ellagitannins as potential natural anthelmintics

Maarit Karonen, Marica Engström, Juha-Pekka Salminen and Hervé Hoste

Tannin-rich plants have lately attracted attention due to their effect against gastrointestinal nematodes in ruminants. They have anthelmintic properties; for example, they can inhibit or delay some key step in the nematode life cycle, such as larval exsheathment or egg production in female adult worms. Most of the current data on the anthelmintic effects of tannins have been obtained on condensed tannins. Information on the antiparasitic effects of hydrolysable tannins is minor. We studied the anthelmintic effects of specific ellagitannins on parasitic nematodes found in ruminants. The *in vitro* tests were performed using pure ellagitannins in order to understand the relationship between the ellagitannin structure and the activity observed. These individual ellagitannins included acyclic and cyclic structures, ellagitannins with different molecular sizes and structural flexibility and ellagitannins with different functional groups. Ellagitannins inhibited the larval exsheathment of *Haemonchus contortus* (the parasite of the abomasum) and *Trichostrongylus colubriformis* (the parasite of the small intestine) larvae. In general, ellagitannins were more efficient at inhibiting the exsheathment of *H. contortus* larvae than those of *T. colubriformis*. The efficiency of inhibition increased as the molecular size of ellagitannins increased. There was no other overall trend in the structural features of ellagitannins that significantly affected the anthelmintic activity on the third-stage infective larvae. Ellagitannins have also activity against the egg hatching and motility of *H. contortus* nematodes. In these activities, the size and overall flexibility of ellagitannins, the types and numbers of functional groups, and the linkage types between monomeric ellagitannins affected the activity differently.

ORAL PRESENTATIONS working group 3

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Is Pasture Management at Fault for Anthelmintic Resistance in Small Ruminants?

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Gastrointestinal nematode (GIN) resistance to anthelmintics in small ruminants, especially goats, causes and, at the same time, results from rising GIN parasitosis. We propose that the rising parasitosis that led to anthelmintic misuse in small ruminants is largely a result of pasture mis-management. Logic supports this premise: goats and sheep co-evolved with GIN for millennia without the latter overwhelming ruminant populations. When allowed to forage at high herbage allowances with adequate plant recovery times between herbivory, small ruminants and GIN coexisted. Only when market pressure for greater production per area increased and historical pasture husbandry practices concomitantly degraded did parasitosis and subsequent resistance to anthelmintics appear. Logic would indicate that correcting the original error will help resolve the issues that arose from it. We propose that anthelmintic resistance in small ruminants could become tolerable, or even irrelevant, if pasture management returns to biologically sound precepts. These include managing pastures for 1. correct plant species for given animal species; 2. plant species diversity compatible with animal preferences; 3. multiple ruminant species grazing/browsing that lead to stable pasture ecosystems; 4. herbage allowances that foster proper grazing selection; 5. improved animal nutrition, primarily crude protein quantity and digestibility; 6. between-herbivory rest periods favoring forage health while suppressing GIN larval populations and; 7. forages high in GIN-suppressing compounds such as condensed tannins. Drawbacks of this "going back to the future" approach could include declining animal production per area and greater pasture husbandry complexity. We postulate that benefits outweigh costs.

ORAL PRESENTATIONS working group 3

Joint COMBAR- ACSRPC meeting: “Anthelmintic Resistance in ruminants: Who cares?”

Weather and the development of anthelmintic resistant *Haemonchus contortus*

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Anthelmintic resistance AR is widespread in several ruminant gastrointestinal (GI) nematodes worldwide and livestock industry guidelines, built on an increasing body of empirical and theoretical research, recommend farmers adopt strategies that maximise the proportion of the parasite population that is left untreated (population *in refugia*) to slow the development of AR. However, GI nematode population dynamics are highly weather-dependent, affecting the seasonal risk of infection and potentially the efficacy of refugia-based control strategies in delaying the development of AR.

As part of the BUG Consortium (Building Upon the Genome), a model simulating the weather-dependent population dynamics of the free-living stages of a range of GI nematode species infecting ruminants was extended to include AR and host management. The model was applied to *Haemonchus contortus* infecting sheep in temperate regions to simulate the development of AR between 1987 and 2016 at sites throughout Europe, representing a range of climate zones. The timing of treatment and host grazing management was varied to reflect the “dose & move” strategy, whereby the host animal is moved to “clean” grazing with no parasite population *in refugia* immediately after treatment, and varying interpretations of the recommended “dose, delay, move” strategy, where there is a delay of several days between host treatment and moving to clean grazing.

The predicted change in resistant allele frequency following treatment varied within and between years. The output provides an insight into the future sustainability of recommended GI nematode control practices and observed seasonal variability in the results of in vivo AR detection methods.

OVERVIEW SHORT TERM SCIENTIFIC MISSIONS

Joint COMBAR- ACSRPC meeting: “Anthelmintic Resistance in ruminants: Who cares?”

	Presenter	Titel
1	A. Amadesi	A Short Term Scientific Mission at Ghent University for the development of the Mini-FLOTAC Automated System
2	P. Baltrusis	Resistance to Benzimidazoles identification in sheep parasite <i>Haemonchus contortus</i> via Pyrosequencing and droplet digital PCR
3	M. Komáromyová	Genotyping of <i>Haemonchus contortus</i> L1 larvae in the Egg Hatch Test via droplet digital PCR
4	M. Mickiewicz	Introduction to most common in vitro techniques to detect anthelmintic resistance of parasites of domestic animals – Institute of Parasitology, Slovak Academy of Sciences 17-26.06.2018
5	M. E. Morgoglione	Mini-FLOTAC and DNA-based technology for detection of helminths, protozoa and microbiome in large ruminants
6	C. Smith Bonde	Short-term scientific mission at CSIC-Universidad de León: Potent <i>in vitro</i> Anthelmintic effects of the seaweed <i>Saccharina latissima</i> against <i>Teladorsagia circumcincta</i>

SHORT TERM SCIENTIFIC MISSIONS

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

A Short Term Scientific Mission at Ghent University for the development of the Mini-FLOTAC Automated System

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The Mini-FLOTAC automated system is a diagnostic platform to automate faecal egg counting (FEC) of livestock gastrointestinal nematodes (GIN) using automation and artificial intelligence. The purpose of the Short Term Scientific Mission (STSM) at the Department of Virology, Parasitology and Immunology of Ghent University (Belgium) was to improve personal knowledge of laboratory techniques for the copromicroscopic diagnosis of GIN in cattle used in Ghent, in order to perform a comparison with the Mini-FLOTAC automated system. Moreover, another aim of the STSM was to learn the techniques of pasture larval counts and coproculture on cattle faeces. For these purposes, during the STSM period the following activities were performed: i) Training on the Standard Operating Procedures (SOPs) for the faecal egg count (FEC) techniques for the diagnosis of GIN in cattle; ii) Participation in the analysis of cattle faecal samples using Mini-FLOTAC technique; iii) Training on SOPs for coprocultures to identify larvae of GIN in cattle faeces; iv) Training on the SOPs for pasture larval count technique. This experience was important for a personal and scientific growth, because it was a great opportunity to learn and improve techniques for diagnosis of GIN in cattle. Moreover, it was very important to have the opportunity to participate in both field and laboratory activities. Future collaborations with Ghent University on validation of the Mini-FLOTAC automated system is envisaged. In particular, an evaluation of detection limit, accuracy and repeatability of the Mini-FLOTAC will be performed, using cattle faecal samples spiked with different levels of GIN eggs.

SHORT TERM SCIENTIFIC MISSIONS

Joint COMBAR- ACSRPC meeting: “Anthelmintic Resistance in ruminants: Who cares?”

Resistance to Benzimidazoles identification in sheep parasite *Haemonchus contortus* via Pyrosequencing and droplet digital PCR

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Haemonchus contortus, a notorious endoparasite in small ruminants, has become ‘a thorn in the side’ for farmers across the world. Even though most gastrointestinal nematode (GIN) infections in livestock are mixed (multiple species parasitizing the same animal), *H. contortus* stands out as the most pathogenic and abundant species. To date, mainly three broad spectrum drug classes are utilized to treat GIN infections in ruminants – benzimidazoles (BZ), levamisole (LEV), and macrocyclic lactones (ML), all of which reduce the existing worm burdens and if used persistently decrease pasture contamination and prevent the establishment of ingested infective-stage larvae (L₃). Benzimidazole drugs, introduced in the 1960s, are still of key importance in common de-worming routines conducted with small ruminants.

However, resistance to the drugs of this class has emerged and has been growing due to a plethora of reasons. Thus, a joined scientific attempt was made to compare two molecular tools, pyrosequencing and droplet digital PCR, in diagnosing the presence of resistance conferring alleles, based on one of the most commonly encountered SNP in the 200th codon (TTC->TAC) in the isotype 1 beta tubulin gene in *Haemonchus contortus*. In summary, although both methods present their own sets of challenges and difficulties, congruent results between the two types of assays were obtained.

SHORT TERM SCIENTIFIC MISSIONS

Joint COMBAR- ACSRPC meeting: “Anthelmintic Resistance in ruminants: Who cares?”

Genotyping of *Haemonchus contortus* L1 larvae in the Egg Hatch Test via droplet digital PCR

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Anthelmintic resistance represents severe menace to animal health and has become a major factor causing production and financial losses in ruminants industry. The aim of this STSM was to isolate the hatched *Haemonchus contortus* L1 larvae at the discriminating doses in the Egg Hatch Test (EHT) and identify as well as quantify their overall genotypic status in terms of the presence of sensitive and benzimidazole-resistant alleles by using the previously established digital droplet PCR (ddPCR) assay. The project aim to verify or to disprove the following hypothesis: In an *in vitro* Egg Hatch Test at the discriminating dose of the anthelmintic, only larvae with resistant genotype will hatch. The larvae of *Haemonchus contortus* hatched in concentrations of tiabendazole 0.05, 0.1 and 0.3 µg/µl were tested. According to ddPCR assay we proved that concentration of a wild-type allele copies is decreasing while concentration of a mutant-type allele copies is increasing respectively at the above listed concentrations. The results of this study could help in diagnosing benzimidazole drug resistance in field samples, using a single concentration in the EHT by elucidating the precise estimate of the proportion of resistant parasite worm DNA in a tested population.

The study was supported by COST COMBAR Action number CA16230.

SHORT TERM SCIENTIFIC MISSIONS

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Introduction to most common in vitro techniques to detect anthelmintic resistance of parasites of domestic animals – Institute of Parasitology, Slovak Academy of Sciences 17-26.06.2018

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In Poland very little data on the anthelmintic resistance in gastrointestinal nematodes of sheep and goats population is available. The anthelmintic resistance (AR) in vitro detection methods are not implemented in routine use in AR diagnostic in Poland. The STSM lasted 10 days and took place in June 2018. The aim of my STSM was to learn how to perform larval development test, micro-agar larval development test and egg hatch test, properly interpret of obtained results and implement them to studies in our laboratory. Furthermore, during STSM I had the opportunity to perform these in vitro tests on faecal samples from three goat herds from Poland in which resistance to ivermectin and benzimidazoles was previously detected in our laboratory by faecal egg count reduction test.

During the STSM I was familiarized and trained in mentioned in vitro methods. Results of performed tests confirmed the resistance of gastrointestinal nematodes to ivermectin, benzimidazoles and levamisole in samples from three goat herds from Poland. The results obtained at this STSM will be part of my PhD thesis which will be published as three manuscripts in scientific journals. Knowledge and skills acquired during this STSM enabled me to introduce new anthelmintic resistance diagnostic methods to our laboratory. During last year after the STSM I had examined 40 goat herds by LDT and EHT, which was part of the work on assessment of the prevalence of AR in goat herds in Poland.

This work is based upon work from COST Action COMBAR CA16230, supported by COST (European Cooperation in Science and Technology).

SHORT TERM SCIENTIFIC MISSIONS

Joint COMBAR- ACSRPC meeting: “Anthelmintic Resistance in ruminants: Who cares?”

Mini-FLOTAC and DNA-based technology for detection of helminths, protozoa and microbiome in large ruminants

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The existence of a complex network of interactions occurring between Gastrointestinal (GI) parasites (helminths and protozoa) and the gut commensal bacteria is well established; nonetheless, little is known in large ruminants, given that GI infection compromises animal health with serious consequences for their welfare and production status. The purpose of the Short Term Scientific Mission (STSM) at Laboratory of Immunology and Parasitology, Veterinary School, Faculty of Health Science, in Langford (Bristol, UK) was to investigate the helminth-microbiota interactions in cattle based on the use of conventional copromicroscopy (Mini-FLOTAC) and DNA-based technology for helminths, protozoa and the microbiome. For these purposes, during the STSM period the following activities were performed: i) Training on DNA-based technology for the analysis of microbiome and GI parasites (helminths and protozoa); ii) Collection of faecal collection samples in a cattle farm located in Salisbury (UK) according to the standard operating procedures (SOPs) for coprological and molecular analysis; iii) Coprological analysis using Mini-FLOTAC technique; iv) DNA high-throughput sequencing of bacterial 16S rRNA and GI parasites 18S amplicons, followed by bioinformatics and statistical analyses of sequence data. This STSM represented a meaningful experience for my personal and scientific growth. Moreover, the STSM was a great opportunity to learn new skills for novel approaches to GI parasite control in large ruminants. Future collaboration with the Laboratory of Immunology and Parasitology, Veterinary School, in Bristol, also for the aims of my PhD project on Water Buffalo in Italy is envisaged.

SHORT TERM SCIENTIFIC MISSIONS

Joint COMBAR- ACSRPC meeting: “Anthelmintic Resistance in ruminants: Who cares?”

Short-term scientific mission at CSIC-Universidad de León: Potent *in vitro* Anthelmintic effects of the seaweed *Saccharina latissima* against *Teladorsagia circumcincta*

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Seaweed contains an abundance of bioactive compounds, and some seaweed species have been used for livestock feeding and as natural deworming agents for centuries. However, there is a lack of scientific evidence. In this study, we investigated the *in vitro* anthelmintic (AH) activity of extracts of seaweed from cold (Nordic) waters. We prepared three different extracts with hexane, dichloromethane:methanol (DCM), or water:methanol (WM) of dried and milled seaweed from four species: *Saccharina latissima*, *Laminaria digitata*, *Ascophyllum nodosum*, and *Palmaria palmata*. Both fermented (addition of *Lactobacillus* spp.) and non-fermented material (N=24) was included. The AH activity was assessed using an 48 hour *Teladorsagia circumcincta* first stage larval (L1) mortality assay (1 mg DM/mL dissolved in DMSO). The results showed a high AH activity of all six *S. latissima* extracts and fermented *L. digitata* extracts, and no activity of *A. nodosum* extracts. An egg hatch assay (EHA), using the same extracts (1 mg/mL, 48 h) and *T. circumcincta* eggs, showed >95% inhibition by the WM extracts of fermented and non-fermented *S. latissima*, and fermented *L. digitata* and *P. palmata*. We conclude that the seaweed *S. latissima* has a strong *in vitro* AH effect against eggs and L1 of the common sheep nematode *T. circumcincta*, and that fermentation of the other seaweeds may increase their ability to inhibit egg hatching.

OVERVIEW POSTER PRESENTATIONS

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

WG	Presenter	Titel
1	WG1 V. Castilla Gómez de Agüero	New tool for the discrimination between <i>Fasciola hepatica</i> and <i>Fasciola gigantica</i> flukes collected from domestic ruminants.
2	WG1 C. Byaruhanga	Molecular detection of <i>Fasciola</i> and snail intermediate hosts, and their environmental DNA, for sustainable management of fasciolosis in South Africa
3	WG1 C. Chartier	Benzimidazole resistance survey in gastrointestinal nematodes of dairy and beef cattle in western France using Egg Hatch Assay
4	WG1 E. Claerebout	Anthelmintic resistance and common worm control practices in sheep farms in Belgium
5	WG1 A. Hendrickx	Towards dynamic risk maps for helminth parasites in ruminants: A case study in Italy
6	WG1 O. M. Keane	Anthelmintic resistance among gastrointestinal nematodes of sheep and cattle in Ireland
7	WG1 M. Komáromyová	Comparison of <i>in vivo</i> and <i>in vitro</i> response in identical parasitic infection in goats and sheep
8	WG1 O. M. Dourado Martins	A proposal of MiniFlotac Market Analysis applied to Italy
9	WG1 T. Letra Mateus	Suspected resistance to ivermectin in gastrointestinal nematodes of ruminants from northern Portugal
10	WG1 M. Mickiewicz	The prevalence of anthelmintic resistance in goat herds in Poland detected by egg hatch test and larval development test
11	WG1 G. Mitchell	Evidence of Triclabendazole resistant <i>Fasciola hepatica</i> on multiple sheep farms in West of Scotland using faecal egg counting and coproantigen ELISA testing
12	WG1 A. Morrison	Use of Multiplexed Tandem PCR in a Scottish Deer Survey
13	WG1 C. Paraud	Anthelmintic resistance in gastrointestinal nematodes of French Angora goats
14	WG1 J. Gilleard	Comparing different metabarcoding approaches to investigate <i>Eimeria</i> species diversity and community structure in cattle.

15	WG1	N. Reslová	What can egg-proteases tell us about the pathogenesis caused by trematodes?
16	WG1	S. Schoenian	Anthelmintic resistance on commercial sheep farms in the southeastern US
17	WG1	S. Simin ¹	First report of ivermectin resistance in goat gastrointestinal nematodes in Serbia
18	WG1	L. Škorpíková	High Resolution Melting Assay: a powerful method usable for species differentiation as well as for detection of polymorphisms associated with anthelmintic resistance
19	WG1	P. Skuce	The response of the saliva proteome in sheep infected with liver fluke (<i>Fasciola hepatica</i>)
20	WG1	S. Stuen	Anthelmintic ResisTance In Sheep helminThs (ARTIST) in Norway.
21	WG1	B. Hinney	Resistance of sheep trichostrongylids against Moxidectin in Austria
22	WG1	J. S. Gilleard	A bespoke nematode ITS-2 rDNA database formatted for multiple common bioinformatic pipelines to support nemabiome amplicon sequencing approaches
23	WG2	J. Charlier	The costs of parasitic helminth infections to the ruminant livestock industry in Europe and allocated research budgets: intermediate results
24	WG2	C. Chylinski	Current use and need for anthelmintics in organic livestock in Europe
25	WG2	Linda Coffey	Partnering for a Better Outcome
26	WG2	E.N. Escobar	Folktales fostering GIN parasites anthelmintic resistance in sheep and goats.
27	WG2	J. Ježek	Parasite treatment practices in small ruminant flocks in Slovenia
28	WG2	M. Komáromyová	Wild ruminants as a potential risk factor for transmission of abomasal nematode <i>H. contortus</i>
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POSTER PRESENTATIONS: working group 1

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

New tool for the discrimination between *Fasciola hepatica* and *Fasciola gigantica* flukes collected from domestic ruminants.

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Fasciolosis is a food-borne trematode infection caused by the liver flukes *Fasciola hepatica* and *Fasciola gigantica*. This disease is considered a zoonosis, mainly infecting wild and domestic ruminants, causing a considerable socio-economic impact in endemic countries. Traditionally, the distinction between these two species has been based on morphological criteria, although accurate recognition of the two flukes is usually difficult because of substantial variations in the morphological features. Therefore, the main objective of this study was to develop a PCR-based assay for discrimination between *F. hepatica* and *F. gigantica* flukes collected in sheep and cattle from Nigeria. A total of 47 animals, 33 cattle and 14 sheep, were sampled and a single adult fluke was collected from each animal. DNA was extracted from flukes and primers were designed based on mitochondrial DNA sequences to amplify a 304 bp fragment for the identification of *F. hepatica* and 752 bp for *F. gigantica*. Moreover, PCR products from 12 flukes were sequenced for phylogenetic analysis. Out of 47 flukes, 39 were identified as *F. hepatica* and 18 as *F. gigantica*. Within each host the percentage of each fluke species was as follows: in cattle 18/33 (54.5%) and 15/33 (45.5%) were *F. hepatica* and *F. gigantica*, respectively, and in sheep 11/14 (78.6%) and 3/14 (21.4%). The phylogenetic analysis confirmed these results. Although the number of flukes collected in sheep was limited, it seems that *F. hepatica* is more prevalent in sheep than *F. gigantica*; whereas the percentage of each species was similar in cattle. To the best of our knowledge, this is the first study which confirms the presence of *F. hepatica* in Nigeria.

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Molecular detection of *Fasciola* and snail intermediate hosts, and their environmental DNA, for sustainable management of fasciolosis in South Africa

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Fasciolosis due to infection with *Fasciola* parasites leads to reduced production and economic losses in domestic ruminants, and is considered as a re-emerging neglected zoonosis. In South Africa, there is lack of data regarding the potential of the intermediate snail species to harbour *Fasciola*, presenting a challenge to identify infection risk areas. This study uses nucleic acid-based analysis aimed at: (i) confirming snail species on farms; (ii) identifying *Fasciola hepatica* infection in snails; and (iii) detecting snail- and worm-derived antigen in the environment of both field and laboratory-bred snails in relation to their presence, prevalence and seasonal cycling. Fresh water snails, mud and water samples were collected from different farm marshy spots and laboratory tanks. Detection of DNA from both snail and immature stages of *F. hepatica* in both snails themselves and their environment (eDNA) was done using the ITS-2 gene PCR and quantitative PCR, followed by cloning and sequencing of amplicons. PCR and sequence analysis confirmed the identity of *Galba truncatula* (n=10) and *Pseudosuccinea columella* (n=3) snail species. *F. hepatica* DNA was detected in nine out of the ten *G. truncatula* (90%) and in all three *P. columella* (100%) snails. Snail antigens were detected in 66.6% and 50% of water and mud samples respectively, in which *G. truncatula* was raised. *P. columella* sequences showed heterogeneity and were 99 to 100% identical to isolates from Colombia. eDNA techniques may therefore be useful in identification and quantification of *Fasciola* and snail host, and may support management decisions to disrupt parasite life-cycle.

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Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Benzimidazole resistance survey in gastrointestinal nematodes of dairy and beef cattle in western France using Egg Hatch Assay

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There is few data regarding anthelmintic resistance in Europe in cattle, although lack of efficacy of macrocyclic lactones in several European countries have been reported recently. Concerning benzimidazoles (BZ), only one survey on 12 herds in Germany and Sweden is available and indicated a full effectiveness of albendazole. Our study aimed at assessing the anthelmintic efficacy of BZ in dairy/beef cattle in western France using the Egg Hatch Assay (EHA). During summer 2018, 17 cattle farms were selected as a convenience sample. Individual faecal samples were collected from approximately 10 first or second grazing season animals per farm and stored anaerobically. Faecal Egg Count (FEC) was performed with Mini-FLOTAC® to select the 2-3 faeces with the highest FEC and to make a composite sample. Egg Hatch Assay was performed as follows: i) eggs were isolated by sieving, centrifugation and flotation in saturated NaCl solution and collected with a sucrose step gradient centrifugation, ii) eggs were then tested using 7 concentrations of thiabendazole (TBZ) from 0.01 to 0.5 µg/mL to determine EC₅₀ (cut-off: 0.1 µg/mL). In the 17 farms, EC₅₀ values ranged from 0.027 to 0.051 µg TBZ/mL and indicated the susceptibility of the worm populations. Bulk coprocultures results showed a predominance of *Cooperia* L3. The allele frequency of BZ-resistance associated beta-tubulin isotype 1 single-nucleotide-polymorphisms within pooled samples of each of the 17 examined will be documented using a pyrosequencing-based assay. The results of this study suggest, in contrast to small ruminants, that BZ anthelmintics are fully effective in cattle.

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Anthelmintic resistance and common worm control practices in sheep farms in Belgium

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In contrast to many other European countries, no data were available on the presence of anthelmintic resistance in gastrointestinal nematodes in sheep in Belgium. A faecal egg count reduction test was performed in 26 sheep farms (29 flocks) in Flanders, Northern Belgium. Results indicated widespread resistance against benzimidazoles (albendazole, fenbendazole and mebendazole), with treatment failure (FECR < 95 %) in all 8 flocks investigated. *Haemonchus contortus* and *Teladorsagia circumcincta* were the predominant species after treatment failure. Amino acid substitutions associated with benzimidazole resistance were detected at the codon positions 167 (8%) and 200 (92%) of the isotype-1 beta tubulin gene in *H. contortus*, codon positions 198 (47%) and 200 (43%) in *T. circumcincta* and position 200 (100%) in *T. colubriformis*. Resistance against macrocyclic lactones (ivermectin, doramectin and moxidectin) (FECR < 95%) was recorded on 7 out of 20 flocks, mainly in *H. contortus* and *T. circumcincta*. Treatment failure was also observed for closantel (in combination with mebendazole) (FECR 53%) and for monepantel (FECR 44%), on one farm each. *Trichostrongylus* spp. and *Cooperia curticei* were implicated with resistance against monepantel.

A questionnaire survey on farm management and worm control measures indicated that worm control was often not sustainable. Ewes and lambs were treated frequently (on average 2.6 and 3.2 times per year), mostly without weighing. Only few sheep farmers (9%) regularly used faecal egg counts to monitor worm infections. Despite the FECRT showing treatment failure, most of the farmers perceived the efficacy of anthelmintics as good (54%) to very good (29%). Only 12% and 4% evaluated the anthelmintic efficacy as mediocre or insufficient, respectively.

In conclusion, anthelmintic resistance is widespread in Belgian sheep flocks. There is an urgent need to efficiently promote sustainable worm control practices to sheep farmers and veterinarians.

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Joint COMBAR- ACSRPC meeting: “Anthelmintic Resistance in ruminants: Who cares?”

Towards dynamic risk maps for helminth parasites in ruminants: A case study in Italy

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Dicrocoelium dendriticum, *Fasciola hepatica* and *Paramphistomum* are trematodes that occur in ruminant livestock. Although they have a different economic impact their occurrence can lead to important economic loss. Modelling the spatial distribution of these parasites can help to improve management in higher risk regions. Until now static models are the golden standard, i.e. models that combine information from several years without highlighting differences between years. The aim of the work reported here is to develop dynamic risk maps to evaluate the change of occurrence throughout the years. This may not only be important to help improve management decisions but is also of particular interest because annual differences in weather conditions may be increased due to climate change.

A georeferenced dataset for the three parasites under study, covering Italy and ranging from 1999-2018, was provided by CReMoPAR (University of Naples). First, for each parasite ‘normal’ distribution models were computed using random forest approaches applied to all years combined. Based on this output the minimal sample size required for modelling individual years was computed. Finally, annual models were constructed and compared to the ‘normal’ model output. Results are discussed with a focus on *Dicrocoelium dendriticum* and pathways for future research are highlighted.

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Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Anthelmintic resistance among gastrointestinal nematodes of sheep and cattle in Ireland

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Grazing livestock are naturally exposed to gastrointestinal nematodes (GIN). Therefore, the availability of efficacious anthelmintic products is of great importance in grass based beef and lamb production systems such as in Ireland. While anthelmintic resistance, including resistance to macrocyclic lactones has been confirmed on a small number of Irish sheep and beef farms, little is known about the extent of drug resistance.

In 2017 and 2018 the efficacy of fenbendazole, levamisole, ivermectin and moxidectin was tested on 16, 11, 17 and 10 dairy calf to beef herds respectively by the faecal egg count reduction test (FECRT). In 2018, the efficacy of fenbendazole, levamisole and ivermectin was tested on 15 sheep farms by the faecal egg count reduction test (FECRT). For cattle, 20 calves per treatment group were utilised along with pre and post dosing FEC. For sheep, 15 lambs per treatment group and a control group were utilised. The FECRT showed evidence of reduced efficacy of ivermectin on 100% of cattle farms tested and on 40% of sheep farms. There was resistance to moxidectin on 70% of cattle farms tested. On 69% of cattle farms and 100% of sheep farms tested fenbendazole failed while levamisole failed on 18% of cattle farms and 20% of sheep farms tested.

Larval identification from faecal cultures showed that for all anthelmintic classes, *Cooperia* and *Ostertagia* were found after treatment. Larval identification for sheep GIN is currently underway.

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Comparison of *in vivo* and *in vitro* response in identical parasitic infection in goats and sheep

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The objective of the study was to compare *in vivo* faecal egg count reduction test (FECRT), *in vitro* Egg Hatch Test (EHT) and molecular determination of codon 200 benzimidazole-resistance associated allele frequencies in *H. contortus* obtained from infected goats and sheep. In this study 54 lambs and 54 goat kids were each infected with mixture of susceptible and benzimidazole-resistant *H. contortus* isolates. Composite infection doses consisted of 10, 20, 30, 40, 60 and 80 % of the respective resistant isolate. The results of the *in vivo* FECRT showed that albendazole treatment caused infection reduction which correlated with eggs per gram values in goats. The results of ED₅₀ values in the *in vitro* EHT varied from 0.12±0.01 to 28.05±20.87 µg/ml TBZ in sheep and from 0.09±0.01 to 15.63±12.10 µg/ml TBZ in goats. The results of PyrosequencingTM revealed that the percentages of the susceptible (TTC) and resistant (TAC) alleles in *H. contortus* L3 larvae derived from different groups of sheep and goats were closely related with the respective composition of larvae which have been used for infection. The results of the *in vivo* and *in vitro* tests in both species confirmed solid base for an estimation of clinical resistance *in vivo*. Surprisingly, comparable results obtained after treatment with the same dose rate indicate a similar bioavailability of the drug in sheep and goats.

The study was supported by Slovak Research and Development Agency Project APVV-14-0169.

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A proposal of MiniFlotac Market Analysis applied to Italy

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The countries that are members of the COMBAR Project research have many differences. In order to identify the differences or similarities between countries, the research aims to develop a macro qualitative analysis of market. The first step is to identify the main relevant influences that might affect the market. In other words, the context that the product intends to be inserted (surrounding environment). Thus, it is important to identify political, economic, social, technological, environmental and legal factors for each country. This first phase of the methodology develops a PESTEL analysis to identify possible scenarios for the strategic plan. The market analysis aims also to evaluate the product or company, as well as the environment. So, the second phase is identifying its internal aspects, strengths or weaknesses of the product, as well as the external aspects, called opportunities or threats in the in the surrounding environment. In order words, it needs to develop a SWOT analysis. The objective remains the same: supporting the strategic or business plan. Finally, the plan needs to overpass the barriers to entry. The plan can also define other objectives like: i) reach a certain percentage of profitability or market, ii) diversify products, iii) develop innovation, etc. The most important part is identifying partners to form a collaborative network. This proposal has been applied to Italy. It is suggested that it be replicated to the other countries of the COMBAR Project.

POSTER PRESENTATIONS: working group 1

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Suspected resistance to ivermectin in gastrointestinal nematodes of ruminants from northern Portugal

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Ruminant production is important for the economy of northern Portugal, thus the organizations of livestock farmers promote the use of anthelmintics once a year. Nevertheless this is made without any previous diagnosis or faecal egg count (FEC), so very little is known about efficacy of drugs and anthelmintic resistance (AR) in gastrointestinal nematodes (GIN) in Portugal. The aims of this study are to assess the diversity, intensity and FEC reduction tests (FECRT) of GIN in ruminants raised in extensive system in the north of Portugal. For this purpose samples were collected in 6 (2 cattle and 4 sheep) farms, on the day the ruminants have been dewormed with ivermectin (subcutaneous) and 14 days after. FECRT were performed on pooled samples using the Mini-FLOTAC technique. Coprocultures were also run for the identification of GIN L3 genera. The intensity was very low, ranging between 5 – 2260 eggs per gram (EPG) with only one sheep farm with intensity of more than 150 EPG. The efficacy of ivermectin ranged between 71.8% to 100%. One sheep farm showed a FECR of 71.8% suggesting resistance to ivermectin. Coproculture revealed the presence of *Teladorsagia* spp. and *Trichostrongylus* spp. in this farm, with *Teladorsagia* spp. being more prevalent after the deworming. The same happened in a second sheep farm (FECR = 97.5%) where *Haemonchus* spp. and *Chabertia* spp. have also been identified, suggesting that *Teladorsagia* spp. may be more resistant to ivermectin. This is an ongoing study and these preliminary data need to be further confirmed.

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The prevalence of anthelmintic resistance in goat herds in Poland detected by egg hatch test and larval development test

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Introduction: The emergence of anthelmintic resistance among gastrointestinal nematodes over the last three decades is the main threat to goat farming around the world. Data on the prevalence and spread of anthelmintic resistance of gastrointestinal nematodes in Poland are still insufficient. The aim of the study was to assess the prevalence of anthelmintic resistance in goats in Poland by in vitro methods: egg hatch test (EHT) and larval development test (LDT).

Materials and methods: The study was carried out in 39 goat herds from all over Poland. The faecal samples were collected directly from the recta of adult goats by owners and delivered to the laboratory within 24 hours. The EHT and LDT were performed in 37 herds (in 2 cases only EHT or LDT). Infectious third-stage larvae in the wells with highest concentrations of each anthelmintic were isolated, and species were identified.

Results: The resistance to thiabendazole was indicated by EHT in 33 herds (89%), and in 28 herds (76%) by the LDT respectively. Most common species present in the highest concentration of thiabendazole was *H. contortus*. Resistance to ivermectin aglycone was indicated in 28 herds (76%), and most prevalent nematodes species developed in the highest drug concentrations were *Trichostrongylus spp.* Resistance to levamisole was indicated in 3 herds (8%) and most prevalent nematodes species developed in the highest drug concentrations were *Trichostrongylus spp.*

Discussion and conclusion: Anthelmintic resistance is common in goats gastrointestinal nematodes in Poland. The most common is resistance to the benzimidazoles which are most commonly used in Poland.

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Evidence of Triclabendazole resistant *Fasciola hepatica* on multiple sheep farms in West of Scotland using faecal egg counting and coproantigen ELISA testing

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The trematode parasite *Fasciola hepatica*, liver fluke, has a considerable impact on livestock production worldwide. Disease control relies heavily on anthelmintics, with Triclabendazole (TCBZ) the only chemical capable of killing immature and adult fluke. Although widespread TCBZ resistance is acknowledged in many countries, including Scotland, the extent is largely unknown.

Ten sheep farms were selected in Argyll with a range of suspected fluke risk and resistance statuses; all routinely treating with varying frequencies. The fluke burden was tracked through composite faecal egg counts (FEC) and coproantigen ELISAs (cELISA) from September 2018 - May 2019. Farmers administered treatments, collecting samples for individual FECs and cELISAs at treatment and 21 days later. Of the animals FEC positive (mean = 19.07 eggs per gram (epg), median = 5.67 epg), 84% remained positive 21 days post-treatment (mean = 16.83 epg, median = 2.75 epg); of cELISA positives pre-TCBZ (mean = 55.79 optical density (OD), median = 41.18 OD), 75% remained positive post-treatment (mean = 40.57 OD, median = 25.94 OD). Five farms administered further treatment with Closantel, one Oxyclozanide and one Albendazole. 6% remained FEC, 19% cELISA positive, with the majority showing 100% reduction (discrepancies occurring when post-treatment samples taken early).

The fluke risk being "low and late" in 2018, cELISA was a better indicator of infection than composite FEC and results indicated that farmers may have historically treated too early in this region. Fluke was identified on all farms involved and reduced efficacy of TCBZ was observed in all eight farms using TCBZ.

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Use of Multiplexed Tandem PCR in a Scottish Deer Survey

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Habitat loss and farm intensification have been suggested as factors involved in changes in host-parasite interactions and infection levels in livestock. One area of interest is the interactions between livestock and wildlife, and how these contacts may influence the dissemination of livestock parasites and anthelmintic resistance; now and in the future. Deer are ubiquitous throughout Scotland and their abundance and/or geographical distribution is considered to be increasing, bringing them in to greater contact with livestock.

During 2017-2018 a Scottish deer health survey (n=1087) was conducted to look at the presence of key livestock pathogens. The majority of samples were collected from Red (46%) and Roe (41%) deer although Fallow and Sika were also examined. Faecal samples were examined for Strongyle and *Nematodirus* egg counts as well as with Multiplexed Tandem-PCR (AusDiagnostics cattle and sheep parasite kits) to identify eight economically important nematode genus/species.

A total of 645 faecal samples were examined for egg counts; ~80% had positive strongyle egg counts, average 33 eggs per gram [EPG; range 0–2376 EPG]. Around 10% of the samples contained small numbers of *Nematodirus* eggs [average=1; range 0-54 EPG].

260 DNA samples (from samples >10 EPG) were analysed, the commonest species/genera identified were *Oesophagostomum*, *O.ostertagi* and *Trichostrongylus*. Other species such as *T.circumcincta*, *Haemonchus* and *Cooperia* were also present but at a lower prevalence. Deer could be responsible for the dissemination of multiple nematode species to livestock; play a role in the spread of anthelmintic resistance or provide a source of refugia.

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Anthelmintic resistance in gastrointestinal nematodes of French Angora goats

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Anthelmintic resistance (AR) in gastrointestinal nematodes (GIN) is very frequent in dairy goats, especially to benzimidazoles. Considering that the use of anthelmintics can be quite different between dairy and fiber goats, an AR survey was conducted in 15 French Angora flocks located all over the country. The size of these flocks ranged from 20 to 100 goats, all of them used natural pastures or rangeland. The main anthelmintics used were moxidectin followed by oxfendazole, with a low number of annual treatments. On each farm, goats were randomly allocated into several groups: an untreated control group, a group that received orally oxfendazole (10 mg/kg body weight (BW)) and a group that received orally a moxidectin drench (0.4 mg/kg BW). Individual faecal egg counts and pooled larval cultures were done 14 days after anthelmintic treatment. AR was present when the % of Faecal Egg Count Reduction (%FECR) was <95 % and the lower limit of the 95% confidence interval <90 %. Two farms were excluded because the control group was absent. For the 12 flocks where oxfendazole was tested, FECR ranged from zero to 83%, meaning that resistance to benzimidazoles was present in all flocks. In the 11 flocks where moxidectin was tested, resistance was present in 3 of them with FECR ranging from 69 to 94%, *Teladorsagia/Trichostrongylus* was always the predominant post-treatment culture larval type. This study confirms the extremely high prevalence of resistance to benzimidazoles for GIN in French Angora goats and demonstrates that resistance to moxidectin is developing.

POSTER PRESENTATIONS: working group 1

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Comparing different metabarcoding approaches to investigate *Eimeria* species diversity and community structure in cattle.

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Bovine coccidiosis, caused by protozoal parasites of the genus *Eimeria*, is an important cause of clinical disease and sub-clinical production loss in cattle with control being largely dependent on the routine administration of anticoccidial drugs. Whilst *E. zurneii* and *E. bovis* are considered to be the most important, over 20 different species of *Eimeria* have been described in cattle and relatively little is known regarding their pathogenesis, prevalence, drug sensitivity or interactions with other pathogens. We have undertaken a metabarcoding approach to explore bovine *Eimeria* species diversity and community structure. Paired-end short read sequencing (Illumina Miseq) was used to target three variable regions of the SSU 18S rRNA coding sequence as well as the ITS-1 rDNA region. The full length SSU 18S rRNA coding sequence was also targeted with long-read sequencing (PacBio Sequel SMRT). The different approaches were compared to evaluate their ability to successfully estimate the species proportions on a variety of different samples. These included mock communities of known *Eimeria* species proportions and field samples from beef and dairy cattle across Western Canada in which ~100 sporulated oocysts had been morphologically identified per sample. The strengths and weaknesses between the different loci and between short- and long- read metabarcoding approaches is discussed.

POSTER PRESENTATIONS: working group 1

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

What can egg-proteases tell us about the pathogenesis caused by trematodes?

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Eggs released by female schistosomes are considered to be the primary cause of symptoms connected with schistosomiasis. Discharge of molecules (mainly proteases) by eggs facilitate their migration through the tissue of the host. Nevertheless, as a side-effect they cause severe pathogenesis manifested by inflammation, granuloma formation and subsequent fibrosis of the tissue. The eggs of *Fasciola hepatica*, trematode which inhabits the bile ducts of number of ruminants and other vertebrate species, are not supposed to have such detrimental effects on its host although the eggs related proteolytic activity was recorded. The detailed protein composition of *F. hepatica* eggs' products remains unrevealed. Comparison of proteins from the excretory-secretory products of eggs from highly pathogenic vs. non-pathogenic species could represent a step towards better understanding of biology of *F. hepatica*, pathogenesis associated with *S. mansoni* infections and thus contribute to effective drug treatment.

In our newly launched scientific project we aim to compare proteolytic cocktails present in E/S products of *S. mansoni* and *F. hepatica* eggs. We focus particularly on the identification of proteases by functional proteomics, evaluation of their physiological role by genomic studies, and their recombinant expression and biochemical characterization.

Acknowledgements: This study was supported by Czech Science Foundation (GA19-17269S).

POSTER PRESENTATIONS: working group 1

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Anthelmintic resistance on commercial sheep farms in the southeastern US

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In 2016, the Let's Grow Committee of the American Sheep Industry Association funded a study to determine anthelmintic resistance on 30 commercial sheep farms in the southeastern US: Maryland (MD), Virginia (VA), and Georgia (GA). Pooled fecal samples were collected from 10 farms in each state and submitted to the University of Georgia for FEC, larvae ID, and DrenchRite® larval development assay (LDA). Percentage and FEC data were log transformed for analysis/inference, but LSMEANS are reported. FEC averaged 5080, 3015, and 3695 epg (SEM=487; MD=GA>VA, P<0.05), respectively for MD, VA, and GA farms. Coprocultures identified *Haemonchus contortus* as the predominant worm species: 89.4, 73.3, and 83.9% (SEM=3.1%; MD=GA>VA, P<0.02), respectively for MD, VA, and GA farms. All farms had high resistance to fenbendazole (SEM=9.4%). Resistance to ivermectin was similar among states, averaging 80, 100, and 100%, respectively for MD, VA, and GA. Resistance to moxidectin and levamisole was lower in MD as compared to the more southern states (P<0.04). All farms in VA and GA had resistance to three of the four anthelmintics tested. Multiple drug resistance in MD was lower, with only 80% of farms having resistance to two drugs and 60% having resistance to three drugs. The percentage of farms with resistance to all tested drugs was 0, 60, and 40%, respectively for MD, VA, and GA farms. While anthelmintic resistance varied by state and farm, all farms had resistance to at least one anthelmintic, which underscores the importance of anthelmintic resistance testing on individual farms.

POSTER PRESENTATIONS: working group 1

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

First report of ivermectin resistance in goat gastrointestinal nematodes in Serbia

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Anthelmintic resistance (AR) in gastrointestinal nematodes (GIN) of small ruminants is well known global phenomenon with high economic cost. Despite the risk, sheep and goat producers in Serbia seem to be unaware of AR and control of anthelmintic efficacy is not a usual practice. In Serbia, AR to ivermectin (IVM) has been confirmed in sheep GIN so far. The aim of this report is to present results of faecal egg count reduction test (FECRT) in goat herd where IVM failure has been detected for the first time. FECRT was performed in a flock of grazing dairy goats in village of Male Pijace, North Banat County, Vojvodina province, Serbia, as a part of screening of AR prevalence in small ruminants. According to deworming history of the flock, goats usually receive three or four annual treatments (albendazole (ALB) 2-3 times a year and IVM once during dry period). These two drugs were used in FECRT. Two treatment groups received ALB 10mg/kg orally (9 goats) or IVM 0.4 mg/kg subcutaneously (8 goats). There was no control group. Faecal samples were taken from the rectum at Days 0 and 10. Mini FLOTAC was used for GIN egg counting (analytical sensitivity=5 epg). Percentage of egg reduction ($PR\%=100(1-T_2/T_1)$) and 95% confidence intervals were calculated. Results were interpreted according to the guidelines of the WAAVP. The efficacy of ALB against goat GIN was 100%. For IVM, PR was 92% (95% CIs: 73-98). These results represent the first report of IVM resistance in goat GIN in Serbia.

POSTER PRESENTATIONS: working group 1

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

High Resolution Melting Assay: a powerful method usable for species differentiation as well as for detection of polymorphisms associated with anthelmintic resistance

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High Resolution Melting Assay (HRMA) is post-PCR method which enables to reveal the genetic variation in DNA samples without the necessity of subsequent sequencing. Sequential changes (down to single nucleotide polymorphisms) are detected in amplified sequences according to their disassociation behavior.

In our study, we employed a qPCR-HRMA to differentiate two of the pathogenic nematodes of ruminants – *Haemonchus contortus* and *Ashworthius sidemi* at the species level. The amplification and subsequent melting assay were specifically targeting the polymorphisms in two fragments of internal transcribed spacer 1 (ITS1) sequence. For the evaluation of melting process, a difference plot representing the most transparent expression of species-specific matrix curves were used.

The occurrence of resistance in helminth species against various classes of anthelmintics has been described in a number of publications. It can be the result of a different mechanisms, such as genetic changes in the drug target genes (e.g. point mutations in β -*tubulin* gene associated with benzimidazole resistance in *Haemonchus contortus*). These single nucleotide polymorphisms offer a means of detecting the presence of resistance using the HRMA.

We show that qPCR-HRMA is rapid and reliable method which can be used not just to assign individual parasites taxa but it has also considerable potential for the detection of sequential changes associated with anthelmintic resistance.

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POSTER PRESENTATIONS: working group 1

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

The response of the saliva proteome in sheep infected with liver fluke (*Fasciola hepatica*)

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Introduction: Diagnostic options for early detection of liver fluke disease are limited, especially for sheep. Here we attempt to identify changes in the salivary proteome of sheep with fasciolosis as a precursor for development of a putative non-invasive diagnostic test for fluke.

Materials & methods: Saliva samples (n=6) were collected weekly over 14 weeks of an experimental model of liver fluke infection in sheep. Proteomics was performed by reduction, alkylation, digestion and labelling of samples with TMT reagents. High resolution LC-MS/MS analysis of TMT-labelled peptides was carried out using an Ultimate 3000 RSLCnano system coupled to a Q Exactive Plus MS. Acquired MS/MS spectra were analysed by Proteome Discoverer and then with RStudio.

Results: Overall, 3232 peptides were identified with further analysis yielding 237 peptides significantly different when comparing pre-infected to post-infected samples. Examination of the relative changes following infection identified 20 proteins that showed notable change in saliva of sheep following liver fluke infection. Two of these proteins, namely complement C3 and bactinecin, were also detected in sheep with a naturally acquired fluke infection.

Conclusions/future work: Infection of sheep with liver fluke leads to alteration of the salivary proteome with increases in proteins known to be part of the host response to infection (complement and bactinecin). Whether these proteome changes represent viable biomarkers for fasciolosis now need assessment with samples from sheep with natural co-infections e.g. GI nematodes and rumen fluke, and before/after flukicidal treatment.

POSTER PRESENTATIONS: working group 1

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Anthelmintic Resistance In Sheep helminths (ARTIST) in Norway.

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Parasitic helminths causes production losses in sheep production all over Europe, and Norway is no exception. Norwegian sheep farmers rely on anthelmintic treatment to keep infection at acceptable levels. Routine treatment may increase the chance of inappropriate treatment (e.g., under-dosing) and unnecessary drug use, which, in turn, may lead in development anthelmintic resistance. Although the prevalence of helminths with anthelmintic resistance in Norway does not seem to be high compared with that in other countries, previous investigations indicate that this may become a growing challenge.

The Norwegian University of Life Sciences, in collaboration with the sheep industry in Norway, is starting a research project with focus on two important parasitic helminths in sheep, *Haemonchus contortus* and *Fasciola hepatica*, between 2019-2023. The project, ARTIST, aims to investigate the effects that these parasites have on the sheep production systems in Norway and how they are currently being controlled. In addition, ARTIST aims to identify tools to optimize future control strategies.

This presentation will give an update on current knowledge, and knowledge gaps, about GI helminths in Norwegian sheep production and an outline of the planned project.

POSTER PRESENTATIONS: working group 1

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Resistance of sheep trichostrongylids against Moxidectin in Austria

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Anthelmintic resistance (AR) in sheep nematodes is increasingly reported worldwide. Few studies have been conducted in Austria and while AR against benzimidazoles (BZ) seem to be frequent, AR against macrocyclic lactones (ML) has not been clearly identified yet. As severe treatment failures were increasingly reported from sheep flocks we aimed to monitor the efficacy of BZ and ML by fecal egg count reduction tests (FECRT) in selected farms. In total 11 farms were examined, three from Styria, eight from Tyrol. Overall, 500 animals were included. At the first farm visit feces was taken and examined by Mini-Flotac (detection limit $EpG=5$). Animals with an $EpG \geq 100$ were included. A larval culture was set up from pooled fecal samples before treatment and in case of a positive FEC also after treatment. Animals were treated with Moxidectin (Cydectin®) (MOX, group 1) Fenbendazol (Panacur®) (BZ, group 2) or left untreated as controls (group 3). All three groups could be tested on one farm. Moxidectin was applied on 10 farms, Fenbendazol on three. FECRT indicated a reduced sensitivity of trichostrongylids against Mox on two farms (78%, 79%) and against BZ on two farms (48%, 89%). The predominant nematode genera before treatment were *Haemonchus* spp., followed by *Trichostrongylus* spp. and the Chabertiinae. After treatment predominantly *Haemonchus* spp. and to a lesser extent *Trichostrongylus* spp. were found. The apparent lack of efficacy of the ML in sheep flocks with visible clinical consequences indicates the urgency of implementing sustainable control practices in Austria.

POSTER PRESENTATIONS: working group 1

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

A bespoke nematode ITS-2 rDNA database formatted for multiple common bioinformatic pipelines to support nemabiome amplicon sequencing approaches

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Progress in next-generation sequencing technologies is revolutionizing our ability to use molecular approaches to identify and quantify the presence of different parasite and other pathogen species in fecal, environmental and other samples. However, there are a variety of challenges to the accurate identification of taxa, particularly to the species level, including marker selection, bioinformatic pipeline choice and parameterization, reference sequence availability and database selection and curation. High quality databases are essential for accurately identifying taxa in samples using marker genes such as the ribosomal small subunit (SSU) and the internal transcribed spacer (ITS). Although large publicly available database exist for both the large and small ribosomal subunits, such as SILVA, there are few options for other marker regions such as ITS-2 rDNA that may be more suitable for particular projects. Furthermore, large databases often have long delays between release cycles. We have developed markerDB, a pipeline to build databases of any chosen marker gene sequence for a given taxonomy built in R and snakemake. Currently it supports ITS-2 and 18S rDNA sequences but can be easily expanded to be use for any gene. The pipeline retrieves sequences from NCBI annotated with the provided marker, identifies the correct region using a hidden markov model (using barrnap, <https://github.com/tseemann/barrnap>) or covariance model (using infernal) and formats them for common pipelines like RDP, dada2 and mothur. This pipeline provides speed and flexibility beyond what is available in public databases. markerDB is available at <https://github.com/ucvm/markerDB>.

POSTER PRESENTATIONS: working group 2

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

The costs of parasitic helminth infections to the ruminant livestock industry in Europe and allocated research budgets: intermediate results

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Gastrointestinal nematode, lungworm and liver fluke infections are highly prevalent in ruminant livestock and an important cause of both visible (mortality, weight loss) and invisible (reduced growth, milk yield, fertility) production losses. We combined literature data with expert consultation to (i) identify knowledge gaps in the prevalence and production impact of helminth infections and anthelmintic resistance (AR); (ii) use the available data to estimate the costs of helminth infections in 17 COST Member countries and (iii) compare those costs with allocated public research and control budgets. Intermediary data of this ongoing effort will be presented. Many data gaps to appropriately estimate the cost of helminth infection and AR were identified and vary on the country level. Data gaps are present at all levels including accurate country-level data on animal numbers, levels of helminth infection and AR and associated production impacts as well as information on applied control strategies and associated costs. Data gaps were imputed based on available data from countries in similar bio-climatic zones allowing and intermediate results on estimated costs on country and European level will be presented and compared with public research budgets.

POSTER PRESENTATIONS: working group 2

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Current use and need for anthelmintics in organic livestock in Europe

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Reducing the input of anthelmintics in livestock farming is an integral step to containing the spread of anthelmintic resistance. Arguably, organic farmers are under even greater pressure to reduce their use, where synthetic anthelmintics are regulated in accordance with organic principles and parasite control is largely driven by prevention through planning and management. Currently however, there is no data available on the use and need for anthelmintics in European organic livestock production. This research aimed to address these gaps in the knowledge by conducting a EU-wide survey of organic experts. Data were obtained for 16 European countries, covering 17,719 organic farms. The results indicate: i) differences in the policing of organic regulation and the recording of anthelmintic use across the EU; ii) country-specific differences in the level of parasite threat and control. Estimates suggest that overall anthelmintic treatments do not exceed one treatment per animal per year, although exceptions were noted. Extrapolated estimates suggest some 45.5 million anthelmintic treatments are entering the European organic farming system annually. Certain countries demonstrated minimal anthelmintic requirements; closer investigation into the parasite management strategies of these countries may highlight areas to improve parasite control. More detailed surveys carried out in Britain showed that 61% of UK organic farmers used anthelmintics in the last 12 months, with 79% of all the supplementary requests for anthelmintics required specifically for sheep. These results provide the first benchmark against which evolving patterns of anthelmintic inputs may be compared to gauge progress across the farming community.

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Partnering for a Better Outcome

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Scientists work very hard to conduct research to explore problems and find answers. Yet, if those answers are only known in the scientific world, the benefits are largely lost. NCAT's ATTRA information service works to translate the knowledge held by researchers into farmer-friendly publications, videos, and other educational resources. In this way, we can provide farmers with practical, science-based information that can help them be more successful.

Staff from NCAT/ATTRA have joined with the American Consortium for Small Ruminant Parasite Control (ACSRPC) to assist with producer education and outreach. Interaction between researchers and educators benefits both. Educators transfer farmer questions to researchers, and researchers provide sound scientific knowledge for the educators. Over the years, we have produced 18 titles together, resulting in 127,576 downloads from the ATTRA website (www.attra.ncat.org) alone. We have also produced webinars, narrated PowerPoints, and short videos, reaching thousands more. Much of this work has been funded by grants awarded to Consortium members. ATTRA publications point readers to the Consortium website (www.wormx.info) and relevant research, and ATTRA publications are also featured on the Consortium website.

Outreach and education are a critical component of the work to battle anthelmintic resistance and support the farmers who are raising ruminants. It is vital to have practical, trust-worthy information readily available to farmers and educators. Farmers, researchers, and educators benefit from teamwork, resulting in better animal health and welfare.

POSTER PRESENTATIONS: working group 2

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Folktales fostering GIN parasites anthelmintic resistance in sheep and goats.

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Some sheep and goat producers consider easily-accessible and free cyber information (Internet) as validated knowledge. Cooperative Extension educators face the challenge to convey science-based practices to their clientele to manage worm burdens, competing, unfortunately, with a huge and ever-growing body of Internet bogus information. Sheep/goat helminthosis is a problem in all agroclimatic zones. Additionally, anthelmintic resistance (AR) is a global problem threatening small ruminants' welfare and productivity. AR is identified when a previously used anthelmintic ceases to kill an exposed worm population at the therapeutically recommended dosage. AR erodes the productivity of small ruminants, affecting the survival of small family farms. The objective of this exploratory analysis is to identify widespread-cyberspace examples of folktales/myths which potentially increase GIN parasites' AR in a flock or herd. Cooperative Extension educators may use this information to enhance and complement their integrated parasite management teaching toolbox.

Examples of the folktales/myths are:

- 1.-All livestock have parasites, even humans, ... parasites are not a problem for sheep and goats...it is normal... sheep and goats are tough
- 2.- "Natural dewormers" are successfully used as a thoughtful blend and in harmony with nature...
- 3.-Increasing or decreasing the recommended dose per animal is irrelevant: *if 2 cc are prescribed, then 3 cc are better, ... or ...*
- 4.-... *use 1 cc instead... saves money!!*
- 5.-Treat sheep/goats with anthelmintics on a schedule... i.e., every month...
- 6.-Use diatomaceous earth to treat internal parasites in sheep/goats.
- 7.- Soak grains, forage, etc., in a lye solution and give it to the sheep/goats to kill the worms...

POSTER PRESENTATIONS: working group 2

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Parasite treatment practices in small ruminant flocks in Slovenia

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Farmers most often control parasites in sheep and goat flocks using anthelmintic drugs. Unfortunately, inappropriate use of them leads to anthelmintic resistance. A survey was conducted to identify the common parasite control practices among sheep and goat farmers in Slovenia.

Questionnaires about parasite control practices were sent to farmers. Two hundred twenty-five farmers participated in the study. Results showed that most farmers treat their animals twice (57%) or three and more (15%) times a year. Most farmers treat based on experiences (68%) or poor body condition score (30%). About half of the farmers (48%) send faecal samples for a parasitological examination, but only 28% treat based on the parasitological report. The survey also revealed that treatment is usually done on the entire flock (69%). Most of the farmers (77%) perform treatment themselves, 23% call veterinarian. Farmers mostly (71.5%) dose anthelmintic according to the body weight of an individual animal, but only 9.3% weigh animals. Some farmers (21.8%) dose regarding average weight of animals, only 6.7% of farmers dose according to body weight of the largest animal. Farmers that rear both, sheep and goats more often treat goats with the same dose of anthelmintic than sheep (65.2%). The anthelmintic drug of choice is still largely advised by the local veterinarian (69%).

The results show that it would be necessary to provide more information for farmers about the problem of parasites and about appropriate suppression methods, as this is crucial for prevention of anthelmintic resistance of parasites and sustainable small ruminant breeding.

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Wild ruminants as a potential risk factor for transmission of abomasal nematode *H. contortus*

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Experimental roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and mouflons (*Ovis musimon*) were infected with the MHco3 sensitive strains and the MHco4 resistant strains of *H. contortus*. The dynamics of the intensity of parasitic infection was observed in all three wild ruminant species. The numbers of eggs per gramme (EPG) of faeces were quantified using a modified McMaster technique for each sampling day. The highest intensity of infection in mouflons was detected on day 58 post infection (EPG 26 500±150) with resistant strain. The roe and fallow deer had very low infection intensity until day 58. The level of benzimidazole resistance in mouflons and sheep were analysed with egg hatch test (EHT) and larval development test (LDT). In mouflons and sheep the ED₅₀ values in EHT (0.043±0.005-0.064±0.008) of susceptible *H. contortus* strain correlated with a low level of resistance obtained by LDT (LD₅₀ 0.007±0.002-0.009±0.001 mg/ml TBZ). In contrast, higher ED₅₀ (0.091±0.001 to 0.111±0.004 mg/ml TBZ) in EHT and LD₅₀ values (0.021±0.002 to 0.031±0.001 mg/ml TBZ) in LDT were determined both in mouflons and sheep in the *H. contortus* resistant strain. Both *in vitro* tests confirmed the same level of benzimidazole resistance for both species.

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Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Caring isn't enough

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The embeddedness of anthelmintics in flock management programmes is testament to the importance that farmers place on controlling gastro-intestinal parasites of sheep. The loss of these tools would have serious ramifications for both animal health, performance and well-being, outcomes that farmers care deeply about. Yet out with farming communities the response of farmers to the threat of anthelmintic resistance (AR) is commonly viewed as inadequate and their attitudes called into question. This paper considers human motivation theory and examines the relative role that attitudes can play in determining the choices made by farmers, highlighting that caring is often not enough to stimulate people to act. A small study in the UK found that the majority (78%) of farmers had changed their worm control strategies due to concerns about AR. This confirms that they do care and are responding to the risk of AR although the changes made are not as great as those considered desirable by external stakeholders. Closing the gap between what is perceived to be the desired level of change in farm practice and its reality in light of the study findings is discussed.

POSTER PRESENTATIONS: working group 2

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Anthelmintic Resistance, Who Cares: The Farmers Do ... Until Worms Under Control

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The only true crisis in gastrointestinal nematodes (GIN) management followed intensification of grazing ruminant production, which practically precluded profitable small ruminant production, e.g. due to *Haemonchus contortus*. However, akin to antibiotic resistance, effective anthelmintics brought relief for animal production, until they flooded the market.

Globally, the drugs were ushered in by South Africa, with apparently the first non-herbal anthelmintic, the "Government Wireworm Remedy" being launched in 1918, after intensive pressure by farmers on the Onderstepoort Veterinary Research Institute (founded in 1908). The resultant haemonchicide, containing arsenic and copper sulphate, was sufficiently effective against *H. contortus* to serve farmer purpose, especially when later modified by addition of nicotine, which improved the safety margin of the formulation and extended its usefulness into the 1960's.

Unfortunately, however, the myriads of anthelmintic products progressively accumulating on the market created the impression with both farmers and research fund grantors that the supply would be endless. Thus, given the complexity and labour-intensity of holistic sustainable GIN management and that even a single remaining effective drug largely masks the importance of anthelmintic resistance (AR) for farmers, the latter have remained unconvinced by the collective evidence of the looming crisis regarding GIN resistance.

The primary challenge is thus to convince farmers of the importance of AR, to get them to comply. Since all previous 'conventional' attempts have failed, we need urgently to review and ponder every candidate novel, including sociological technological possibility, including machine learning, for farmer training, the single most challenging hurdle facing AR and sustainable GIN management.

POSTER PRESENTATIONS: working group 3

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Testing sustainable nematode control strategies on farm

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Over the last 15 years, several projects have been commissioned to investigate sustainable ways of controlling parasites in organic and low input livestock production systems. Although these projects have largely delivered the underpinning scientific research, progress to implement control strategies has been hindered by limitations of research at a farm systems level. In the UK, commercial organic farmers were given a variety of options to consider for sustainable parasite control on farm. Participating farmers were asked to select one or more of these options, use it in one group of animals, and then compare the results with another group managed in a "usual" manner on their farm. Protein supplementation around parturition, the use of different protein source and using weight loss to apply targeted selective treatment (TST) were three alternatives that were tested by organic sheep farmers. Soya supplementation reduced FEC in lactating ewes, when compared to unsupplemented ewes managed on the same farm. On a second farm, drenching the animals that lost weight (TST approach) did not appear to be affecting total FEC, as these were not necessarily the animals with the highest FEC. Protein supplementation with undegradable dietary protein around parturition did not further improve FEC of organic ewes in a third organic farm, when compared with soya supplemented ewes. In conclusion, on farm trials are a useful tool to identify the relevance of research work for the farming community. Larger scale on farm demonstrations should be planned for the future.

POSTER PRESENTATIONS: working group 3

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Effect of water quality irrigation on the composition and quality of secondary metabolites of *Salix* plants used for the control of parasites nematodes

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Willows (*Salix*) are found worldwide where the foliage has been proposed as a fodder for both small and large ruminants. The biomass of willow is rich in nutritive value for grazing ruminants. Bioactive ingredients from plants can depend on water quality and ecotype and we hypothesized that this may cause variability in anthelmintic bioactivity. Four *Salix* ecotypes were cultivated and irrigated either using tap water or secondary effluents. Plant leaves were collected twice a year and the extracts were evaluated to test this hypothesis. The 70% ethanolic extract yield ranged between 19-30% for both harvests and ecotypes. Polyphenol content of extracts ranged between 23-35% for tap water and 18-39% for water effluent. Flavonoid content varied between 13-54% for tap water and 15-50% for water effluent. Salicylic acid content ranged between 6-13% for tap water and 7-10% for water effluent. Crude polyphenol extracts from both harvests of Kishon ecotype irrigated either using tap water or secondary effluents resulted consistently in more than 80% exsheathment inhibition when applied to L3 larvae, where the other three ecotypes resulted in lower inhibition rates. So far the water quality doesn't significantly affect chemical as well as biological activity of the ecotypes. Consumption of *Salix* foliage by ruminants could lead to an overall decrease in the intestinal load of nematodes, however, ecotype variation in anthelmintic activity should be taken into account. Using HPLC-PDA showed that chemical constituent of the various ecotypes is different and is composed of a mixture of flavonoid and phenolic acid derivatives.

POSTER PRESENTATIONS: working group 3

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

The impact of acute *Fasciola Hepatica* infection on the ovine gastrointestinal microbiome; a potential role in the immunopathology of infection?

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There is a plethora of evidence supporting a role for the gastrointestinal (GI) microbiome in the mammalian immune response to helminth infection. Overall, evidence from experimental nematode infections suggests an immune-modulatory role of the GI microbiota which may facilitate the establishment of chronic infection. On the other hand, data from experimental models of trematode infection suggests a more sinister role for gut bacteria, i.e. the promotion of immunopathology. Indeed, the co-administration of antibiotics with *Schistosoma mansoni* cercariae in mice significantly reduced pathology and was associated with improved outcomes for infected animals. The trematode *Fasciola hepatica* causes significant production losses in the sheep industry, and acute outbreaks can have a high mortality rate; furthermore, resistance to the anthelmintic triclabendazole is becoming increasingly widespread. It is therefore timely and relevant to explore the response of the ovine GI microbiota to acute *F. hepatica* infection and establish whether gut bacteria play a role in the pathogenesis of infection. In this study, for the first time, we profiled the faecal microbiota of sheep experimentally infected with *F. hepatica* over a 14 week period and compared it to uninfected control animals. We used next generation sequencing of the bacterial 16S rRNA gene, followed by bioinformatics and biostatistical analysis to identify differences in microbial alpha and beta diversity and the abundance of specific bacterial taxa between infected versus uninfected groups, and across different time-points. The relevance of our findings for the immunopathology of *F. hepatica* and implications for future control will be discussed.

POSTER PRESENTATIONS: working group 3

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Integrating genetic selection with selective anthelmintic treatment to manage gastrointestinal nematodes in Katahdin sheep in the United States.

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Gastrointestinal nematodes (GIN) continue to plague the small ruminant industry throughout the world due to the prevalence of anthelmintic resistance. Producers are encouraged to utilize tools to manage GIN, which include selective deworming based on FAMACHA to slow resistance and genetic selection of individuals within a flock/herd. The National Sheep Improvement Program (NSIP; USA) collaborates with Sheep Genetics (MLA, Australia) to provide estimated breeding values (EBV) for parasite resistance based on fecal egg counts (FEC) collected at weaning (42-90 days of age) and post-weaning (91-150 days of age) for sheep and goat breeds in the USA. The Katahdin breed is a composite hair sheep with variable levels of genetic resistance to GIN. We have been working with Katahdin producers (21 farms; 2009-2013 and 2017-2019) enrolled in NSIP to collect phenotypic data (body weights, maternal traits, FEC and FAMACHA scores, and incidence of deworming to post-weaning measures). The objective is to better understand consequences of selection for GIN resistance or resilience. In this presentation, we will summarize relationships between FEC EBVs, FAMACHA scores and incidence of deworming to post-weaning (9.6% of 2,613 lambs) and results of a high-density genome scan of progeny-tested Katahdin sires to demonstrate the value of having genetic resistance within flocks. Parasite resistant lambs with balanced EBVs become valuable replacement sheep within seedstock and commercial operations.

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POSTER PRESENTATIONS: working group 3

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Modelling the anthelmintic activity of proanthocyanidins by the hydrolysable tannin library

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Regarding alternative control of nematodes, most of the studies have focused on proanthocyanidins as these are commonly present in legume species. As a tannin researcher, a common question to face is why bother with hydrolysable tannins when it comes to alternative parasite control? In addition to their comprehensive bioactivities, the benefit of hydrolysable tannin is that unlike proanthocyanidins, individual hydrolysable tannin structures can be isolated for accurate structure–activity -comparisons. Thus, among the other bioactivities of hydrolysable tannins, we have studied their *in vitro* anthelmintic activities in detail. With the data obtained thus far, we now know the main structure–activity -relationships between structures of hydrolysable tannins and their *in vitro* anthelmintic activity. Now we aim to measure a library of chemical properties for these known active compounds so that the same properties could be easily measured also for the more complex proanthocyanidin-rich samples. With the known compounds we can then predict what type of a library of chemical properties is expected from the active vs. inactive tannins. This knowledge could be utilized for any tannin-containing sample – hopefully with less sophisticated methods – to select plant species or tannin-based plant products with optimal properties to be used as ruminant feeds or feed additives for the control of the nematodes.

POSTER PRESENTATIONS: working group 3

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Anthelmintic activity of Tunisian chamomile (*Matricaria recutita* L.) against *Haemonchus contortus*

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The chemical treatment of gastrointestinal parasitic diseases has been undermined by increasing resistance and high toxicity.

There is an urgent need to search for alternative natural sources for the treatment of such parasites. In this respect, the present study aims to quantify phenolic compounds of chamomile (*Matricaria recutita* L.) and to study their *in vitro* anti-oxidant and anthelmintic activities in solvents with increasing polarity. *In vitro* determination of anti-oxidant capacity was carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical cation methods. *In vitro* anthelmintic activity was investigated on egg-hatching inhibition and loss of motility of adult worms of *Haemonchus contortus* from sheep. The results showed that methanolic and aqueous extracts contain more total polyphenols, total flavonoids and condensed tannins than chloroformic and hexanic extracts. ABTS and DPPH assays showed that methanolic extracts had the highest anti-oxidant potency (IC₅₀=1.19µg/ml and 1.18µg/ml, respectively). *In vitro* anthelmintic activity showed that both methanolic (IC₅₀ =1.559mg/ml) and aqueous (IC₅₀ =2.559mg/ml) extracts had the greatest effect on egg hatching and motility of worms (100% after 8h post exposure at 8mg/ml). A significant and positive correlation between DPPH and ABTS tests was observed for all tested extracts. Therefore, total phenolic, total flavonoid and condensed tannin values were correlated with IC₅₀ from both ABTS and DPPH, and with inhibition of egg hatching. To our knowledge, this report is the first of its kind to deal with *in vitro* anthelmintic activities of chamomile extracts.

POSTER PRESENTATIONS: working group 3

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Agroindustrial by-products containing tannins as potential natural resources to control gastro intestinal nematodes in ruminants: What ? Why ? How ?

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Because of the widespread, worldwide development and diffusion of resistances to synthetic chemical anthelmintics (AHs) in populations of gastro intestinal nematodes (GINs) of sheep and goats, there has been a strong impetus to explore alternative solutions including the AH properties of some bioactive plant materials (related to their content of plant secondary metabolites = PSMs). A few temperate Legume forages containing condensed tannins (CTs) [e.g. Sulla (*Hedysarum coronarium*), Sainfoin (*Onobrychis viciifolia*) and Sericea cuneata (*Lespedeza cuneata*)] have been the first plants used as scientific models to describe and to analyze the AH properties. CTs belong to the phytochemical group of polyphenols with known high level of bioactivity. Further studies have then been expanded to CT-rich tropical browse Legumes. More recently, there has been an increasing interest to explore worldwide the possible use of tannin-rich by-products from a wide range of agro-industries (nuts, wood, carob, coffee, cocoa, wine etc.). Besides the use to control GINs, employing by-products can help to reduce industrial wastes and pollution. Based on *in vitro* and *in vivo* results obtained on by-products of "nuts", our aim is to illustrate the pros- and cons- to apply such resources against GINs of small ruminants, and to describe the conditions for further developing their application in farm conditions.

POSTER PRESENTATIONS: working group 3

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Strategic plan for control of resistant gastrointestinal helminths in grazing ruminants in the Czech Republic

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The current trend in ecological farming of ruminants creates favourable conditions for the spread of various helminth parasites (e. g. *Fasciola hepatica*, *Dicrocoelium dendriticum*, *Moniezia* sp., *Haemonchus contortus*, *Teladorsagia* sp., *Trichostrongylus* spp., *Ostertagia* sp., *Cooperia* spp.). The emergence of anthelmintic resistance of these parasites threatens their effective control management globally. Czech Republic, being no exception, lacks coordinated and systematic strategic plan to tackle this problem.

In the framework of our new project we aim firstly, to apply modern molecular diagnostic methods in monitoring the parasite load at selected farms in the Czech Republic. Secondly, we intent to define the risk factors and particular mechanisms of anthelmintic resistance, e.g. via the adoption of advanced methods of molecular biology in order to define sequential gene polymorphisms and epigenetic modifications. Last, we plan to suggest the appropriate grazing management for Czech farmers to ensure effective parasite control and sustainable use of anthelmintics.

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POSTER PRESENTATIONS: working group 3

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Resistance or resilience? - very low burden of gastrointestinal parasites in cattle of autochthonous breeds from the north of Portugal

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Cattle production is an important factor for the survival and sustainability in the north of Portugal. Although gastrointestinal parasites may cause an impact on health and productivity of these animals, most of the farms do not have a protocol of deworming and there is no tradition of doing diagnosis before it. So resistance to anthelmintic maybe expected. On the other side, over-deworming may also be occurring. The aim of this study was to assess the need of deworming in cattle farms where deworming had not been done for at least one year. For this purpose we collected samples from farms with cattle raised in extensive systems, on the day the animals have been dewormed and performed faecal egg counts using Mini-FLOTAC. Samples have been collected from 54 farms and at least one egg has been found in 37 farms (68.5%), namely strongyle (29/54), *Moniezia* spp. (5/54), *Toxocara vitulorum* (2/54) and *Capillaria* spp. (1/54). The highest burden (525 eggs per gram – EPG) was found in a sample with *Toxocara vitulorum*. The second highest burden was 170 EPG (strongyle). From the results obtained so far, we can suggest that none of the farms should have been dewormed. This may be explained by the fact that 85% of the cattle of these farms are from Barrosã, Minhota and Cachena autochthonous breeds, which are cattle very resilient and well adapted to the region. Also, these cattle feed on an enormous diversity of wild plants and there are reports on its anthelmintic activity in ethnoveterinary practices.

POSTER PRESENTATIONS: working group 3

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Ozone and animal health

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Ozone, O₃, is the most powerful oxidising agent and has high activity against bacteria and viruses even at very low concentrations (0.05ppm). In water it has a short half-life of about 20 mins at room temperature and breaks down to give oxygen so there are no problems with undesirable by-products. With the invention of a boron-doped diamond electrode that produces ozone in solution in water its potential use in animal health can be explored. In England given continuously in drinking water to young calves at about 0.3 ppm scouring stopped, calves grew faster than untreated animals and less antibiotics were used. In Italy when young organic water buffalo calves, sick with *Cryptosporidium* and *Giardia*, each drank 3l of ozonated water the infections were cured. So would drinking ozone water affect nematode burdens? The results of tests in sheep being conducted in the summer of 2019 will be described. Successful tests would suggest we might be able to overcome the problem of anthelmintic resistance.

POSTER PRESENTATIONS: working group 3

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

A 3.5-year field trial involving biological control of strongyles in wild captive ruminants

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Wild captive ruminants (Family: Bovidae) belonging to the subfamilies Antilopinae (*Antilope cervicapra*, *Gazella cuvieri*), Caprinae (*Ovis orientalis musimon*, *Capra aegagrus hircus*) and Bovinae (*Bison bison*, *Taurotragus oryx*) grazing in parcels with herbage almost all year long were provided commercial pelleted feed enriched with a blend of spores of a filamentous fungus with ovicide activity (*M. circinelloides*) and other larvicide (*D. flagrans*). Pellets were added 10^4 - 10^5 spores of each fungus / kg meal during the mixing phase of industrial manufacturing. All animals were dewormed at November 2013, and the efficacy assessed by examining their feces 14 days later.

Eggs of strongyles were detected in the feces of the ruminants involving the genera *Trichostrongylus*, *Nematodirus*, *Chabertia*, *Haemonchus* and *Trichuris*. Eggs of pinworms (*Trichuris* spp.) were occasionally detected throughout the assay. The anthelmintic efficacy ranged between 96% and 100%. In the Antilopinae, egg-output values near to 300 EPG were observed during the first 15 months, then dropped below 200 EPG till the end. After the anthelmintic treatment, Caprinae and Bovinae species attained counts of eggs of gastrointestinal nematodes lower than 150 EPG until the end of the study. It is concluded that addition of parasiticide fungi to the feed is a results in the destruction of the stages in the feces (eggs and larvae), thus contamination of grassland is prevented and the risk of nematode infection lessens.

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POSTER PRESENTATIONS: working group 3

Joint COMBAR- ACSRPC meeting: "Anthelmintic Resistance in ruminants: Who cares?"

Helminth infection downregulates methanogens throughout the gastrointestinal tract of equines; a potential role for helminth infection in regulation of greenhouse gases?

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It is well established that the gastrointestinal (GI) microbiota regulate immune responses to GI pathogens. In particular, data from our group and others demonstrate that immunomodulatory GI microbes promote both immunopathology from, and facilitate chronic infection with, GI helminths. A deeper understanding of the mechanisms which govern these interactions will pave the way to designing novel approaches to helminth control, aimed at promoting an optimal immune response to infection whilst limiting pathology. Here we systematically described the impact of helminth infection on the GI microbiota in a herbivore-helminth system by comparing GI microbiota between acutely and chronically infected equine livestock and uninfected controls; and furthermore, we triangulated this data by profiling luminal and mucosal microbiota taken from throughout the GI tract at post-mortem in infected *versus* uninfected horses. The results revealed similarities between acute helminth infection models in mice and equines, suggesting that helminths may employ ubiquitous mechanisms in regulating host gut microbiota. Furthermore, the microbial signature of helminth infection was distinctly different between acutely and chronically infected animals, indicating that host adaptive immunity plays an important role in helminth-microbiota interactions. At post-mortem, data supported that from chronically infected animals, and also showed that the most profound impact of infection upon the GI microbiota was a significant down-regulation of methanogen populations. The implications of this finding for helminth control in the context of climate change will be discussed.

POSTER PRESENTATIONS: working group 3

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Development of an online FAMACHA® Certification Program

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The barber pole worm (*Haemonchus contortus*) is the most economically important parasite affecting small ruminant production on pasture and the most common cause of anemia during the grazing season in the U.S. The FAMACHA® system is a critical tool that allows small ruminant producers to make deworming decisions based on an estimate of the level of anemia associated with *H. contortus* infection. However, farmers and non-veterinarians must complete training before they can purchase and use of the FAMACHA® card. One common route for training is attendance at a workshop. Many stakeholders are constrained by busy schedules that prohibit them from attending these workshops, therefore in response to the need for a self-paced option for obtaining FAMACHA® certification, the University of Rhode Island, in partnership with the American Consortium for Small Ruminant Parasite Control (ACSRPC) and with funding from the U.S. Dept. of Agriculture, has developed an online FAMACHA® Certification program which includes the use of online training videos and assessment as well as stakeholder production of a video to demonstrate proper FAMACHA® technique.

Since March 2016, participants from 48 states and Canadian provinces have completed this program. Farmers and professionals from 14 countries and regions outside the U.S. have also requested participation. Since its inception, the program website has been visited by new users from 148 countries (89% from the U.S. and Canada).

POSTER PRESENTATIONS: working group 3

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A comparison of two different anthelmintic treatment regimens against natural gastrointestinal nematode infections on two Lithuanian sheep farms

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Reasons for applying targeted treatment (TT) and targeted selective treatment (TST) approaches is to effectively control nematode-induced production impacts, while preserving anthelmintic efficacy by maintaining a pool of untreated parasites in refugia. According to TT, the whole flock is dewormed based on knowledge of the risk, or parameters that quantify the mean level of infection, whereas according to TST, only individual animals within the grazing group are treated, based on parasitological, production and/or morbidity parameters. The aim of this study was to compare two different treatment protocols on sheep farms in Lithuania. The study was conducted from April to October on three sheep farms. On the TT (the whole flock) and TST (with FECs ≥ 300 , respectively) farms all adult animals were treated orally with fenbendazole irrespective of EPG counts before the grazing season. The second treatment was applied with injectable ivermectin on both farms. However, on the TT farm all sheep were also treated on 2nd August regardless of their EPG counts, while on the TST farm only those animals with an EPG ≥ 300 were treated on 1 July using a threshold of ≥ 300 EPG. No treatments were administered on the control farm ($n = 1$) during the study.

Spring treatment of ewes significantly reduced nematode faecal egg counts (FEC) both on the TT and TST farms, with the benefit of lowering pasture contamination with infective L3 stage larvae at the start of grazing season, while it remained significantly higher on the control farm. The positive effect of the spring treatment of ewes was reflected by increased body weight gains (BWG) in lambs in the first half of the grazing season. Following the second treatment, the weight gains in lambs on the TST farm were higher compared to lambs on the TT farm, while BWG in the control lambs started to decrease. The difference was also substantiated by the body condition scores and dag scores of lambs, which were highest on the TST farm compared with those on the control and TT farms.

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The rational simplification of a recombinant cocktail vaccine to control the parasitic nematode *Teladorsagia circumcincta*

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Using data from five independent vaccine trials, which employed a subunit cocktail vaccine containing eight recombinant proteins to protect sheep against *Teladorsagia circumcincta*, a strategy was developed to simplify antigen complexity of the vaccine. Across trials there was a correlation between percentage reduction in cumulative faecal egg count (FEC) and sera IgG antibody avidity to vaccine antigen *T. circumcincta* apyrase-1 (Tci-APY-1). In addition, IgG and IgA within the serum and abomasal mucus of control (parasite challenged) lambs strongly recognised Tci-APY-1 and *T. circumcincta* metalloproteinase-1 (Tci-MEP-1) but only weakly bound the other six antigens, indicating Tci-APY-1 and Tci-MEP-1 are most effectively recognised by the parasite-induced antibody response. On the basis of these findings, a two-protein vaccine comprising Tci-APY-1 and Tci-MEP-1 was tested in a direct comparison with the original eight-component vaccine. A further group was immunised with Tci-MEP-1 in combination with a mutated form of Tci-APY-1 (mTci-APY-1), which had no enzymatic activity. Across the trial, the mean FEC levels of the eight-antigen recipients were lower than those of the adjuvant only control group and the mean FEC of the mTci-APY-1 and Tci-MEP-1 recipients was lower, although not statistically significantly, than that of the adjuvant-only control group. Mean cumulative FEC levels were reduced by 43% in lambs immunised with mTci-APY-1 plus Tci-MEP-1 compared with the controls.

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A combined, automated high-throughput system for the quantification of the viability of the free-living nematode *Caenorhabditis elegans*

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Resistance to anthelmintics is a growing problem in livestock industry. Few pathogenic nematodes are available to perform *in vitro* assays as an indicator of viability for anthelmintic drug discovery. The free-living nematode *Caenorhabditis elegans* is used as a model because it can be cultured in the absence of a host through their whole lifecycle.

A new *in vitro* assay to show the worm's viability using synchronized fourth-stage larvae (L4) *C. elegans* is based on combining larval migration inhibition (LMI) and XTT assays. The LMI assay consists of a migration system which enables the differentiation of mobile from non-mobile larvae through the use of sieves. The XTT viability assay is based on the absorbance of the activity of mitochondria enzymes in living worms which is readily reduced to a highly water-soluble orange colored product. The transgenic *C. elegans* strain VP596 carries two fluorescent constructs to monitor paralysis (*dop-3::RFP*) and to assess oxidative stress (*gst-4::GFP*).

With this combined method, we distinguish non-paralyzed living worms, paralyzed living worms and dead worms. We also assess the GFP/RFP ratio by fluorescence in 384 well-plates to show death mechanism through *dop-3* and/or *gst-4*. The proposed assay was evaluated by studying toxicity of several well-known anthelmintics and proved to be linear in the range 0-100% using mixtures of populations of killed and living L4 *C. elegans*.

This approach allows for sensitive measurement of worm viability in an easy, economical, reproducible and no time-consuming manner.

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Evaluation of the Nutritional Value and Bioactivity of *Lespedeza* Accessions from a Germplasm Collection

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Interest in the tannin-containing legume sericea lespedeza (*Lespedeza cuneata*) as a nutraceutical forage for livestock has been increasing in the United States, particularly with 'AUGrazer', a grazing-tolerant type with well-documented anti-parasitic activity. Other *Lespedeza* species/accessions have not been adequately evaluated. A study was completed to determine nutritional and bioactive potential of *Lespedeza* from a germplasm collection from the USDA Plant Genetic Resources Conservation Unit in Griffin, GA. Seventy accessions of 20 *Lespedeza* species were planted in small plots at Fort Valley State University in Fort Valley, GA on June 22, 2016 after two-leaf germination was achieved in the greenhouse. After establishment, forage was harvested in July, 2017, and June, 2018, freeze-dried, ground, and analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF), *in vitro* true digestibility (IVTD), protein precipitable phenolics (PPP), total phenolics (TP), and protein bound (PB) to PPP. *Lespedeza cuneata* cultivars established and grew better in the field than other species in this study. AUGrazer, the *L. cuneata* cultivar most commonly used as a pasture and hay crop in the United States, had NDF, ADF, and IVTD values of 33.0%, 23.3%, and 65.7%, respectively, and PPP, TP, and PB values of 85.4 mg/g, 105.3 mg/g, and 1.1 mg/g, respectively. Several other *Lespedeza* species/accessions, including several native species, had similar or greater nutritional and bioactivity values as AUGrazer, suggesting good nutraceutical forage potential. Data from this study can be used by plant breeders to develop superior nutraceutical forages to support livestock nutrition and health in the future.

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Can use of medicinal plants in lambs' nutrition influence their parasitological status?

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Medicinal plants and their using in the prevention and treatment of gastro-intestinal parasitism of sheep has origin in traditional veterinary medicine. This study assessed the efficacy of four medicinal plant mixtures (MPM₁, MPM₂, MPM₃, MPM₄) against experimental infections with *H. contortus* in lambs. MPM 1-4 contained selected medicinal plants common in Central Europe. Ultra-performance liquid chromatography and tandem mass spectroscopy was used for quantifying main bioactive compound groups in MPMs. During the three years experiment the female lambs 3-4 months of age were infected orally with approximately 5000 L₃ larvae of a strain of *H. contortus*. Eggs per gram (EPG) of faeces were quantified during the 70 d post-infection period and mean of abomasal worm counts were assessed. The mean EPG counts were significantly lower between 30 and 60 d post-infection from 45% to 60% for the infected lambs receiving MPMs. Mean worm counts were significantly lower in MPM supplemented groups. Mean cumulative gain in live weight of lambs was higher only when MPM₁ was used as diet supplement ($P < 0.01$). We can conclude that a direct anthelmintic impact was not sufficient for the elimination of *H. contortus*. However, the MPM contributed to slowing the dynamics of infection and likely indirectly contributed to the increase in the resistance of the lambs to nematode infection. This study was supported by funds from the Slovak Research and Development Agency (APVV 14-0169) and VEGA 2/0099/19.

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Prevention of infection in grazing cattle by means of nutritional pellets containing parasiticide fungi

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Four groups of Friesian cattle naturally infected by trematodes and nematodes were maintained under a leaders/followers four-day rotation for 24 months. Commercial pellets were given to leaders (G-L1; milking cows) and followers (G-F1; heifers) in assay 1 (G-L1 and G-F1), and the leaders in assay 2 (G-L2; dried cows). Followers in assay 2 (G-F2) received pellets containing a blend of parasiticide fungi (3×10^5 spores of *Mucor circinelloides* and *Duddingtonia flagrans* / Kg pellet). Heifers were dewormed at the beginning of each assay, and cows at the starting of the drying period. Coprological analyses showed the anthelmintic treatment was successful. After 24 months, the values of *Calicophoron daubneyi* egg-output reduced by 5% in G-L1, 42% in G-F1, 83% in G-L2 and 100% in G-F2. The numbers of eggs of gastrointestinal nematodes dropped by 13%, 18%, 72% and 88%, respectively. Clinical signs concerning digestive system, respiratory apparatus and reproductive system, the skin or fur were not detected in the G-F2. Data obtained points that ingestion of spores of parasiticide fungi enhances the valuable effect of rotational grazing for diminishing the helminths egg-output counts, which contributes to reduce the risk of infection among grazing animals.

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High-throughput screening of anthelmintic drugs

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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. 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).

Status of the project.

Recently we were awarded a grant for screening 30.000 compounds and expect to start in the second half of 2019. 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.