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Development and performance of an automated fecal egg count system for small ruminant strongylids

Paul Slusarewicz[a,b], Joanna H. Slusarewicz[c], Martin K. Nielsen[b,*]

[a] MEP Equine Solutions, 3905 English Oak Circle, Lexington, KY, 40514, USA
[b] M.H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, 1400 Nicholasville Road, Lexington, KY, 40546, USA
[c] Department of Engineering and Public Policy, Carnegie Mellon University, 5215 Wean Hall, Pittsburgh, PA, 15213, USA

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ABSTRACT

An automated equine fecal egg count test, known as the Parasight System, was modified for use with small ruminants. Modifications included the introduction of a short centrifugation step in a flotation medium, an adjustment in post-stain sample filtering, and training of an image analysis-based egg counting algorithm to recognize and enumerate trichostrongylid eggs. In preliminary assessments the modified method produced trichostrongylid egg counts comparable to manual McMaster analyses of the same samples from both ovine and caprine sources. The coefficient of determination (R^2) for the linear correlation between McMaster and automated counts from these samples was 0.948, and there were no significant differences when comparing counts using feces from either sheep or goats. More extensive comparison utilized ovine samples split into three groups based on trichostrongylid egg content: Low (200–500 EPG), Medium (501–1000 EPG) and High (1001 or greater EPG). Each group contained 5 samples, each of which was used to produce individual slurries that were counted 8 times each using both McMaster and the automated method. This, again, showed no difference in accuracy between the techniques, but revealed significantly higher precision, as assessed by coefficients of variation (CoV), for the automated method for determining egg counts in the Low and Medium groups. The CoV of the McMaster method was 2.2, 2.5 and 1.3 times greater than the automated in the Low, Medium and High groups, respectively. Overall, the automated egg counting system showed good linear agreement with trichostrongylid egg counts determined with the McMaster method, and demonstrated significantly better precision. This technology reduces operator error, and the results presented here illustrate its utility for determination of small ruminant trichostrongylid fecal egg counts.

1. Introduction

Routine fecal egg count (FEC) and FEC reduction (FECR) analyses are an integral part of parasite management strategies in small ruminants (Kahn and Woodgate, 2012; Rinaldi et al., 2019; Burke and Miller, 2020). These have become all the more important since the evolution and spread of anthelmintic resistance in trichostrongyle parasites (Waller, 1994; Jackson and Coop, 2000; Čerňanská et al., 2006; Howell et al., 2008; da Cruz et al., 2010; Herrera-Manzanilla et al., 2017; Ploeger and Everts, 2018; Lambertz et al., 2019). Parasite load has been shown to adversely affect productivity (Miller et al., 2012; Abbas and Hildreth, 2019) and the development of anthelmintic resistance, therefore, represents a growing concern to the small ruminant industry.

Numerous methods are available for conducting FECs and FECRs. These include the Stoll (Stoll, 1923), McMaster (Gordon and Whitlock, 1939), Wisconsin (Cox and Todd, 1962), FLOTAC (Cringoli et al., 2010) and Mini-FLOTAC (Cringoli et al., 2017) methods. These tests are based on the lower density of ova relative to the non parasitic elements of the bulk feces, and ova are separated by flotation when a fecal sample is suspended in a dense medium (usually between 1.2 and 1.3 g/L). This separation facilitates the identification and counting of the ova by reducing the background produced by the non parasitic components of the feces.

A common theme in all these tests is the need for manual count of the ova under a microscope in order to obtain quantitative results. As a result, FECs can be affected by the expertise or training status of the analyst (Slusarewicz et al., 2019; Cain et al., 2020). These concerns have been addressed by the development of a variety of new technologies.

* Corresponding author.
E-mail address: martin.nielsen@uky.edu (M.K. Nielsen).

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The FECPAK^{G2} system (Rashid et al., 2018) collects ova in a conical meniscus (Cooke et al., 2015), where they can be photographed through multiple focal planes by a specialized camera; the composite images are then uploaded to a cloud server, where they are manually read remotely by a trained analyst (thereby eliminating the need for one on site), albeit at the cost of a more complex and laborious sample preparation process. Yet other systems have circumvented the possible variability introduced by manual examination by introducing automated counting systems that utilize advanced computer vision algorithms produced using deep-learning neural networks (Elghryani et al., 2020; Nagamori et al., 2020; Cringoli et al., 2021). These systems, however, require high resolution images as inputs to their algorithms in order to produce acceptable diagnostic specificity and sensitivity. In practice this requires the collection of a large number of high resolution images in order to capture the entire sample, and so is a trade-off between optical magnification and field-of-view. As a result, such systems need to take multiple images (using motorized stages) to capture the entire sample, which increases test times by between 6 and 42 min (Elghryani et al., 2020; Nagamori et al., 2020; Cringoli et al., 2021).

We have developed an alternative automated method that circumvents this dilemma by producing a single low magnification image with a broad field-of-view that can nevertheless be analyzed computationally (Slusarewicz et al., 2016; Scare et al., 2017). This is achieved by fluorescently labelling eggs by means of the chitin present in their shells using a derivatized recombinant chitin-binding protein (Slusarewicz et al., 2016). Imaging in fluorescence mode facilitates the production of high contrast, low background images with sufficient fidelity for automated identification even at low magnification. As a result, only a single image is needed for each sample, and it is counted in less than a minute, while a four minute sample preparation and processing procedure is simplified by means of an automated device. For determination of equine strongylid fecal egg counts, this system has been shown to perform with an accuracy comparable to manual McMaster tests while being significantly more precise (Slusarewicz et al., 2019; Cain et al., 2020).

In this paper, we describe the development of a version of this system intended for use with small ruminants, and report on its performance for determining trichostrongylid egg counts in caprine and ovine samples compared to the McMaster technique.

2. Materials and methods

2.1. Materials

The properties of the McMaster and automated techniques used in this study are presented in Table 1. The FECA MED 35.6 % (w/w) sodium nitrate flotation medium (specific gravity 1.27) was from VEDCO Inc. (St. Joseph, Missouri, USA). Double-chamber McMaster slides were from FEC Source (Bank, Oregon, USA). An Eclipse E200 microscope (Nikon, Mellville, New York, USA) was used for McMaster egg counting.

The Parasight automated system was provided by the manufacturer (MEP Equine Solutions, Lexington, KY) and its various components are illustrated in Fig. 1. The system consists of a Sample Preparation Tool (SPT), a reusable Reagent Dispensing Unit (RDU), disposable Egg Chambers (ECs), and an Imaging Unit (IU) with an Android tablet running an app that both downloads the image from the IU and runs the egg counting algorithm. The SPT itself consists of a silicone bottle and an interchangeable spring loaded plunger for sample homogenization and a filter cap for sample filtration prior to processing in the RDU. The filter cap contains three filters in a series of decreasing U.S. mesh sizes of 16 (1190 μm), 40 (420 μm), and 70 (210 μm).

Samples were centrifuged using a 15 mL centrifuge tube in a CF-800−1 fixed-angle centrifuge (Hardware Factory Store Inc., Azusa, CA). The fixed angle centrifuge was chosen to represent a type present in many veterinary practices.

2.2. Samples

Ovine and caprine fecal samples were collected during June-July 2020 from various regions of the United States, namely Pennsylvania, Georgia, Arkansas, and California and shipped overnight and on ice from each source. Samples were collected by a mixture of veterinary practices, parasitology research laboratories, and reference laboratories, with some samples being collected rectally and others from freshly deposited feces on the ground. Samples were stored in sealed plastic bags and at 4 °C until analyzed (within 5 days of receipt).

2.3. *Algorithm development*

Images for algorithm training were produced from the samples described above. Samples were resuspended in FECA MED at a ratio of 1 g : 9 mL and filtered through two layers of cheesecloth. The filtrate was then divided into 8 mL portions in 15 mL centrifuge tubes and spun at 2200 *g* for 3 min. Each supernatant was then processed through the automated system's RDU, and images captured with the IU. Full (18 megapixel) resolution images were then retrieved and individual strongylid eggs copied into individual images to produce a training set of approximately 5000 images, which were then used to train a TensorFlow-based deep learning neural network (www.tensorflow.org).

The original automated system was designed to count strongylid eggs from equines. Since the lengths of the minor axes of equine strongylid eggs range between 40 and 65 μm (Foreyt, 2001; Slusarewicz et al., 2016), they can all be trapped on the 37 μm mesh integrated into the EC that is used to capture eggs in the equine system. However, ruminant trichostrongyle eggs are significantly smaller, with minor axial lengths ranging between 34 and 50 μm (Foreyt, 2001). Due to this smaller size, we used equine ECs fitted with 25 μm meshes to produce images for training. This would prevent possible loss of smaller eggs during sample processing (particularly since the process utilizes a substantial suction force at several stages to drain reagents between treatment steps), and therefore their possible under representation in the training set.

2.4. *FEC methods*

Preliminary comparison to McMaster counts used 13 ovine and 12 caprine samples analyzed in triplicate, while further comparison of automated and McMaster counting used 15 samples with a broad range of strongylid egg counts to produce fifteen slurries that were then analyzed eight times each by both methods.

To reduce biologic FEC variability when comparing the automated method with the McMaster technique, both tests were conducted on the same slurries rather than on independent slurries produced from different fecal subsamples. Furthermore, slurry subsamples were counted either three or eight times with both methods to, at least partially, reduce subsampling variation. In some cases, samples were analyzed using multiple sequential expulsions of slurry from the SPT, and in other cases slurry from one or multiple SPTs were filtered through the tool and pooled prior to multiple analyses (see below). The McMaster method used in this study has been described previously (Slusarewicz et al., 2019).

The equine automated system makes use of an SPT consisting of a silicone bottle attached to a series of increasingly fine filters designed to

Table 1
Properties of the two fecal egg counting techniques evaluated in the study.

	McMaster	Automated
Volume of flotation medium (mL)	54	54
Fecal weight (g)	6	6
Volume of suspension counted (mL)	0.3	8
Chamber area counted	2 × 10 mm X 10 mm	30 mm X 24 mm
Multiplication factor	33.3	2.5

Fig. 1. Components of the automated system. The system consists of: a reusable Sample Preparation Tool (SPT), which itself consists of a silicone bottle, a handle for sample homogenization and a dispensing cap with integrated filters; disposable Egg Chambers (ECs) in which samples are chemically treated and imaged; a Reagent Dispensing Unit (RDU); an Imaging Unit (IU); and an Android tablet with an App for capturing images from the IU and for counting eggs in the images.

removes as much of the fecal debris as possible prior to trapping eggs on a fine mesh filter (Scare et al., 2017; Slusarewicz et al., 2019; Cain et al., 2020, 2021). Initial attempts to use the equine system with sheep and goat feces encountered two problems. Firstly, many samples tended to clog the filter system of the SPT, sometimes making complete dispensing of the sample problematic. Secondly, many samples also either clogged the EC, thereby preventing proper draining of chemical reagents during automated processing, or contained sufficient fecal debris to obscure substantial numbers of eggs. We addressed the first problem by removing the final (finest) filter from the SPT filter stack, and the second by resuspending the sample in a sodium nitrate (specific gravity 1.25–1.30) flotation medium (FM) and introducing a post-filtration centrifugation step (see below) to remove the bulk of the fecal particles that could foul the EC membrane.

The automated equine method was therefore adapted for ruminants as follows. Samples (6 g) were suspended in 54 mL of FECA-MED using the SPT. In the case of pelletized samples, feces were placed in a plastic bag and kneaded into a paste prior to weighing. Eight mL of suspension was poured into a 15 mL tube through the filter assembly of the SPT and centrifuged at 2200 g for 3 min. The supernatant was then poured into an EC placed on the RDU, drained and then processed with the same cycle used for equine samples. Samples were decanted immediately after centrifugation, because the pellet loosened substantially to the point that significant amounts could dislodge during pouring. When multiple samples were centrifuged simultaneously, all supernatants were decanted immediately afterward into fresh tubes so that they could later be processed at leisure.

2.5. Method comparison

Initial experiments sought to determine the extent of any correlation between triplicate McMaster and automated counts of the same samples. Upon discovering that small ruminant feces have a propensity to block filters, we were concerned that this might lead to egg loss during filtration. More specifically, using the SPT (even with the finest mesh removed) to produce sufficient combined slurry for both manual and automated triplicate analyses might lower egg counts due to progressive clogging during passage through the meshes. We therefore decided to use cheesecloth as a filtration medium. However, we were also concerned that results produced using cheesecloth may not represent those obtained when using the SPT. We therefore produced a slurry in the SPT, dispensed 8 mL directly into a centrifuge tube, spun the sample, and used this for the first automated count. The remaining material in the SPT was then filtered through two layers of cheesecloth and the filtrate returned to the SPT and harvested through the integrated filters. This material was then used to conduct all three McMaster and the second and third automated counts.

While the second and third automated counts were, on average, 4.9 and 10.0 % greater than the first (n = 26), respectively, these differences were not significantly different at the 0.05 significance level according to a Wilcoxon signed-rank test (see Statistical Analyses, below). Furthermore, the third automated counts were 13.7 % greater than the second but also not significantly different. These data indicate that the approach of pre-filtering through cheesecloth prior to the SPT meshes did not produce slurries with significantly differing counts to the first dispense from the SPT (i.e., the intended in-use condition).

To assess test precision we adopted a similar experimental design to that described previously (Slusarewicz et al., 2019). Samples were divided into three groups based on trichostrongylid egg count in eggs per gram (EPG): Low (201–500 EPG), Medium (501–1000 EPG) and High (>1000 EPG). Five samples were assigned to each group after screening using triplicate McMaster counts of the same slurry, and each sample was coded to hide its identity from the analyst. Slurries were prepared using two SPTs and then successively filtered through cheesecloth and then the SPT filters. The pooled filtrate was then counted eight times by both the McMaster and automated methods.

2.6. Statistical analyses

Statistical calculations were done using R version 4.1.0 (The R Foundation) with the "nlme" package installed or with SPSS build 1.0.0.1447 (IBM, Armonk, NY). A mixed model was used to determine whether dislodging the lower density (LD) pellet had a significant effect on egg count, with sample ID as a random effect. To determine the significance of the difference in relationship between automated versus McMaster test results for ovine and caprine fecal samples, linear regression models were created regressing mean egg count per sample on test method and animal species with an interaction term between test method and animal species. The p-value of this interaction term coefficient determined whether animal species had a significant effect on fitted slope. Differences between counts of samples prepared by different filtration methods were assessed using a Wilcoxon signed rank test.

Differences between coefficients of variation (CoVs) were assessed using independent t-tests after confirming normality both by inspection of Q—Q plots and by using Shapiro-Wilk tests. Levene's Test for Equality of Variances was used to assess the homogeneity of variance; when variances were determined to be unequal (which only occurred when the CoVs of all egg level groups were combined), a heteroscedastic t-test was performed, and in the case of unequal variance, a homoscedastic t-test was used. Differences between egg counts were assessed using

paired t tests after confirming normality of the differences both by inspection of Q - Q plots and by using Shapiro Wilk tests.

Differences were considered significant at P < 0.05.

3. Results

3.1. Method optimization

Centrifugation of samples in flotation medium produced two pellets: a larger one of high density (HD) material at the bottom of the tube and distal to the center of rotation, and a smaller one of LD material towards the top of the slurry meniscus and proximal to the center of rotation (Fig. 2). The latter was also sometimes accompanied by a faint smear of material along the length of the tube.

We were concerned that the LD material might contain some eggs that had adhered to the side of the tube and thus result in some egg loss during sample processing. However, we observed that when the supernatant was decanted while the LD pellet faced downward so that the liquid washed over it, the majority of this material was dislodged and deposited into the egg chamber. In contrast, if the tube contents were decanted after rotating the tube 180 degrees around its longitudinal axis so that the LD pellet faced upward and the supernatant did not flow over it, substantially less or no material was dislodged (data not presented).

To determine whether dislodging the LD pellet had any effect on the resulting count, three slurries prepared from different samples were counted using the two pouring methods (i.e., with the LD pellet facing upward or downward) to produce 8 counts per slurry per method. There was no significant difference in mean egg count between the two methods. In contrast, the LD pellet-depleted samples produced CoVs that were, on average, 2.4-fold larger than the LD-enriched samples (Table 2) and this difference was significant (P < 0.05). As a result, all subsequent automated FECs were conducted with the LD enrichment procedure.

Once the ovine system had been built, we tested the possibility that some ovine eggs might be lost through the pores of the 37 µm mesh of equine ECs by running quintuplet automated counts of the same slurry using ECs fitted with either 25 or 37 µm screens (Table 3). The counts generated with the larger mesh size screens were, on average, 21.0 % lower than those generated with the smaller screens, a difference that was statistically significant (P < 0.001). As a result, 25 µm screens were used for the remainder of the study.

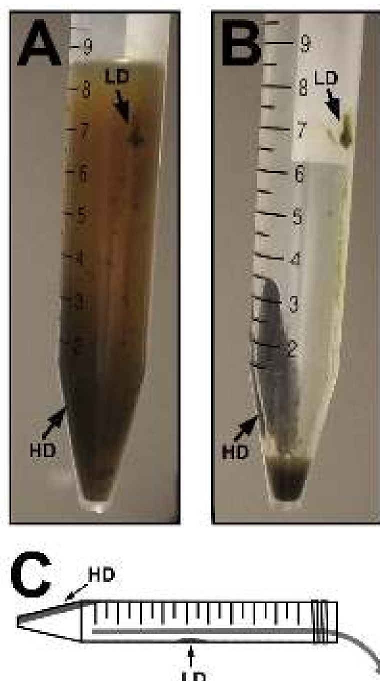

Fig. 2. Sample appearance post-centrifugation. (A) Sample centrifugation produced a large, high density (HD) pellet at the bottom of the tube on the side distal to the axis of rotation, and a smaller low density (LD) pellet on the opposite side of the tube that was often accompanied by a thin smear of material below it. (B) Appearance of the same tube after carefully decanting supernatant with the LD pellet facing upward to minimize its disturbance. (C) Schematic diagram of tube orientation for decanting supernatant while maximizing retrieval of the LD pellet material.

3.2. Method performance

The correlation of the McMaster and automated methods was assessed by comparing triplicate counts from the same slurry. Both ovine and caprine samples were analyzed in triplicate using manual and automated counts from single slurries of each. The combined manual and automated counts for both species exhibited a strong positive correlation with a coefficient of determination (R^2) of 0.958 and produced a line with a slope approaching unity (1.05; Fig. 3). When treated separately, the ovine and caprine data produced lines with slopes of 0.954 and 1.08, and R^2 values of 0.884 and 0.972, respectively. The difference between the ovine and caprine slopes was not statistically significant.

The precision of the two methods was next compared using only ovine samples (3 groups based on egg count, with 5 samples per group and each counted 8 times). After averaging replicate counts, these data produced a line with a slope of 0.971 and an even stronger correlation (R^2 = 0.991), presumably due to the increased number of replicates (Fig. 4). There was no significant difference between the counts within each group nor in all the groups combined. Precision (as assessed by the CoVs) was superior for the automated method in all 3 groups (Table 4), but was significant only in the low and medium count groups (P < 0.01 and P < 0.05, respectively). The CoV for McMaster was 2.2, 2.5 and 1.3 fold greater than for the automated method in the Low, Medium and High groups, respectively. In all the groups combined, the McMaster CoV was 2.0-fold higher than that of the automated method, and this difference was also statistically significant (P < 0.001).

4. Discussion

The purpose of this study was to modify an automated equine FEC

Table 2
Effect of egg chamber filter pore size on egg counts. A single fecal slurry was counted in quintuplicate using egg chambers designed for use with equine samples (37 µm) or fitted with a finer mesh (25 µm). Mean results for each group are shown.

	Mesh Size	
	37 µm	25 µm
Mean EPG	91[illegible]	1,160
S.D.	77.9	89.[illegible]
CoV	8.5	7.[illegible]

S.D. = standard deviations (S.D.).
CoV = coefficient of variation.
EPG = eggs/g.

Table 3
Effect of post centrifugation enrichment of the low density (LD) pellet on egg counts. Slurries produced from three fecal samples (A, B and C) were counted after decanting the supernatants to either enrich or deplete the LD pellets (6 counts per group). Results are reported as mean counts for each group.

	Without LD Pellet			With LD Pellet		
Sample	A	B	C	A	B	C
Mean EPG	622.3	1275.5	441.6	594.8	1102.4	460.1
S.D.	126.1	245.2	134.3	52.1	143.1	19.2
CoV	20.3	19.2	30.4	8.8	11.0	4.2

S.D. = standard deviations (S.D.).
CoV = coefficient of variation.
EPG = eggs/g.

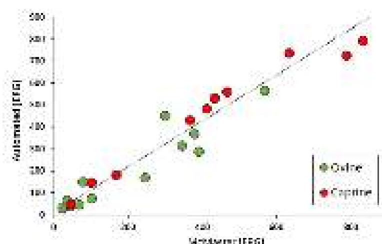

Fig. 3. Correlation of triplicate ovine and caprine automated counts with triplicate McMaster counts. Thirteen ovine and 12 caprine samples were used to produce slurries that were then counted in triplicate by the McMaster and automated methods. The averaged results were then plotted together. The line represents the best least squares fit to the combined data. The slope is 1.05 with a coefficient of determination (R^2) of 0.938.

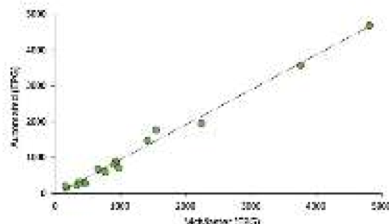

Fig. 4. Correlation of octuplet ovine automated counts with octuplet McMaster counts. Fifteen ovine fecal samples with a broad range of counts were used to produce slurries that were then counted eight times each by the automated and McMaster methods. The averaged results were then plotted together. The line represents the best least squares fit to the data. The slope is 0.974 with a coefficient of determination (R^2) of 0.991.

system for use with small ruminants and to compare its performance to manual McMaster counting of trichostrongylid eggs. We found that, when analyzing ovine samples, the modified system performs comparably to the McMaster with respect to accuracy while operating with a higher precision.

It should be acknowledged that this study did not differentiate the strongylid eggs to genera or species, and more work is needed to ensure that the algorithm will reliably recognize and count all eggs of interest. While we assume that a majority of eggs present in the sample material were of trichostrongyle origin, some could be from strongyle species, such as *Oesophagostomum* spp., and it is possible that this could add to variability in size and shape.

Sample processing for small ruminants was very different than that for equids in that the filters used for the latter were rapidly clogged when using fecal matter from the former. We attribute this behavior to a difference in particle sizes in the feces of small ruminants versus equids (Clauss et al., 2015). The former engage in fore-gut fermentation (rumination), which is both more efficient and time consuming than the hind-gut fermentation of the latter, leading to more complete degradation of plant matter and therefore a smaller particle size distribution in their feces. In fact, the reported mean particle sizes for ovine and caprine feces are smaller than pores of the first two filters of the SPT (Clauss et al., 2015), meaning that many of the fecal particles could potentially gain access to the final filter where they could overwhelm and eventually block it. Furthermore, the greater relative abundance of smaller particles meant that a greater number could eventually be deposited on the EC mesh, thereby clogging this too. While the former problem could be addressed by modifying the pore sizes in the filter stack of the SPT, the latter could not. We ameliorated SPT clogging by removing the final, finest mesh filter from the SPT and we also addressed both problems with a single solution – that is the use of a brief, high speed centrifugation step in a flotation medium to separate the bulk of the fecal debris from the eggs. It should be noted that, unlike FEC methods such as the Stoll and Wisconsin, the purpose of this spin is not to float the eggs to the surface of the liquid, but rather to sediment the fecal particles to the bottom of the tube. We used an FM consisting of sodium nitrate, because both zinc and magnesium sulfate, which are commonly used for FMs, would precipitate as metal oxides during the bleaching step of sample processing and block the EC mesh, thereby preventing correct reagent exchange. Other FMs such as Sheather's solution, salt and sugar/salt mixtures that do not contain divalent cations whose oxides are insoluble could also be used for this purpose.

Initial experiments uncovered that two types of pellet formed during centrifugation (Fig. 2). One, containing the bulk of the fecal solids sedimented to the bottom of the tube and therefore contained material that was denser than the FM (approximately 1.25 g/mL). However, a smaller, but significant amount of material that was less dense than the FM floated inward during centrifugation and was deposited along the inside wall of the tube. We found that the stability of the lower density pellet was dependent on the orientation of the tube when the supernatant was decanted, and that test precision was enhanced when the pellet was washed into the EC prior to the test, suggesting that it may have contained some trapped eggs whose presence or absence have an effect on the final result. However, these observations were made on a limited number of samples and so any conclusions at this point should remain tentative pending more rigorous investigation. Such investigations could be warranted because the possibility of egg loss in the lower density pellet could be relevant to all centrifuge methods utilizing fixed-angle rotors.

As with the equine test, the automated small ruminant system produced counts that exhibited a strong, positive correlation with manual counting and produced counts comparable to the McMaster (Slusarewicz et al., 2019; Cain et al., 2020). Since the algorithm had been trained with a mixture of both ovine and caprine trichostrongylid images, it could detect both with apparent equal accuracy, since the slopes of the lines of automated vs. McMaster counts were not statistically different.

The precision of the method was, in broad terms, similar to that of the equine method, as reported previously (Slusarewicz et al., 2019; Cain et al., 2020), in that the automated method performed with approximately twice the precision of the McMaster (half the CoV) in the low and medium egg level groups. In contrast, the automated method exhibited only a 30 % improvement in precision in the high group, a difference that was not statistically significant, while in previous studies this difference was also two-fold and significant. This may have been partially due to an over performance of McMaster relative to previous

Table 4
Precision of manual and automated methods using ovine samples. Fifteen ovine fecal samples were placed into three groups (high to medium to low) based on their egg content (5 per group), and were used to produce slurries that were then counted eight times each by the automated and McMaster methods. The mean results in eggs/gram for each group of eight counts are shown to the left, as are the determined coefficients of variation. The average count and CoV for each group are shown to the right (upper numbers) along with the 95 % confidence intervals for each mean (lower ranges).

	Mean EPG		CoV		Mean EPG/95% CI		Mean CoV/95% CI	
	McMaster	Automated	McMaster	Automated	McMaster	Automated	McMaster	Automated
Low	331.5	291.6	38.0	15.5				
	463.7	250.2	14.1	11.4	298.0	245.0	31.0	14.3
	167.1	165.1	37.5	9.9				
	154.0	216.2	35.4	9.8	177.4–408.7	190.3–301.6	22.9–41.1	9.8–14.3
	374.6	324.8	33.4	21.1				
Medium	763.9	816.8	45.4	9.5				
	849.4	875.5	23.3	6.2	779.2	744.7	25.5	10.2
	728.2	698.7	26.4	8.3				
	582.5	621.0	15.5	13.4	724.6–933.7	645.0–844.4	14.0–37.0	7.3–13.0
	969.9	711.5	14.5	13.5				
High	1405.0	1452.6	15.3	11.4				
	1544.5	1750.8	13.5	17.1	2751.4	2759.0	15.1	11.2
	3758.7	3978.3	12.0	10.7				
	2235.0	1956.6	17.0	8.4	1456.1–4046.7	1491.1–3926.5	11.7–18.4	8.1–14.4
	4803.5	4656.6	10.3	8.5				

CoV = coefficient of variation.
CI = 95 % confidence interval.
EPG = eggs/g.

studies because the high group contained two samples with substantially higher egg counts than in previous equine studies (approximately 3500 and 5000 EPG). Such high counts are not uncommon in sheep (Niezen et al., 1998; Le Jambre et al., 2007), and these samples also produced the lowest McMaster CoVs, presumably because stochastic subsampling variation decreases with increasing egg concentration (Torgerson et al., 2012). Further studies using sample groups stratified at higher egg count levels could clarify whether manual and automated count precision continue to converge at extremely high egg counts. However, it should be acknowledged that an accurate and precise determination of these high counts probably has limited clinical implications, whereas performance in the lower count ranges would be more relevant, especially for older animals/ewes.

In this study, precision was investigated only using ovine fecal samples. However, the facts that the algorithm was trained on a combination of ovine and caprine samples and that caprine and ovine samples formed statistically indistinguishable lines when compared to McMaster suggests that the ovine results presented here would be recapitulated when using caprine samples, although this would need to be confirmed by a specific study. Furthermore, this study did not assess test performance on eggs from large ruminants, which will be the subject of future studies. Furthermore, the algorithm can be trained to also recognize other parasite egg types such as *Nematodirus* and *Trichuris* spp., which should be pursued as well.

In summary, we have described the development of a rapid, automated egg counting procedure for use with small ruminants and evaluated its performance relative to manual McMaster counting using sheep feces. There was no significant difference in the accuracy of the manual and automated methods (i.e., they produced, on average, equivalent counts). However, the automated method was significantly more precise than McMaster counting at egg levels below 1000 EPG, and equally precise at higher counts.

CRediT authorship contribution statement

M.K. Nielsen and **P. Slusarewicz** conceptualized the study administered and supervised the project. **P. Slusarewicz** collected and curated the data, and **J. Slusarewicz** conducted the statistical analyses. **P. Slusarewicz** wrote the manuscript with input from all co-authors.

Declaration of Competing Interest

PS and MKN both hold stock in MEP Equine Solutions, LLC and PS is an employee of this company, which is commercializing an automated image-based parasite egg counting technology.

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