JOINT COMBAR WG MEETING

ANTHELMINTIC RESISTANCE IN RUMINANTS: FROM RESEARCH TO RECOMMENDATIONS

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

Ana Afonso [1], Maria Salomé Gonçalves [2], Tiago Perloiro [3], Pedro Vieira [4], Inês Sarraguça [2], Pâmela Valente [2], Maria Alexandra Basso [2], Telmo Nunes [2], Catarina Oliveira [5], Jacinto Gomes [1,2], Ana Cristina Ferreira [1], Luís Telo da Gama [2], Andreia Amaral [2], Helga Waap [1,2]

Introduction

Targeted selective treatment (TST) using the FAMACHA© method was proposed as an alternative method for the management of GIN in small ruminants to slow down anthelmintic resistance.

The aim of the study: to evaluate the accuracy of the FAMACHA© method for TST in Black and White varieties of Merino sheep in the Alentejo region under field conditions.

Methods:

Study area-Alentejo region

Sampling: 09/2019 – 09/ 2020

Coproculture

Haemonchus L3 identification

Merino Black

n= 203 in 10 farms

Merino White

n=302 in 16 farms

PCV

Two thresholds: scores ≥ 3 and ≥4.

Clinical anemia: defined as PCV ≤27%.

Results:

- A significant correlation between FAMACHA scores and PCV was observed for both breeds (r_s=-0,262; p<0,05);
- PCV was significantly correlated with FEC (r_s=-0,167; p <0,05), but not with FAMACHA scores (r_s=-0,007; p>0,05);
- Sensitivity for detecting anaemic animals was better considering scores ≥3 (79.1% for White Merino and 71.4% for Black Merino sheep), but specificity was low (52.1% and 32.3%, respectively);
- Overall sensitivity and specificity in detecting animals needing anthelmintic treatment (defined as FEC ≥500EPG) was 57.1% and 40.8%, respectively;
- Within-herd prevalence of *Haemonchus* was 6.7-20% in 32% of farms but it was the less representative species.

Conclusion: The FAMACHA© method may not be suitable for TST in the Alentejo region due to an apparent low prevalence of *Haemonchus*

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

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INTRODUCTION

- Gastrointestinal parasitic nematode (GIN) infections cause significant economic losses in livestock and affect animal welfare *(Fitzpatrick et al, 2013; Charlier et al, 2014)*.
- Ivermectin (IVM), an anthelmintic from macrocyclic lactones family, is the most widely used today to treat these infections.
- Unfortunately, IVM-resistant parasites have appeared all over the world, compromising the success of GIN control *(Kaplan et al, 2012)*.
- In nematodes, resistance to IVM is polygenic and is associated with an increase in the expression of detoxification genes that contribute to the elimination of the drug *(Kotze et al, 2014; Ménez et al, 2016)*.
- The hypothesis is that worm are resistant because they have enhanced capacity to metabolize and export parental IVM and thus limit its access to the target in the worm.

OBJECTIVES

1) Localize fluorescent IVM in *Caenorhabditis elegans*.
2) Compare fluorescence intensity in sensitive and resistant strains.

MATERIALS ET METHODS

Caenorhabditis elegans strains

- IVM sensitive: N2 Bristol
- IVM resistant: IVR10

IVM-Fluo

- Fluorescent derivative of IVM

$\lambda_{excitation}$: 405 nm/ $\lambda_{emission}$: 415-478 nm

- Stable and non-toxic in *C. elegans* culture conditions

Worm incubation with IVM-Fluo

- Liquid culture S media
- Young Adults
- 96H incubation
- [IVM-Fluo]: 10µg/ml

Confocal imaging

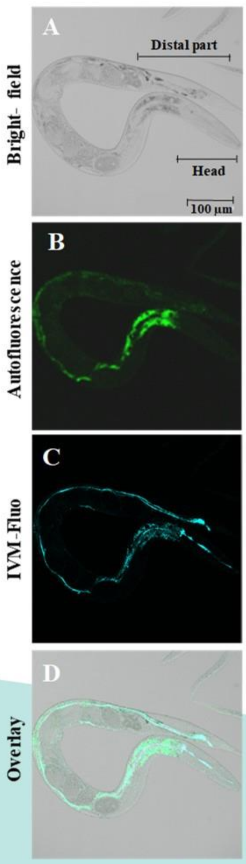
- 4H after washing IVM-Fluo
- Worms paralyzed by Levamisole
- 12-bit images

IVM-Fluo signal quantification with ImageJ

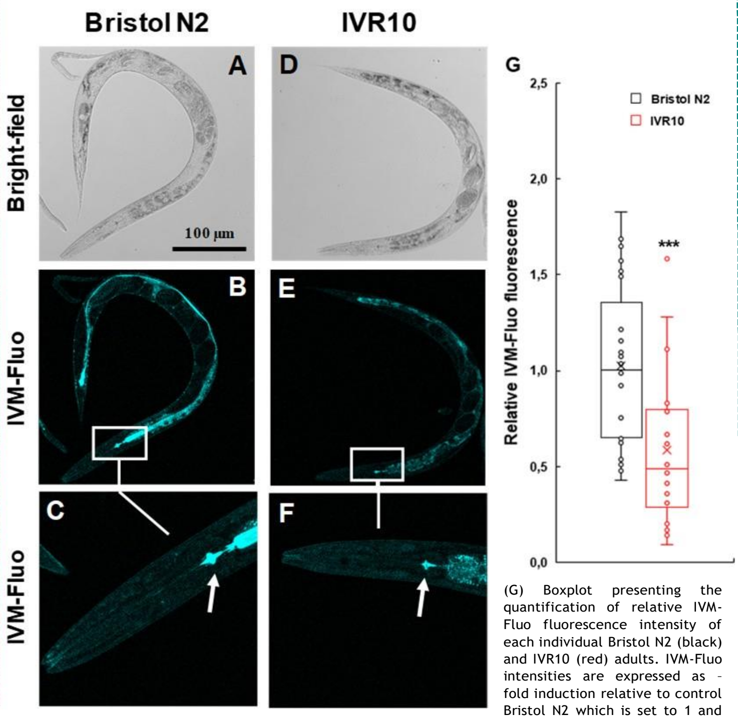
$$\frac{\text{IVM-Fluo Area}}{\text{Total worm area}}$$

RESULTS

1 In *C. elegans*, IVM-Fluo is mainly located in the pharynx, all along the digestive tract and in some spots of the distal parts.



2 IVM-Fluo intensity decreased in IVM-resistant strain IVR10, particularly in the pharynx when compared to parental N2 Bristol strain.



(G) Boxplot presenting the quantification of relative IVM-Fluo fluorescence intensity of each individual Bristol N2 (black) and IVR10 (red) adults. IVM-Fluo intensities are expressed as -fold induction relative to control Bristol N2 which is set to 1 and are reported as the mean ± S.D; n = 20-30 worms/group. *** p<0.001 vs wild-type Bristol N2.

CONCLUSIONS

- IVM-Fluo is a powerful probe, stable and suitable in *C. elegans*.
- IVM-Fluo is less accumulated in resistant strain, in line with the hypothesis of an enhanced efflux and/or metabolism.

PERSPECTIVES

- Investigate time-kinetic and stage dependence of IVM-Fluo distribution in sensitive and resistant worms.
- Study IVM-Fluo / Protein interactions using super-resolution microscope.
- Study the effect of important genes on macrocyclic lactone resistance (*P-gps, cyps, nhr-8, Dyf-7…*).
- Investigate IVM distribution in natural drug resistant parasitic nematode *(H. contortus…)*.
- Develop moxidectin and eprinomectin fluorescent probes.

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INTHERES

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

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INTRODUCTION

Gastrointestinal nematode infections are a constraint in meat and dairy herds and cause a decrease in animal health, productivity and farm profitability. In order to assess current deworming practices and improve our knowledge on anthelminthic resistance (AHR), a questionnaire was sent to 105 Livestock Producers Organizations (LPO). Our main goal was to do a preliminary assessment on the knowledge, attitudes and practices of small ruminant veterinarians concerning anthelmintic resistance.

MATERIAL AND METHODS

The questionnaire was done by e-mail and telephone and included fifteen questions, divided into three sections: 1) general information on sheep flocks, such as size, breeds and geographical location; 2) criteria underlining deworming practices like the kind of anthelminthic used and why, drenching guns, dosing measurement, quarantine strategies and pasture management; 3) general knowledge on anthelmintic resistance development.

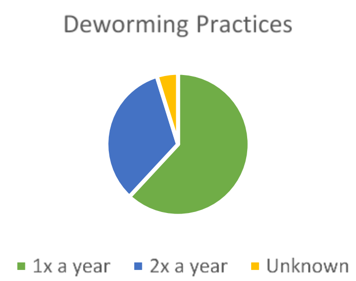


Fig. 1: Deworming Practices.

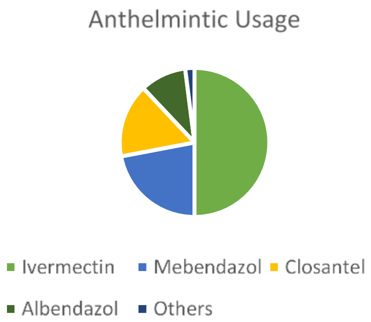
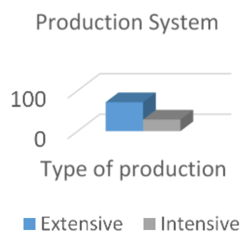


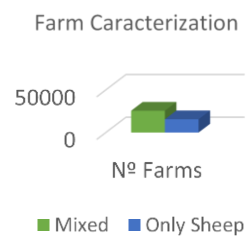
Fig. 2: Anthelmintic Usage.

Fig. 3: Regions covered by the participant LPO.

RESULTS

- 25524 production systems, where 15507 are both sheep and goat farms and 71,4% work on an extensive system;
- The mean number of sheep by farm is 39 and 61,9% of the farms produce both meat and milk;
- 61,9% of the LPO practice deworming once a year, 33,3% do it twice a year and 4,8% claim the veterinarian does not take part in this action (Fig.1);
- Ivermectin was the substance most frequently used - 71,4% of herds (Fig. 2);
- Dosage calculation per animal is made by estimating the average weight through visual observation in 2/3 of the farms;
- Pasture rotation was mentioned as the most common control method for helminth infections;
- More than half never had recommended a FECRT;
- Drenching guns are only calibrated daily on 28,6% of the cases;
- 57,1% of veterinarians say producers are not aware of the consequences of anthelmintic resistance.





DISCUSSION

Only 20% of the LPO fully replied to the questionnaire (Fig.3). The most critical points are the ones related to anthelmintic dosage and administration, specifically regarding the rough determination of weight and the lack of calibration of drenching guns, both essential factors for the deworming effectiveness. Quarantine of new animals is recommended as well as pasture rotation after deworming, although both measures are not usually applied by producers.

CONCLUSION

The absence of FECRT performance and monitoring of anthelmintic efficacy are frequent. These are key points that should be implemented as a regular habit. All veterinary practitioners surveyed said to be aware of anthelmintic resistance and almost half of them already doubted under some circumstances of anthelmintic efficacy.

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

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ABSTRACT

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

INTRODUCTION

The Genus *Salix* (Family Salicaceae) is a widespread herbal species in natural habitats, also cultivated in many countries worldwide. It has high rates of evapotranspiration, and high tolerance to saturated soils that lead to oxygen shortage in the root zone. The leaves and fine stems of willow (*Salix* spp.) are richer in nutritive values for grazing ruminants than low quality summer pasture. In addition to nutritious forage, the trees provide nutraceutical compounds such as anthelminthic, antioxidants, and analgesic secondary metabolites. Bioactive ingredients from plants can control parasitic nematodes in animals; however, the concentration of these bio-active compounds can be both seasonal, and species-dependent. Willow tree irrigated using secondary treated wastewater used as a source for goat fodder showed that willow biomass can serve as medium-quality forage for dairy goats. Specific secondary compounds such as phenolic and salicylic acid found in willow fodder may be of significant value in maintaining and improving the health and welfare of dairy goats

OBJECTIVES

The main aim is to evaluate the effects of irrigation type on seasonal variation of growth of different willow ecotypes, as well as their production of secondary metabolites such as phenols. The effects of leaves crude extracts on the exsheathment of L3 gastrointestinal nematodes inhibition as well as the minimum concentration with maximum biological activity of subsequent fractions were evaluated as well.

MATERIALS & METHODS

Three different willow ecotypes, which are also found as different genotypes, were collected from different regions of Israel and cultivated at Ramat Hanadiv Nature Park. The area is characterized by a Mediterranean climate with hot and dry summers and average annual rainfall of 570 mm. Seedlings of the 3 willow ecotypes were grown under ambient temperature in the open field for two years in 50 liters pots (one seedling per pot, with 6 replicates for each ecotype) filled with soil collected from Ramat Hanadiv. The plants were irrigated using two different water types (TWW or FW). The soil used in the experiment was a sandy silt soil with a high level of clay. Leaf biomass from each plant was harvested and weighed to determine total fresh weight. Dry matter (DM) weight of plant leaves was determined by drying at 50°C for 48 h in an air-forced oven. In order to evaluate quantity and quality of secondary metabolites in willow leaves, 70% ethanol extract were prepared using dry leaves from the different ecotypes. Total phenolic acids content in plant extracts were evaluated and calculated as equivalent of Gallic acid or Quebracho according to the Folin-Ciocalteu method. The effect of plant extracts from 3 different willow ecotypes irrigated with either FW or TWW were tested on the exsheathment process using the LEIA assay on L3 of the nematode *Trichostrongylus colubriformis*.

RESULTS

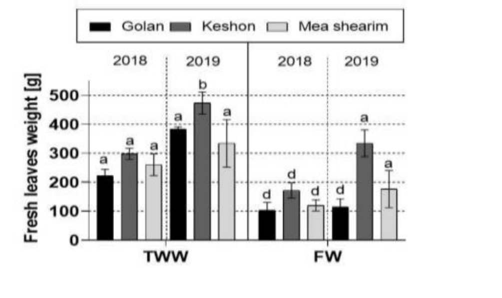


Figure 1: Fresh leaves weight [g] of 3 willow ecotypes irrigated with fresh tap water (FW) compared with treated wastewater (TWW) collected during July or October 2018 (Harvest 1, 2) and October 2019 (Harvest 3).

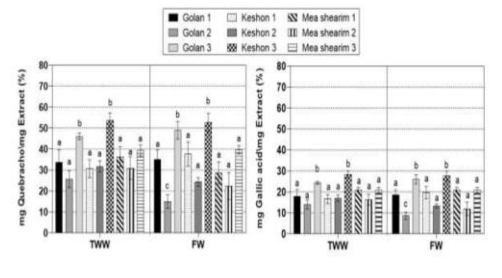


Figure 2: Phenol content as mg Quebracho (left) or mg Gallic acid (right) or as per mg plant crude extract of 3 willow ecotypes irrigated with fresh tap water (FW) compared with treated wastewater (TWW) collected during July, or October 2018 (Harvest 1,2) and June 2019 (Harvest 3).

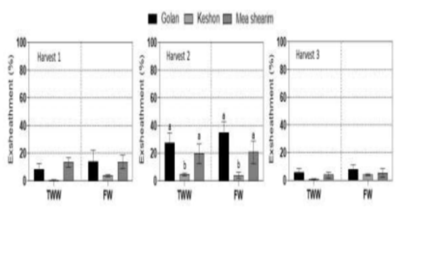


Figure 3: Effect of extracts of 3 different willow ecotypes collected during 2018 (July and October) and June 2019, termed "harvest 1", "harvest 2" and "harvest 3" respectively at 1200 ppm on the exsheathment of parasitic nematodes process during 60 min incubation compared to control.

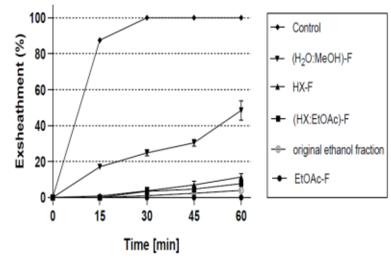


Figure 4: Effect of different fractions of the ethanol fraction of the Kishon willow ecotype at 1200 ppm on the exsheathment process during 60 min incubation compared to the control and the original ethanol crude fraction. Mean of 3 replicates ± SE. water:Methanol Fraction (H_2O:MeOH-F); Hexane Fraction (HX-F); Hexane:Ethyl Acetate Fraction (HX: EtOAc-F); Ethyl Acetate Fraction (EtOAc-F).

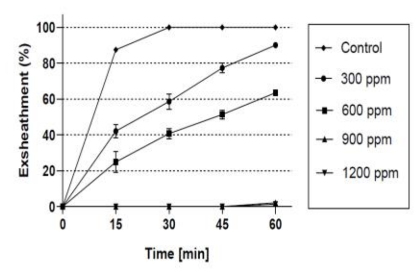


Figure 5: Effect of different concentrations (300, 600, 900, and 1200 ppm) of ethyl acetate fraction from Kishon ecotype compared to control on the exsheathment process during 60 min of incubation.

CONCLUSIONS

*** Irrigating of *Salix* with secondary treated wastewater doubled willow biomass yields**

**** Willow ecotypes contents of phenols and flavonoids depend on the cultivated ecotype**

***** Crude ethanol extract of Keshon ecotype resulted in ≥ 95% exsheathment inhibition**

****** Sub-fraction of ethyl acetate of Keshon ecotype showed ≥ 90% exsheathment inhibition**.

ACKNOWLEDGEMENS

We thank the Middle East Regional Cooperation (MERC) Program framework fund for support, Project #33-018.

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

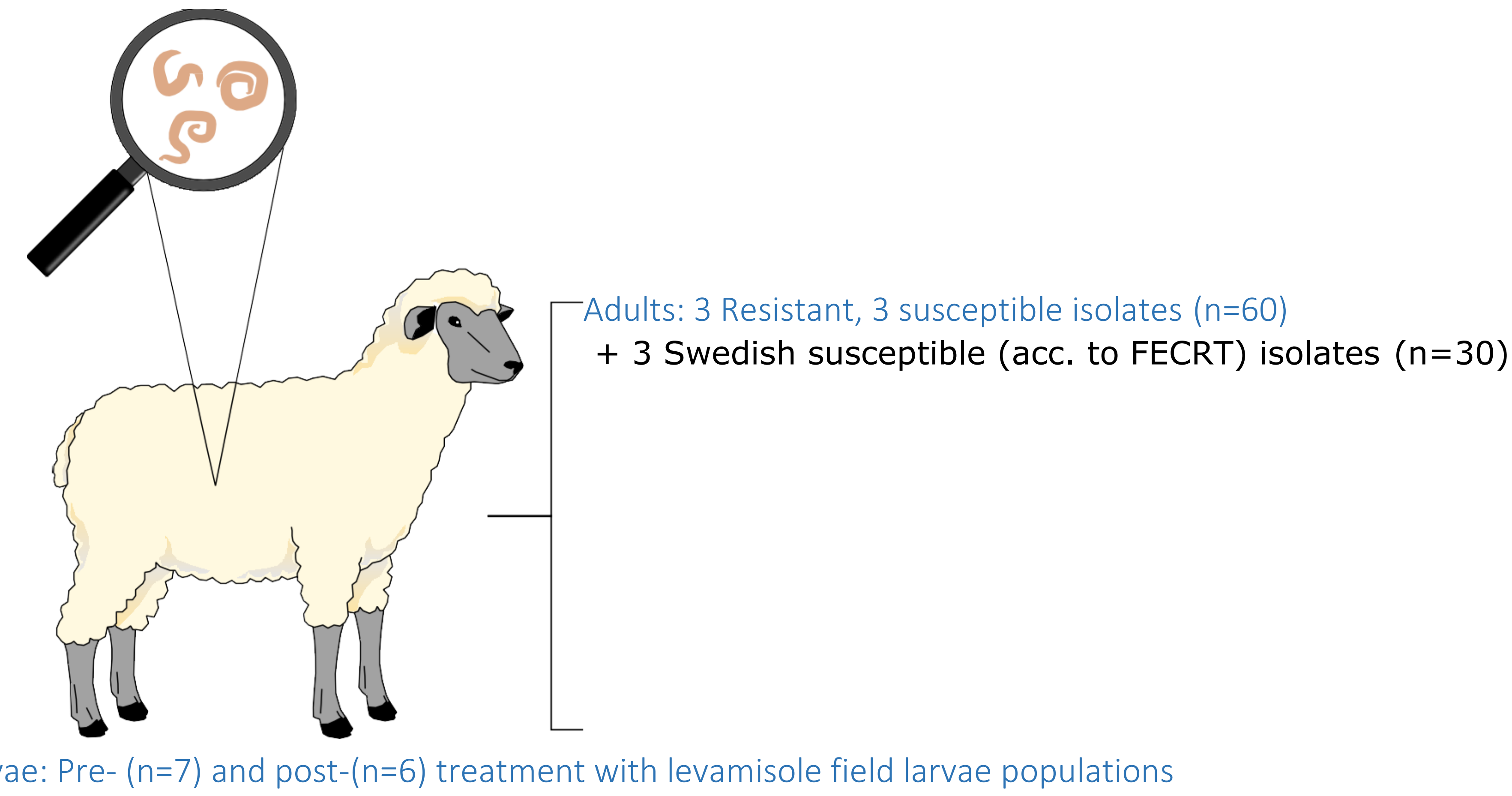
Paulius Baltrušis, C. Charvet, Peter Halvarsson, Johan Höglund

1. Introduction and aims

The nematode *Haemonchus contortus* is one of the most prevalent and pathogenic parasites in small ruminants, usually controlled using anthelmintics.
Increasing treatment costs and deteriorating farm animal health are both direct consequences of the rise in anthelmintic resistance in this worm. To reduce the spread and progression of anthelmintic resistance development, up-to-date molecular screening techniques need to be proposed.

Here, we describe a series of experiments evaluating the suitability of ddPCR to assess the 63 bp deletion in the *hco-acr-8* (= *hco-acr-8b*) as a marker for the levamisole resistance diagnosis in *H.contortus*.

Figure 1. *H.contortus* adult-stage and larvae populations studied by sequencing, conventional and ddPCR



2. Results

Adult *H. contortus*:

Significant difference (p=0.01) between the susceptible (S1-S3) and resistant (R1-R3) isolate categories in terms of the genotype frequencies was found (Figure 2; row 1 and 2).

However, homozygous for the deletion (i.e. RR) genotype was the most common, even in isolates phenotypically susceptible to levamisole.

Swedish adult *H. contortus*, derived from farms where levamisole is effective, varied genotypically from mostly homozygous for the deletion (Farm A 2018) to mostly heterozygous (Farm B) to a mix of different genotypes (Farm A 2020) (Figure 2; row 3).

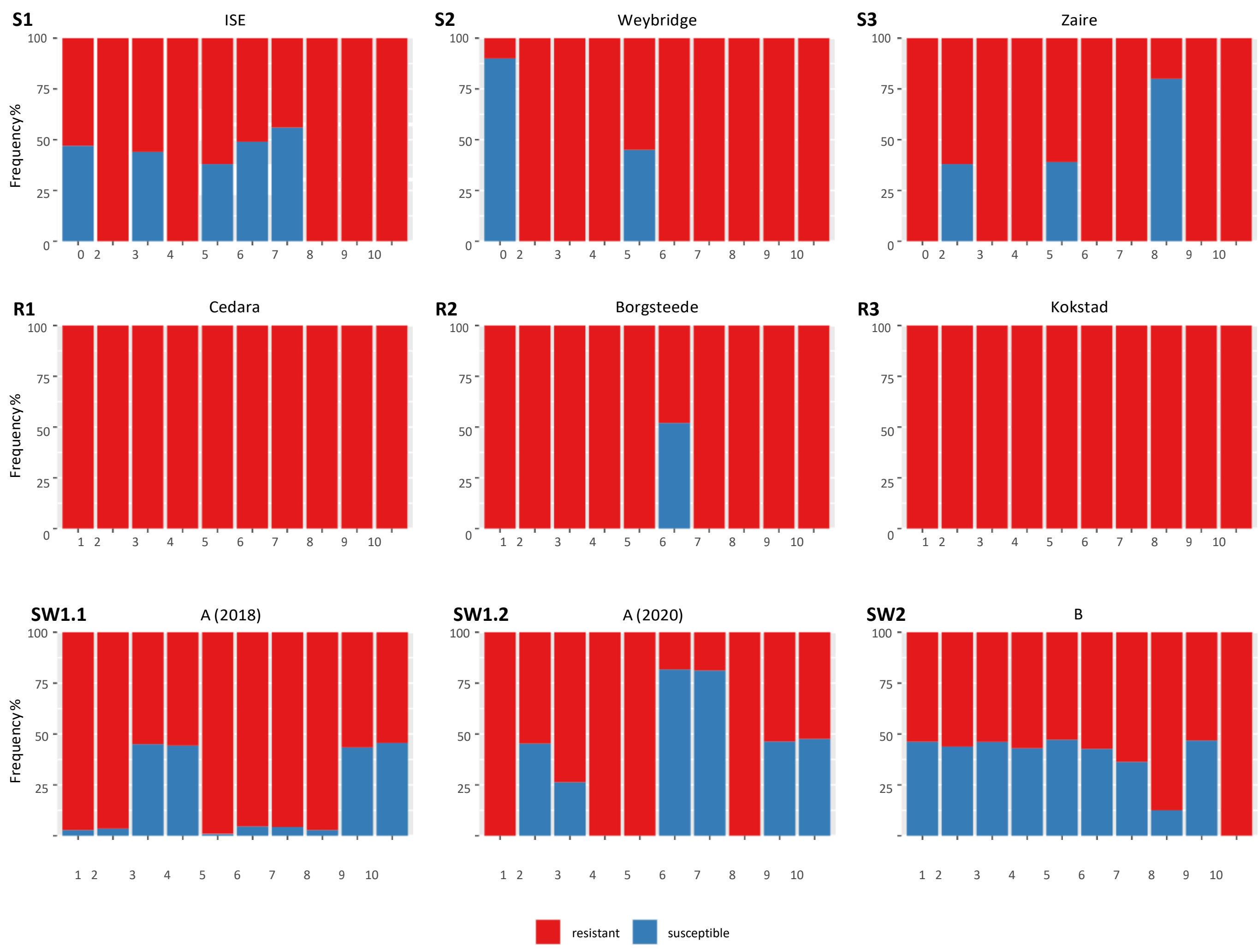


Figure 2. The frequencies (%) of "susceptible" (full- length intronic 2 sequence; in blue) and "resistant" (63 bp deletion in the intron 2 in the hco-acr-8; in red) alleles in various individual, adult H. contortus isolates (Susceptible: S1-S3; Resistant: R1-R3 as well as Swedish susceptible: SW1.1, SW1.2 and SW2)

Larval culture populations:

The 63bp deletion was detected in all tested, pre- treatment larvae culture samples at ratios ranging
35-80%, however all post-treatment samples contained no trace of *H. contortus* amplicon DNA (Figure 3).

The fecal egg counts in the pre-treatment samples varied between 195±152 and 6340±4510 epg, whereas all post-treatment samples were negative (Table 1).

Figure 3. The frequencies (%) of "susceptible" and "resistant" alleles in larvae cultures, recovered from different farms (A, B, C, D, E, F, G*) pre- and post-treatment with levamisole

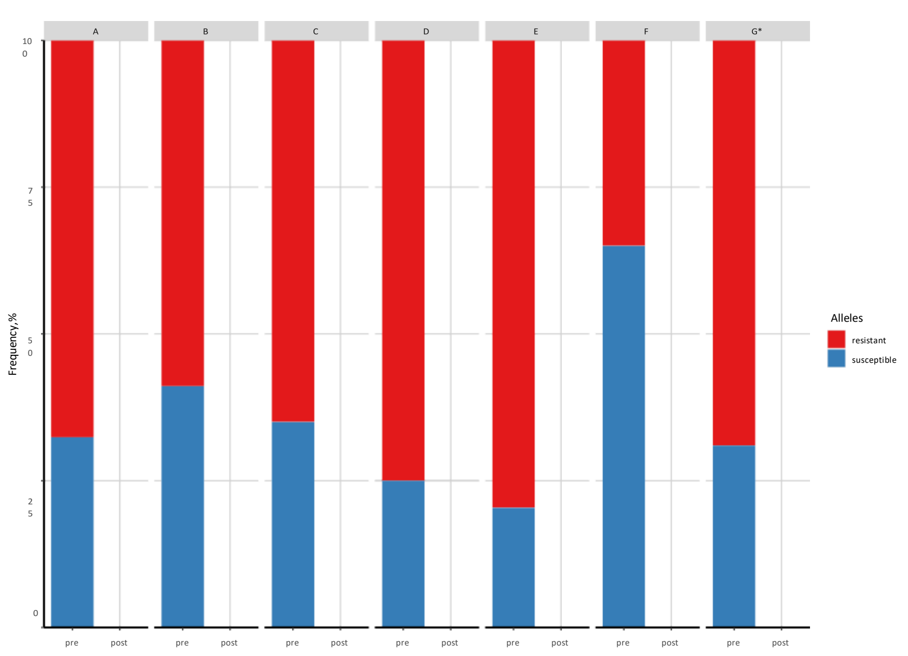


Table 1. Strongylid eggs per gram of feces counts obtained from sheep (n=10-15) on farms (A-G*), from which the before and after (7-10 days) levamisole treatment, H. contortus field larvae populations were derived

Farm	Average pre-treatment EPG count	Standard deviation	Average post-treatment EPG count	Efficacy of treatment (%)
A	6340	4510	0	100
B	5356	3787	0	100
C	394	308	0	100
D	6340	4510	0	100
E	419	827	0	100
F	195	152	0	100
G*	292	228	NOTTESTED	-

3. Conclusions

1 The 63bp deletion in *hco-acr-8* cannot always be associated with levamisole resistance in *H. contortus*

2 Robustness and precision of PCR-based assays rely on the homogeneity in the primer- binding sites, thus, making changes in intronic regions generally unattractive as resistance markers

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THE PREVALENCE OF ANTHELMINTIC RESISTANCE ON LITHUANIAN SHEEP FARMS

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INTRODUCTION

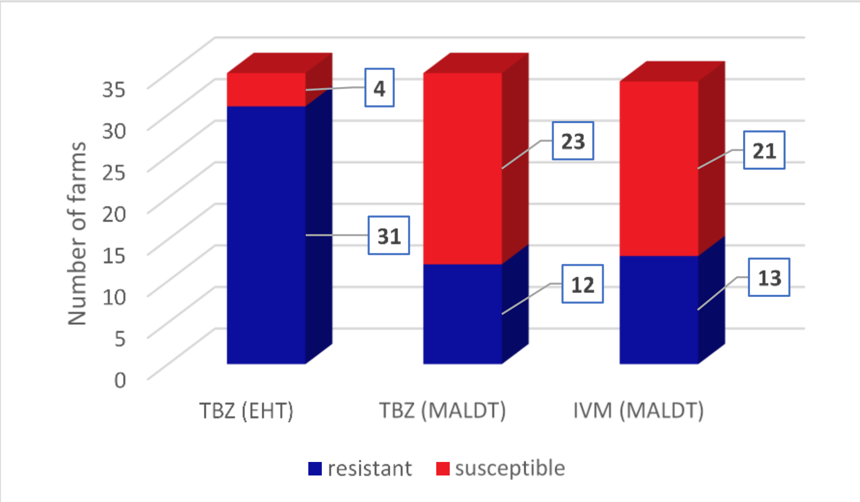
Anthelmintic resistance (AR) of gastrointestinal nematodes (GIN) of sheeps is the main problem worldwide, including Lithuania, where the number of sheep was increasing the last 15 years. The intensive and incorrect use of anthelmintics led to the development of AR. The aim of the study was to evaluate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

MATERIALS AND METHODS

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

RESULTS

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



DISCUSSION AND CONCLUSION

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

LITHUANIAN UNIVERSITY OF HEALTH SCIENCES

COMBAR combatting anthelmintic resistance in ruminants

University of Copenhagen
Faculty of Health and Medical Sciences
Department of Veterinary and Animal Sciences

The effects of seaweed (*Saccharina latissima*) in feed on nematode burdens in pigs

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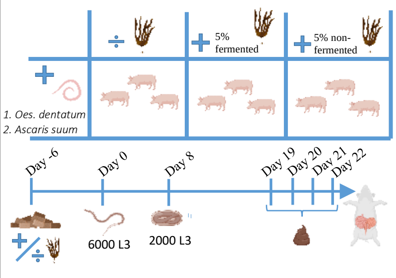
Introduction

Seaweed contains an abundance of bioactive compounds, and some seaweed species have been used as natural deworming agents for centuries in traditional Chinese medicine (1). Fermentation is believed to, amongst others, increase digestibility of feed components and enhance the bioactivity of several herbal medicines (2). Previous studies on extracts from Nordic seaweeds in our group have shown promising effects against *Ascaris suum* larvae (L3) *in vitro*. Our current aim was thus to examine if this could translate into similar effects in *vivo*.

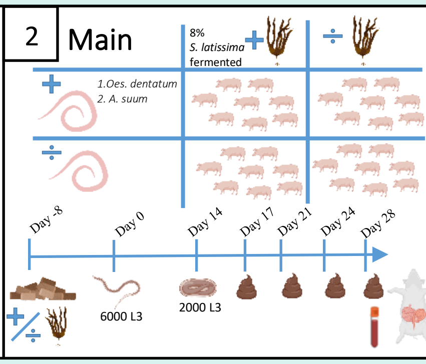
Material and Methods

1 Pilot



Design
- 1x3 study (n=3) all infected
- Inclusion of diet (-/+ of fermented or non-fermented seaweed) on day -6 p.i.
- Infection with *Oesophagostomum dentatum* L3 on day 0 p.i.
- Infection with *Ascaris suum* on day 8 p.i.
- Collection of feces for egg excretion on day; 19,20,21,22 p.i.
- Necropsy of pigs and harvest of intestinal worms on day 22 p.i.

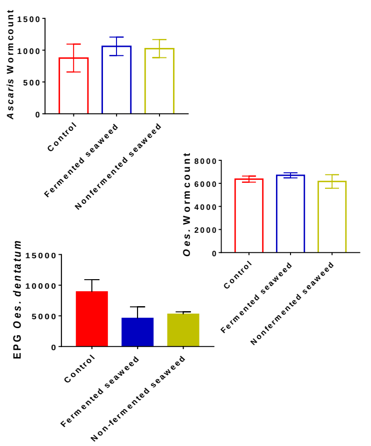
2 Main



Design
- 2x2 study (n=8)
- Inclusion of diet (+/- fermented seaweed) on day -8 p.i.
- Infection with *Oesophagostomum dentatum* on day 0 p.i.
- Infection with *Ascaris suum* on day 14 p.i.
- Collection of feces for egg excretion on day; 17,21,24,28 p.i.
- Necropsy of pigs and harvest of intestinal worms on day 28 p.i.

Results

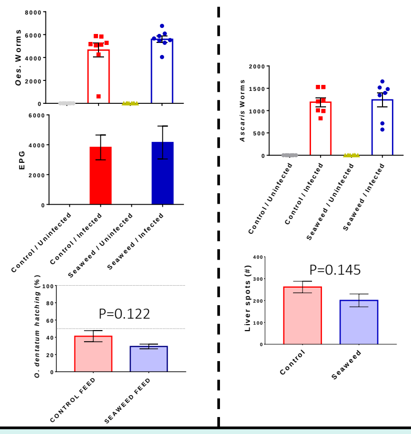
1 Results from pilot



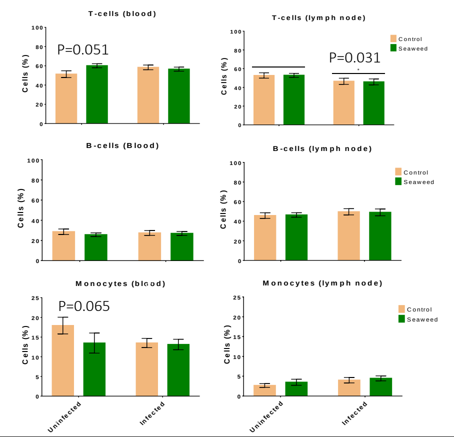
2 Results from main study

Oes. dentatum

Ascaris suum



Flow cytometry



Conclusion

- We found no significant differences in *A. suum* and *O. dentatum* worm counts or related parameters. However, *S. latissima*-fed groups had a:
  - reduction in faecal egg counts (*O. dentatum*) of ~40% (only Study 1) in both fermented and non-fermented seaweed fed pigs
  - reduction (16%) in hatching percentages in larval culture (*O. dentatum*)
  - fewer liver milk spots (-23%)
- Seaweed feed inclusion does not seem to alter immune response to parasites in pigs in short term feeding studies, however we did observe:
  - increase in T-cells and decrease in monocytes in blood (P>0.05) in control fed vs. seaweed fed uninfected pigs

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gudp

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

Introduction: With intensive use of anthelmintic drugs over the past decades, anthelmintic resistance (AR) in horse nematodes is becoming a growing issue in many countries. However, little information is available about the parasites, treatment practices or AR in the horse population in Lithuania. The aim of this study was to assess the current situation of AR on horse farms in Lithuania.

Materials and Methods: The aim of this study was to assess the current situation of AR on horse farms in Lithuania. The study was conducted in 25 stables. On each farm a faecal egg count reduction test (FECRT) was performed after deworming tests with ivermectin (IVM), (Bimectin 0,2 mg/kg, Ireland) and pyrantel (PYR), (Embotape 19 mg/kg, Ireland).

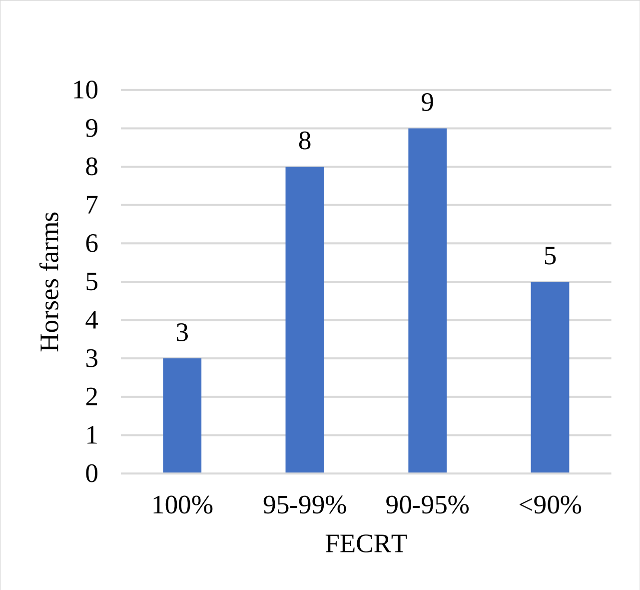
Results and Discussion:

This study showed that strongyle infections were highly prevalent in horses residing in stud farms in Lithuania, with high infection intensities according to an over dispersed distribution pattern. Of all tested subjects (n=707) 93% exceeded the value of 200 strongyle EPG, which is considered the cut-off limit with in the new equine strongyle control strategy based on targeted selective treatment.

This study demonstrated a very high efficacy of IVM on Lithuanian horse farms. As expected, the mean percentage efficacy of IVM in eliminating strongyle eggs from animals faeces ranged from 99.7% to 100% for IVM in all stud farms. On three out of 25 farms, each individual horse had a FEC of 20 EPG on day 14. The observed FECR on these three farms were with 99.9%, 99,9% and 99.7%, respectively, highly indicative for susceptibility. For the FECRT with PYR, horses were divided into treatment (n=250) and control (n=156) equines. On three out of 25 farms (12%) the FECR was 100%. On eight farms (32%), the FECR was between 95 and 99%, and nine farms (36%) had a FECR between 90 and 95%. On five farms (20%), FECR was <90% (Figure 1). PYR treated horses also shown significantly (P<0.01) lower egg output than untreated groups after treatment.

All third stage larvae derived from strongyle egg positive faecal samples, which were collected after IVM/PYR treatment and could be designated to species by morphological criteria were cyathostomin larvae.

Figure 1. Faecal egg count reduction test with pyrantel embonate.



Conclusions: This in vivo study showed that horse farms in Lithuania already have problems with AR and the main growing problem, regarding our study, is resistance to pyrantel.

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

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Introduction

Gastrointestinal nematodes such as *Haemonchus* spp., *Cooperia* spp., *Trichostrongylus* spp., *Teladorsagia* spp., *Ostertagia* spp. and others often appear in mixed infections. They cause a poor general health condition in ruminants with weight-loss, diarrhoea and anemia as main symptoms.

In Europe, anthelmintic resistance is increasingly recognized as a problem regarding animal welfare and economic production (Rose et al. 2015; Geurden et al. 2015). This project aims to monitor nematode prevalence and drug efficacy in the north-eastern area of Germany.

A faecal egg count reduction test (FECRT) has been conducted on 11 sheep farms using fenbendazole, ivermectin and moxidectin. The initial results, covering 10 of the 11 sheep farms, are presented here.

Methods

Study design:

- Farms were included if last anthelmintic treatment was at least 8 weeks ago and at least 20 lambs were available per treatment group
- If only 20 lambs were available, only moxidectin was used
- FECRT according to the instructions described in Kaplan (2020, Box 1)
- Animal weight was determined with a mobile scale or if existent, the farmers own scale
- Individual egg per gram (epg) counts were performed before and 14 days after treatment using Mini- FLOTAC
- Number of Mini-FLOTAC devices counted was adjusted according to the epg to have at least 200 raw egg counts (sensitivity of 2.5-5 epg)
- FECR with 95% CI calculated using R package eggCounts allowing individual efficacies (Wang et al., 2018)
- Results interpreted following the WAAVP guidelines (Coles et al. 1992; Levecke 2018)
- Pools of L1 were prepared for future nemabiome studies (Avramenko et al. 2019)
- Pools of L3 were purified from cultures for larval migration inhibition assays

Classification of nematode populations according to WAAVP guidelines:

- Resistance: FECR < 95% and 95% CI < 90%
- Suspected resistance: either FECR < 95% or CI < 90%
- Otherwise normal efficacy

Results

- Farms included meat, milk, wool production, landscape preservation, as well as hobby and sideline keepings with a wide range of sheep breeds: Suffolks, German black-headed meat sheep, East frisian milk sheeps, Skuddes, Landrace of Bentheim, Pomeranian coarsewool, Gotland sheep, Coburg fox sheep and Mules. Animal weights ranged from 3.7 to 75 kg.
- Results are shown in Figure 1 – 3 for the drugs fenbendazole, ivermectin and moxidectin, respectively.
- Reduced efficacy of fenbendazole and ivermectin was found on 6 of 8 farms tested while moxidectin resistance was encountered on 3 of 10 farms tested plus 1 farm with suspected resistance.

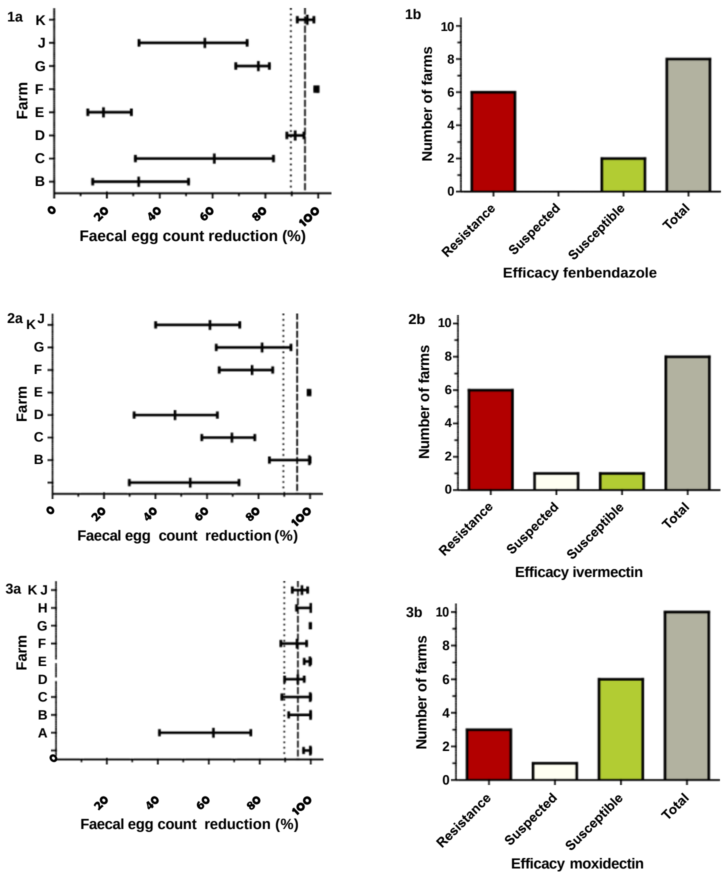

Fig.1a – 3a: Results of Faecel egg count reduction in percent (%) with Certainty intervals (CI), 1a) Fenbendazole, 2a) Ivermectin, 3a) Moxdectin results
Fig. 1b – 3b: Results of faecal egg count reduction in total numbers, 1b) Fenbendazole, 2b) Ivermectin, 3b) Moxdectin

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Summary & Outlook

- Offer a second faecel egg count testing with monepantel on farms where all tested drugs showed reduced efficacy
- FECRT on cattle using fenbendazole and ivermectin in spring 2021 applying FLOTAC with 1 epg sensitivity
- Questionnaire addressing farm veterinarians in Germany to identify complications in communication
- Larval migration inhibition assays using ivermectin, moxidectin and levamisole
- Determine nematode communities before and after using the nemabiome next-generation sequencing approach
- Quantify single-nucleotide polymorphisms in the isotype 1 β-tubulin gene associated with benzimidazole resistance on species level using deep amplicon sequencing
- Results of our research will contribute raising the awareness of an increasing anthelmintic resistance on farms and to transfer scientific findings into practice.

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

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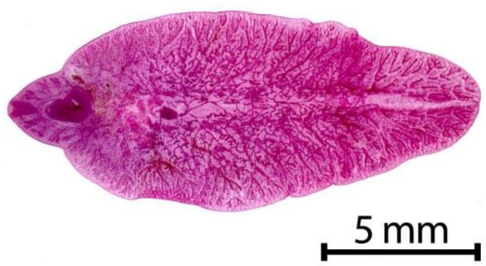
INTRODUCTION

Fasciola hepatica is a worldwide-distributed trematode parasitizing in the liver of mammals. The spatial distribution in Europe depends on the occurrence of the intermediate host *Galba truncatula*, a freshwater snail that commonly appears in wetlands close to coast regions.

Infections of livestock with *Fasciola hepatica* are of considerable economic significance causing financial losses of € 635 million per year in Europe due to lost production and treatment costs[1]. The drug of choice for the treatment of fasciolosis is triclabendazole (TCBZ), a halogenated benzimidazole that is effective against all fluke stages; however, the exact mode of action is still unclear. The widespread use of TCBZ for many years led to the development of resistant *Fasciola* populations. The first report of resistance was published in 1995 in Australia[2]. Since then, TCBZ-resistance has been reported in several countries all over the world during the last decades, but data concerning the susceptibility of German *Fasciola* populations are lacking.

Therefore, the aim of our study is to evaluate the efficacy of TCBZ against *F. hepatica* in German sheep flocks.

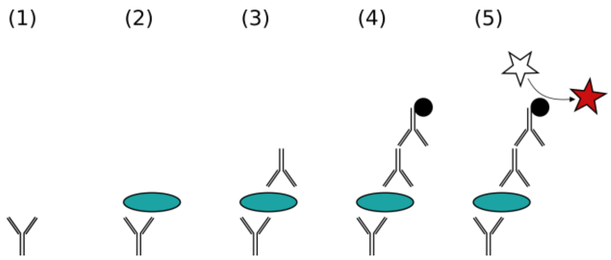


MATERIAL/METHODS

Faecal samples will be collected from 10-15 sheep farms in the north German state Lower Saxony. On each farm, 20-30 sheep will be individually sampled and treated with TCBZ subsequently. The collection of the second set of samples will be conducted 14 days after treatment. To compare the fluke burden pre- and post-treatment, we will use two methods to evaluate the efficacy of TCBZ.

Firstly, we will perform a faecal egg count reduction test (FECRT) using a composite sedimentation method that has been validated at the University of Liverpool[3]. If there is a faecal egg count reduction of at least 95% at 14 days post-treatment, we define that *F. hepatica*-isolate as TCBZ-susceptible.

Secondly, we will conduct a coproantigen reduction test (CRT) with the commercial BIO K 201-ELISA kit (Bio-X Diagnostics S.A., Belgium). This ELISA is based on the detection of *F. hepatica*-antigens in faecal material during the infection and is able to detect an infection after 7-9 weeks p.i[4]. We define the *F. hepatica*-isolate as TCBZ-susceptible, if no coproantigens are detectable 14 days post treatment.



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As the resistance mechanisms are not revealed yet, we will search for resistance markers by Next Generation Sequencing (NGS) to identify possible regions for resistance genes in the genome of TCBZ-resistant *F. hepatica*-isolates from our field trials.

Furthermore, we want to establish the Fasciola Egg Hatch Test (FEHT) in order to assess how albendazole (ABZ) affects the egg hatch rates in German *Fasciola*-isolates as this test does not work with the highly lipophilic TCBZ. For testing the ovicidal activity of ABZ, we will isolate *Fasciola*-eggs from our field samples and incubate them for 12 hours in different concentrations of ABZ. After another incubation period for 15 days, the ratio between hatched and non-hatched eggs will be determined. In general, the FEHT is a simple and low-cost in vitro-method to determine lower ovicidal activity in ABZ- resistant isolates.

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THE KUBIC FLOTAC MICROSCOPE: A NEW TOOL FOR FAECAL EGG COUNT OF GASTROINTESTINAL NEMATODES

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THE KFM

In this study we performed a first validation of the Kubic FLOTAC Microscope (KFM) on faecal egg count (FEC) of gastrointestinal nematodes (GINs) in cattle. The KFM is a compact, low-cost and portable digital microscope designed to analyse faecal specimens prepared with the Mini-FLOTAC/FLOTAC in both field and laboratory settings (Fig.1). The KFM can be remotely controlled via software by an external device, i.e. smartphone, tablet or PC (Fig.2) to perform the visual identification and counting of parasitic structures (Fig.3).

Figure 1. The Kubic FLOTAC Microscope (KFM) used with the Mini-FLOTAC (A) and FLOTAC devices (B).

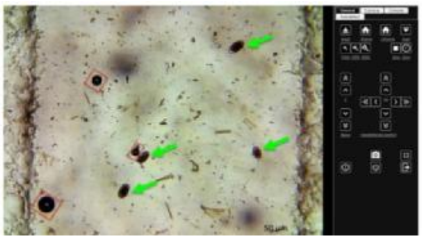

Figure 2. An image of a part of a Mini-FLOTAC chamber, captured by PC connected with the microscope that shows GIN eggs (green arrows) and air bubbles (red sa uares).

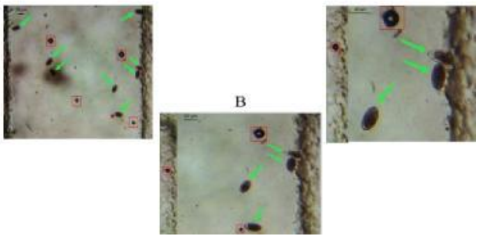

Figure 3. Digital imaging of GIN eggs (green arrow) and air bubbles (red square) using the KFM with a digital zoom 100X (A) 200X (B) and 300X (C).

MATERIALS AND METHODS

Thirty faecal samples were collected from cattle experimentally infected by GINs and analysed at the Faculty of Veterinary Medicine, Ghent University, Belgium, using the Mini-FLOTAC technique (Cringoli et al., 2017). Then, a comparison was performed between a traditional optical microscope (OM) and the KFM (Fig.4).

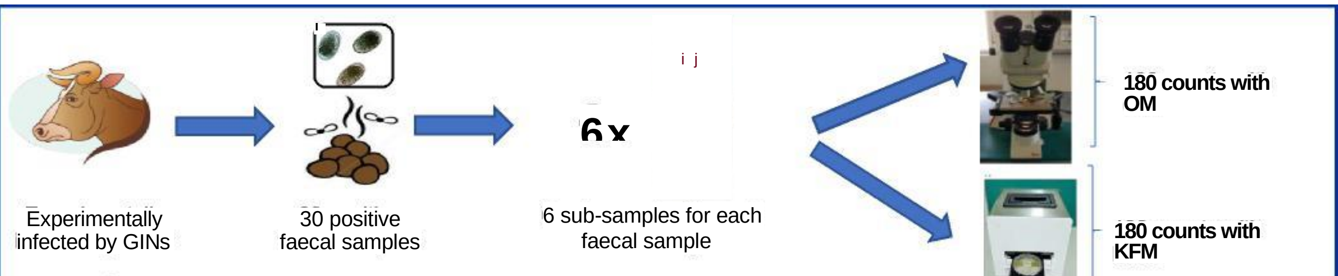

Figure 4. Study design

RESULTS AND DISCUSSION

The results showed a substantial agreement with a low discrepancy (-0.425 ± 7.370) between the GIN counts obtained (Fig.5). Moreover, captured images by KFM showed a quality comparable with the view provided by the OM. This is very important for the real-time identification, quantification of parasitic structures and for the Artificial Intelligence software development, that is actually in progress. Moreover, these pictures can be transferred via internet to other laboratories for a remote diagnosis as expected by Tele-Parasitology. Therefore, the KFM is a promising automated system for an accurate and efficient Faecal Egg Count to improve parasite diagnosis and to assist a new generation of operators in veterinary and public health.

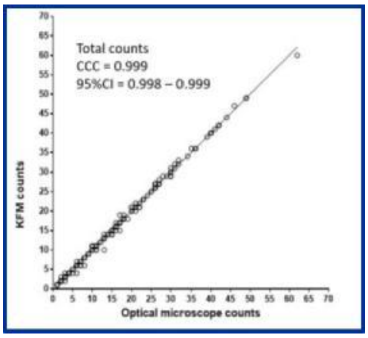

Figure 5. Study design

Reference- Cringoli G, Maurelli MP, Levecke B, Bosco A, Vercruysse J, Utzinger J and Rinaldi L (2017) The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. *Nature Protocols* 12, 1723-1732.

COMBAR WG MEETING
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COMBAR combatting anthelmintic resistance in ruminants
ANTHELMINTIC RESISTANCE IN RUMINANTS: FROM RESEARCH TO RECOMMENDATIONS

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

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INTRODUCTION

The gastrointestinal nematodes (GINs) affect the health and productivity in grazing livestock. The increasing development of resistance to anthelmintics (AH) has stimulated the search for alternatives to control GINs. The study of medicinal plants (MP) in recent decades has increased and with it, the desire to validate and preserve ancestral knowledge for future generations. The validation of AH effect using *invitro* test, is a preliminary option to evaluate the bioactive properties of MP. Within the Cyperaceae family the species *Kyllinga odorata Valh* (*Fig1*. A) was mentioned as multiple purposes in traditional medicine (Ricardo et al, 2017), the roots (*Fig1*.B) are used as AH treatment in humans in South America (Meza et al, 2019), but it is practically unknown in the scientific field. The distribution of this plant is worldwide (*Fig1*.C).

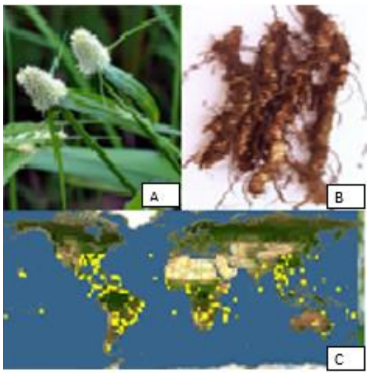

Fig1. K. odorata (A), Root of K. odorata (B), Distribution of K. odorata (C)

OBJECTIVES

To evaluate the AH effects of methanolic extracts (ME) from roots of *K. odorata Valh* on eggs and infective larvae (L3), and to determine the role of tannins/polyphenols in the AH effects.

MATERIALS AND METHODS

Faeces collected from small ruminants with monospecific infection of *H. contortus* and *T. colubriformis* were used to obtain Fresh eggs and L3.
Decreasing concentration of ME (1200 to 150 µg/ml) with 4 replicates each one, were used in EHA and LEIA (Jackson and Hoste, 2010, *Fig2*). The highest concentration of extract was used in another set with and without tannin inhibitor: polyvinylpolypyrrolidone (PVPP) in 1:50. The percentage of inhibition (%INH) and the effective concentration 50% (EC50) were calculated for both assays.

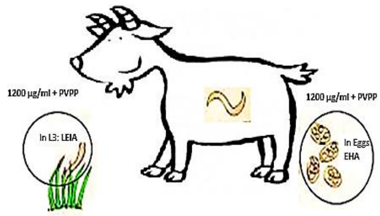

Fig.2. Invitro test used in L3 and Eggs

RESULTS

The percentage of inhibition %INH
- Exsheathment and Hatching process **+>75%** in both species at 1200 *µg/ml.*

EC50 values
- In LEIA were 565.06 µg/ml (Hc), and 610.61 µg/ml (Tc)
- In EHA were 176.99 µg/ml (Hc) and 143.82 µg/ml (Tc).

Results %INH with and without PVPP (Fig3).
- %INH in LEIA were 27 and 37 % *less effective* with the addition of PVPP, the polyphenols were mainly responsible for the AH effect in L3, in contrast %INH in EHA *was higher* with the addition of PVPP in both species, especially in Tc.

The addition of PVPP in EHA showed that polyphenols were not responsible for AH effect.

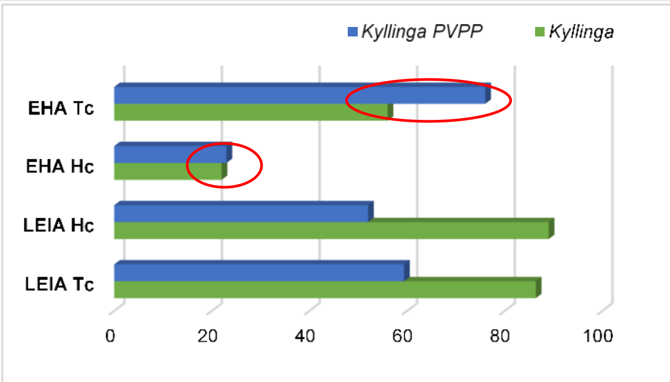

Fig 3. %INH of exsheathment and hatching process with and without PVPP

CONCLUSION

Potential use of *K. odorata* as an alternative to control GINs. The results suggest that tannins ***are not the solely class of metabolites*** responsible for the AH effects. **As far as we know**, it is the first study to evaluate the AH effect of this plant. The current results suggest potential for future use, but in *vivo* studies are needed.

ACKNOWLEDGMENTS

BECAS DON CARLOS ANTONIO LÓPEZ Excelencia es futuro
cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY
Funded by the Horizon 2020 Framework Programme of the European Union

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

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INTRODUCTION

Sheep farming is of great importance in generating income to smallholders in semi-arid areas worldwide. Gastro-intestinal helminths (Haemonchus etc.) cause significant damage to the animals with poor welfare conditions, impacting in local economies. Parasite resistance is common in the semiarid region of Paraiba (Silva el al. 2018). The **OBJECTIVE** of this work was to evaluate the FAMACHA method / FMC in a herd of 60 Santa Inês sheep from Paraíba, Brazil, and compare the data of previous production years.

MATERIAL AND METHODS

In order to detect anemic sheep, FAMACHA chart was used in the evaluations bi-weekly for 12 months by trained personnel. Blood and fecal samples were collected monthly to determine packed cell volume (PCV), and parasite fecal egg count/FEC respectively (Fig 1). The animals were divided into: dry, pregnant and lactating ewes, lambs and rams.

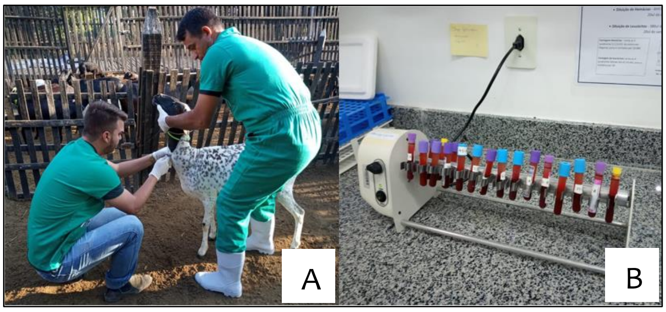

Fig. 1. A) Blood collection. B) Blood samples to obtain the PCV.

Management data were gathered from the last 5 years: 763 doses/year, 27% mortality, clinical signs during rainy period. Levamisole LEV, was used during the period with 100% effic.

RESULTS

Haemonchus was the predominant parasite (94.7%) (Table 1).

Genus	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
Haem.	86	96	93	92	96	95	94	99	92	95	91	89
Trich.	14	2	5	6	3	3	5	-	8	4	7	8
Oesop.	-	2	2	2	1	2	1	1	-	1	2	3

Haem: Haemonchus sp.; Trich: Trichostrongylus spp.; Oesop: Oesophagostomum sp.

Table 1. Helminth genera (%) observed in coprocultures of Santa Inês sheep, in the semi-arid region of Paraíba, Brazil.

There were up to 97% of success on the interpretation of FMC with 90 to 100% efficacy in the treatment decision. Among the 1356 evaluations, in only 40 (3%) were required deworming, being 27 during the rainy season and 13 during the long dry season (Fig. 2). A statistically significant correlation ($P < 0.01$) was observed between FEC and PCV for the dry, lactating, and pregnant categories.

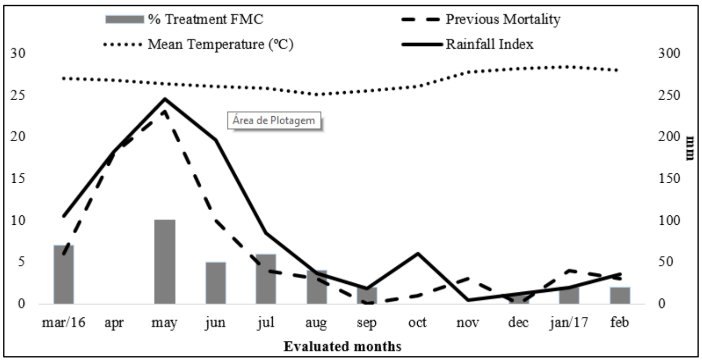

Fig. 2. Percentage of FMC treatment, previous mortality, mean temperature and rainfall index at the Sheep Unit Farm, semiarid of Paraíba, Brazil.

The highest FEC were only during the rainy period. The correctness of the conduct to use FMC reached 100%. This high rate was related to the method's ability to differentiate resilient from the more susceptible animals, when Haemonchus sp. is predominant. During the actual experimental period, we noted that the FMC method was able to identify the animals that needed deworming and, by using an effective drug, the death rate was reduce to zero in the evaluated herd (Fig. 3). LEV kept a high efficacy at 96%.

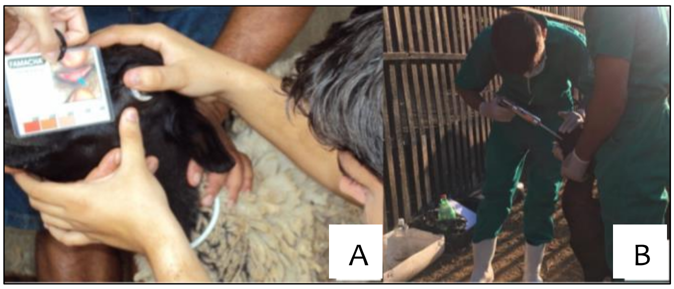

Fig. 3. A) Evaluation of the ocular conjunctiva of a lamb through the FMC. B) Animal deworming due to FMC (> 2).

CONCLUSION

The use of FMC and monitoring the performance data significantly reduced the need for treatments in animals of various categories, allowing sustainable farming in the semiarid region of Brazil, when comparing to previous years.

* Vilela, V. et al. Sustainable agriculture: Use of FAMACHA method in Santa Ines sheep in the Semi-arid region of Brazil. *Semina*. 2020.
Silva, FF. et al. Nematode resistance to five ath classes in sheep herds in Northeastern Brazil. *Braz J Vet Parasitol* 27, 423-429. 2018.

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COMBAR
combatting anthelmintic resistance in ruminants

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

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INTRODUCTION

Helminth control is performed in cattle two or three times a year, using mostly macrocyclic lactone products. In Brazil, cattle raising is divided in *Bos indicus* (Red Angus, Brangus, Charolais, Hereford), found mostly in the South and *B. taurus* (Nelore, Brahma, Gir, Guzera), distributed in the Central, South East and Northeast regions. Pure breed and their crosses are affected by multi-species parasite infections. Since early 2000', the first reports of multi-drug (macrolactones/Fig. 1, benzimidazols, levamisole), and multi-spp. (*Haematobia* sp., *Haemonchus* spp., *Cooperia* spp., *Dictyocaulus sp.*, *Rhipicephalus* sp.) resistance were published (Mello et al. 2006; Molento et al., 2006). The **OBJECTIVE** of this study was to evaluated the data from the literature regarding drug resistance in helminths of cattle (12 articles) and to correlate to parasite control practices based on a questionnaire given to 32 farmers.

MATERIAL AND METHODS

A search was made using SCOPUS and PubMed and the terms Brazil, cattle, parasite resistance, and anthelmintics. The articles were selected if using fecal egg count reduction test/FECRT or DNA based diagnostic with a clear scientific protocol. Some returns did not specify their methods or did not have sufficient statistical support and were removed.

Composto químico	Dia 0	Dia 7		Dia 14		Dia 21	
	OPG	OPG	% Red.	OPG	% Red.	OPG	% Red.
G1. Ivermectina 1%	433	467	0	425	2	350	19
G2. Ivermectina 1%	450	525	0	450	0	50	89
G3. Ivermectina 1%	1208	608	50	1300	0	850	30
G4. Ivermectina 1%	642	608	5	442	31	558	13
G5. Ivermectina 3,1%	865	515	40	395	57	631	27
G6. Abamectina 1%	317	392	0	375	0	508	0
G7. Abamectina 1%	425	270	36	442	0	967	0
G8. Ivermectina 2,25% e abamectina 1,25%	400	67	83	158	60	133	67
G9. Doramectina 1%	500	400	20	933	0	1167	0
G10. Moxidectina 1%	20	15	25	75	0	280	0

1. Ivermectinaâ (Ouro Fino); 2. Ivermectinaâ (Jofadel); 3. Imectinâ (Irfa); 4. Dectiverâ (Lapisa); 5. Ivomec Goldâ (Merial); 6. Abactinâ (Biofarm); 7. Abamectinaâ (Jofadel); 8. Solutionâ (Intervet); 9. Dectomaxâ (Pfizer); 10. Cydectin NFâ (Fort Dodge).

Figure 1. FECRT of macrolactones against nematodes of cattle.

A questionnaire was designed to determine the details regarding beef cattle management and parasite control. Thirty-two farmers were asked to answer the questions during a practical field course in 2019 in Santa Maria, Brazil. The data were analyzed based on the reports from the published articles (city, local management, parasites, drug efficacy), and correlated to the information from questions (city, local management, parasites and parasite control).

RESULTS

Most of the studies have reported nematode resistance (10/12) to macrolactones. The FECRT reports came from the central and southern states (Fig. 1). Half of the articles lack some information, such as coproculture or EPG after 21 and 28 days. All farmers rotated the products at every treatment and the animals were treated at maximum of 40-day interval (Fig. 2). All treatments were based on visual evaluation (body condition score, tick or fly counts), and 94% of the farmers treat all animals with combinations. All farmers used ivermectin, abamectin, doramectin or moxidectin in different concentrations in more than 80% of the time to control endo- and ectoparasites. Sixty percent of farmers had some knowledge about drug resistance. The most important factor for parasite selection was the high level of drug exposure to control the cattle-tick (6 to 19 times a year) and horn-fly (*Haematobia irritans*).

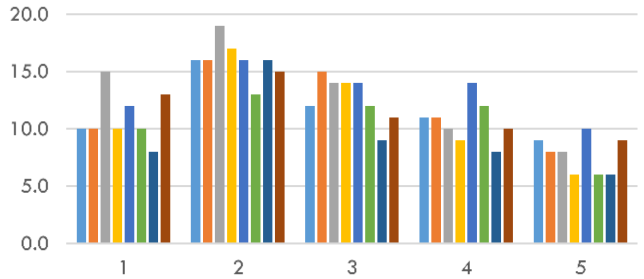

Figure 2. Number of treatments per year for tick control in nine areas (columns) in a cattle farm during 2013 and 2017.

DISCUSSION

Treatment frequency to control nematode parasites of cattle does not have a great selection pressure, but parasites are being exposed to a much higher chemical exposure, imposed by tick control. Most of the articles included "drug resistance" in the title or some other adjective, advocating an alarming situation. In only one article the authors have mentioned the possibility that tick control could have contributed to nematode resistance. Although FECRT was used to confirm the lack of efficacy to ML, it did not represent the real cause of resistance (genetically or phenotypically). From another perspective, nematode parasites of cattle may not have presented such a fast selection if the animals were treated as advised (2-3x/year). Thus, ML multi-parasite resistance should be attributed to the desperate tick control protocols (>10x/y).

CONCLUSION

Nematode resistance to MLs should be considered secondary due to the suppressive use of these products to control ticks, and to a lesser extent to horn-flies, in cattle. Attention must be made when reporting ML resistance.

References

Mello, M. et al. Side-resistance to macrolactones in cattle nematodes. *Arch. Vet. Sci.* 11, 8-12. 2006
Molento, MB et al. Suppressive treatment of abamectin against *Dictyocaulus viviparus* and the occurrence of resistance in first-grazing calves. *Vet. Par.* 141, 373-376. 2006.

Experimental haemonchosis in lambs: effect of medicinal herbs on histopathology

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Background 1

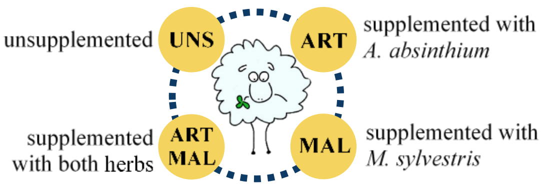
Haemonchosis, an infection causes by gastrointestinal nematode *Haemonchus contortus*, is a prevalent parasitic disease of small ruminants. Adult parasites live in the abomasum where they attach to the abomasal mucosa and feed on blood. Medicinal herbs are currently rising attention as natural alternatives for controlling this infection. The diet supplementation with dry medicinal herbs containing bioactive substances can be used to indirectly improving animal resistance to this infection.

Aim 2

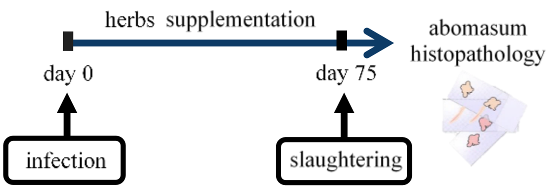
The aim of my STSM at Warsaw University of Life Science was to learn how to perform histopathology and to evaluate the effect of dry medicinal herbs (*Artemisia absinthium* and *Malva sylvestris*) as feed supplements on abomasum histopathology and mucosal immunity of *H. contortus* infected lambs.

Material and Methods 3

Twenty-four parasite-free female lambs (Improved Valachian) 3-4 months of age with initial body weight of 18.67 ± 0.55 kg were randomly divided into four experimental groups and infected orally with 5000 L3 larvae of *H. contortus*. Each animal was fed oats (500 g dry matter (DM)/d) and meadow hay (ad libitum).



A. absinthium 1 g/sheep/day | *M. sylvestris* 15 g/sheep/day | mix 16 g/sheep/day



Results and Discussion 4

Histological changes were detected mainly in mucosal membrane of abomasum, with the presence of inflammatory infiltrates in MAL, ART, and ARTMAL (Fig. 1). Increase mucus production was observed in the ARTMAL (Fig. 2). In some cases, the formation of lymphatic nodules was seen, mainly in MAL group.

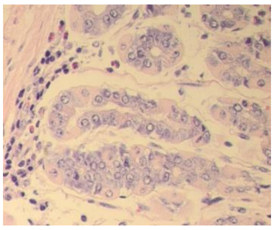


Fig. 1 Inflammation (400×)

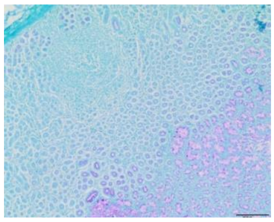


Fig. 2 Increase mucus production (40×)

Natural immune responses against *H. contortus* in sheep are connected with the infiltration of immune cells into the infected tissue. Eosinophils were not affected by different treatment (Fig. 3A). The number of mast cells was significantly higher in UNS and ART than MAL (Fig. 3B). Plasma cell numbers were higher in ARTMAL than MAL (Fig. 3C). Abomasal tissue regenerated more frequently in ARTMAL than the other experimental groups (Fig. 3D).

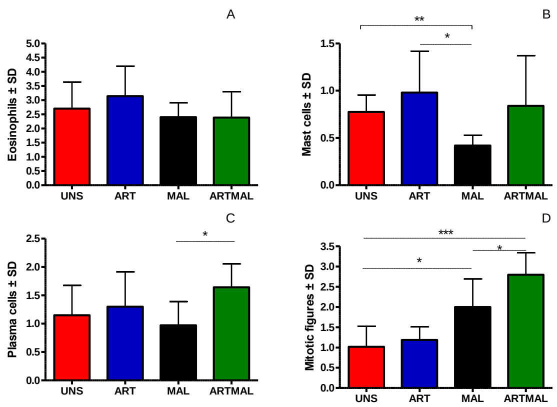


Fig. 3 Mean numbers of immune cells and mitotic figures in abomasum of infected lambs at the end of experiment (n=6).

Conclusion 5

During my STSM I learnt how to perform histopathological analysis and I found out that treatment with *A. absinthium* and *M. sylvestris* affected local immune response in the abomasum of *H. contortus* infected lambs. Supplementation by medicinal herbs, therefore, could improve sheep immunity against parasites, but further research is needed.

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

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INTRODUCTION

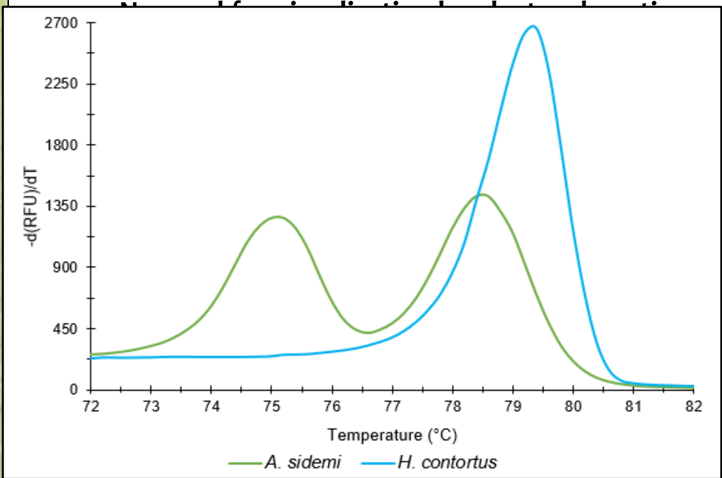
Infection of ruminants by helminth parasites, especially gastrointestinal nematodes (GINs), has a considerable social and economic impact throughout the world. Correct identification of parasitic taxa is crucial in many parasite research areas, including monitoring of occurrence, epidemiological studies, or assessment of anthelmintic efficacy. However, according to morphological features, differentiation of some nematode species is complicated, e.g., hematophagous strongylids *Haemonchus contortus* and *Ashworthius sidemi*, the pathogenic GINs of domestic and wild ruminants.

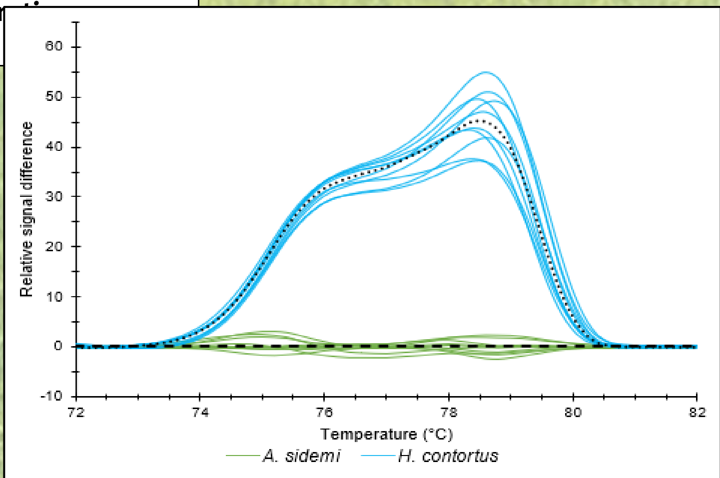
HIGH RESOLUTION MELTING (HRM) ANALYSIS

- Single-tube method using a single pair of universal primers
- Detection of sequence alterations via differences in melting profile of dsDNA amplicons
- Genotyping, mutation scanning, sequence matching, taxa affiliation
- **Easy, rapid, unambiguous, and cost-effective identification tool**

Fw: 5'-GCAATTCGTGGTAAATAACGCAG-3'
Rev: 5'-GGGTGTATTGCTAWAATACTGCCTC-3'

The pair of universal primers targeting the *internal transcribed spacer 1* (*ITS-1*) sequence of the ribosomal region.





RESULTS

The results of our study based on the *ITS-1* sequence allow a rapid and reliable differentiation of parasitic GINs *H. contortus* and *A. sidemi*. Based on specific melting curves, we identified a total of 45 specimens of adult nematodes that originated from a wide range of domestic and wild ruminant species living in various parts of the Czech Republic. We also confirmed that HRM analysis is applicable for the infective larval stages of the nematodes and therefore promises a significant improvement of intravital diagnostics.

Further information:
Skorpikova, L., Reslova, N., Magdalek, J. et al. The use of high resolution melting analysis of *ITS-1* for rapid differentiation of parasitic nematodes *Haemonchus contortus* and *Ashworthius sidemi*. Sci Rep 10, 15984 (2020). https://doi.org/10.1038/s41598-020-73037-9

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4th COMBAR JOINT WG MEETING
ANTHELMINTIC RESISTANCE IN RUMINANTS: FROM RESEARCH TO RECOMMENDATIONS
9-10 DECEMBER 2020

combatting anthelmintic resistance in ruminants

EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

OVICIDAL POTENTIAL OF ESSENTIAL OILS OF *JUNIPERUS COMMUNIS* AND *ACHILLEA MILLEFOLIUM* TO CONTROL GASTROINTESTINAL NEMATODES OF SHEEP

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INTRODUCTION: The growing problem of anthelmintic resistance and consequent economic losses requires a search for alternative strategies. Within this context, medicinal herbs and their products such as essential oils (EOs) are considered as sustainable option. The aim of this study was to evaluate the *in vitro* ovicidal potential of three samples of EOs against ovine gastrointestinal nematode (GIN) eggs.

MATERIAL AND METHODS: In the present study, one sample of *Juniperus communis* EO and two samples of *Achillea millefolium* EO were used. Qualitative and semi-quantitative chemical characterization of EOs were done by gas chromatography (GC) and mass spectrometry (MC). *In vitro* ovicidal activity of tested samples was evaluated using egg hatch test (EHT), which was performed at six different concentrations (50; 12.5; 3.125; 0.781; 0.195 and 0.049 mg/mL) for each EO. In addition, coproculture examination was conducted to determine the genera of GINs *in vitro* tested on the efficacy of EOs.

RESULTS: GC-MS analyses revealed a rich composition of the tested EOs with the total number of identified compounds in *J. communis* and two chemotypes *A. millefolium* of 28, 28 and 27, respectively. Some differences in identified compounds between two chemotypes of *A. millefolium* were recorded, which can be related to the different environmental factors under which the plants were grown as well as EO isolation methods. In the EHT, the most potent activity was shown by *J. communis* EO with inhibitory effect on egg hatchability that varied from 81 to 96.75% depending on the concentration. The activity of two samples of *A. millefolium* EO were 46.5% to 99.5% and 69.6% to 97.25%, respectively, but chemotype 1 showed better performances in the context of a higher number of concentrations that inhibited egg hatching by more than 90%. The results of coproculture showed the presence of four genera of sheep GINs: *Haemonchus* (53%), *Trichostrongylus* (29.5%), *Teladorsagia* (14.5%) and *Chabertia* (3%).

Table 1. *Chemical composition of tested essential oils determined by GC-MS (five most represented compounds)*

J. communis	*A. millefolium* chemotype 1	*A. millefolium* chemotype 2
α-Pinene, 40.46%	1,8-Cineole, 41.69%	β-Pinene, 28.53%
Sabinene, 14.04%	Camphor, 8.37%	β-Caryophyllene, 18.71%
Myrcene, 8.87%	cis-Chrysanthenyl acetate, 4.90%	1,8-Cineole, 11.69%

Table 2. *Efficacy (mean percentage) of tested essential oils and controls against sheep nematode egg hatching*

Concentration (mg/mL)	Inhibition of hatchability (%)		
	J. communis	*A. millefolium* 1	*A. millefolium* 2
50	96.75^{Aa}	99.5^{Aa}	97.25^{Aa}
12.5	95.5^{ABab}	98^{Aa}	90^{Ab}
3.125	94.75^{ABa}	95.25^{Aa}	73^{Bb}
0.781	91^{Ba}	87.5^{Ba}	72.75^{Bb}
0.195	85.5^{Ca}	49^{Cb}	71.25^{Bc}
0.049	81^{Ca}	46.5^{Cb}	69.50^{Bc}

CONCLUSION: Essential oils of *J. communis* and *A. millefolium* have great potential to be part of the integrated approach designed to control sheep GINs. Moreover, this study highlights the possible importance of medicinal herb products in combating anthelmintic resistance.

DISCUSSION: The results of the present study showed the high ovicidal, anthelmintic potential of *J. communis* EO and two chemotypes of *A. millefolium* EO against sheep gastrointestinal nematodes. To our knowledge, this is the first report about anthelmintic activity of these essential oils. There was only a report about the anthelmintic efficacy of crude aqueous and crude ethanolic extracts of entire *A. millefolium* against the sheep GINs (Tariq et al., 2008). The high anthelmintic potential of the tested EOs can be related to their chemical composition, bearing in mind that these compounds were also represented in significant percentages in other essential oils with proven anthelmintic efficacy. For instance, 1,8-cineole (eucalyptol) was well represented in *Piper aduncum* (55.8%), *Rosmarinus officinalis* (42.11%) and *Artemisia lancea* (34.56%) EOs, α-pinene also in *R. officinalis* (14.76%) EO, β-pinene in *Citrus aurantifola* (11.86%) EO, etc (Zhu et al., 2013; Oliveira et al., 2014; Ferreira et al., 2018; Pinto et al., 2019). Therefore, we consider *J. communis* and *A. millefolium* EOs suitable for further *in vivo* trials in the context of the possible discovery of new anthelmintic agents.

FACULTY OF VETERINARY MEDICINE
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RISK FACTORS FOR LUNGWORM INFECTIONS AND ASSOCIATED PRODUCTION LOSSES IN GRAZING DAIRY CATTLE

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Introduction

Infections with lungworms (*Dictyocaulus viviparus)* have a major impact on the health and productivity in dairy cows. The epidemiology of *D. viviparus* is volatile and pasture infection levels can rise rapidly if weather conditions are favourable, causing unexpected lungworm outbreaks. So far, the reliable prediction of clinical lungworm outbreaks has remained elusive and infections are treated only therapeutically. This may lead to animal welfare issues and production losses due to subclinical or pre-patent infections.

Objectives

The aims of this project are to identify risk factors for and to predict disease outbreaks and production losses due to lungworm infections in dairy cattle, by combining parasitological, herd management and production data.

Materials and methods

Farm selection: Electronic invitation of all Flemish dairy farmers

Bulk Tank Milk (BTM) sampling
- Twice monthly, April – October, 2018-2019
- Short questionnaire → management

ELISA:
- Major Sperm Protein (MSP), Fiedor et al. (2009)[1]
- Cut-off 0.41 Optical Density Ratio (ODR)

Production data
- Mean milk yield (kg/cow/day)
- Mean milk protein%
- Mean milk fat%

Statistical analysis
- ODR – outbreak: logistic regressions
- ODR – production/linear and logistic mixed effects models

Results BTM ELISA (Figure 1)

Cut-off 0.41 Optical density ratio (ODR)

2018:
- 12% samples positive
- 54% farms positive

2019:
- 6% samples positive
- 30% farms positive

Fig. 1. Heat map showing the distribution of the ODR values per sampling and per farm for the 2018 and 2019 grazing season. Dark green = lowest values; dark red = highest values. Farms are ranked from top to bottom by decreasing mean ODR values in 2018.

Results (Figure 2)

Time between all positive ODR's and outbreak

- 50-59% positive ODR's before outbreak
- 42-47% positive ODR's within 12 w before outbreak
- 33-37% positive ODR's within 8 w before outbreak

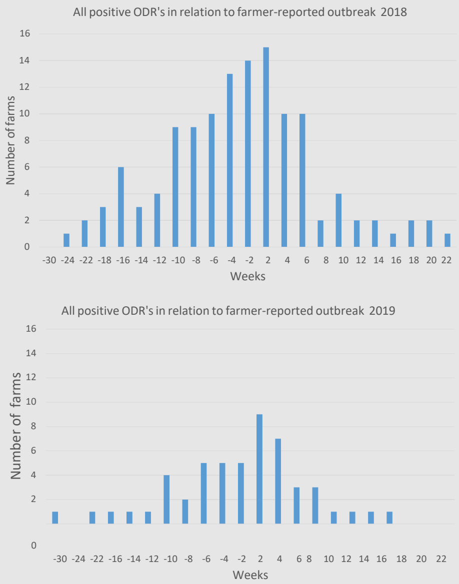

Fig. 2. Histogram of all at which each farm with a farmer-reported outbreak became positive on their BTM, in relation to the outbreak (Week 0), in 2018 (a) and 2019 (b).

Results: Association ODR - Production

Linking each test day of milk production to closest BTM: Increase ODR from 25th (0.12) to 75th (0.25) percentile
- Mean milk production = -0.26kg/cow/day
- Mean % fat = -0.02%

At least 1 positive BTM during grazing season:
- Mean milk production: -0.17 to -0.70 kg/cow per day
- Mean % fat: -0.01 to -0.03%
- Mean % protein: 0.00 to -0.01%

At least 2 consecutive positive BTM during grazing season :
- Mean milk production: -0.58 to -1.74 kg/cow per day
- Mean % fat: 0.00 to -0.01%
- Mean % protein: 0.00 to -0.01%

Results: Association ODR – Risk factors

One Positive BTM: No significant associations

Two consecutive positive BTM (Table 1)
- Positive association :
  - Frequent purchase of animals
  - Higher % grazing season covered by anthelmintics in FGSC
- Negative association: Mowing at least 50% of pastures

Variable	At least two consecutive positive BTM samples		
	OR	SD	P-value
Intercept	0.18	1.64	≤ 0.001
Year (Baseline = 2018)			
2019	0.62	1.61	0.31
Mowing pastures (Baseline = ≤ 50% mowed)			
> 50% pastures mowed	0.57	1.42	0.10
Frequent animal purchase (Baseline = no)			
Yes	2.68	1.70	0.06
Anthelmintic coverage FGSC (Baseline 0-25%)			0.01
26-50%	2.15	1.68	0.14
51-100%	3.88	1.78	0.04
Year:AnthelminticCoverageFGSC			0.02
2019:26-50%	0.77	1.98	0.71
2019:51-100%	0.11	2.55	0.02

Table 1. Results of the model of best fit for the risk of having at least two consecutive positive BTM samples.

Results: Association ODR – outbreaks (Table 2)

Higher risk for outbreak when:
- ODR above cut-off
- Two consecutive positive samples
- **Positive in August/October**

Outbreak i.f.o.	Year	OR	95% CI OR	P-value
1 pos. 0.41	2018	2.0	[0.97 : 4.47]	0.07
	2019	2.3	[1.22 : 4.43]	< 0.01
2 pos. 0.41	2018	5.5	[2.65 : 11.77]	< 0.001
	2019	3.2	[1.47 : 6.85]	< 0.01
Aug pos. 0.41	2018	4.4	[2.38 : 8.08]	< 0.001
	2019	2.9	[1.10 : 7.13]	< 0.05
Oct pos. 0.41	2018	3.7	[1.92 : 7.10]	< 0.001
	2019	1.8	[0.49 : 5.62]	0.32

Table 2. Results of the univariate logistic regressions between reported outbreaks and one positive BTM ODR during the season (cut-off 0.41 ODR), at least two consecutive positive BTM ODR's (cut-off 0.41 ODR) and a positive BTM ODR in August or October (cut-off 0.41 ODR). CI = Confidence Interval; OR = Odds Ratio.

Conclusions

A positive BTM could have a predictive value for an outbreak of lungworm disease. Implementation of risk factors and meteorological data could enhance the predictive value.

Frequent purchase of animals and a higher % of the grazing season covered by anthelmintics are risk factors for an outbreak or for having two positive BTM ODR's, while mowing at least 50% of the pastures diminishes this risk.

An increase in ODR from the 25th to 75th quartile resulted in a loss of 0.26kg milk/cow per day and 0.02% in the mean percentage of fat. Having at least two consecutive positive BTM samples during the grazing season resulted in a loss of 0.58 to 1.74 kg milk/cow per day.

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[1] Fiedor, C., Strube, C., Forbes, A., Buschbaum, S., Klewer, A.-M., von Samson-Himmelstjerna, G., Schnieder, T., 2009. Evaluation of a milk ELISA for the serodiagnosis of Dictyocaulus viviparus in dairy cows. Veterinary Parasitology 166, 255-261.

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

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1. **The purpose of this study was to determine the effect of *C. nematophagum* bacterie on first stage larvae of *Haemonchus contortus* (L) in micro plots**

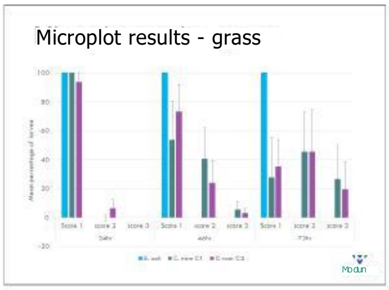
- Pots with sown grass seed in triplicate
- adding ca. 1.800 *Haemonchus contortus* Land either *E. coli* OP50-1 or two concentration of *C. nematophagum*
- incubate for 24, 46, 72 hours

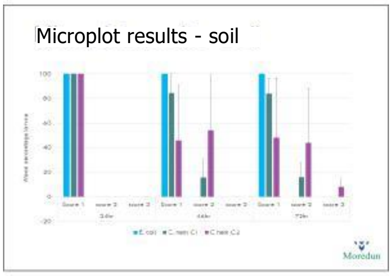
2. **The effect of *C. nematophagum* bacterie on naturally occurring free-living nematodes present on grass samples**

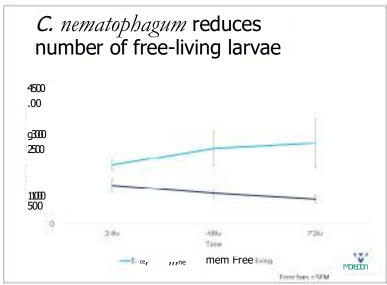
- 140 g fresh pasture in triplicate
- equal volume *E. coli* or *C. nematophagum* added
- incubated 24, 48, 72 hours

Results

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







L_1 larvae *Haemonchus contortus* - healthy and after exposure

Condition score

1. Larvae look healthy, cuticle intact, internal structures look normal
2. Deformed cuticle, granulation of internal structure, can still see shape of larvae
3. Cuticle still visible, pronounced vaculoation throughout internal structure, larvae have pale appearance

ANALYSIS OF BLOOD PARAMETERS IN SNEEP PROVIDED SPORES OF PARASITICIDE FUNGI

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Control of Parasites Research Group (COPAR, GI-2120). Department of Anima] Pathology; Faculty of Veterinary. University of Santiago de Compostela. 27002-Lugo (Spain).

INTRODUCTION

Since several decades, the administration of spores of certain filamentous fungi have been shown highly useful for reducing the risk of gastrointestinal infections on grazing sheep. Nevertheless, there is a lack of information about the possible effect on their health status.

RESULTS

During the examination of blood samples taken from two flocks in two years, normal (physiological) values were obtained for the red blood parameters while sheep took the spores of the filamentous fungi. Regarding the white blood cas, values belonging to physiological counts were also recorded.

YEAR

Parameter	RBC	HGB	HCT	VCM	MCH	MCHC
Reference values	9-15 la' mL	10-15 g/dL	27-42	24-32 fL	8-12 pg	32-42 g / dL
Aug	13.33	13	36.33	28	9.67	35.33
Sep	10	10	28.75	28.25	10.5	37.5
Nov	10.3	10.5	29.12	27.5	9.5	38
Jan	9.88	10	28.5	29.25	11.13	37.63
feb	9.56	9.22	27.33	29.44	10.89	36.67
Mar	9.38	10.1	28.75	29.88	11	37.13
Apr	10	2	29.4	30.4	11.6	38.2
May	10.5	10.8	28.75	27.13	9.88	36.5
YEAR						
Oct	10.17	9.33	15.83	15.33	9.33	60.33
Nov	9.45	11.73	12.18	30.45	8.91	28.18
Dec	11.25	10.25	11.25	29	10.25	32.75
Feb	10.43	12.29	10	30.57	9.71	31.43
Apr	10.51	10.8	32.2	29.65	10.4	37.1

Parameter	WBC	LYM	GRA	MON
Reference values	5-14 10^6 / mL	40-70	10-63 %	0-6 %
Aug	9	68.67	28.13	3.2
Sep	6	63	34.25	2.75
Nov	8.5	56.15	41	2.85
Jan	10.38	69.13	27.5	3.37
Feb	12.56	67.44	30.11	2,45
Mar	11.75	66.38	29.52	4.1
Apr	7	59.6	37.4	3
May	8.88	63	35.25	1.75
Oct	6.17	65.33	30.8	3.87
Nov	8.82	63.44	34.89	1.67
Dec	12	64.25	32	3.75
Feb	11.57	66	31.7	2.3
Apr	7.2	59.2	38	2.8

& METHODS

Over a two-year period, two flocks of twelve pasturing sheep in2 Sakona Farm (Azpeitia, Basque Country) were provided a dosage of 10^6 spores of Mucor cirdnelloides and *Duddingtonia flagrans twice* a week. Blood samples were sheep were passing eggs of gastrointestinal nematodes at the beginning of each season (Autumn), collected and analyzed by means of an automated coulter-counter. Due to the anthelmintic treatment was administered.

These results confirm the healthiness of developing biologica! control measures based on the long-term administration of fungi to grazing livestock, for reducing the risk of infection by helminths.

ГONCLUSION

It is concluded that the integrated control of helminths on grazing sheep can be improved by giving them spores of filamentous fungi without any risk for their health.

USC

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

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2 Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre, Lethbridge, AB, Canada

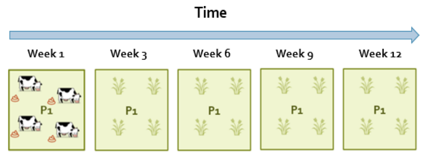
Background

- Two reasons make Alberta an important region to study gastrointestinal nematode (GIN) epidemiology:
1. Extreme climate (-30°C in winter and 30°C in summer)
2. Large beef cattle production industry (42% of Canada)
- However, the epidemiology of GIN in western Canada is not well-understood, not to mention the application of sustainable parasite control
- Aims of the studies:
1. Determine the extent to which the different bovine GIN species overwinter in western Canada
2. Investigate the pasture contamination pattern of GIN during the grazing season
3. Develop empirical sustainable grazing strategies based on the above findings

Methods

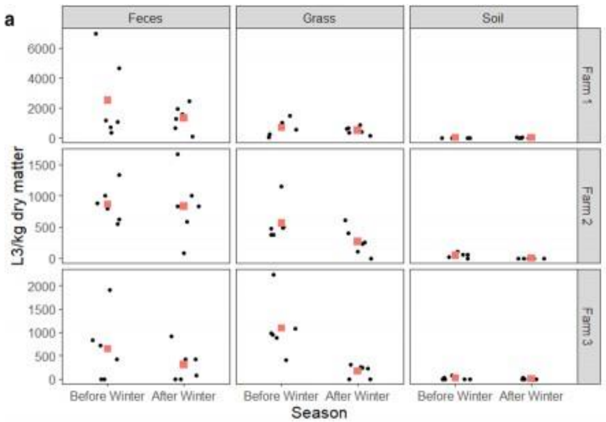
- **Study 1:** Overwinter of GIN larvae on western Canadian Pasture (3 farms were included)
  Approach one: Environmental sampling before and after winter
  Approach two: Tracer calve trail after winter

- **Study 2:** Pasture L3 dynamic during the grazing season
- Pastures were grazed only for a few days at the beginning of the grazing season. Grass samples were then collected from these pastures every 3 weeks.
- 9 pastures were included in total.



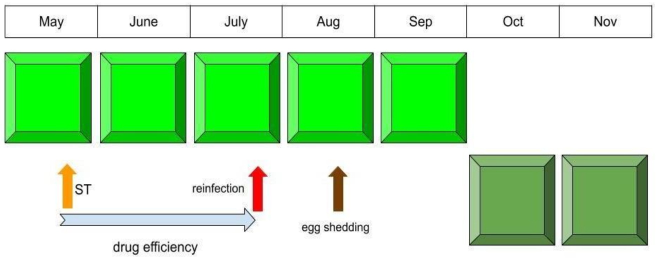
Results

- **Study 1:** GIN L3 were able to overwinter on western Canadian pasture (except for *H. placei*) and develop to adult stage inside spring grazing cattle.



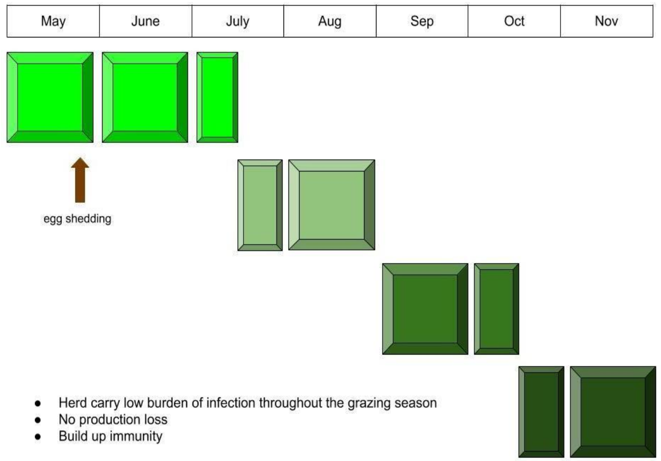
- **Study 2:** Grass L3 count peaked at 9th week after egg deposition.

Grazing management



- Herd start grazing (pick up overwinter L3) from early May
- Apply strategic treatment 3 weeks later
- Depending on the type of treatment, drug efficiency may last for 3 to 10 weeks
- Herd is reinfected at mid-July by the surviving overwinter L3 (fewer than spring)
- 3-week prepatent period and shed eggs on mid-August
- Move on to Pasture 2 at the end of September

Anthelmintic treatment: 1 strategic treatment (ST) in spring
Rotational pasture needed: 2



- Herd carry low burden of infection throughout the grazing season
- No production loss
- Build up immunity

Anthelmintic treatment: 0
Rotational pasture needed: 4