Veterinary Parasitology 327 (2024) 110146

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Faecal egg count reduction test in goats: Zooming in on the genus level

Anna Maurizio [a,*], Lucie Škorpíková [b], Jana Ilgová [b], Cinzia Tessarin [a], Giorgia Dotto [a], Nikol Reslová [b], Jaroslav Vadlejch [c], Erica Marchiori [a], Antonio Frangipane di Regalbono [a], Martin Kašný [b], Rudi Cassini [a]

[a] Department of Animal Medicine, Production and Health, University of Padova, Viale dell'Università, 16, 35020 Legnaro, Italy
[b] Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno 611 37, Czech Republic
[c] Department of Zoology and Fisheries, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamycka 129, Suchdol, Prague 165 00, Czech Republic

ARTICLE INFO

Keywords:
Gastrointestinal tract
Caprine nematodes
Species richness
Coprology
QPCR
Anthelmintic resistance

ABSTRACT

The faecal egg count reduction test (FECRT) is the most widely used method to assess treatment efficacy against gastrointestinal nematodes (GIN). Information on genera composition of the GIN community is not available with this test and it is commonly obtained by identifying cultured third-stage larvae (L3) or through molecular assays in the post-treatment survey, but results provided are usually only qualitative or semi-quantitative. The updated WAAVP guidelines now recommend assessing anthelmintic efficacy for each GIN genus/species separately (genus-specific FECRT), but this approach is poorly employed in Europe and in goats especially. For this reason, four FECRT trials were conducted using oxfendazole and eprinomectin in two Italian goat farms. Samples were processed individually using the McMaster technique and then pooled to create two samples from faeces of 5 animals each. Pooled samples were analysed using the McMaster and cultured for seven days at 26°C to obtain L3s. The genus-specific FECRT was based on larval identification, integrating coproculture and FEC results. Larvae were identified as *Haemonchus*, *Trichostrongylus*, *Teladorsagia*, *Oesophagostomum* / *Chabertia* and *Bunostomum*. Molecular assays (a multiplex real-time PCR and two end-point PCRs) were also implemented on pooled samples to support the morphological identification. The Spearmann Rho test confirmed a high correlation between the two approaches (Rho = 0.941 and Rho = 0.914 respectively for *Haemonchus* and *Trichostrongylus*, the two most common genera). Both oxfendazole and eprinomectin were effective in one farm, while none in the other farm (FECR = 75.9% and 73.3% respectively). In the second farm, the genus-specific FECRT highlighted a different response to treatment among genera: oxfendazole lacked efficacy against both *Haemonchus* and *Trichostrongylus* spp., eprinomectin only against *Haemonchus*, while all other genera were susceptible to both drugs. This study brings new attention on the importance of adopting a genus-specific approach to identify and quantify differences in susceptibility to anthelmintics among genera in goats, providing support for FECRT interpretation, anthelmintic resistance evaluation and evidence-based GIN control.

1. Introduction

Worldwide, sheep and goats are constantly exposed to gastrointestinal nematodes (GIN). Infections caused by these parasites may threaten the animal health and welfare and affect the economic sustainability of the ruminant livestock production. The consistent reliance on anthelmintics for GIN control has led to the development of drug resistance, which is now spreading at worrying pace worldwide, including in Europe (Rose Vineer et al., 2020). Small ruminants can be parasitized by several genera of nematodes, most commonly in mixed-species infections. These genera differ not only in pathogenicity, biology and epidemiology, but also in their ability to develop anthelmintic resistance (AR) (Kotze and Prichard, 2016). For instance, *Haemonchus contortus* is a blood-feeding species of high concern for its pathogenicity and ability to develop AR (Elmahalawy et al., 2018). It is adapted to warmer environmental conditions compared to other genera such as *Teladorsagia circumcincta* and *Trichostrongylus* spp. Depending on the circumstances these species may be also pathogenic but, being non-haemotophagous, they affect the host in a different way.

Traditionally, GIN burden monitoring in live animals relies on

* Corresponding author.
E-mail address: anna.maurizio@phd.unipd.it (A. Maurizio).

https://doi.org/10.1016/j.vetpar.2024.110146
Received 30 October 2023; Received in revised form 7 February 2024; Accepted 7 February 2024
Available online 8 February 2024

coproscopic observation and enumeration of parasite eggs in faecal samples (i.e. Faecal Egg Count, FEC), and so does the evaluation of treatment efficacy (i.e. FEC Reduction Test, FECRT) for the detection of anthelmintic resistance (McIntyre et al., 2018; Vercruysse et al., 2018). In the FECRT, the percentage of egg reduction following treatment is used to calculate anthelmintic efficacy. Since GIN genera cannot be differentiated solely based on egg morphology (except for *Nematodirus* spp. and *Marshallagia marshalli*), the FECRT provides only information on the reduction of the overall number of eggs. As such, data on susceptibility of particular GIN genera to anthelmintics (i.e., shifts in the relative presence of certain genera) are not provided by this approach.

The reliability and accuracy of GIN identification has increased significantly with the emergence and advancement of DNA based technologies (Avramenko et al., 2015; Elmahalawy et al., 2018; Ljungström et al., 2018; Reslova et al., 2021; Roeber et al., 2017). Molecular tests are indeed faster, highly sensitive and more specific compared to the more traditional approach based on faecal examination followed by coproculture and microscopic identification of cultured third-stage (L3) larvae. Coproculture suffers from several disadvantages, related to the time and expertise required, to the variability in development rate/-mortality of larvae of specific GIN genera and to the low specificity due to the overlapping morphological and morphometric traits of some species and genera (Knoll et al., 2021; Roeber and Kahn, 2014; Rossanigo and Gruner, 1996). Nevertheless, since molecular methods are more complex to set up and they are not available in all laboratories, coprocultures still represent the most widely applied method for GIN differentiation (Do Amarante, 2011; Jacobson et al., 2020).

The identification of GIN genera in combination with the FECRT has been recommended by the World Association for the Advancement of Veterinary Parasitology (WAAVP) for the last three decades (Coles et al., 2006, 1992; Kaplan et al., 2023) to support the interpretation of the FECRT results in small ruminants, but only the last version (Kaplan et al., 2023) strongly recommends to estimate the FECRT separately for each GIN genus/species. Several publications implementing a genus-specific assessment of anthelmintic efficacy are available in cattle (Leathwick et al., 2016; Leathwick and Miller, 2013; Waghorn et al., 2006a) and sheep (Falzon et al., 2013; Lyndal-Murphy et al., 2014; Playford et al., 2014; Rendell, 2010; Waghorn et al., 2006b), mainly in Australasia, where McKenna (1997, 1996, 1990) first developed and promoted the approach. In Europe, only a handful of studies with this approach are present in sheep (Bartley et al., 2006; Bull et al., 2022; McMahon et al., 2013) and in cattle in Belgium (De Graef et al., 2012; El-Abdellati et al., 2010), while its use in goats is hardly reported worldwide (Mahieu et al., 2014). Indeed, in most cases the focus has historically been on the qualitative observation of the species that survived the treatment (Bordes et al., 2020; Čerňanská et al., 2006; Lambertz et al., 2019; Mickiewicz et al., 2020; Vadlejch et al., 2014; Zanzani et al., 2014). The aim of this study was therefore to bring attention to the genus-specific approach to FECRT in Europe, while testing its use on goats. In this study, carried out in dairy goat farms of Northern Italy, GIN genera were identified using coprocultured larvae, but molecular analyses were also implemented to confirm the identification and the estimated relative proportion of each genus.

2. Materials and methods

2.1. Study design, sample collection and anthelmintic treatment

The study was carried out between November 2018 and January 2019 in two dairy goat farms of Northern Italy. Farm 1 was located in the easternmost part of the Po valley (Friuli-Venezia Giulia region), while Farm 2 was located in a hilly area of Veneto region. Both farms reared about 50 lactating goats of Chamois Coloured goat breed in a semi-intensive way, with year-round access to a contiguous pasture and in-loco cheese production.

The genus-specific assessment of anthelmintic efficacy was evaluated with four FECRT trials, testing two different drugs in each farm:

- Oxfenil© (oxfendazole, OXF): oral suspension, Virbac S.A., Carros, France (Farm 1_OXF and Farm 2_OXF);
- Eprinex Multi© (eprinomectin, EPR): pour-on, Boehringer Ingelheim Animal Health Italia S.p.A., Milan, Italy (Farm 1_EPR and Farm 2_EPR).

Trials were carried out on the occasion of the treatment scheduled by the respective veterinary practitioner. The animals did not receive any anthelmintic treatment for at least three months prior to entering the study. Treatments were performed according to the drug manufacturer's instructions; however, double the ovine dose was administered to goats. For each farm, on the same day OXF was administered at a dosage of 10 mg/kg body weight to one group and EPR was given at a dosage of 2 mg/kg body weight to the second group of animals. Body weight was estimated visually, but in each farm some individuals were also weighed using a dynamometer and a weighing harness to confirm the accuracy of the estimation.

Ten animals were included in each trial. Faecal samples were collected at day 0 (D0) before treatment and again from the same animals 14 days post-treatment (D14). Animals were identified individually using the ear tag codes. Samples were collected from the rectum, kept under cold chain and analyzed at the Parasitology Laboratory of the Department of Animal Medicine, Production and Health of the University of Padova within a maximum of 48 hours after collection. Samples were processed both individually and pooled. For pooled samples, each treatment group was divided in two subgroups of five animals each, pooling together 6 g from each individual sample (30 g per pool in total). The same division of animals in sub-groups was maintained for D14 pools. Pooled samples were thoroughly homogenized and used to perform McMaster analysis, coproculture and molecular analysis.

2.2. Laboratory analyses

2.2.1. Faecal egg counts

Individual samples were analyzed using the McMaster technique (MAFF, 1986) with the limit of detection of 20 EPG. Briefly, 5 g of faeces were diluted to reach a volume of 30 ml of saturated sodium nitrate solution with specific gravity 1.3 in a 50 ml Falcon tube. The content was gently mixed, homogenized, filtered through a double-layer gauze and applied into the chambers of a McMaster slide. Eggs were left to float for a few minutes and then counted in both chambers. In this study, only gastrointestinal strongylid eggs were considered. In addition, 5 g from each composite sample were also analyzed with the same technique, and the Falcon tube content was further processed to obtain the sample for DNA extraction, as described in the next paragraphs.

2.2.2. Coproculture and morphological identification

The remaining faeces from each pool (25 g) were used to prepare larval cultures for the morphological identification of strongylid genera. Samples were moistened and mixed with vermiculite to prevent fungal contamination and facilitate ventilation. Faeces were incubated for seven days at 26 ºC and moistened every day. Third-stage larvae (L3) were then collected using the Baermann technique and stored in a fridge (± 4 °C) until identification. Larvae were observed under the microscope (Olympus BX40F-3, Japan) at a total magnification of 100× or 400×. The first 50 larvae recovered for each pool were identified by keys currently used at the Laboratory of Parasitology of the University of Padova (see supplementary materials S1); these identification keys are based on data proposed by MAFF (1986), van Wyk et al. (2004), Knoll et al. (2021) and experiences gained at our lab. If there were fewer than 50 larvae present, all of them were identified. Larval identification was facilitated by using the NIS-Elements imaging software (Nikon Corporation, Japan).

2.2.3. Isolation of genomic DNA from strongylid eggs

Eggs from pooled samples were subjected to molecular analysis to identify present strongylid genera. Genomic DNA extraction was performed according to Bott et al. (2009). Briefly, following the McMaster analysis, the double-layer gauze was removed from the Falcon tube and the remaining content was further processed to collect the remaining eggs. The Falcon tubes were centrifuged at 1000× g for 10 min and 5 ml of upper layer containing eggs were transferred into a new 50 ml Falcon tube, diluted with 45 ml distilled water, and centrifuged again at 1000× g for 10 min. Subsequently, the pellet was resuspended in 0.5 ml of purified water and used to extract the genomic DNA. The extraction was carried out using PSP®Spin Stool DNA Kit (Stratec, Lot: JA160005 Ref: 1038100300), according to the manufacturer's manual. The purified DNA was then stored at −20 ºC until molecular analysis.

2.2.4. Multiplex real-time PCR assay

A multiplex real-time PCR assay employing genus-specific TaqMan probes was used for the molecular semi-quantitative assessment of strongylid eggs (Reslová et al., 2021). This highly specific and sensitive assay allows approximate quantification of ovine and caprine parasites belonging to genera *Haemonchus* spp., *Teladorsagia* spp., *Trichostrongylus* spp. and *Chabertia ovina*. The target regions amplified during qPCR were ITS2 in case of *Haemonchus* sp. and *Teladorsagia* sp.; ITS1 in case of *Trichostrongylus* sp. and CO1 in case of *Chabertia ovina*. Detailed composition of reaction mixture, sequences of primers and TaqMan probes, cycling conditions and semi-quantitative data evaluation are described in Reslova et al. (2021).

All samples were tested in duplicates on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) in 96-well PCR plates. Calibration curve was calculated using Ct values, which corresponded to serial dilution of *Haemonchus contortus* plasmid construct with a defined number of plasmid copies (5×10^7, 5×10^6, 5×10^5, 5×10^4, 5×10^3), which were run on the same plate as faecal DNA samples. The results are presented as plasmid copy numbers corresponding to the genus-specific DNA amount in the pooled faecal specimen.

2.2.5. End-point PCR for Oesophagostomum and Bunostomum

Primers on ITS rDNA described by Bott et al. (2009) and Wang et al. (2012) were used to detect *Oesophagostomum* spp. (OEV, forward: 5'-TGAAATGAGACAACCGTAGTCG-3' and NC2, reverse: 5'-TTAGTTTCTTTTCCTCCGCT −3') and *Bunostomum* sp (p1, forward: 5′-GATTACGTCCCTGCCATTTGT-3′ and p2, reverse: 5′-GTTCACTCGCCGTTACTAAGG-3′) respectively. The amplification protocols are indicated below.

The amplification mixture of final volume 30 µl included 1X of Buffer, 2 mM of $MgCl_2$, 0.25 mM of dNTPs, 0.5 µM of each forward and reverse primers, 1 U of Taq Polymerase (Platinum ™ Taq DNA Polymerase, ThermoFisher Scientific, Cat. No: 10966026) and 3 µl of DNA. PCRs were performed in thermal cycler (Applied, Biosystems 2720 Thermal Cycler), using the following thermal profile: for *Oesophagostomum* spp., initial denaturation (94 ºC, 5 min); 35 cycles of denaturation (94 ºC, 30 s), annealing (55 ºC, 30 s) and elongation (72 ºC, 30 s); and final elongation (72 ºC, 7 min). For *Bunostomum* sp., annealing temperature was changed to 50 ºC and elongation time to 40 s. The PCR products were run onto agarose gel 2% in TBE buffer stained with SYBR Safe DNA Gel Stain (Invitrogen, ThermoFisher Scientific, USA, Lot: 1911823). The amplicons were sequenced by Sanger technology (Macrogen, Spain) and compared with those already published in GenBank.

2.3. Data analysis

The concordance between morphological and molecular identification of genera was investigated both qualitatively (presence/absence) and semi-quantitatively. For the latter, the number of eggs of each genus was estimated by multiplying EPG of each pool (calculated by the pooled McMaster analysis) by the relative proportion of each genus observed during larval identification. This estimation was compared with the number of plasmid copies obtained by the multiplex real-time PCR (see supplementary material S2). The two estimations were categorized into three classes (low level = +; medium level = ++; high level = +++) according to percentiles and their agreement in the semi-quantitative estimation was descriptively assessed. In addition, the Spearman Rho Correlation test was performed on non-transformed data to assess the concordance between the two approaches using the statistical software R version 4.2.1 (R Core Team 2022). For the purposes of comparison, each pooled sample was treated independently, regardless of the farm/subset it belonged.

FECR and 90% confidence intervals (90% CI) were calculated for both general FECRT and genus-specific FECRT, according to one of the methods (Dobson et al., 2012) indicated in the recently revised WAAVP guidelines (Kaplan et al., 2023). Anthelmintic treatment efficacy was then interpreted according to the classification outlined by Denwood et al. (2023) and subsequently integrated in the WAAVP guidelines. The minimum efficacy target and expected efficacy respectively were fixed at 90% and 95%, in line with the previous guidelines (Coles et al., 1992). Hence, the classification was:

- resistant (R) when the upper limit of the 90% CI (CI_U) < 95%.
- low resistant (LR, a sub-category of the previous) when the lower limit of the 90% CI (CI_L) ≥ 90%;
- inconclusive (INC) when $CI_U \geq 95\%$ and $CI_L < 90\%$;
- susceptible (S) when $CI_U \geq 95\%$ and $CI_L \geq 90\%$.

For genus-specific FECRT, the relative proportion of each genus estimated by larval identification was converted to the absolute number of eggs, considering the total number of eggs counted (prior transformation to EPG) among the 10 animals of the trial as reference amount (see supplementary materials S3). Genus-specific FECRTs and associated efficacy were calculated and interpreted as above described for the overall FECRT.

3. Results

3.1. Comparison of morphological and molecular identification

A total of 16 pooled samples (eight pooled samples collected on both D0 and D14) was subjected to both morphological identification of L3 and to molecular analysis, and the results of the two analyses were compared (Table 1). It was possible to obtain a molecular quantitative estimation based on the numbers of plasmid copies for the genera *Haemonchus*, *Trichostrongylus*, *Teladorsagia* and *Chabertia*, using a multiplex real-time PCR, while for genera *Oesophagostomum* and *Bunostomum*, only the presence or absence was confirmed by the qualitative end-point PCR. The comparison between the two approaches showed an overall fair agreement in the results: out of 80 comparisons, only 8 were inconsistent in qualitative terms, while in 42, both morphological and molecular outputs were negative and in 30, both outputs were positive. For the latter (30 positive results), considering only *Haemonchus*, *Trichostrongylus* and *Teladorsagia* (20/30), semi-quantification agreed in 17 out of 20 cases, and it disagreed by one class and by two classes in 2 and 1 cases, respectively. *Haemonchus* and *Trichostrongylus* were commonly identified as the two dominant genera in the first farm (Farm 1_Ox and Farm 1_Ep) and *Haemonchus* and *Oesophagostomum/Chabertia* in the second one (Farm 2_Ox and Farm 2_Ep), with *Oesophagostomum* being the genus mostly contributing to this, according to the results of the molecular analysis. Both methods confirmed the sporadic and limited presence of *Teladorsagia* and *Bunostomum* in these two farms. The good level of similarity in the estimation was further confirmed for both *Haemonchus* and *Trichostrongylus* by the high value of correlation at the Spearman's Rho test (Rho = 0.941 and Rho = 0.914 respectively).

Table 1
Semi-quantitative estimation of GIN genera according to log-transformed data from morphological (FEC) and molecular (real-time PCR) identification (low level = +; medium level = ++; high level = +++). Results from end-point PCRs for *Oesophagostomum* and *Bunostomum* are also presented as positive (POS) or negative (NEG). Two pools from the same trial are denoted as A and B.

ID	*Haemonchus*		*Trichostrongylus*		*Teladorsagia*		*Oesoph./Chabertia*		*Oesoph.*	*Bunostomum*	
	FEC	Real-time PCR	FEC	Real-time PCR	FEC	Real-time PCR	FEC	Real-time PCR	End-point PCR	FEC	End-point PCR
1A_Ox_D0	+++	+++	+++	+++	++	++	+++	NEG	POS	NEG	NEG
1B_Ox_D0	+++	+++	+++	+++	NEG	NEG	+++	NEG	POS	++	NEG
1A_Ox_D14	++	++	+++	++	NEG	NEG	NEG	NEG	NEG	NEG	NEG
1B_Ox_D14	++	+++	+++	+++	NEG	NEG	NEG	NEG	NEG	NEG	NEG
1A_Ep_D0	+++	+++	+++	+++	NEG	NEG	++	NEG	POS	NEG	NEG
1B_Ep_D0	+++	+++	+++	+++	++	NEG	++	+	POS	NEG	NEG
1A_Ep_D14	++	++	+	+++	NEG	NEG	NEG	NEG	NEG	++	NEG
1B_Ep_D14	+++	+++	+	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
2A_Ox_D0	+++	+++	NEG	NEG	NEG	NEG	+++	++	POS	++	POS
2B_Ox_D0	+++	+++	NEG	NEG	NEG	NEG	+++	+	POS	NEG	POS
2A_Ox_D14	NEG	++	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
2B_Ox_D14	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
2A_Ep_D0	+++	+++	NEG	NEG	NEG	NEG	+++	NEG	POS	++	NEG
2B_Ep_D0	++	++	NEG	NEG	NEG	NEG	+++	++	POS	++	POS
2A_Ep_D14	NEG	++	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
2B_Ep_D14	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG

3.2. Overall and genus-specific FECRT results

Trials conducted at the first farm showed a reduced efficacy towards OXF and EPR, while both tested anthelmintics were fully effective at the second farm (Table 2). Concerning the differences in treatment response among genera, results of the genus-specific FECRT are presented in Table 3. It should be noted that while *Haemonchus* and *Trichostrongylus* were also found in the D14 samplings of both Farm 1 trials, *Oesophagostomum/Chabertia* egg shedding was consistently reduced to zero levels after treatment. In Farm 1_Ep, *Bunostomum* was found at D14 despite not being detected at D0. Results of Farm 1, where neither treatment was effective, are further presented in Fig. 1.

4. Discussion

The precise identification of the causative agents of an infection is pivotal to nematode control and has growing implications in a context of AR spreading. As goats usually harbour mixed nematode infections, species/genus-specific diagnosis is important, in order to assess their specific contribution to these infections (Roeber and Kahn, 2014). Morphological and molecular tools are commonly used and continuously improved for this purpose, also within FECRT trials (Bosco et al., 2020; Cazajous et al., 2018; Halvarsson and Höglund, 2021; Roeber et al., 2012). Several studies in sheep (Bartley et al., 2006; Bull et al., 2022; Falzon et al., 2013; Lyndal-Murphy et al., 2014; McMahon et al., 2013; Playford et al., 2014; Rendell, 2010; Waghorn et al., 2006b) and cattle (De Graef et al., 2012; El-Abdellati et al., 2010; Leathwick et al., 2016; Leathwick and Miller, 2013; Waghorn et al., 2006a) assessed the susceptibility of individual GIN genera to anthelmintic treatment in quantitative terms, as now suggested by the new WAAVP guidelines (Kaplan et al., 2023), while goats, unsurprisingly, are overlooked by the scientific literature. Sheep and goats share the same major nematode species, but the host immune response and the pharmacokinetics of anthelmintics impose a significantly higher selection pressure for AR in goats (Hoste et al., 2011; Sargison, 2011), which can then be a source of resistant GIN also for sheep. This study tested the use of genus-specific FECRT in this species, whose role in AR development and spread require even more refinement in GIN monitoring and control.

The efficacy of two anthelmintics, OXF and EPR, was tested at two dairy goat farms, in a total of four trials. Coproculture was selected to differentiate among GIN genera for practical purposes, as this technique, regardless of its well-known limitations is still the most easily conceivable in diagnostic laboratories. The use of coproculture also enabled to overcome the problem of quantifying *Oesophagostomum*, not included in our multiplex real-time PCR assay (Reslová et al., 2021), even though in our larval identification approach it was not possible to reliably differentiate *Oesophagostomum* and *Chabertia* larvae, so the contribution of each genus was not determined. Coproculture results were nevertheless substantiated to a good extent by three separate molecular tests, a multiplex real-time PCR for *Haemonchus*, *Trichostrongylus*, *Teladorsagia* and *Chabertia* and end-point PCRs for *Oesophagostomum* and *Bunostomum*. Results provided by both morphological and molecular approach are just estimates, and therefore carry a level of uncertainty around them, which can be considered a limitation in the sound interpretation of the agreement between the two methods. The good correlation between molecular and morphological estimates was anyway confirmed in our study by the Spearman's Rho correlation.

Both OXF and EPR were fully effective to control nematode burden at farm 2 and our approach indicated a 100% FECR for all the GIN genera present. The multiplex real-time PCR and the two end-point PCRs at D14 also provided negative results for all GIN genera, except for a very low amount of *Haemonchus* DNA detected in half (2/4) of the pooled samples by real-time PCR. This could be potentially explained by the exposure to +4°C temperatures during storage, which are known to negatively impact *Haemonchus* larvae development (McKenna, 1998). However, the absence of *Haemonchus* eggs in the remaining pooled samples

Table 2
FECR and 90% confidence intervals in trials of the first phase of the study. FEC at D0 and D14 refer to total counted eggs (prior transformation to EPG) among the 10 animals. [a] Oxfenil®, Virbac; [b] Eprinex Multi®, Boehringer Ingelheim. BZ = benzimidazoles; ML = macrocyclic lactones; PO = peroral. R = resistant; S = susceptible.

ID	Drug	Class	Admin. route	FEC		FECR (%)	90% CI	Efficacy
				D0	D14			
Farm 1_Ox	Oxfendazole[a]	BZ	PO	1156	279	75.9	*73.7 – 77.9*	R
Farm 1_Ep	Eprinomectin[b]	ml	pour-on	876	234	73.3	*70.7 – 75.7*	R
Farm 2_Ox	Oxfendazole[a]	BZ	PO	391	0	100	*99.2 – 100*	S
Farm2_Ep	Eprinomectin[b]	ml	pour-on	628	0	100	*99.5 – 100*	S

Table 3
FECR and 90% confidence intervals, calculated for overall strongylids and for each strongylid genus (see Supplementary materials for the calculation). FEC at D0 and D14 refer to total counted eggs (prior transformation to EPG) among the 10 animals. –, not calculable; n.d., not detected. R = resistant; S = susceptible; INC = inconclusive; LR = low resistant.

ID	Genus	FEC		FECR%	90% CI	Efficacy
		D0	D14			
Farm 1_Ox	**Overall strongylids**	**1156**	**279**	**75.9**	**73.7–77.9**	**R**
	Haemonchus	474.0	86.5	81.8	78.6–84.5	R
	Trichostrongylus	485.5	192.5	60.3	56.6–63.9	R
	Teladorsagia	11.6	0.0	100	78.8–99.6	INC
	Oesophag./Chabertia	173.4	0.0	100	98.3–100	S
	Bunostomum	11.6	0.0	100	78.8–99.6	INC
Farm 1_Ep	**Overall strongylids**	**876**	**234**	**73.3**	**70.7–75.7**	**R**
	Haemonchus	219.0	201.2	8.1	5.6–11.8	R
	Trichostrongylus	604.4	21.1	96.5	95.0–97.5	S
	Teladorsagia	17.5	0.0	100	85.1–99.7	INC
	Oesophag./Chabertia	35.0	0.0	100	92.0–99.9	S
	Bunostomum	0.0	11.7	–	–	R
Farm 2_Ox	**Overall strongylids**	**391**	**0.0**	**100**	**99.2–100**	**S**
	Haemonchus	215.1	0.0	100	98.6–100	S
	Trichostrongylus	0.0	0.0	–	–	n.d.
	Teladorsagia	0.0	0.0	–	–	n.d.
	Oesophag./Chabertia	172.0	0.0	100	98.3–100	S
	Bunostomum	3.9	0.0	100	54.3–99.0	INC
Farm 2_Ep	**Overall strongylids**	**628**	**0.0**	**100**	**99.5–100**	**S**
	Haemonchus	251.2	0.0	100	98.8–100	S
	Trichostrongylus	0.0	0.0	–	–	n.d.
	Teladorsagia	0.0	0.0	–	–	n.d.
	Oesophag./Chabertia	345.4	0.0	100	99.1–100	S
	Bunostomum	31.4	0.0	100	91.2–99.8	S

suggests its very limited presence at D14, likely detected only by molecular methods given their higher sensitivity compared to the traditional copromicroscopy. The detection of such low amount of *Haemonchus* has anyway little practical meaning, highlighting the importance of quantitative assays for a better interpretation of results. At farm 1, lack of efficacy was detected in both trials (FECR was 75.9% for OXF and 73.3% for EPR) and the changes of the nematode community following treatment were investigated. We interpolated coproculture and FEC results, at both D0 and D14, similarly to how previously done by previous studies carried out mainly in other species, as above-mentioned. The absolute egg quantification was obtained from relative percentages of abundance for each GIN genus, so that differences in susceptibility of each separate genus could be observed without the influence of other GIN response to treatment. For instance, at Farm 1_Ox trial, OXF was fully effective against *Teladorsagia*, *Oesophagostomum/Chabertia* and *Bunostomum*, while results for *Haemonchus* and *Trichostrongylus* showed an insufficient reduction (FECR was 81.8% for *Haemonchus* and 60.3% for *Trichostrongylus*). And again, in Farm 1_Ep, eprinomectin was classified as non-effective overall, though according to coproculture, all GIN were susceptible to it except for *Haemonchus*. Interestingly, *Trichostrongylus* still represented approximately 10% of L3 at D14, though with a FECR of 96.5% it was considered susceptible to EPR.

In this trial also *Bunostomum* was classified as resistant to EPR because it was detected in low amount at D14 but not at D0, drawing attention to one of the main limitations of this approach, when applied to genera with low pre-treatment burdens (McKenna, 1996). The requirement for the minimum number of eggs counted remains the same (approximately 200 eggs counted in total) for each separate genus-specific FECRT as indicated in general FECRT guidelines (COMBAR, 2021; Kaplan et al., 2023). This means that, when the genus-specific approach is applied, higher FEC are required to obtain statistically sound results, which were not achieved for *Bunostomum* and neither for *Teladorsagia* and *Oesophagostomum / Chabertia* in this trial. Moreover, the new classification system outlined by Denwood et al. (2023) and now adopted by the new WAAVP guidelines, established the CI_L and the CI_U (rather than the percentage of FECR) as the main criteria to interpret FECRT results. Since in our study, as also commonly done in research and clinical trials, genus identification was carried out on pooled samples, the 90%CI of genus-specific FECRTs were calculated based on the assumption of no variation in relative species abundance among the individual animals (Kaplan et al., 2023). This approach could be considered questionable and its results should be interpreted accordingly, but it represents a compromise as the only alternative is to have no CI. Notwithstanding these limitations, the determination of changes in the parasite community following treatment can still provide substantial help in confirming, or questioning diagnosis of AR. A number of pharmacological, technical, parasite and host-related factors could indeed confound the result of the FECRT (Morgan et al., 2022), but similar effects would be expected for all genera. On the contrary, it is realistic for only one/few species or genera to develop resistance at once. Differences in the FECRT estimation among GIN genera may more easily suggest the presence of AR and inferences could be made regarding which species may be resistant (e.g., *Haemonchus* at Farm 1_Ep trial) and which not (e.g., *Trichostrongylus* at Farm 1_Ep trial). In addition, high levels of AR in minority species or species with low fecundity can be missed by FECRT unless a genus/species-specific assessment is included (McKenna, 1990). Similarly, this approach also allows to overcome the complexity dictated by the differences in prolificity among genera (e.g. one female of *H. contortus* producing thousands of eggs/day in contrast to few hundreds/day for females of other genera (Roeber and Kahn, 2014).

Lastly, the four trials indicated a clear difference in drug efficacy between the two farms, with both anthelmintics effective in farm 2 and none in farm 1. The purpose of this study was methodological and not to detect and report the presence of AR, so further confounding factors (see Morgan et al., 2022) which might have influenced FECRT results and which might explain the differences between farms have not been thoroughly investigated. However, the presence of AR in the area where these farms are located (Trentino, Veneto and Friuli-Venezia Giulia, in Northern Italy) has never been reported and our results advocate for further investigations.

5. Conclusions

Coproculture can be very complex and time consuming to apply in

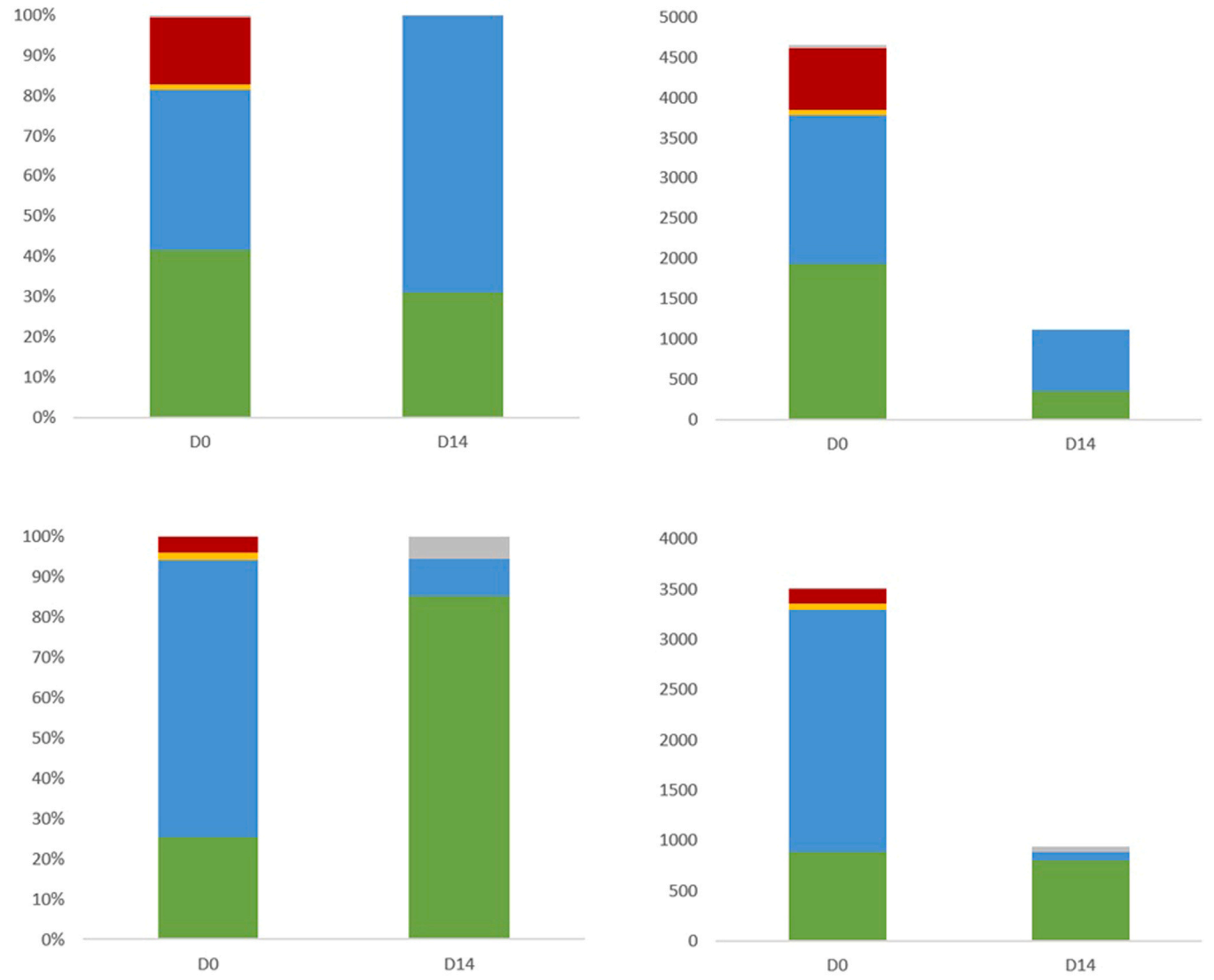

Fig. 1. Genera contribution to overall GIN burden (based on morphological identification) in Farm 1_Ox (oxfendazole, on the top) and Farm 1_Ep (eprinomectin, on the bottom) trials with data presented in terms of relative percentage (on the left) and absolute EPG numbers (on the right). *Haemonchus* = green; *Trichostrongylus* = blue; *Teladorsagia* = yellow, *Oesophagostomum / Chabertia* = red; *Bunostomum* = grey.

both large-scale research and daily diagnostics, and it is likely that new molecular tools for rapid and accurate GIN species identification will provide a more viable path to widely available the genus/species-specific FECRT. However, coproculture may still represent an interesting solution for many laboratories, especially in low-income countries where the availability of modern and more expensive technologies is limited. This study, based on coproculture but substantiated by molecular analyses, implemented the genus-specific assessment of anthelmintic efficacy in goats, up to now overlooked. The quantification of each genus/species in both pre- and post-treatment could indeed allow for more evidence-based decisions in GIN control, which is pivotal in this species for its unfortunate predisposition towards AR development. The considereably improved FECRT interpretation obtained from our results calls for new attention and efforts towards the use of this approach in the European context, where it is still not commonly employed in FECRT-based studies. Lastly, the results obtained at farm 1 indicated the possible presence of AR in the area, suggesting the need for a wider survey.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Anna Maurizio: Data curation, Formal analysis, Investigation, Visualization, Writing - original draft; **Lucie Škorpíková:** Formal analysis, Investigation, Writing - review & editing; **Jana Ilgová:** Formal analysis, Investigation, Writing - review & editing; **Cinzia Tessarin:** Investigation, Resources; **Giorgia Dotto:** Investigation, Resources; **Nikol Reslová:** Methodology, Investigation, Writing - review & editing; **Jaroslav Vadlejch:** Supervision, Writing - review & editing; **Erica Marchiori:** Validation, Writing - review & editing; **Antonio Frangipane di Regalbono:** Conceptualization, Supervision, Writing - review & editing; **Martin Kašný:** Methodology; **Rudi Cassini:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetpar.2024.110146.

References

Avramenko, R.W., Redman, E.M., Lewis, R., Yazwinski, T.A., Wasmuth, J.D., Gilleard, J. S., 2015. Exploring the gastrointestinal "nemabiome": deep amplicon sequencing to quantify the species composition of parasitic nematode communities. PLoS One 10, 1–18. https://doi.org/10.1371/journal.pone.0143559.

Bartley, D.J., Donnan, A.A., Jackson, E., Sargison, N., Mitchell, G.B.B., Jackson, F., 2006. A small scale survey of ivermectin resistance in sheep nematodes using the faecal egg count reduction test on samples collected from Scottish sheep. Vet. Parasitol. 137, 112–118. https://doi.org/10.1016/j.vetpar.2005.12.014.

Bordes, L., Dumont, N., Lespine, A., Souil, E., Sutra, J.F., Prévot, F., Grisez, C., Romanos, L., Dailledouze, A., Jacquiet, P., 2020. First report of multiple resistance to eprinomectin and benzimidazole in Haemonchus contortus on a dairy goat farm in France. Parasitol. Int. 76, 102063 https://doi.org/10.1016/j.parint.2020.102063.

Bosco, A., Kießler, J., Amadesi, A., Varady, M., Hinney, B., Ianniello, D., Maurelli, M.P., Cringoli, G., Rinaldi, L., 2020. The threat of reduced efficacy of anthelmintics against gastrointestinal nematodes in sheep from an area considered anthelmintic resistance-free. Parasites Vectors 13, 1–12. https://doi.org/10.1186/s13071-020-04329-2.

Bott, N.J., Campbell, B.E., Beveridge, I., Chilton, N.B., Rees, D., Hunt, P.W., Gasser, R.B., 2009. A combined microscopic-molecular method for the diagnosis of strongylid infections in sheep. Int. J. Parasitol. 39, 1277–1287. https://doi.org/10.1016/j.ijpara.2009.03.002.

Bull, K., Glover, M.J., Rose Vineer, H., Morgan, E.R., 2022. Increasing resistance to multiple anthelmintic classes in gastrointestinal nematodes on sheep farms in southwest England. Vet. Rec. 190 https://doi.org/10.1002/vetr.1531.

Cazajous, T., Prevot, F., Kerbiriou, A., Milhes, M., Grisez, C., Tropee, A., Godart, C., Aragon, A., Jacquiet, P., 2018. Multiple-resistance to ivermectin and benzimidazole of a Haemonchus contortus population in a sheep flock from mainland France, first report. Vet. Parasitol. Reg. Stud. Rep. 14, 103–105. https://doi.org/10.1016/j.vprsr.2018.09.005.

Čerňanská, D., Várady, M., Čorba, J., 2006. A survey on anthelmintic resistance in nematode parasites of sheep in the Slovak Republic. Vet. Parasitol. 135, 39–45. https://doi.org/10.1016/j.vetpar.2005.09.001.

Coles, G.C., Bauer, C., Borgsteede, F.H.M., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P. J., 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44, 35–44. https://doi.org/10.1016/0304-4017(92)90141-U.

Coles, G.C., Jackson, F., Pomroy, W.E., Prichard, R.K., Von Samson-Himmelstjerna, G., Silvestre, A., Taylor, M.A., Vercruysse, J., 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 136, 167–185. https://doi.org/10.1016/j.vetpar.2005.11.019.

COMBAR, 2021. Faecal egg count reduction test (FECRT) protocol. Gastrointestinal nematodes - SHEEP and GOATS 1–5.

De Graef, J., Sarre, C., Mills, B.J., Mahabir, S., Casaert, S., De Wilde, N., Van Weyenberg, M., Geldhof, P., Marchiondo, A., Vercruysse, J., Meeus, P., Claerebout, E., 2012. Assessing resistance against macrocyclic lactones in gastrointestinal nematodes in cattle using the faecal egg count reduction test and the controlled efficacy test. Vet. Parasitol. 189, 378–382. https://doi.org/10.1016/j.vetpar.2012.04.040.

Denwood, M.J., Kaplan, R.M., McKendrick, I.J., Thamsborg, S.M., Nielsen, M.K., Levecke, B., 2023. A statistical framework for calculating prospective sample sizes and classifying efficacy results for faecal egg count reduction tests in ruminants, horses and swine. Vet. Parasitol. 314, 109867 https://doi.org/10.1016/j.vetpar.2022.109867.

Do Amarante, A.F.T., 2011. Why is it important to correctly identify Haemonchus species? Rev. Bras. Parasitol. Veter-.-. ária 20, 263–268. https://doi.org/10.1590/s1984-29612011000400002.

Dobson, R.J., Hosking, B.C., Jacobson, C.L., Cotter, J.L., Besier, R.B., Stein, P.A., Reid, S. A., 2012. Preserving new anthelmintics: A simple method for estimating faecal egg count reduction test (FECRT) confidence limits when efficacy and/or nematode aggregation is high. Vet. Parasitol. 186, 79–92. https://doi.org/10.1016/j.vetpar.2011.11.049.

El-Abdellati, A., Geldhof, P., Claerebout, E., Vercruysse, J., Charlier, J., 2010. Monitoring macrocyclic lactone resistance in Cooperia oncophora on a Belgian cattle farm during four consecutive years. Vet. Parasitol. 171, 167–171. https://doi.org/10.1016/j.vetpar.2010.03.003.

Elmahalawy, S.T., Halvarsson, P., Skarin, M., Höglund, J., 2018. Droplet digital polymerase chain reaction (ddPCR) as a novel method for absolute quantification of major gastrointestinal nematodes in sheep. Vet. Parasitol. 261, 1–8. https://doi.org/10.1016/j.vetpar.2018.07.008.

Falzon, L.C., Menzies, P.I., Shakya, K.P., Jones-Bitton, A., Vanleeuwen, J., Avula, J., Stewart, H., Jansen, J.T., Taylor, M.A., Learmount, J., Peregrine, A.S., 2013. Anthelmintic resistance in sheep flocks in Ontario, Canada. Vet. Parasitol. 193, 150–162. https://doi.org/10.1016/j.vetpar.2012.11.014.

Halvarsson, P., Höglund, J., 2021. Sheep nemabiome diversity and its response to anthelmintic treatment in Swedish sheep herds. Parasites Vectors 14 (1), 12. https://doi.org/10.1186/s13071-021-04602-y.

Hoste, H., Sotiraki, S., Torres-Acosta, J.F., de, J., 2011. Control of Endoparasitic Nematode Infections in Goats. Vet. Clin. North Am. - Food Anim. Pract. 27, 163–173. https://doi.org/10.1016/j.cvfa.2010.10.008.

Jacobson, C., Larsen, J.W., Besier, R.B., Lloyd, J.B., Kahn, L.P., 2020. Diarrhoea associated with gastrointestinal parasites in grazing sheep. Vet. Parasitol. 282, 109139 https://doi.org/10.1016/j.vetpar.2020.109139.

Kaplan, R., Denwood, M., Nielsen, M., Thamsborg, S., Torgerson, P., Gilleard, J., Dobson, R., Vercruysse, J., Levecke, B., 2023. A new World Association for the Advancement of Veterinary Parasitology (WAAVP) guideline for diagnosing anthelmintic resistance using the faecal egg count reduction test (FECRT). Vet. Parasitol. 318, 109936 https://doi.org/10.1016/j.vetpar.2023.109936.

Knoll, S., Dessì, G., Tamponi, C., Meloni, L., Cavallo, L., Mehmood, N., Jacquiet, P., Scala, A., Cappai, M.G., Varcasia, A., 2021. Practical guide for microscopic identification of infectious gastrointestinal nematode larvae in sheep from Sardinia, Italy, backed by molecular analysis. Parasites Vectors 14 (1), 14. https://doi.org/10.1186/s13071-021-05013-9.

Kotze, A.C., Prichard, R.K., 2016. Anthelmintic Resistance in Haemonchus contortus. History, Mechanisms and Diagnosis., Advances in Parasitology. Elsevier Ltd. https://doi.org/10.1016/bs.apar.2016.02.012.

Lambertz, C., Poulopoulou, I., Wuthijaree, K., Gauly, M., 2019. Anthelmintic resistance in gastrointestinal nematodes in sheep raised under mountain farming conditions in Northern Italy. Vet. Rec. Open 6, 1–7. https://doi.org/10.1136/vetreco-2018-000332.

Leathwick, D.M., Miller, C.M., 2013. Efficacy of oral, injectable and pour-on formulations of moxidectin against gastrointestinal nematodes in cattle in New Zealand. Vet. Parasitol. 191, 293–300. https://doi.org/10.1016/j.vetpar.2012.09.020.

Leathwick, D.M., Miller, C.M., Sauermann, C.W., Candy, P.M., Ganesh, S., Fraser, K., Waghorn, T.S., 2016. The efficacy and plasma profiles of abamectin plus levamisole combination anthelmintics administered as oral and pour-on formulations to cattle. Vet. Parasitol. 227, 85–92. https://doi.org/10.1016/j.vetpar.2016.07.031.

Ljungström, S., Melville, L., Skuce, P.J., Höglund, J., 2018. Comparison of four diagnostic methods for detection and relative quantification of Haemonchus contortus eggs in feces samples. Front. Vet. Sci. 4 https://doi.org/10.3389/fvets.2017.00239.

Lyndal-Murphy, M., Ehrlich, W., Mayer, D., 2014. Anthelmintic resistance in ovine gastrointestinal nematodes in inland southern Queensland. Aust. Vet. J. 92, 415–420. https://doi.org/10.1111/avj.12250.

MAFF, 1986. Manual of Veterinary Parasitological Laboratory Techniques, Third ed. H. M.S.O., London.

Mahieu, M., Ferré, B., Madassamy, M., Mandonnet, N., 2014. Fifteen years later, anthelmintic resistances have dramatically spread over goat farms in Guadeloupe. Vet. Parasitol. 205, 379–384. https://doi.org/10.1016/j.vetpar.2014.07.029.

McIntyre, J., Hamer, K., Morrison, A.A., Bartley, D.J., Sargison, N., Devaney, E., Laing, R., 2018. Hidden in plain sight - Multiple resistant species within a strongyle community. Vet. Parasitol. 258, 79–87. https://doi.org/10.1016/j.vetpar.2018.06.012.

McKenna, P.B., 1998. The effect of previous cold storage on the subsequent recovery of infective third stage nematode larvae from sheep faeces. Vet. Parasitol. 80, 167–172. https://doi.org/10.1016/S0304-4017(98)00203-9.

McKenna, P.B., 1997. Further potential limitations of the undifferentiated faecal egg count reduction test for the detection of anthelmintic resistance in sheep. N. Z. Vet. J. 45, 244–246. https://doi.org/10.1080/00480169.1997.36038.

McKenna, P.B., 1996. Potential limitations of the undifferentiated faecal egg count reduction test for the detection of anthelmintic resistance in sheep. N. Z. Vet. J. 44, 73–75. https://doi.org/10.1080/00480169.1996.35938.

McKenna, P.B., 1990. The detection of anthelmintic resistance by the faecal egg count reduction test: an examination of some of the factors affecting performance and interpretation. N. Z. Vet. J. 38, 142–147. https://doi.org/10.1080/00480169.1990.35640.

McMahon, C., Bartley, D.J., Edgar, H.W.J., Ellison, S.E., Barley, J.P., Malone, F.E., Hanna, R.E.B., Brennan, G.P., Fairweather, I., 2013. Anthelmintic resistance in Northern Ireland (I): Prevalence of resistance in ovine gastrointestinal nematodes, as determined through faecal egg count reduction testing. Vet. Parasitol. 195, 122–130. https://doi.org/10.1016/j.vetpar.2013.01.006.

Mickiewicz, M., Czopowicz, M., Kawecka-Grochocka, E., Moroz, A., Szaluś-Jordanow, O., Várady, M., Königová, A., Spinu, M., Górski, P., Bagnicka, E., Kaba, J., 2020. The first report of multidrug resistance in gastrointestinal nematodes in goat population in Poland. BMC Vet. Res. 16, 1–12. https://doi.org/10.1186/s12917-020-02501-5.

Morgan, E.R., Lanusse, C., Rinaldi, L., Charlier, J., Vercruysse, J., 2022. Confounding factors affecting faecal egg count reduction as a measure of anthelmintic efficacy. Parasite 29. https://doi.org/10.1051/parasite/2022017.

Playford, M.C., Smith, A.N., Love, S., Besier, R.B., Kluver, P., Bailey, J.N., 2014. Prevalence and severity of anthelmintic resistance in ovine gastrointestinal nematodes in Australia (2009-2012). Aust. Vet. J. 92, 464–471. https://doi.org/10.1111/avj.12271.

Rendell, D.K., 2010. Anthelmintic resistance in cattle nematodes on 13 south-west Victorian properties. Aust. Vet. J. 88, 504–509. https://doi.org/10.1111/j.1751-0813.2010.00648.x.

Reslova, N., Skorpikova, L., Kyrianova, I.A., Vadlejch, J., Höglund, J., Skuce, P., Kasny, M., 2021. The identification and semi-quantitative assessment of gastrointestinal nematodes in faecal samples using multiplex real-time PCR assays. Parasites Vectors 14 (1), 12. https://doi.org/10.1186/s13071-021-04882-4.

Roeber, F., Kahn, L., 2014. The specific diagnosis of gastrointestinal nematode infections in livestock: larval culture technique, its limitations and alternative DNA-based approaches. Vet. Parasitol. 205, 619–628. https://doi.org/10.1016/j.vetpar.2014.08.005.

Roeber, F., Larsen, J.W.A., Anderson, N., Campbell, A.J.D., Anderson, G.A., Gasser, R.B., Jex, A.R., 2012. A molecular diagnostic tool to replace larval culture in conventional faecal egg count reduction testing in sheep. PLoS One 7. https://doi.org/10.1371/journal.pone.0037327.

Roeber, F., Morrison, A., Casaert, S., Smith, L., Claerebout, E., Skuce, P., 2017. Multiplexed-tandem PCR for the specific diagnosis of gastrointestinal nematode infections in sheep: an European validation study. Parasites Vectors 10, 1–11. https://doi.org/10.1186/s13071-017-2165-x.

Rose Vineer, H., Morgan, E.R., Hertzberg, H., Bartley, D.J., Bosco, A., Charlier, J., Chartier, C., Claerebout, E., De Waal, T., Hendrickx, G., Hinney, B., Höglund, J., Jez Ek, J.I., Kašný, M., Keane, O.M., Martínez-Valladares, M., Mateus, T.L., McIntyre, J., Mickiewicz, M., Munoz, A.M., Phythian, C.J., Ploeger, H.W., Rataj, A.V., Skuce, P.J., Simin, S., Sotiraki, S., Spinu, M., Stuen, S., Thamsborg, S.M., Vadlejch, J., Varady, M., Von Samson-Himmelstjerna, G., Rinaldi, L., 2020. Increasing importance of anthelmintic resistance in European livestock: creation and meta-analysis of an open database. Parasite 27. https://doi.org/10.1051/parasite/2020062.

Rossanigo, C.E., Gruner, L., 1996. The length of strongylid nematode infective larvae as a reflection of developmental conditions in faeces and consequences on their viability. Parasitol. Res. 82, 304–311. https://doi.org/10.1007/s004360050118.

Sargison, N.D., 2011. Pharmaceutical control of endoparasitic helminth infections in sheep. Vet. Clin. North Am. - Food Anim. Pract. 27, 139–156. https://doi.org/10.1016/j.cvfa.2010.10.014.

Vadlejch, J., Kopecký, O., Kudrnáčová, M., Čadková, Z., Jankovská, I., Langrová, I., 2014. The effect of risk factors of sheep flock management practices on the development of anthelmintic resistance in the Czech Republic. Small Rumin. Res. 117, 183–190. https://doi.org/10.1016/j.smallrumres.2014.01.003.

Vercruysse, J., Charlier, J., Van Dijk, J., Morgan, E.R., Geary, T., Von Samson-Himmelstjerna, G., Claerebout, E., 2018. Control of helminth ruminant infections by 2030. Parasitology 145, 1655–1664. https://doi.org/10.1017/S003118201700227X.

Waghorn, T.S., Leathwick, D.M., Rhodes, A.P., Jackson, R., Pomroy, W.E., West, D.M., Moffat, J.R., 2006a. Prevalence of anthelmintic resistance on 62 beef cattle farms in the North Island of New Zealand. N. Z. Vet. J. 54, 278–282. https://doi.org/10.1080/00480169.2006.36711.

Waghorn, T.S., Leathwick, D.M., Rhodes, A.P., Lawrence, K.E., Jackson, R., Pomroy, W. E., West, D.M., Moffat, J.R., 2006b. Prevalence of anthelmintic resistance on sheep farms in New Zealand. N. Z. Vet. J. 54, 271–277. https://doi.org/10.1080/00480169.2006.36710.

Wang, C.R., Gao, J.F., Zhu, X.Q., Zhao, Q., 2012. Characterization of Bunostomum trigonocephalum and Bunostomum phlebotomum from sheep and cattle by internal transcribed spacers of nuclear ribosomal DNA. Res. Vet. Sci. 92, 99–102. https://doi.org/10.1016/j.rvsc.2010.10.024.

Wyk, J.A.Van, Cabaret, J., Michael, L.M., 2004. Morphological identification of nematode larvae of small ruminants and cattle simplified, 119, 277–306. https://doi.org/10.1016/j.vetpar.2003.11.012.

Zanzani, S.A., Gazzonis, A.L., Di Cerbo, A., Varady, M., Manfredi, M.T., 2014. Gastrointestinal nematodes of dairy goats, anthelmintic resistance and practices of parasite control in Northern Italy. BMC Vet. Res. 10, 1–10. https://doi.org/10.1186/1746-6148-10-114.