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Comparison of traditional copromicroscopy with image analysis devices for detection of gastrointestinal nematode infection in sheep

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ABSTRACT

Sustainable parasite control practices are necessary to combat the negative effects of gastrointestinal nematodes on animal health and production while reducing the selection pressure for anthelmintic resistance. Parasite diagnostic tests can inform treatment decisions, the timing and effectiveness of treatment and enable livestock breeding programmes. In recent years new diagnostic methods have been developed, some incorporating machine learning (ML), to facilitate the detection and enumeration of parasite eggs. It is important to understand the technical characteristics and performance of such new methods compared to long standing and commonly utilised methods before they are widely implemented. The aim of the present study was to trial three new diagnostic tools relying on image analysis (FECPAK^{G2}, Micron and OvaCyte) and to compare them to traditional manual devices (McMaster and Mini-FLOTAC). Faecal samples were obtained from 41 lambs naturally infected with gastrointestinal nematodes. Samples were mixed and separated into 2 aliquots for examination by each of the 5 methods: McMaster, Mini-FLOTAC, FECPAK^{G2}, Micron and OvaCyte. The techniques were performed according to their respective standard protocols and results were collected by trained staff (McMaster and Mini-FLOTAC) or by the device (FECPAK^{G2}, Micron and OvaCyte). Regarding strongyle worm egg count, McMaster values varied from 0 to 9,000 eggs per gram (EPG). When comparing replicate aliquots, both the Mini-FLOTAC and Micron methods displayed similar repeatability to McMaster. However, we found FECPAK^{G2} and OvaCyte significantly less precise than McMaster. When comparing parasite egg enumeration, significant positive linear correlations were established between McMaster and all other methods. No difference was observed in EPG between McMaster and Mini-FLOTAC or FECPAK^{G2}; however, Micron and OvaCyte returned significantly higher and lower EPG, respectively, compared to McMaster. The number of eggs ascribed to other parasite species was not sufficient for performing a robust statistical comparison between all methods. However, it was noted that FECPAK^{G2} generally did not detect *Strongyloides papillosus* eggs, despite these being detected by other methods. In addition, *Moniezia* spp and *Trichuris* spp eggs were detected by OvaCyte and Mini-FLOTAC, respectively, but not by other methods. The observed variation between traditional and new methods for parasite diagnostics highlights the need for continued training and enhancing of ML models used and the importance of developing clear guidelines for validation of newly developed methods.

1. Introduction

Gastrointestinal parasites are a worldwide threat to animal health and production (Charlier et al., 2020). The use and misuse of anthelmintic drugs to control parasitic infection has resulted in multi-drug resistant parasites to even the newest molecules, constituting a major challenge for the livestock industry (Keegan et al., 2015; Herrera-Manzanilla et al., 2017; Bartley et al., 2019; Bordes et al., 2020; Dey et al., 2020; Kelleher et al., 2020; Sauermann et al., 2023). Today, sustainable parasite control practices are essential to delay the further

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development of anthelmintic resistance (Vercruysse et al., 2018). That is why coprologic diagnostic tests are of critical importance in this field. These tests have a multitude of uses including in parasitic disease control programmes, carrying out targeted (selective) treatments, selective breeding and monitoring anthelmintic resistance in nematode populations using the faecal egg count reduction test (FECRT) (Charlier et al., 2018; Keegan et al., 2018; Hayward, 2022).

Many coprologic diagnostic tests are based on the principle of floating the parasite elements with a solution of higher density than them, with variations in sample quantities and procedure (Ballweber et al., 2014). Quantitative techniques, such as the McMaster method, are useful tools to determine strongyle gastrointestinal nematode faecal egg counts (FECs). This longstanding and reliable method is the most commonly used diagnostic test and has a multiplication factor of 50 eggs per gram (EPG) or less when certain adjustments to the technique are employed (Gordon and Whitlock, 1939; MAFF, 1989; Cringoli et al., 2010). Newer methods based on similar floatation principles have been developed, such as the Mini-FLOTAC technique, which is a modified version of FLOTAC, presenting a multiplication factor of 5 EPG (Cringoli et al., 2017). However, such methods still require a trained technician to 'read' or collect the data. In an attempt to simplify the procedure, make it more user-friendly and return results more quickly for veterinarians and farmers a number of automated or semi-automated image analysis methods have been recently developed, into which a machine learning (ML) approach has been introduced (Nagamori et al., 2021; Slusarewicz et al., 2021; Bucki et al., 2023; Elghryani et al., 2023). These methods generally involve manual preparation of faecal samples in floatation solution followed by sample submission to a device for image capture, detection and quantification of parasite eggs. The detection algorithm has been trained through ML to discern between eggs of different species or groups of parasites and to discriminate them from debris found in the sample. While this technology has great potential, several challenges arise because parasite eggs can show differences in their characteristics (size, colour, shape) and can be difficult to discern from debris. Therefore, comprehensive training and validation of the ML model is crucial (AlDahoul et al., 2023). Currently available devices for automated gastrointestinal nematode egg detection include FECPAK^{G2} (Techion Group Ltd), Micron kit (Micron AgriTech) and OvaCyte (Telenostic Ltd).

Veterinarians, farmers and other end-users are beginning to adopt automated egg counting methods in ruminant livestock, although there is limited published information about their performance, including how their results compare to traditional microscopy-based methods (Slusarewicz et al., 2021). Despite having a relatively high multiplication factor, McMaster is the most widely used FEC method (Cringoli et al., 2010; Nielsen, 2021) and was previously recommended by the World Association for the Advancement of Veterinary Parasitology (WAAVP) for the detection of anthelmintic resistance (Wood et al., 1995). Hence, many published livestock parasitology studies are based on it, including many recent ones (Flota-Burgos et al., 2023; Gonzaga et al., 2023; Werne et al., 2023). Therefore, the performance of automated methods in comparison to McMaster is of particular interest.

A study comparing the FECPAK^{G2} method to a modification of the McMaster method for counting nematode eggs in alpaca faeces reported moderate to good agreement between the methods (Rashid et al., 2018). By contrast, a study of ovine faecal strongyle egg counts comparing the Micron kit to McMaster found that it returned significantly higher EPGs (Bucki et al., 2023). Studies comparing the OvaCyte device with McMaster and Mini-FLOTAC for the estimation of FEC in cattle and horses reported strong correlations between the three techniques, albeit OvaCyte classified a higher percentage of equine samples as strongyle positive than Mini-FLOTAC, although the latter had a lower multiplication factor (Elghryani et al., 2020; Elghryani et al., 2023).

Parasite diagnostic tests need to be as accurate as possible, so they can be relied on for good animal health, welfare and management, to treat livestock appropriately and mitigate anthelmintic resistance. Given the growing popularity of tests based on image analysis, the present study was carried out to compare the performance of the traditional FEC methods McMaster and Mini-FLOTAC, with the automated techniques FECPAK^{G2}, Micron and OvaCyte using faecal samples from sheep naturally infected with gastrointestinal nematodes.

2. Materials and methods

2.1. Animals and sample collection

All procedures involving animals were carried out with approval from the Teagasc Animal Ethics Committee (protocol number TAEC2022–361).

In October 2023, naturally voided faecal samples were collected over 2 hours into faecal collection bags from 30 Belclare lambs of approximately 7 months of age. The lambs were from the Teagasc sheep research farm in Athenry, Co. Galway and were naturally infected at pasture with a variety of gastrointestinal nematode species. As the egg counts of these samples were high, in April 2024 an additional 11 faecal samples were collected from a group of mixed breed yearling rams with a low group FEC. All faecal samples were taken to the laboratory at Teagasc Athenry, mixed and 2 separate aliquots (A and B) removed to perform each of the 5 FEC methods; McMaster, Mini-FLOTAC, FECPAK^{G2}, Micron and OvaCyte. A total of 40 g was required to complete the 2 assays for each method (Table 1). For one sample, an insufficient quantity of faeces was recovered (36.6 g) and so the McMaster and Mini-FLOTAC B aliquots were prepared with 2.07 g and 2.48 g respectively with the FEC adjusted accordingly. All egg counts for all methods were completed within 7 days of the samples being collected and data collectors were blinded to the results of other techniques until all data had been collected and collated.

2.2. Aliquot preparation and FEC determination

2.2.1. McMaster

On the day of sample collection McMaster aliquots were prepared by homogenising 3 g of faeces with 42 ml cold water. The slurry was passed through a 150 μm mesh sieve and 15 ml of filtrate collected and centrifuged at 432 x *g* for 3 min at 4 °C. The supernatant was removed and the pellet stored at 4 °C until use. Immediately before use, the pellet was resuspended up to 15 ml in saturated salt solution (specific gravity (SG) 1.2). The slurry was mixed by inverting at least 3 times before a sub-sample was removed to fill the first chamber of a McMaster slide (Chalex LLC). The slurry was mixed by inverting again before another sub-sample was removed to fill the second chamber of the slide. The number of eggs within the marked grids was counted at 10X magnification on a Nikon E200 Eclipse microscope. *Nematodirus* spp, strongyle and *Strongyloides papillosus* eggs were enumerated separately. The presence of other species (*Moniezia* spp, coccidia, *Trichuris* spp) was noted but not counted.

2.2.2. Mini-FLOTAC

A modification of the Mini-FLOTAC procedure was used (Cringoli et al., 2013). On the day of sample collection, Mini-FLOTAC aliquots were prepared by homogenising 5 g of faeces with 45 ml cold water. The slurry was passed through a 150 μm mesh sieve and 15 ml of filtrate

Table 1
Details of faecal egg counting methods compared.

	McMaster	Mini-FLOTAC	FECPAK^{G2}	Micron	OvaCyte
Volume of sample (g)	3	5	6	3	3
Multiplication Factor	50	5	35	30	10.9–15.4 35.5–47.6[a]

[a] Values for 2 samples that were run with standard scan only.

collected and centrifuged at 432 x *g* for 3 min at 4 °C. The supernatants were removed and the pellets transported to the laboratory at Teagasc Grange, Dunsany, Co. Meath where they were stored at 4 °C until use. Immediately before use the pellet was resuspended up to 15 ml in saturated salt solution (SG 1.2). The slurry was mixed by inverting at least 3 times before a sub-sample was removed to fill the first chamber of a Mini-FLOTAC slide (University of Naples Federico II, Naples, Italy). The slurry was mixed by inverting again before a second sub-sample was removed to fill the second chamber of the slide. The slide was then rested on the bench for 10 minutes to allow the eggs to float. After 10 minutes the key was turned to remove the floated eggs to the reading chamber. The number of eggs within the marked grids was counted at 10X magnification on an Olympus BX41 or CX43 microscope. *Nematodirus* spp, strongyle and *S. papillosus* eggs were enumerated separately. The presence of other species (*Moniezia* spp, coccidia, *Trichuris* spp) was noted but not counted.

2.2.3. FECPAK^{G2}

FECPAK^{G2} aliquots were prepared in accordance with the protocol provided by the manufacturer (Techion Group Ltd). In summary, 18 ml of water was added to 6 g of faeces and homogenised to form a slurry. This was added up to the 'slurry' line on the sedimenter (~12 ml) and water subsequently added up to the 'water' line. The lid was attached and the slurry mixed by inverting 5 times. The sedimenter was then left to stand for at least 30 min at room temperature. The supernatant was then poured out from side A of the sedimenter as far as the 'flush' line and saturated salt (SG 1.2) added up to the 'sheep saline' line. The mixture was then poured from side B of the sedimenter into the provided cylinder. The lid with 2 sieves (600 and 425 μm) was attached. The cylinder was inverted 3 times to mix and immediately a 460 μl sub-sample was removed and added by reverse pipetting into the first well of a pre-wetted cassette. The cylinder was again inverted three times and the second sub-sample removed and added to the second well of the cassette. The cassette was left to rest for 6 minutes before being loaded into the FECPAK^{G2} Micro-I. Images were uploaded to the Techion cloud server where they were initially analysed by a ML model and subsequently double checked by a trained technician who can make any necessary changes to the counts (i.e. the counts are ML-assisted). The EPGs for strongyles, *Nematodirus* spp and *S. papillosus* were received by e-mail from Techion within approximately 1 hour.

2.2.4. Micron

Micron aliquots were prepared in accordance with the Micron kit protocol. Briefly, 3 g of faeces was added to 42 ml water, broken up with a spatula and mixed well. The slurry was inverted 5 times to mix and then poured through the provided sieve (mesh size 1190 μm) and the filtrate collected. Subsequently, the sample was inverted 5 times and two 5 ml sub-samples were removed using a syringe and placed into 5 ml tubes. A handheld centrifuge was used to pellet the samples (~40 spins). After centrifugation, the supernatant was removed and the pellets re-suspended up to the 5 ml mark with a saturated salt solution (SG 1.2). The two sub-samples were combined and inverted 5 times to mix. Using a syringe, 3.3 ml of the prepared slurry was added to the microscope slide, which was allowed to rest for 2 minutes. A phone camera (Samsung Galaxy S10) was attached to the reader as per Micron Agritech instructions and the slide was pushed in to initiate processing. Using the Micron kit app, a video was recorded and uploaded to the cloud based service. The videos were analysed by a trained technician and EPGs for strongyles, *Nematodirus* spp and *S. papillosus* were returned via the Micron app as well as a semi-quantitative value for coccida oocysts (high, moderate, low, not detected). The presence of *Trichuris* spp or *Moniezia* spp eggs was also noted. The images from 3 aliquots were initially considered too dark for egg enumeration. It was advised by Micron to repeat these samples with increased centrifugation (~80 spins) and allowing the slide to rest for 10 minutes. This amended method resulted in an egg count for one of the aliquots. However, the remaining two aliquots had to be removed from the study as an egg count was not obtained following two further attempts.

2.2.5. OvaCyte

OvaCyte aliquots were prepared in accordance to the OvaCyte™ protocol provided with the kit. On a weighing scale, 3 g of faeces was placed into a labelled container to which 47 ml of the provided floatation solution (saturated salt SG 1.2) was added. To create a slurry, the faeces was mixed with the floatation solution using a spatula for approximately 15 seconds and the resulting slurry poured through the provided sieve. To avoid bubble formation the filtrate was gently swirled and a syringe used to extract 5 ml. The syringe was then inserted into the vertically held OvaCyte™ cassette and the slurry added. The cassette was left to rest for 7 minutes before placing onto the device and the run started using the extended scan. The extended scan was completed for all except 2 aliquots where the extended scan failed to complete within 30 min and so the runs were terminated to prevent distortion of eggs from being in salt solution for too long. EPGs for Strongyles, *Nematodirus* spp, *S. papillosus, Moniezia* spp and coccidia oocysts were returned within the device and on the OvaCyte™ portal. The returned results include the scan ID, which allows the user to view, via the OvaCyte™ portal, all images relating to that sample. These images include annotations of features counted as eggs, thus allowing the user to manually review the egg count. For 8 samples that were deemed positive for *Moniezia* spp the features annotated as eggs from this genus were reviewed.

2.3. Statistics

If no eggs were detected, FEC was set as zero. A value of 25 was added to all FEC values to facilitate data transformation. A Box-Cox data transformation was applied to the data to identify suitable transforms which promoted homogeneity of variance, which was determined by Levene's test, and all statistical analysis was subsequently carried out on log transformed data ($\log_{10}$(FEC+25)).

The coefficient of variation was calculated for each sample/method combination. The average of the A and B aliquots was determined and used for the comparison between methods. For strongyles, correlation of FEC with that of McMaster was determined using Pearson's correlation coefficient. ANOVA for the effect of the different methods of FEC determination was blocked by stool sample and counts for each method were compared to the standard McMaster technique using Dunnett's test. The repeatability of each FEC method was determined by calculating Lin's Concordance Correlation Coefficient between the A and B aliquots. The concordance was tested for significant difference from that of the McMaster method using Fisher's z-test. It was not possible to normalise the data for *Nematodirus* spp. or *S. papillosus* across all five tests and attempts to analyse the data using parametric techniques resulted in failure of the assumptions of ANOVA. The data were therefore converted to binomial form (presence/absence of eggs) and Pearson's chi-squared test applied. Semi-quantitative coccidia counts were recoded 0–4. Statistical analysis was carried out with SAS v9.4 and NCSS 2022.

3. Results

Five different methods for determining sheep faecal egg count were compared; two manual microscopy methods and three methods that involve image analysis to identify parasite eggs. The sample requirements and multiplication factor for each method are shown in Table 1.

3.1. Strongyle eggs

For each sample (n = 41), each method was performed on two separate aliquots, resulting in 82 measurements per method. However,

for one method (Micron) the assay failed for two aliquots despite being repeated three times. Therefore, a replicate value is missing for two animals for this method. Summary statistics for each of the FEC methods are listed in Table 2.

3.1.1. Data distribution

The distribution of strongyle EPGs was plotted for each method. All methods returned data that had a positive skew (Fig. 1). The data was successfully normalised by a log transformation to promote homogeneity of variance.

3.1.2. Repeatability

Repeatability (precision) was assessed by plotting FEC for the A against the B aliquot and calculating Lin's Concordance Correlation Coefficient (CCC). Concordance was compared to that of McMaster to identify methods that were significantly more or less precise than the McMaster method. McMaster, Mini-FLOTAC and Micron showed very high concordance (> 0.98) between the A and B aliquots (high repeatability) and repeatability for Mini-FLOTAC and Micron was not significantly different from McMaster. FECPAK^{G2} and OvaCyte presented lower concordance correlation coefficients (lower repeatability) and showed significant differences compared with McMaster ($P < 0.001$; Fig. 2).

3.1.3. Comparison of methods

ANOVA of the different methods of determination of FEC, blocked by stool sample, showed a significant effect of both method and of stool sample ($P < 0.0001$). Dunnett's test was used to compare FEC methods, with McMaster as the control group. The FEC values generated by the Mini-FLOTAC and FECPAK^{G2} methods did not differ significantly from those of McMaster ($P = 0.26$ and $P = 0.81$ respectively); however, significant differences were found for the Micron ($P < 0.0001$) and OvaCyte ($P = 0.0002$) methods, which returned significantly higher and lower EPGs respectively than McMaster (Fig. 3).

As the analysis of variance showed that the Micron and OvaCyte methods returned significantly different EPGs to McMaster, the correlation of FEC from each method with that of McMaster was determined in order to assess whether a linear relationship existed between the results. The correlation analyses are shown in Fig. 4. All methods had a significant positive correlation with McMaster.

3.1.4. Nematodirus spp

Nematodirus spp egg counts were very low and hence the samples were not well suited for comparison of the FEC methods with McMaster for this genus. Lin's Concordance Correlation Coefficient showed low repeatability for every method, including McMaster (Table 3). Mini-FLOTAC demonstrated the highest repeatability, presumably due to the low multiplication factor of this test (5 EPG).

There were significant differences between methods in terms of classifying samples as positive or negative using Pearson's chi-square test ($P = 0.0014$). McMaster identified fewer *Nematodirus* spp positive samples than the other tests, particularly Mini-FLOTAC and Micron (Fig. 5).

Table 2
Summary statistics for strongyle eggs for each faecal egg counting method. FEC range, median and inter-quartile range is for the average of the A and B aliquots.

	McMaster	Mini-FLOTAC	FECPAK^{G2}	Micron	OvaCyte
No of A aliquots	41	41	41	41	41
No of B aliquots	41	41	41	39	41
FEC Range	0–8,963	0–8,745	0–7,193	0–25,155	0–4,661
FEC Median	1,600	1,990	1,855	3,720	1,121
Interquartile range	2,050	2,675	2,450	5,445	1,690
CV (%)	17.1	11.3	38.3	19.1	28.9

3.1.5. Strongyloides papillosus

FECPAK^{G2} detected *S. papillosus* eggs in only 2 samples despite them being detected in 32 samples by the McMaster method and hence was excluded from the analysis. An invalid *S. papillosus* result was returned for two aliquots with the Micron method; despite these aliquots having valid strongyle and *Nematodirus* spp counts. The count of *S. papillosus* eggs was also missing for one sample for Mini-FLOTAC. Lin's Concordance Correlation Coefficient showed a very high repeatability for the Mini-FLOTAC and OvaCyte methods for this species. For McMaster, the repeatability was high but significantly less than that of Mini-FLOTAC or OvaCyte. Micron had a lower repeatability than all other methods; this was significantly lower than McMaster (Table 4).

There were no significant differences between methods in terms of classifying samples as positive or negative for *S. papillosus* eggs using Pearson's chi-square test ($P = 0.23$; Fig. 6).

3.2. Other parasite genera

Only the OvaCyte method returns quantitative data for coccidia. There was a reasonable concordance between the A and B aliquots (0.78) for this method. Micron returns semi-quantitative data for coccidia. An "invalid" result for coccidia was returned for 13 aliquots with this method. There was a low concordance ($CCC = 0.40$; $n = 28$) between the A and B aliquots with this method. Coccidia oocysts were detected in all samples by the Mini-FLOTAC method; however, 5, 4 and 6 samples were negative by the McMaster, Micron and OvaCyte methods respectively.

No *Moniezia* spp eggs were detected by the McMaster, Mini-FLOTAC or Micron methods; however, eight samples were positive for *Moniezia* spp eggs by the OvaCyte method, although the counts were low and no sample was positive for both the A and B aliquots. The features annotated as *Moniezia* spp eggs were reviewed by the authors; none were considered to be clearly eggs of this genus although two features were insufficiently clear for a definitive determination.

No *Trichuris* spp eggs were detected by the McMaster or Micron methods, although eggs from this genus were detected in five samples by the Mini-FLOTAC method. However, the number of *Trichuris* spp eggs detected was low and may have been too low for detection by other methods with higher multiplication factors.

4. Discussion

The aim of this study was to provide information about the performance of three new diagnostic tools for faecal egg counting (FECPAK^{G2}, Micron and OvaCyte) and compare them to traditional manual devices (McMaster and Mini-FLOTAC) using faecal samples collected from naturally infected sheep. Strongyle egg count was the most suitable for evaluating the different methods because there were sufficient eggs for comparison and the transformed data followed a normal distribution. The results from all methods demonstrated a significant positive correlation with the McMaster results, indicating a linear relationship between the egg counts obtained with each method and McMaster. However, only the Mini-FLOTAC and FECPAK^{G2} methods returned EPG values that were quantitatively similar to McMaster. The reading of the McMaster and Mini-FLOTAC slides were carried out in separate laboratories by different laboratory technicians so it was notable they were not significantly different. FECPAK^{G2} also returned quantitatively similar results to McMaster. FECPAK^{G2} images are initially analysed by ML and subsequently verified by a trained technician who can make any necessary changes to the count (Techion Group Ltd, pers. comm.). As such, the results from this system are ML-assisted and it is unclear to the end-user what, if any, adjustments have been made to the results by human intervention. The results presented here may therefore not accurately reflect the performance of the ML model alone but rather the performance of the product. Micron and OvaCyte returned significantly higher and lower EPGs, respectively, than McMaster, with the Micron

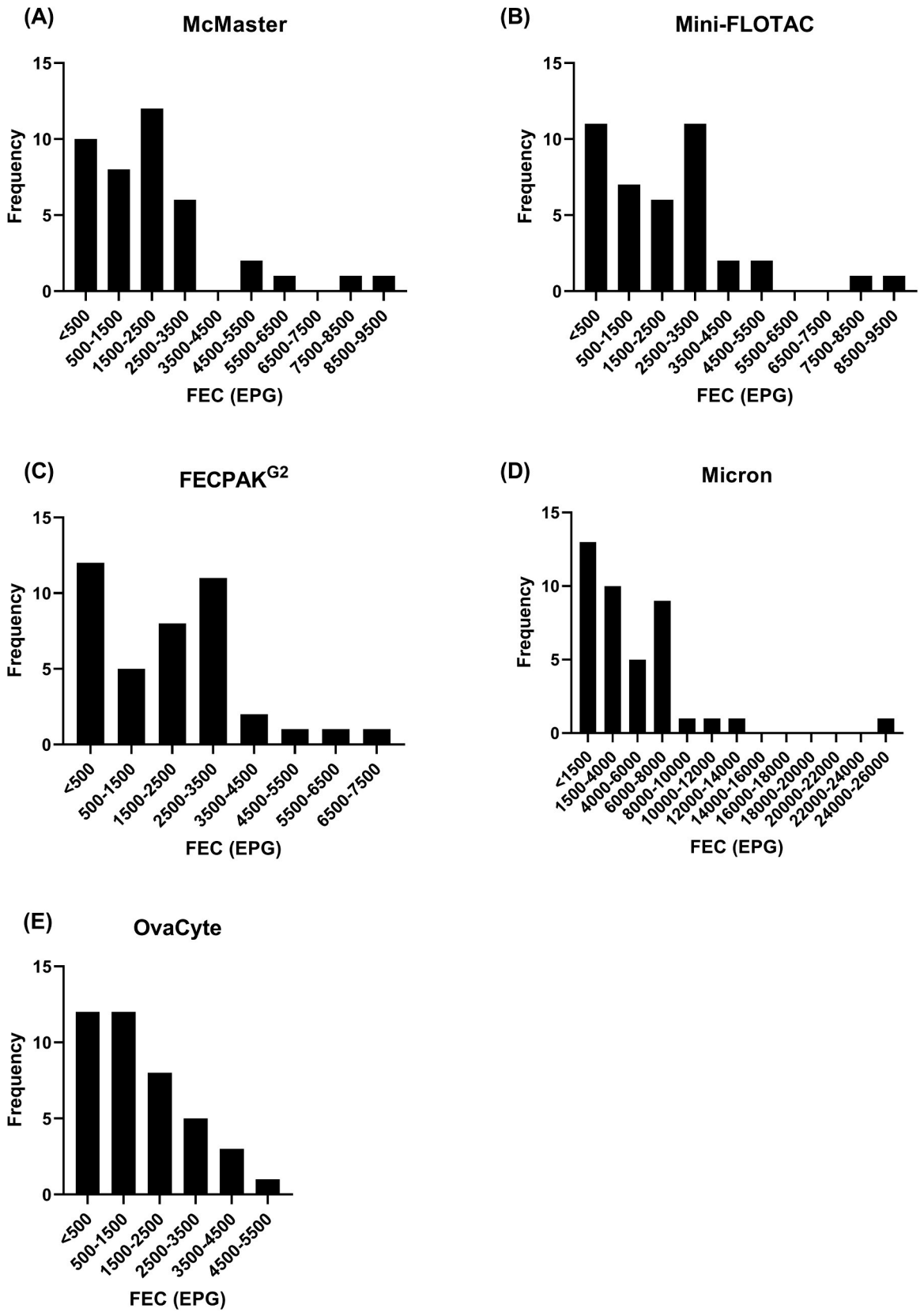

Fig. 1. Frequency distribution of strongyle faecal egg count (FEC) from 41 naturally infected lambs using different egg counting methods. The distribution of the average FEC of the A and B aliquots is plotted for the McMaster (A), Mini-FLOTAC (B), FECPAK^{G2} (C), Micron (D) and OvaCyte (E) methods.

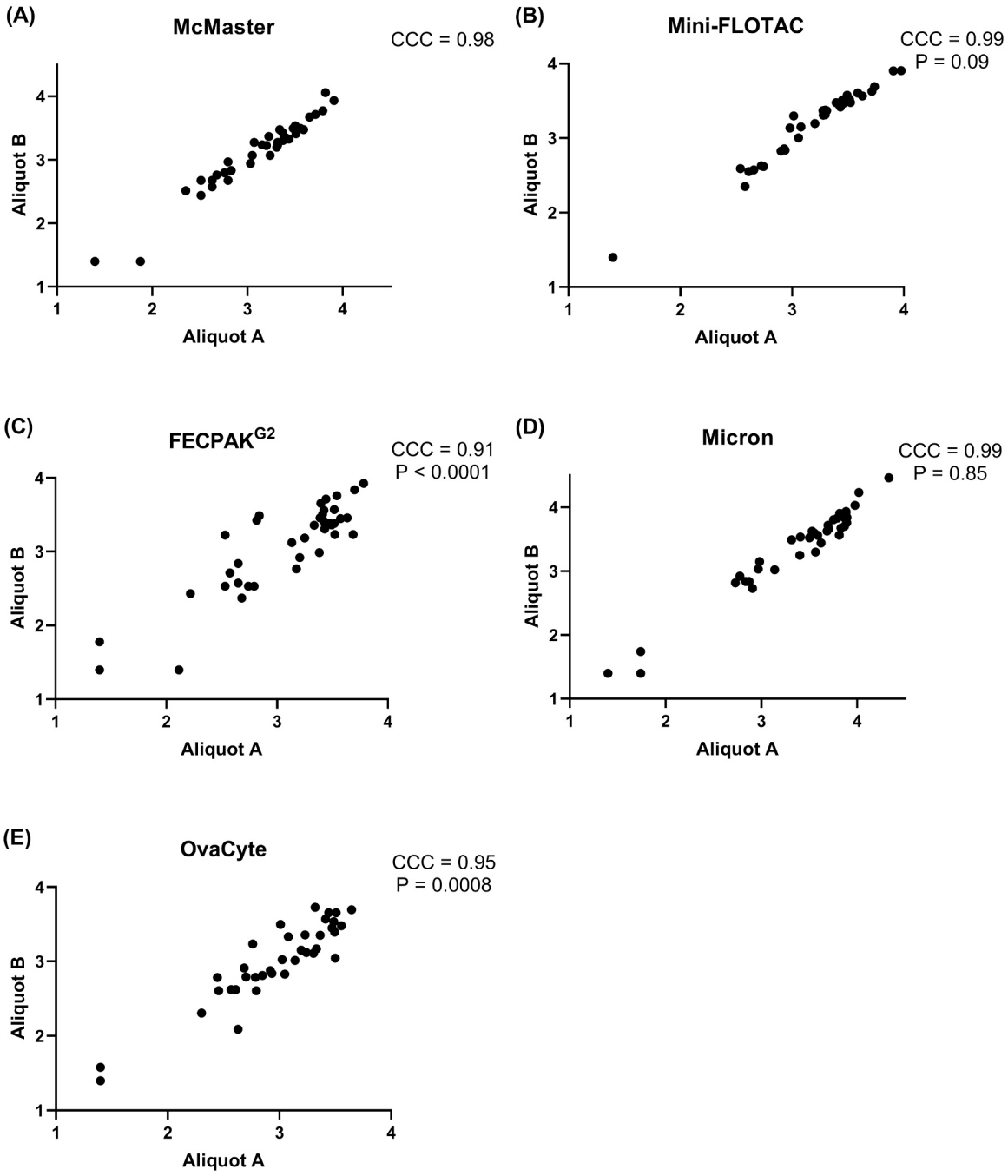

Fig. 2. Scatterplot of strongyle faecal egg count (FEC) from the A and B aliquots for the different FEC methods. The $\log_{10}$(FEC+25) is plotted for McMaster (A), Mini-FLOTAC (B), FECPAK^{G2} (C), Micron (D) and OvaCyte (E) methods. Lin's concordance correlation coefficient (CCC) is shown on the plot in addition to the significance of the repeatability compared to the McMaster method.

values on average over twice the McMaster values. A previous study that compared the Micron and McMaster methods also found that the Micron method resulted in significantly higher EPGs than McMaster (Bucki et al., 2023). Moreover, the McMaster method described by Bucki et al. (2023) did not conform to a commonly accepted protocol and likely under-estimated the true difference between the Micron and McMaster methods. The videos generated by the Micron device can be analysed by a trained technician or the system's ML algorithm (Bucki et al., 2023); however, the product on the market currently, and utilised for this study, used a trained technician to count the eggs. In contrast, results from the OvaCyte device are returned instantly on the screen of the device implying no human intervention. Previous studies evaluating the OvaCyte device used faecal samples from cattle and horses and found high agreement between McMaster and OvaCyte (Elghryani et al., 2020; Elghryani et al., 2023). However, the dilution ratio of the faecal sample varied between the studies of Elghryani et al. (2020, 2023) and the protocol established in the marketed product, which was used in the present study.

All of the methods evaluated are modifications of the basic McMaster principle i.e. floatation of eggs in a saturated salt solution, albeit there are differences between the methods in parameters such as the sieve diameter for the removal of large solid particles and the multiplication factor of the test. Nonetheless, all methods were considered adaptations of the McMaster method. Therefore, the goal of the analysis was to compare manual and automated methods of egg identification and enumeration. Mini-FLOTAC has been reported to have higher sensitivity

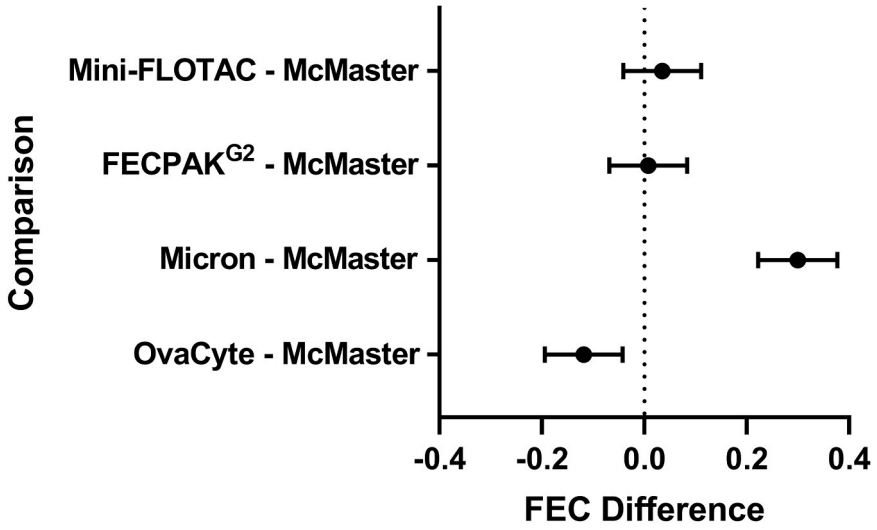

Fig. 3. Difference and 95% confidence interval of the difference for each FEC method compared to McMaster.

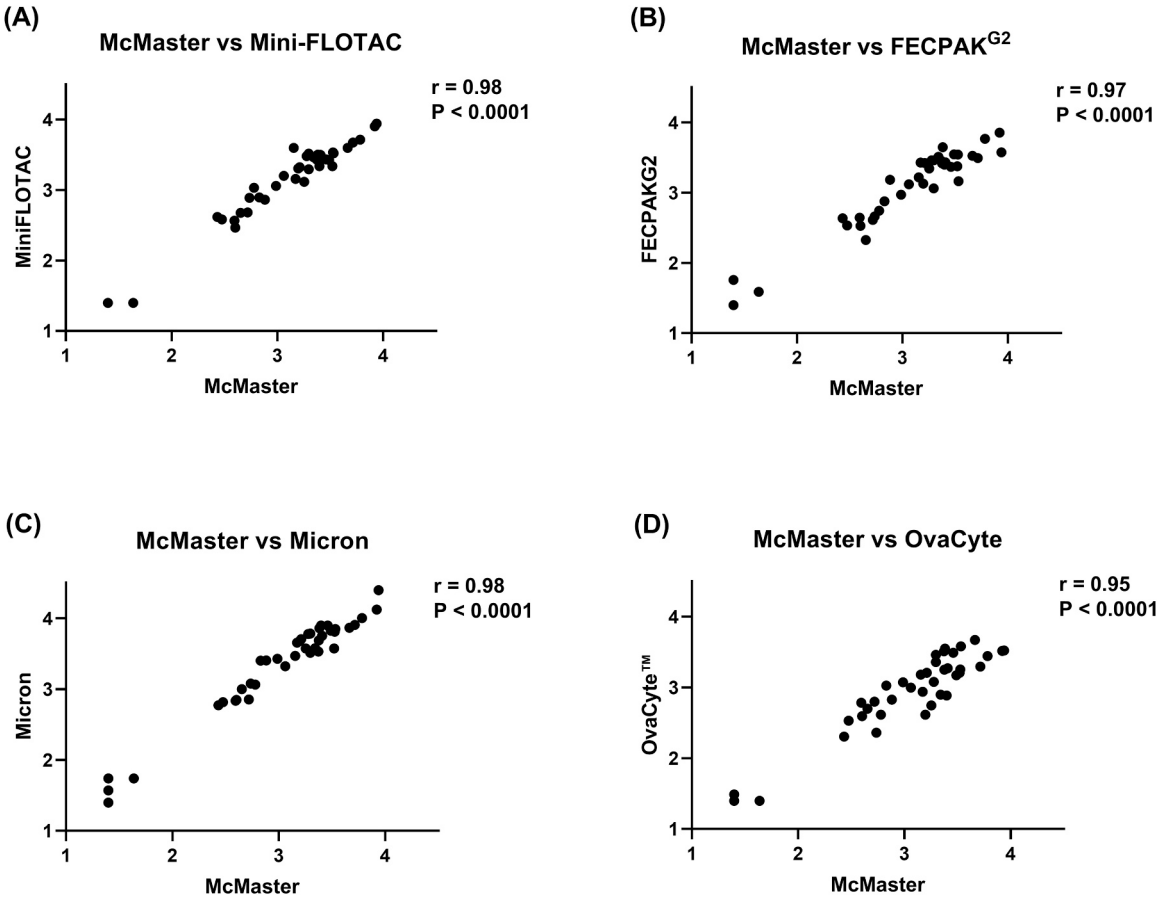

Fig. 4. Correlation of strongyle faecal egg count (FEC) for each method with McMaster. The average $\log_{10}$(FEC+25) for Mini-FLOTAC (A), FECPAK^{G2} (B), Micron (C) and OvaCyte (D) is plotted against the corresponding McMaster value. The correlation (r) and the significance of the correlation are shown on each plot.

and accuracy than McMaster, particularly at low EPGs (Amadesi et al., 2020). Despite this, McMaster was used as the reference method to which all other methods were compared in this study. McMaster was chosen as it is the most widely utilised method and is the method automated devices are most likely to replace in the field. While Micron and OvaCyte over and underestimated the egg count, respectively, in comparison to McMaster, the true egg count of the samples was unknown and hence accuracy of the different methods could not be assessed. Previous studies have reported the accuracy of McMaster to be >80% for sheep faecal samples of ≥200 EPG (Bosco et al., 2018). However, this figure may not apply more broadly given the variation in egg counting methods between laboratories in terms of sample quantity, dilution factor, sieve mesh size, floatation solution and use of centrifugation or not. The use of faecal samples, spiked with a known number of

Table 3
Repeatability of each method for the detection of *Nematodirus* spp eggs.

Method	Lin's Concordance Correlation Coefficient	N	P against McMaster
McMaster	0.41	41	-
Mini-FLOTAC	0.68	41	> 0.05
FECPAK^{G2}	0.42	41	> 0.05
Micron	0.59	39	> 0.05
OvaCyte™	0.33	41	> 0.05

nematode eggs, would have allowed determination of the accuracy of each method. The consequence of the difference in FEC between the McMaster and other methods depends on the purpose for the test. For example, when selecting animals for breeding, ranking animals is generally sufficient and all methods rank animals similarly. However, when employing tests to decide when to administer treatment then the absolute value of FEC becomes important. Interpretation of FEC involves consideration of a variety of animal, management, parasite and climatic factors and EPG thresholds that warrant anthelmintic treatment are intricately linked to these factors. Nonetheless, strongyle EPG thresholds broadly indicative of low, medium and high burden and meriting anthelmintic treatment of lambs have been suggested (Leathwick et al., 2006; Abbott et al., 2009; McRae et al., 2014; Borkowski et al., 2020; Slusarewicz et al., 2021). Such thresholds are historically based on McMaster tests and the application of these thresholds to new automated methods, which inflate or reduce EPGs in comparison to McMaster, could have important practical implications by leading to unnecessary treatments being administered or, conversely, withholding treatment from animals that would benefit from treatment.

Both the Mini-FLOTAC and the Micron methods showed similar repeatability to the standard McMaster method; however, FECPAK^{G2} and OvaCyte were significantly less repeatable than the McMaster method. It should be noted that FECPAK^{G2} was originally designed for on-farm use and the nature of the method, where initially water and subsequently floatation solution are added up to a marked line on the sedimenter, may lead to increased variability in comparison to other methods which rely on weighing scales or graduated cylinders. This may have contributed to the greater variability of this method compared to McMaster. In contrast to the present study, a previous study compared the variance in egg count of 10 replicate samples from 6 horses and found that the variance was lower for FECPAK^{G2} compared to Mini-FLOTAC (Boelow et al., 2022). However, it is unclear if the volumes were exactly measured in that study or if the manufacturer-supplied protocol was used. Variation between repeated measures of the same sample has been reported to depend on the egg count, with lower repeatability at lower egg counts (Nápravníková et al., 2019; Cain et al., 2020; Daş et al., 2020). Repeatability in a diagnostic tool is of vital importance for veterinary medicine, as excessive variability can compromise results when performing FECRT, selective treatment or selecting animals for breeding and repeatability should be assessed at a variety of FEC levels (Nielsen, 2021).

The number of eggs of other parasites found in the samples was not sufficient for robust statistical comparison of the various methods; however, some important aspects were noted. Mini-FLOTAC was the most precise test for detecting *Nematodirus* spp eggs. This is most likely due to a combination of the low number of *Nematodirus* spp eggs present in the samples and the low multiplication factor of this test meaning that low positive samples were more likely to be identified with this method (Boelow et al., 2022). FECPAK^{G2} rarely detected eggs of *S. papillosus*. On the other hand, *Moniezia* spp eggs were identified in 8 samples with OvaCyte, whilst they were not found with any of the other methods. The OvaCyte system includes the useful feature of being able to review the images captured. The features annotated as *Moniezia* spp eggs were reviewed and the majority were clearly not eggs of this genus. This suggests it may be beneficial to review the performance of the model used in this device for the detection of *Moniezia* spp. For *Trichuris* spp, Mini-FLOTAC was the only technique with which it was detected in a low number of samples, possibly because the egg count was low and hence unlikely to be detected with the other methods.

A variety of methods for the comparison of new FEC tests with existing tests have been reported including correlation, Cohen's κ and

Table 4
Repeatability of each method for the detection of *Strongyloides papillosus* eggs.

Method	Lin's Concordance Correlation Coefficient	N	P against McMaster
McMaster	0.90	41	-
Mini-FLOTAC	0.97	40	0.006
Micron	0.76	37	0.04
OvaCyte	0.97	41	0.01

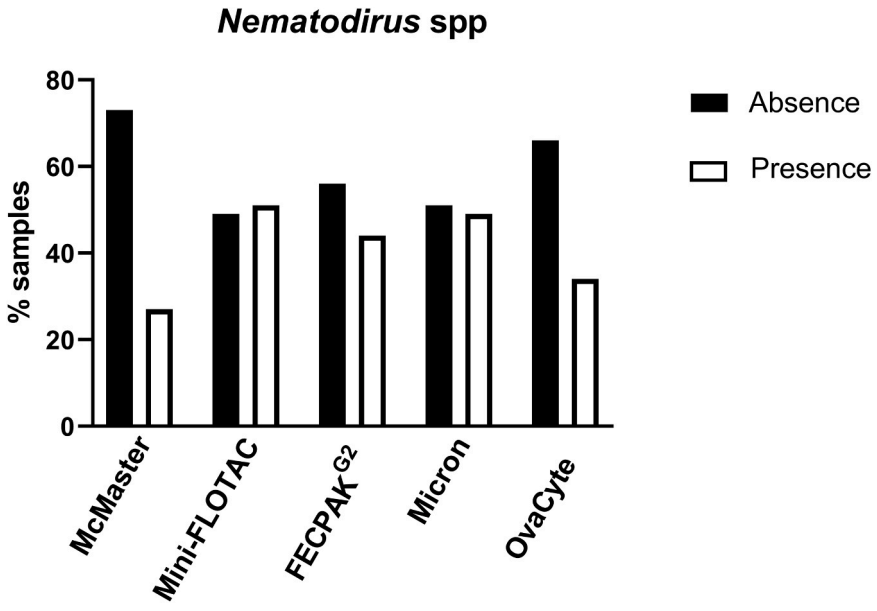

Fig. 5. Percentage of samples which showed presence or absence of *Nematodirus* spp eggs for each faecal egg count method.

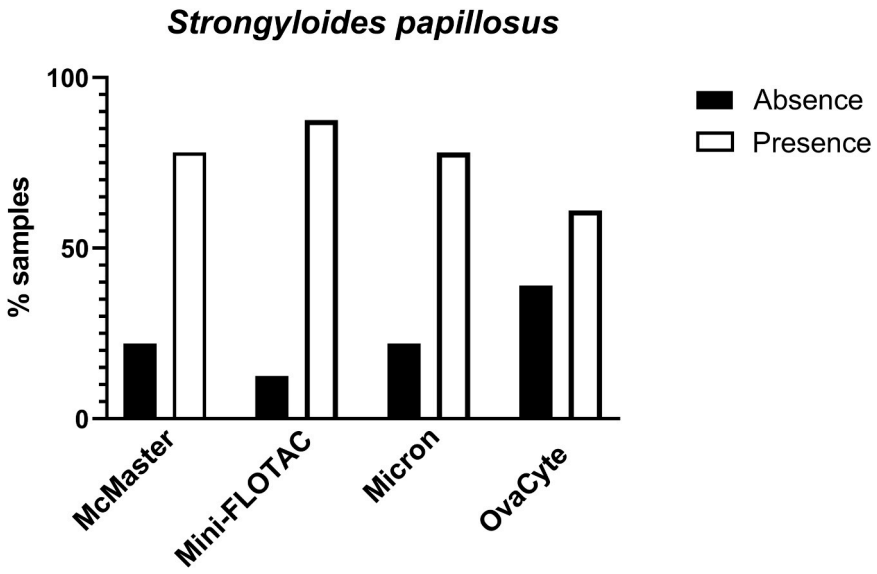

Fig. 6. Percentage of samples which showed presence or absence of *Strongyloides papillosus* eggs for each faecal egg count method.

latent class analysis (Cringoli et al., 2021; Boelow et al., 2022; Elghryani et al., 2023). Results have also been compared using FEC (EPG) or the number of eggs counted under the microscope (Rashid et al., 2018; Cringoli et al., 2021). The results were compared in terms of EPG rather than the number of eggs observed under the microscope although the multiplication factor inflates the variance (Torgerson et al., 2012). This approach was taken as EPG is reported by the automated methods but not the number of eggs counted. In addition, these devices are often used by practitioners as a direct replacement for the McMaster method, and so the performance in terms of EPG is of practical relevance.

A number of tools for the automated detection of parasite eggs in livestock or companion animal faecal samples have been developed or are being developed (Rashid et al., 2018; Li et al., 2019; Cringoli et al., 2021; Nagamori et al., 2021; Slusarewicz et al., 2021; Bucki et al., 2023; Elghryani et al., 2023). A comprehensive effort to evaluate all such methods was beyond the scope of this study and only technologies that were accessible to us were included. All of the automated products included in this study are marketed for use with sheep faecal samples, although there are limited published data on their performance for this species (Bucki et al., 2023).

5. Conclusions

In this study, we endeavour to offer an insight into the performance of some of the methods on the market for parasite egg detection in faecal samples of sheep compared to the industry standard McMaster test. All test methods showed a significant positive linear correlation with McMaster. In our hands, there was no difference in the performance of the manual Mini-FLOTAC method compared to McMaster (equivalent EPGs and repeatability). However, all of the automated methods differed in at least one feature. The magnitude of FEC was significantly higher and lower respectively for the Micron and OvaCyte devices compared to McMaster. This might be acceptable in some scenarios where FEC ranking of animals is required, e.g. selection of resistant animals for breeding programmes. However, it may present problems when working with commonly accepted treatment thresholds, thereby facilitating the development of resistance to anthelmintics by affecting parasites in refugia or negatively impacting animal performance. Both the FECPAK^{G2} and OvaCyte showed lower repeatability than McMaster. In addition, there were some notable differences between methods in the detection of eggs from other parasites such as *Moniezia* spp. and *S. papillosus*. This manifests the need to continue training and improving ML models for parasite egg detection and enumeration. It also demonstrates the need for the establishment of clearly defined criteria for validating new diagnostic tools as they come to market.

Ethics

All procedures involving animals were carried out with approval from the Teagasc Animal Ethics Committee (protocol number TAEC2022–361).

CRediT authorship contribution statement

John P. Dalton: Writing – review & editing, Funding acquisition, Conceptualization. **Michael Parkinson:** Writing – review & editing, Formal analysis, Conceptualization. **Padraig O'Boyle:** Writing – review & editing, Investigation. **Saoirse Ellis:** Writing – review & editing, Investigation. **Orla M. Keane:** Writing – original draft, Investigation, Formal analysis, Conceptualization. **Cynthia Machin:** Writing – original draft, Investigation, Formal analysis. **Amanda McEvoy:** Writing – original draft, Project administration, Investigation.

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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