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Comparing pooled and individual samples for estimation of gastrointestinal strongyles burden and treatment efficacy in small ruminants

Anna Maurizio *, Erica Marchiori, Cinzia Tessarin, Rudi Cassini

Deparment of Animal Medicine, Production and Health, University of Padova, Viale dell'Università, 16, 35020 Legnaro, PD, Italy

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ABSTRACT

Monitoring endoparasite burden (FEC) and treatment efficacy (FECR) is a key element of sustainable parasite control. However, the costs of the analysis often discourage their implementation by farmers and veterinary practitioners. Pooling samples is considered to be a good alternative to reduce time and monetary costs, but limited data are available on the use of pooled samples in small ruminants, especially for goats. In this study, data collected over the years in sheep and goat farms were analyzed, and results obtained from individual and pooled analysis were compared for the purposes of FEC and FECR assessment. A total of 801 individual and 134 pooled samples (composed of 3–12 individual samples) were included. For FECR testing, 2 pools of 5 samples each were created per trial and the same animals were sampled at day 0 (D0 – treatment day) and 14 days after (D14). Samples were analyzed by McMaster technique (limit of detection 20 EPG). Results from pooled and individual FEC were not significantly different (Wilcoxon signed-rank test) and correlation (Spearman's rank test) was high for all sub-categories, although agreement (Lin's concordance correlation) was often classified as poor. Results were not influenced by the pool size (<6 or ≥ 6). Interpretation of treatment efficacy between the two methods was comparable for all sheep trials, while it differed for goats in 4 out of 10 trials. Wilcoxon signed-rank test indicated a non significant difference between pooled and individual FECR. However, correlation and agreement between FECR were considerably better for sheep compared to goats, for which they were very limited, despite the correlation between FEC at D0 and D14 was always high. According to our results, pooled FECR can be a good option but the absence of 95 %CI represents a major drawbacks in the interpretation of results. Further studies on the topic for goats are needed.

1. Introduction

Gastrointestinal strongyles (GIS) are among the main constraints to small ruminant farming. Assessing GIS burden is key for sustainable and effective control of parasites and the current spread of resistance to anthelmintic drugs makes it even more crucial. Targeting treatments more efficiently is critical to preserve the efficacy of anthelmintics on the long term (Kenyon and Jackson, 2012). In a group of animals, this is generally achieved using parasitological techniques to estimate the infection burden. Burden estimation traditionally relies on faecal egg count (FEC) techniques, which are widely employed in parasitological laboratories (Gilleard et al., 2021) and are also recommended (Coles et al., 1992; COMBAR, 2021) to evaluate anthelmintic efficacy through the FEC reduction test (FECRT). In FECR testing, results of a pre-treatment FEC are compared with those of a FEC performed on the same animals 7–21 days post-treatment, the exact period depending on the class of drug used. The efficacy of the drug is subsequently calculated based on the obtained reduction of the FEC. Although other methods are available to test anthelmintic efficacy, currently FECRT is the preferred tool for detecting and monitoring AR in both common practice and research studies (Calvete and Uriarte, 2013; Morgan et al., 2022). Several quantitative copromicroscopic methods with different sensitivity are available, the most common being McMaster (limit of detection: 15–50 Eggs Per Gram - EPG), followed by Mini-FLOTAC (limit of detection: 5 EPG), which is especially indicated when low numbers of EPG are expected (such as in cattle) (Cringoli et al., 2017). When using FEC to assess the parasitic burden at farm level, the accuracy of the estimation is influenced by the number of samples analysed. However, given the subclinical nature of endoparasite infections and the poor perception of the risk of AR, the time and cost required for the analysis often remain perceived as excessive and unnecessary, and treatments are still performed without prior diagnosis (Kenyon and Jackson, 2012).

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

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One possible solution to overcome this major limitation is the use of composite samples, in which samples from several individuals are pooled together and analysed as one. Several studies investigated the correspondence between composite and individual samples for the assessment of the infection burden and anthelmintic efficacy, through both modelling (Morgan et al., 2005) and field studies. In sheep high levels of correlation or agreement were generally found between pooled and individual samples (Morgan et al., 2005; Rinaldi et al., 2014; Ward et al., 1997), regardless of the number of samples included in the pool (n < 20 (Maurizio et al., 2021a)) and the analytic sensitivity of the FEC technique employed. Comparable results were obtained in FECRT trials, also performed in areas with very different frequencies of resistance (Kenyon et al., 2016; Rinaldi et al., 2014). Correspondence of FEC and FECRT was also investigated in cattle, while only preliminary results were published on the topic for FEC in goats (Maurizio et al., 2021c). Hence, the aim of the study was to further evaluate the performance of pooled samples compared to individual sampling, in assessing the parasite burden at farm level in sheep and goats. Additionally, this study also provides the first evaluation on the use of composite samples for FECR testing in goats and additional data on composite FECRT in sheep.

2. Materials and methods

2.1. Study design and sample collection

The present study is inserted in the framework of a wider monitoring survey on endoparasites and AR in small ruminants of North-eastern Italy. Individual faecal samples were collected from the rectum of animals from 19 goat and 27 sheep farms. Samples were individually identified using ear tag codes. Fecal samples were 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 h after collection. For each farm, 1–6 pools were created with faeces from 3 to 12 individuals. Composition and size of pools were based on the number of samples collected per farm (Maurizio et al., 2021b), with respect of in-farm managerial division of animals. In parallel, in 8 out of 19 goat farms and in 6 out of 27 sheep farms a FECRT trial was conducted, so animals were sampled (and treated) on day 0 (D0) and again 14 days post-treatment (D14). These farms were selected with the support of local veterinary practitioners based on risk factors for endoparasites (i.e. use of pasture) and on suspicion of reduced drug efficacy. Depending on the farm, treatment was performed with:

1. Benzimidazoles (BZ):

- Sverminator® (Albendazole) - oral suspension, Fatro (TR6, TR15, TR16)
- Panacur 2,5 %® (Fenbendazole) – oral suspension, MSD (TR5)
- Oxfenil® (Oxfendazole) – oral suspension, Virbac (TR1, TR3, TR9)
- Contruerme® (Albendazole) – oral suspension, Izo (TR7)

2. Avermectins (AVM):

- Ivomec Ovini® (Ivermectin) – solution for subcutaneous injection, Boerhinger Ingelheim (TR11, TR12, TR13)
- Ivomec plus® (Ivermectin and Clorsulon) – solution for subcutaneous injection, Boerhinger Ingelheim (TR8, TR10)
- Eprinex Multi® (Eprinomectin) – pour-on, Boerhinger Ingelheim (TR2, TR4, TR14)

Dosage was doubled for goats (Hoste et al., 2011). In farms included in the FECRT trial, two pools of 5 animals each per farm were created and the same pools where maintained also for sampling at D14. If it was not possible to collect faeces at D14 from an animal included in a pool at D0, the respective post-treatment pool was composed without that sample.

2.2. Laboratory analysis

All pooled and individual faecal samples were analyzed with McMaster method (analytical sensitivity of 20 EPG), in order to compare the results of individual and pooled analyses. Briefly, for each individual sample 5 g of feces were diluted in 30 ml sucrose-sodium nitrate solution with 1300 specific gravity in a falcon tube. The content was gently mixed until homogenization and an aliquot collected with a Pasteur pipette through a double-layer gauze. The aspired liquid was then used to fill the chambers of a McMaster slide. After waiting a few minutes, eggs were counted in both chambers. All type of eggs were recorded separately (Strongylid, *Nematodirus* spp./*Mashallagia* spp., *Strongyloides papillosus, Skrjabinema* spp., *Trichuris* spp., *Capillaria* spp. and Cestoda eggs). Pools were created thoroughly mixing equal amount of faeces from each animal. For each pool, 5 g of feces were analyzed as described for the individual samples. In FECR trials based on individuals samples, if the egg count at D0 did not reach up to at least 200 eggs overall, a second aliquot of each of the 10 samples was re-examined (COMBAR, 2021). The same number of aliquots was examined at D14.

2.3. Data analysis

For the purpose of data analysis and because of their minor relevance, genera other than Strongylids were excluded from the analysis. *Nematodirus* spp./*Marshallagia* spp. eggs were considered together with the Strongylid eggs, hereafter referred to as "GIS" eggs.

The arithmetic mean of individual FEC was calculated and compared with the FEC obtained processing the respective pool. Anthelmintic efficacy was first evaluated according to the COMBAR guidelines (COMBAR, 2021), calculating FECR and 95 % confidence intervals (95 %CI) through an on-line analysis program (R package 'eggCounts' (Wang et al., 2018)). However, since this tool was not designed for pooled FECRT, FECR was also calculated with the formula: FECR % = $((EGGS_{pre} - EGGS_{post}) / EGGS_{pre}) \times 100$ (Coles et al., 1992), which is also recommended to calculate pooled FECR (Kaplan, 2020). In this formula, EGGSpre and EGGSpost indicate the mean number of eggs counted in the two pools or in the 10 individual samples, pre- and post-treatment respectively. For individual FECR, the 95 %CI was also calculated (Levecke et al., 2018).

Anthelmintic treatment efficacy was then interpreted according to Denwood et al. (2023), which anticipated the upcoming revised WAAVP guidelines. Values of 90 % and 95 % have been maintained as minimum efficacy target and expected efficacy respectively (Coles et al., 1992). The classification was resistant (R) when the upper limit of the 95 %CI (95 $\%CI_U$) < 95 %, low resistant (LR, a sub-category of the previous) when the lower limit of the 95 %CI (95 $\%CI_L$) ≥ 90 %, inconclusive (INC) when 95 $\%CI_U \geq 95$ % and 95 $\%CI_L < 90$ % and susceptible (S) when 95 $\%CI_U \geq 95$ % and 95 $\%CI_L \geq 90$. For composite samples, for which 95 % CI are not available, FECR results were interpreted according to Kaplan (Kaplan, 2020): effective with no evidence of resistance (S) when > 95 %, reduced efficacy with suspected resistance (SR) when between 90 % and 95 %, reduced efficacy with likely resistance (RL) when between 80 % and 90 % and ineffective with highly likely resistance (RHL) when < 80 %. For a matter of comparison, we considered in agreement S with S, INC and LR with SR, R with RL/RHL for individual and pooled efficacy respectively.

2.3.1. Concordance and agreement in FEC and FECR

Statistical testing followed the approach previously adopted in the literature (Bosco et al., 2020; George et al., 2017; Rinaldi et al., 2019). The nonparametric Spearman correlation coefficient (rho), and the relative 95 %CI were used to measure the correlation between individual and pooled FEC. Lin's concordance correlation coefficient (CCC), with corresponding 95 %CI, were also calculated to assess the agreement between the two methods of analysis. According to the CCC value, the strength of agreement was classified as poor (<0.9), moderate

(0.90–0.95), substantial (0.95–0.99) or almost perfect (>0.99) (McBride, 2005). Spearman correlation coefficient and Lin's CCC were evaluated overall and then separately for each host species, for different pool sizes (n < 6, n ≥ 6) and, in FECR trials, for FEC at D0 and at D14. The threshold for the pool size was selected to form two classes roughly homogeneous in number, in order to compare the results obtained from pools of smaller and larger size. The correlation and agreement between individual and pooled FECR were assessed as per FEC. Spearman correlation coefficient and Lin's CCC were again evaluated overall and then separately for each species. In addition, Wilcoxon signed rank test with continuity correction was employed to compare the median of individual and pooled analysis for FEC and FECR overall and for the above-mentioned sub-categories (species, pool size, D0, D14). In this test, p-value > 0.05 indicates when the median of two methods is not significantly different. To clarify the effect of the species and pool size (explanatory variables) on the results, a multivariable linear model with log(abs(pool-individual)/mean(pool,individual)) as response variable was performed. Boxplots were also used to display the effect of species and pool size. Finally, the Bland Altman plot, which graphically describes the agreement between the two methods (Bland and Altman, 1999), was also implemented to complement the statistical analyses. The level of significance was set at a p-value < 0.05 for all tests. All statistical analyses were performed in the statistical software R version 4.2.1 (R Core Team 2022).

3. Results

A total of 801 individual and 134 pooled samples were analysed. Fig. 1 summarizes their distribution according to species and pool size and the FECR testing design.

The mean values obtained from individual samples showed high correlation with the corresponding pooled FEC (Table 1). Correlation was slightly lower for goats (rho=0.92) compared to sheep (rho=0.95), and for smaller pool size (rho=0.93) compared to size ≥ 6 (rho=0.95). Wilcoxon signed rank test confirmed that FEC obtained with the two methods were not significantly different (p = 0.44). According to Lin's CCC, agreement was poor (CCC<0.9) for all comparisons except for sheep and pool size ≥ 6 subcategories, for which it was moderate (0.9 < CCC < 0.95). In the Bland Altman plot (Fig. 2) the relation between the two methods with the range of agreement (± 1.96*sd*) is graphically represented. The funnel-shaped graph shows an increase in variability parallel with the increase of the true mean, but differences seem not to tend distinctively neither towards over- nor underestimation. The effect of species and pool size was displayed by plotting the relative difference adjusted to the mean in Fig. 3, which shows comparable boxplots between both species and between pool sizes < 6 and ≥ 6. Moreover, in the multivariable linear model the effect of these two variables was not significant (p = 0.148 and 0.243 respectively), indicating that neither influenced the results.

FECRT results are presented in Table 2. The selection criteria of the farms allowed to achieve a good variability in terms of FEC reduction, ranging from negative values (i.e. increase in FEC compared to the pre-treatment) to 100 % efficacy. Interpretation of treatment efficacy between the two methods was comparable for all sheep trials, while it differed for goats in 4 out of 10 trials (TR2, TR4, TR6 and TR9). Overall correlation between the individual and the pooled approach was 0.66, with huge difference between sheep (rho=1, p < 0.001) and goats (rho=0.40, p = 0.25) (Table 3), even though considering FEC at D0 and at D14, correlation was always higher than 0.78. Indeed, from Wilcoxon signed rank test, FECR obtained from individual analyses was not significantly different (p = 0.95) from the pooled counterpart for both sheep and goats. Agreement was moderate (0.9 < CCC < 0.95) for overall FECR and sheep FECR, poor (CCC<0.9) for goats FECR. The limits of agreement are represented in the Bland Altman plot (Fig. 4), which also emphasizes the different range of values within which sheep and goat FECR are distributed.

FEC	Total	Sheep	Goats
n° of farms involved	46	27	19
n° of individual samples analyzed	801	377	424
n° of pooled samples analyzed	134	57	77

FECRT	Total	Sheep	Goats
n° of FECR trials	16	6	10
n° of individual samples analyzed	317	118	199
n° of pooled samples analyzed	64	24	40

FECRT trials - Goats		D0	D14
TR1	BZ	2 pools of 5 samples each	= D0
TR2	AVM	2 pools of 5 samples each	= D0
TR3	BZ	2 pools of 5 samples each	= D0
TR4	AVM	2 pools of 5 samples each	= D0
TR5	BZ	2 pools of 5 samples each	= D0
TR6	BZ	2 pools of 5 samples each	= D0
TR7	BZ	2 pools of 5 samples each	= D0
TR8	AVM	2 pools of 5 samples each	1 pool of 5 samples 1 pool of 4 samples
TR9	BZ	2 pools of 5 samples each	= D0
TR10	AVM	2 pools of 5 samples each	= D0

N° pooled samples based on the pool size:

	Sheep	Goats
➢ 3 samples		2
➢ 4 samples	2	3
➢ 5 samples	22	52
➢ 6 samples	9	3
➢ 7 samples	6	10
➢ 8 samples	10	6
➢ 9 samples	1	
➢ 10 samples	3	1
➢ 11 samples	1	
➢ 12 samples	2	

FECRT trials - Sheep		D0	D14
TR11	AVM	2 pools of 5 samples each	= D0
TR12	AVM	2 pools of 5 samples each	= D0
TR13	AVM	2 pools of 5 samples each	= D0
TR14	AVM	2 pools of 5 samples each	1 pool of 4 samples 1 pool of 5 samples
TR15	BZ	2 pools of 5 samples each	1 pool of 5 samples 1 pool of 4 samples
TR16	BZ	2 pools of 5 samples each	= D0

Fig. 1. Number of sheep and goat farms, individual faecal samples and pooled samples used for the study. D0 = pre-treatment; D14 = post-treatment; BZ = benzimidazole; AVM = avermectin.

Table 1
Spearman's rank correlation coefficient (rho), Lin's concordance correlation coefficient (CCC) and Wilcoxon signed rank test statistic (W), between FEC from individual and pooled samples in different subsets. Means are reported in counted eggs.

	n° pools	Individual mean *(Dev.St)*	Pooled mean *(Dev.St)*	rho	95 %CI	CCC	95 %CI	W	p-value
OVERALL									
FEC	134	32.3 *(83.0)*	34.0 *(45.6)*	0.94	0.92–0.96	0.80	0.73–0.85	3615	0.435
pool size <6	53	46.0 *(106.6)*	41.7 *(49.3)*	0.93	0.89–0.95	0.76	0.65–0.84	1526	0.236
pool size ≥6	81	19.0 *(46.5)*	22.2 *(36.9)*	0.95	0.91–0.97	0.91	0.86–0.94	428	0.591
FEC at D0	32	80.9 *(152.5)*	77.5 *(57.9)*	0.84	0.70–0.92	0.62	0.36–0.79	268	0.948
FEC at D14	32	17.7 *(42.6)*	16.0 *(21.9)*	0.90	0.81–0.95	0.91	0.84–0.95	221	0.456
GOATS									
FEC	77	37.2 *(101.6)*	33.3 *(48.0)*	0.92	0.88–0.95	0.76	0.66–0.84	1146	0.090
FEC at D0	20	83.6 *(177.7)*	71.7 *(58.2)*	0.80	0.56–0.92	0.57	0.23–0.79	124	0.490
FEC at D14	20	7.9 *(19.4)*	5.7 *(7.5)*	0.78	0.51–0.91	0.80	0.65–0.89	85.5	0.156
SHEEP									
FEC	57	26.9 *(54.5)*	35.0 *(42.7)*	0.95	0.91–0.97	0.90	0.83–0.94	666	0.384
FEC at D0	12	76.5 *(98.1)*	87.1 *(58.7)*	0.80	0.43–0.94	0.78	0.45–0.92	29	0.456
FEC at D14	12	34.3 *(62.1)*	33.2 *(27.3)*	0.96	0.86–0.99	0.89	0.71–0.96	36	0.845

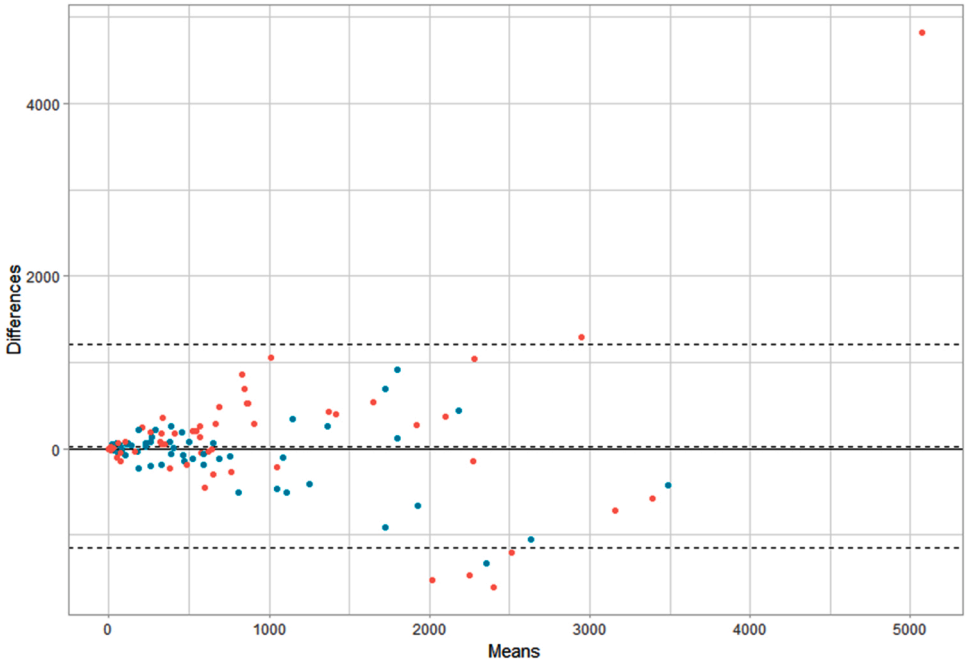

Fig. 2. Bland-Altman plot of difference (y-axis) against mean (x-axis) for FEC of individual and pooled analyses for sheep (green) and goats (red).

4. Discussion

With the increasing spread of anthelmintic resistance (Rose Vineer et al., 2020), cost-saving strategies, such as pooling samples, are needed to encourage the use of FEC and FECRT by farmers, in order to monitor endoparasite burdens, to target treatments more effectively and to detect the onset of resistance at early stages (Rinaldi et al., 2014). Studies investigating the correlation between mean FECs from individual samples and the corresponding pooled results were reviewed by Maurizio et al. (Maurizio et al., 2021a) and showed that correspondence is consistently high, regardless the statistical approach and the pool size ($n < 20$) employed. It is important to note that the present work is based on data collected over the years for different purposes, hence it was not designed from the beginning to test the influence of different pool sizes. A pool size of 5 was selected for FECR trials according to the protocol described by Rinaldi et al. (Rinaldi et al., 2014), and allowed the formation of 2 pools per trial. This resulted in an imbalanced distribution of pool sizes, as Fig. 1 shows. However, our results confirmed that correlation between the two FEC methods is high, even though not as high as other studies observed in sheep (Bosco et al., 2020; Morgan et al., 2005; Rinaldi et al., 2014) and cattle (Rinaldi et al., 2019; Ward et al., 1997). Wilcoxon signed-rank test indicated that the pooled approach can be a valid alternative to individual analysis for FEC. Composite samples of larger size seemed to provide better results, probably because the effect of outliers is reduced, but adjusting the relative difference to the mean (as shown in Fig. 3 and confirmed by our model) this difference seems to disappear. Agreement according to Lin's CCC was mostly poor, which was confirmed by further graphical analysis implemented in the present study (Fig. 2). The increase of variance at higher FECs, although it should not be overlooked, was expected and it might not be even problematic at very high FEC values, since the decision to opt for treatment would be likely clear. Instead, for intermediate FEC values, this lack of agreement poses some issues. Indeed, the main limitation of the use of composite samples remains the lack of a CI, which cannot be calculated as the number of samples is too low to provide a statistically valid CI (George et al., 2017). Hence, a pooled approach does not provide information on the distribution of parasites in the host population and more caution is required, compared to the use of individual samples, in the interpretation of results for the purposes of monitoring (Sargison, 2013). A decrease in the confidence of the result is indeed to be expected (Morgan et al., 2022), even more if pools are formed without weighing the individual faeces (Voigt et al., 2022) or using faeces collected from

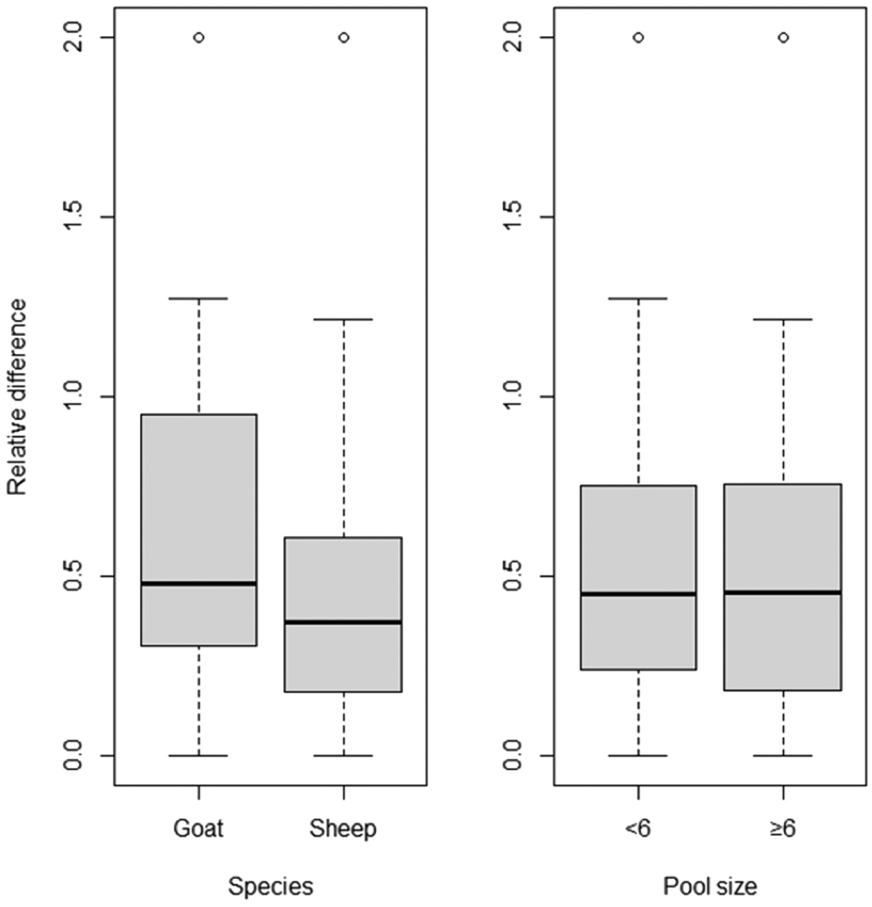

Fig. 3. Relative difference between pooled and individual analyses adjusted to the mean by species and pool size (<6 and ≥6).

Table 2
Comparison between FECR calculation from pooled and individual samples and agreement (Agree.) in the interpretation of efficacy. The classification was according to Denwood et al. (2023) as resistant (R), low resistant (LR), inconclusive (INC) and susceptible (S). For pools reduced efficacy was further classified as reduced with suspected resistance (SR), reduced with likely resistance (RL) and reduced with highly likely resistance (RHL) (Kaplan, 2020). Mean counts at D0 and D14 are also reported. –, not calculable.

			Pools				Indiv					Agree.
	ID	Drug	D0	D14	FECR (%)	Efficacy	D0	D14	FECR (%)	95 %CI	Efficacy	
Goat	TR1	BZ	166.5	18.5	88.9	RL	115.6	27.9	75.9	*63.7–84.0*	R	Yes
	TR2	IVM	158.0	10.0	93.7	SR	87.7	23.4	73.3	*64.8–79.8*	R	No
	TR3	BZ	51.5	0.0	100	S	39.3	0.0	100	–	S	Yes
	TR4	IVM	50.5	6.0	88.1	RL	63.1	0.0	100	–	S	No
	TR5	BZ	22.5	0.0	100	S	61.3	0.7	98.9	*98.0–99.4*	S	Yes
	TR6	BZ	25.5	2.0	92.2	SR	26.0	5.7	78.1	*71.4–83.2*	R	No
	TR7	BZ	101.5	0.0	100	S	160.0	0.0	100	–	S	Yes
	TR8	IVM	12.0	3.0	75.0	RHL	19.2	1.8	90.8	*87.0–93.4*	R	Yes
	TR9	BZ	111.0	17.0	84.7	RL	238.6	19.4	91.9	*91.1–92.6*	LR	No
	TR10	IVM	18.0	0.5	97.2	S	26.3	0.0	100	–	S	Yes
Sheep	TR11	IVM	64.5	68.0	-5.4	RHL	93.7	79.5	15.2	*2.6–26.1*	R	Yes
	TR12	IVM	44.0	54.5	-23.9	RHL	42.8	69.4	-62.2	*-93.2-(−36.1)*	R	Yes
	TR13	IVM	154.5	27.5	82.2	RL	95.0	18.1	81.0	*73.0 – 86.5*	R	Yes
	TR14	IVM	111.0	32.0	71.2	RHL	73.7	18.9	74.4	*55.5–85.2*	R	Yes
	TR15	BZ	141.5	15.5	89.0	RL	151.3	13.4	91.1	*88.2–93.3*	R	Yes
	TR16	BZ	7.0	1.5	78.6	RHL	28.3	6.2	78.1	*62.3–87.3*	R	Yes

the ground (Morgan et al., 2022). Moreover, individual analysis may lead to identify, or at least suspect, the presence of high-shedding individuals or categories, towards which treatments should be preferably targeted. While this is certainly a big advantage considering the current scientific recommendations (Charlier et al., 2014), it is also true that other indicators are more applicable than FEC for individual monitoring in a flock.

Concerning the assessment of FECR, results were dissonant. It is likely that the outcome of statistical analyses have been affected by the limited number of trials, although the sample size was not different from other studies (George et al., 2017; Kenyon et al., 2016; Rinaldi et al., 2014). More specifically, Spearman's correlation among the two methods was poor for goats, despite high correlation of FEC at both D0 and D14 for both species, while it was perfect (rho=1) for sheep,

Table 3
Spearman's rho correlation coefficient (rho), Lin's concordance correlation coefficients (CCC) and Wilcoxon signed rank test statistic (W), between FECR from individual and pooled samples. –, not calculable.

		Spearman's rank correlation		Lin's concordance correlation		Wilcoxon signed rank test	
FECR	n° trials	rho	95 %CI	CCC	95 %CI	W	p-value
Overall	16	0.66	0.25–0.87	0.93	0.83–0.97	54	0.950
Goats	10	0.41	(−0.29)–0.83	0.27	(−0.36)–0.73	16	0.834
Sheep	6	1.00	–	0.94	0.70–0.99	12	0.834

although this result is quite spurious (likely driven by the two outliers–as shown in Fig. 4). For this reason, in addition to statistical tests employed in previous similar studies (Bosco et al., 2020; George et al., 2017; Rinaldi et al., 2019), Wilcoxon signed-rank test was also performed in order to assess whether the two methods were actually different in terms of results, which was indeed rejected.

To ensure reliable conclusions on the drug efficacy, it is now recommended to count under the microscope (before applying a correction factor to obtain the epg) a minimum number of 200 eggs prior treatment for individual samples (Kaplan, 2020; Levecke et al., 2018). For pooled FECRT, indications suggest to examined no less than three McMaster slides per composite sample, and if < 50 eggs are counted in these three slides, additional slides/chambers must be analysed to reach the 50-eggs threshold (Kaplan, 2020). As the main purpose of our study was not to use pooled samples for FECRT, our sample design established the creation and analysis of only two separate pools per FECR trial to be examined once each, so it should be underlined that this is not compliant with the most recent recommendations (George et al., 2017; Kaplan, 2020). Anyway, the absence of a CI increases the margin of error in the interpretation of treatment efficacy when pooled samples are used (Kaplan, 2020; Rinaldi et al., 2019), which is critical especially for FECR values which indicate suspected reduced efficacy and in consideration of the fact that the reliance on CI (rather than on FECR %) for the evaluation of treatment efficacy is expected to increase (Denwood et al., 2023).

Indeed, FECR< 95 % does not necessarily indicate AR, but rather a reduction of the effectiveness of the treatment, which can be due to several other factors (Morgan et al., 2022). Efficacy data can only be correctly interpreted once confounding factors are excluded, which is not always entirely possible (Kaplan, 2020; Morgan et al., 2022). Once the major confounding factors are excluded, a more structured diagnostic approach, such as the one described by Bosco et al. (Bosco et al., 2020), or biomolecular tools (Avramenko et al., 2019; Gilleard et al., 2021; Kotze et al., 2020) are needed to confirm AR in these farms. To our perception and knowledge, major confounding factors (e.g. animal selection, dosage, administration, conservation of anthelmintics) can be excluded in our study, with the exception of one farm (TR1 and TR2) in which underdosage was suspected and subsequently confirmed.

5. Conclusion

As farmers struggle to include monitoring as a routine procedure, there is a pivotal need for approaches that provide reliable information while minimizing financial and technical resources. Pooled samples can reduce dramatically workload and costs of the analyses. This study confirms their usefulness as an alternative to assess FEC in sheep and provides, to our knowledge, the first data on the topic for goats. We underline that, according to our statistical analyses, species and pool size had no effect on the results, suggesting that, when present, differences in correlation and agreement between sheep and goats are likely affected by other factors which may need further investigation. With regards of FECR testing, the individual and pooled approaches provided similar interpretation in most cases and were statistically comparable, but correlation was not as high as expected, especially for goats. This might compromise the interpretation of treatment efficacy, especially when pooled FECR lies in the uncertainty area (FECR around 90 %). According to our results, pooled FECR can be a good option if the alternative is no testing. Otherwise, the drawbacks in the interpretation of results (absence of a CI) do not justify, to our opinion, its use. When relying for FECRT exclusively on pooled samples, we strongly encourage the use of multiple pools to improve the accuracy of the test. Also, further studies on the use of pooled FEC and FECRT for goats are needed to support and expand our results.

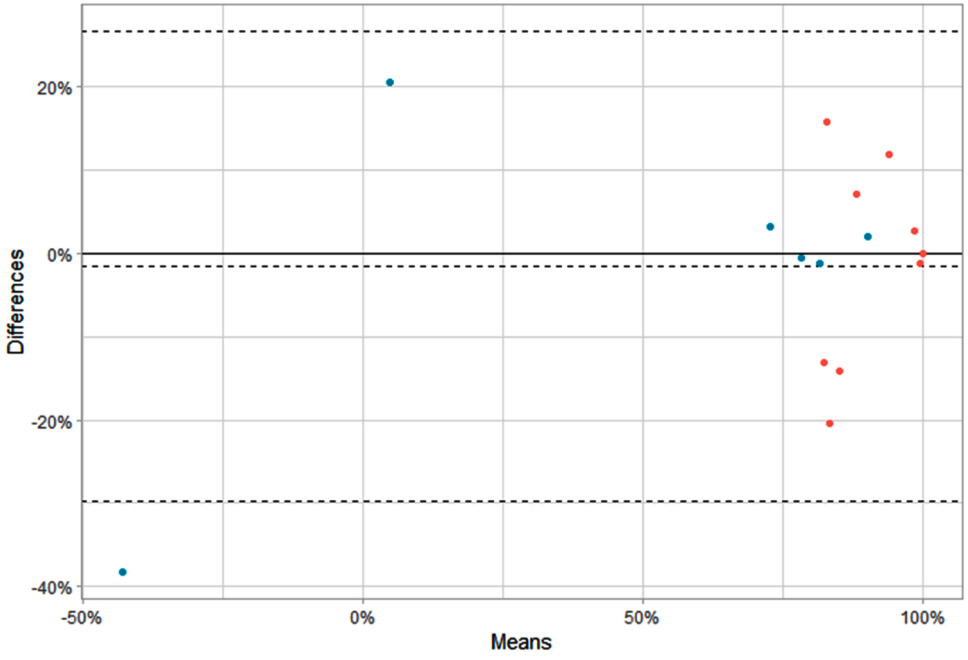

Fig. 4. Bland-Altman plot of difference (y-axis) against mean (x-axis) for FECR of individual and pooled analyses for sheep (green) and goats (red).

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CRediT authorship contribution statement

Anna Maurizio: Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft; Erica Marchiori: Conceptualization, Investigation, Writing – review & editing; Cinzia Tessarin: Investigation, Resources, Writing - review & editing; Rudi Cassini: Conceptualization, Resources, Project administration, Funding acquisition, Supervision, Writing – review & editing.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetpar.2023.109935.

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