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Comparative evaluation of an AI-based counting system (OvaCyte™) and the McMaster counting method for quantification of strongyle eggs in sheep faeces

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ABSTRACT

We compared an artificial intelligence (AI)-based technology (OvaCyte™, OC) for the enumeration of strongyle eggs in sheep faeces with the McMaster method (MM). Initially, two experiments were performed with faeces containing pure *Haemonchus contortus* eggs. In experiment A, faeces containing three egg concentrations were processed using OC (extended and standard mode) in parallel with MM. In experiment B, faeces were spiked with different amounts of eggs. Secondly, samples from naturally infected sheep were analysed. Overall, EPG values in experiment A were consistent across all replicates at each dilution. Accuracy was particularly good for the AI-method (mean OC=72 %, mean MM=45 %), and it also achieved the highest precision (CV 5.6–40 %). In experiment B, as in experiment A, within replicate variability was observed at for both methods all concentrations. Although there were no significant differences between sample means, precision and the number of egg-positive samples was higher for OC. Finally, analysis of both experimental ($r = 0.98$) and field samples ($r = 0.93$) showed a strong positive correlation between OC and MM. OC also yielded a higher proportion of positive samples than MM in the field study OC provided a higher proportion of positive samples than MM. This study is the first comparison of OC and MM using both experimental and field-based data. In contrast to previous studies, our analysis was based on identical sample preparations that were processed in parallel using both methods. Although the results show strong agreement between methods, some limitations of OC were noted. These limitations can probably be overcome by further refinement of the AI model.

1. Introduction

Infections with gastrointestinal nematodes (GIN) pose a significant challenge to pasture-based livestock farming globally, including Sweden, where grazing animals are frequently affected. These infections can lead to reduced productivity and increased veterinary costs, affecting both animal health, welfare and farm profitability (Charlier et al., 2020; Höglund et al., 2013). Conventional diagnostic methods based on flotation of parasitic elements in a higher density solution, such as faecal egg counts (FEC) using the McMaster, FLOTAC or other counting techniques are still the standard for the detection of nematode eggs in faecal samples (Rinaldi et al., 2011). However, these methods require trained personnel, are time consuming and due to subjective factors they can show variations in diagnostic sensitivity, accuracy and precision, limiting their widespread use for large-scale parasite monitoring in livestock systems (Levecke et al., 2012).

Coprological examination for nematode eggs is an important part of diagnostic veterinary parasitology (Nielsen, 2021), but there are many factors in the methodology that can influence the outcome even of one selected technique (Vadlejch et al., 2011). Despite inherent limitations, the quantification of eggs in faeces is a common tool to inform farmers about the parasite status in their livestock and it should be included in parasite management plans. Although the faecal egg count (FEC) only detects patent infections and does not always reflect the total worm burden, it can be used to find out how different animals in a group/flock are contributing to pasture contamination (Guzhva et al., 2024). Thus, the FEC provides useful information for targeted treatment with anthelmintics. Moreover, given the lack of universally accepted molecular

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methods and markers, the evaluation of anthelmintic resistance is still based on FEC. The results of the faecal egg count reduction test – still the most widely accepted method for evaluating the efficacy of anthelmintic treatments on farms – are indeed calculated using FEC values (Kaplan et al., 2023). Accurate, precise and sensitive results are therefore of utmost importance. Automated FEC analysis based on artificial intelligence (AI) can enable widespread use of coprological analyses that are not limited to specialised laboratories and allow timely and targeted treatment with anthelmintics. In countries, where sustainable livestock production and digital farm management systems are increasingly integrated, AI-based diagnostics could be included into precision livestock management approaches to support long-term parasite control strategies and improve animal health outcomes (Rinaldi et al., 2022).

In Sweden as in other countries, sustainable parasite control is becoming an increasing priority, due to the growing concerns regarding anthelmintic resistance on sheep farms (Höglund et al., 2022; Rose Vineer et al., 2020). The focus is on evidence-based approaches that aim to limit the use of anthelmintics by encouraging farmers and veterinarians to rely on diagnosis-based treatments rather than blanket deworming strategies (Kenyon et al., 2009). In a recent study we found that respondents' habits of testing faecal samples for strongyle eggs varied between organic and conventional farms (Halvarsson et al., 2022). Although compliance with diagnostic treatments was high (65 %), it is expected that adoption of an evidence-based approach to anthelmintic treatment can be further increased if diagnostic tools become more accessible. AI-driven or assisted image analysis in microscopy is a promising innovation in this context, which has shown the potential to automate the detection and quantification of nematode eggs, improve efficiency, limit human error and ensure standardised results (Slusarewicz et al., 2016). Currently, there are several devices that integrate AI into microscopy to implement digital imaging capabilities and analyses of faecal samples of veterinary interest. Some of these devices, such as OvaCyte™ (Telenostic Ltd), ParaSight™ (ParaSight Imaging System Inc) and Micron Kit (Micron Agritech) have paved the way for commercial applications in the livestock industry. Others, such as Kubic FLOTAC Microscope (University of Naples) are currently being used in research but not yet available on the market (Boelow et al., 2022; Cain et al., 2024; Cringoli et al., 2021; Elghryani et al., 2023; McEvoy et al., 2024).

Nevertheless, all these devices contribute to the growing field of automated parasite detection, an area in which we expect to see expansion in the future. In addition to improvements in the diagnostic laboratory, AI-assisted diagnostics combined with geospatial strategies could also significantly improve advisory services for farmers and veterinarians around the world (Cringoli et al., 2013). However, it is important to emphasise that while the principles of these technologies are essentially similar when dealing with the detection of parasite eggs in faecal samples, the machine learning models and algorithms need to be trained in a specific context with a focus on a particular host-parasite relationship (Capuozzo et al., 2024). Before AI-based methods can become a part of routine parasitological diagnostics, it is therefore important to validate the different technologies to ensure that the tool works for different populations, geographical settings and sample qualities. If this is the case, these advances could play a crucial role in expanding the range of end-users of coprological analysis and improving in turn the accuracy and accessibility of parasite surveillance.

This study evaluates the performance of an AI-assisted microscopy device (OvaCyte™, OC) against the conventional manual McMaster technique (MM) for detecting and quantifying strongyle nematode eggs in sheep faeces. Given that MM remains a widely recognized standard diagnostic tool while OC offers instant, automatically generated results, our aim is to compare their performance using both experimental and field-generated samples. This research intends to enhance the efficiency and applicability of AI diagnostics in veterinary decision-making and farm advisory services, contributing to precision parasite control strategies in the livestock industry.

2. Material and methods

2.1. Ovine faecal samples

We analysed fresh faecal samples from: i) a pen reared sheep experimentally infected with the ISE isolate of *Haemonchus contortus* (experiment A), ii) an uninfected sheep (experiment B), and iii) sheep of different ages from Swedish farms (field study), as described in 2.2.

2.2. Experimental design

2.2.1. Experiment A

Faecal pellets from the infected sheep were mixed with tap water to obtain a faecal slurry containing ≈ 15000 egg per gramme (EPG) in the undiluted state (1:1). This material was then serially diluted with tap water 1:10 and 1:100 to obtain biological replicates (Fig. 1). In this way we created three sample dilutions (1:1, 1:10 and 1:100) from the same stock (faecal slurry) with an expected concentration of approximately 15000 EPG, 1500 EPG and 150 EPG, respectively. The number of eggs at each dilution was then counted in triplicate (technical replicates) according to the OvaCyte™ (OC) protocol (Telenostic Ltd., Ireland), and in parallel with the McMaster method (MM) as described in 2.3.1. This procedure was repeated three times to obtain three biological replicates. In total, 27 sample dilutions were prepared and analysed using both the AI-based method and conventional microscopy. The whole experiment was repeated twice, i.e. 27 samples were read with the OC extended reading mode (lasting twelve minutes, approximatively, experiment A1) + MM and 27 more samples were examined with the OC standard reading mode (lasting approximately six minutes, experiment A2) + MM.

2.2.2. Experiment B

To further compare accuracy and precision but also egg recovery rates between MM and OC (standard mode), faecal pellets from an uninfected sheep were diluted 1:1 with tap water to obtain a homogeneous slurry. The slurry was then divided into five samples of 20 g each and spiked with different amounts of purified *H. contortus* eggs (A ≈3000, B ≈30000, C ≈60000, D ≈90000, E ≈ 120000) (Fig. 2). The number of eggs added was estimated as follows: eggs obtained by flotation were collected in water to a final volume of 5 mL. The liquid was vortexed and 50 mL were collected in triplicate in which the eggs were counted. The number of eggs detected in 50 µl was then expressed per 5 mL. The spiked samples were then analysed in 6 biological replicates, i.e. aliquots of each concentration were analysed by injecting the OC cassette and MM chamber with the faecal suspension as described in 2.3.1.

2.2.3. Field study

To approach the situation in a routine diagnostic setting, faecal samples (n = 156) from Swedish farms were examined. Firstly, samples sent to a routine laboratory were screened with MM. Secondly, we identified suitable samples covering the entire dynamic range of infection (i.e. representing the full range of FEC levels on Swedish sheep farms). Finally, the selected samples were re-analysed in parallel with both OC (standard mode) and MM. They were then procesed as described in 2.3.2.

2.3. Parasitological analyses

AI-based counts were performed with OC which is a cloud-based device in which parasitic elements are recognised and counted by an AI algorithm. A detailed description of this technology can be found in Elghryani et al. (2020). In the present study, we utilized the latest version of the device (as of 1st March 2025, Ovine Plus).

The analyses of all samples in this study were performed with the aliquots of the same sample preparation (filtrate) analysed in parallel with both counting methods. The multiplication factor for each of the

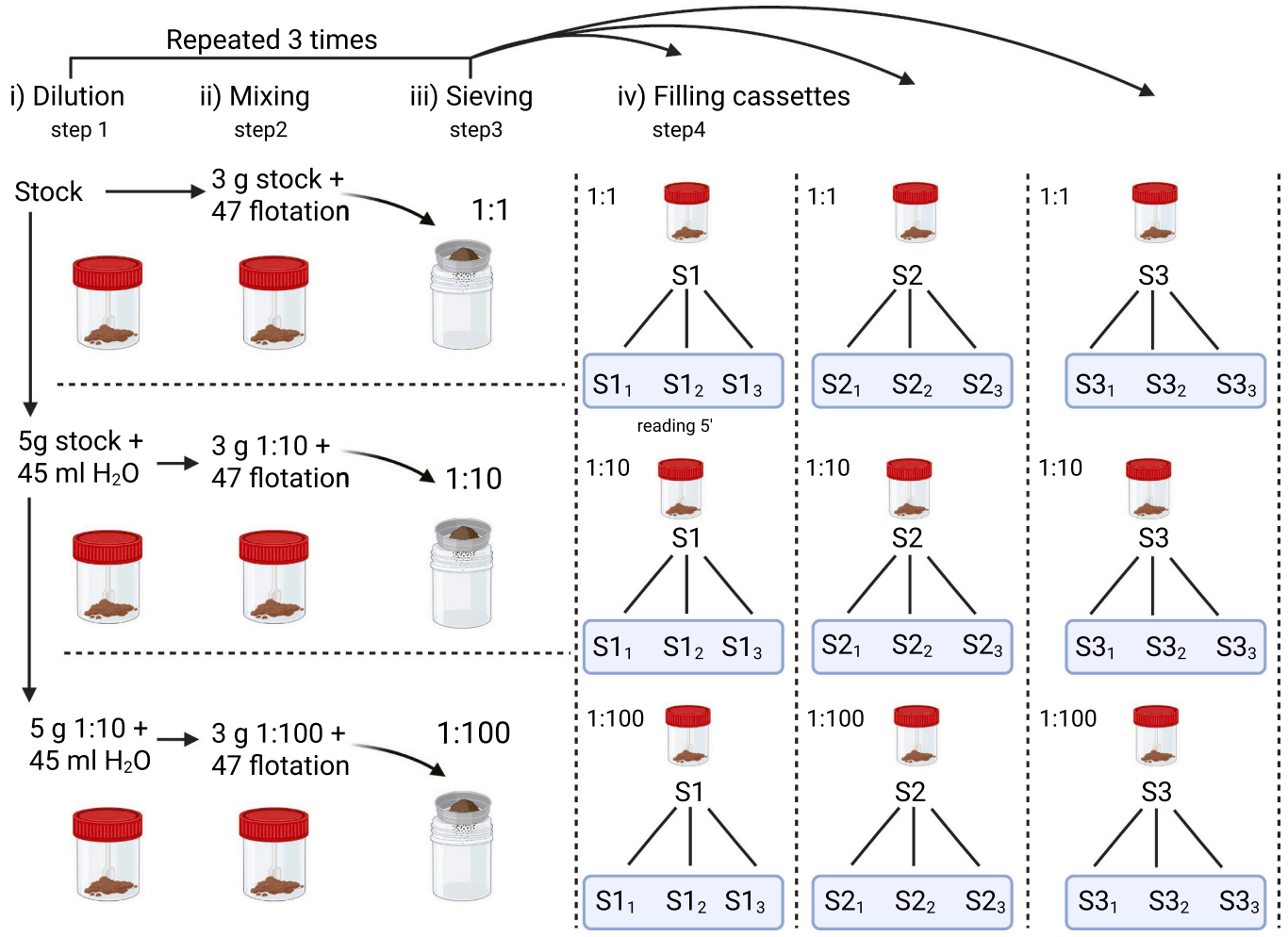

Fig. 1. Illustration of the setup for experiment A.

method used in this study is different. In the MM modification we used, each counted egg is multiplied by a factor of 50 ×, whereas in the OC method each counted egg is multiplied by a factor of 3 × (extended mode) or 7 × (standard mode). As only strongyle eggs were present in the experimental samples, statistical comparisons were limited to this group of nematode eggs.

2.3.1. Experimental study

For each sample, 3 g of faeces were homogenized with 47 g of the flotation solution (specific gravity 1.200) provided by the manufacturer (Telenostic Ltd., Ireland). The sample was mixed thoroughly with the flotation solution and filtered through a metal sieve (mesh size: 0.25 µm, diameter: 52 mm, height: 18 mm) into a sample container. The sieved solution was aspirated into a 10 mL syringe, degassed, and then injected into the OC cassette. The cassette was then placed and fastened on the device. After starting the cassette reading procedure, succussion and calibration steps (approximately 8 min in total) were performed before image capture and upload to cloud storage for automatic AI-based identification. In some cases, a manual review of the pictures was needed shortly before the results and the captured images were made available in the proprietary web-based software. The Ovine Plus model we used required us to use cassettes with a volume of 7.5 mL.

Manual counting of parasitic elements was performed after 5 min using the MM technique. Each compartment of an MM slide was filled with 0.5 mL of the same suspension also used to fill the OC cassette. The eggs were counted under both grids on the slide, each holding 0.15 mL. Since the faeces flotation solution ratio in suspension prepared for MM (1:15) deviates from the one normally obtained according to OC instructions (1:17), OC sample results were corrected by a multiplication factor. The time required for the MM egg count was between 1–3 min, depending on the number of eggs contained in the sample.

2.3.2. Field study

The samples from the sheep farms (n = 156) were processed as follows: 3 g of faecal pellets were homogenised in 42 mL of water using a hand blender (Braun 4179, Braun GmbH, Germany) for ≈ 10 s, filtered through gauze and centrifuged at 450 g for 5 min. The washed pellet was resuspended in an equal volume of saturated NaCl (specific gravity 1.200). This suspension was both injected into the OC cassette and used to fill the MM slide. The OC cassette was processed as described above and with standard read time. In the MM counting technique, strongyle eggs were classified and counted according to the criteria of Ljungström et al., (2018).

2.4. Statistical analysis

Raw data was recorded in Microsoft Excel (version 16.95.1) for Mac. Subsequently, all data from the field samples and experimental replicates were imported into GraphPad Prism (version 10.4.1 532) to perform statistical analyses and create graphs. The Descriptive Statistic platform was used to calculate arithmetic mean, standard deviation (SD), minimum (min) and maximum (max) values, and coefficient of variation (CV). Comparisons between sample means obtained with the two methods for each dilution (Experiment A) and FEC-level

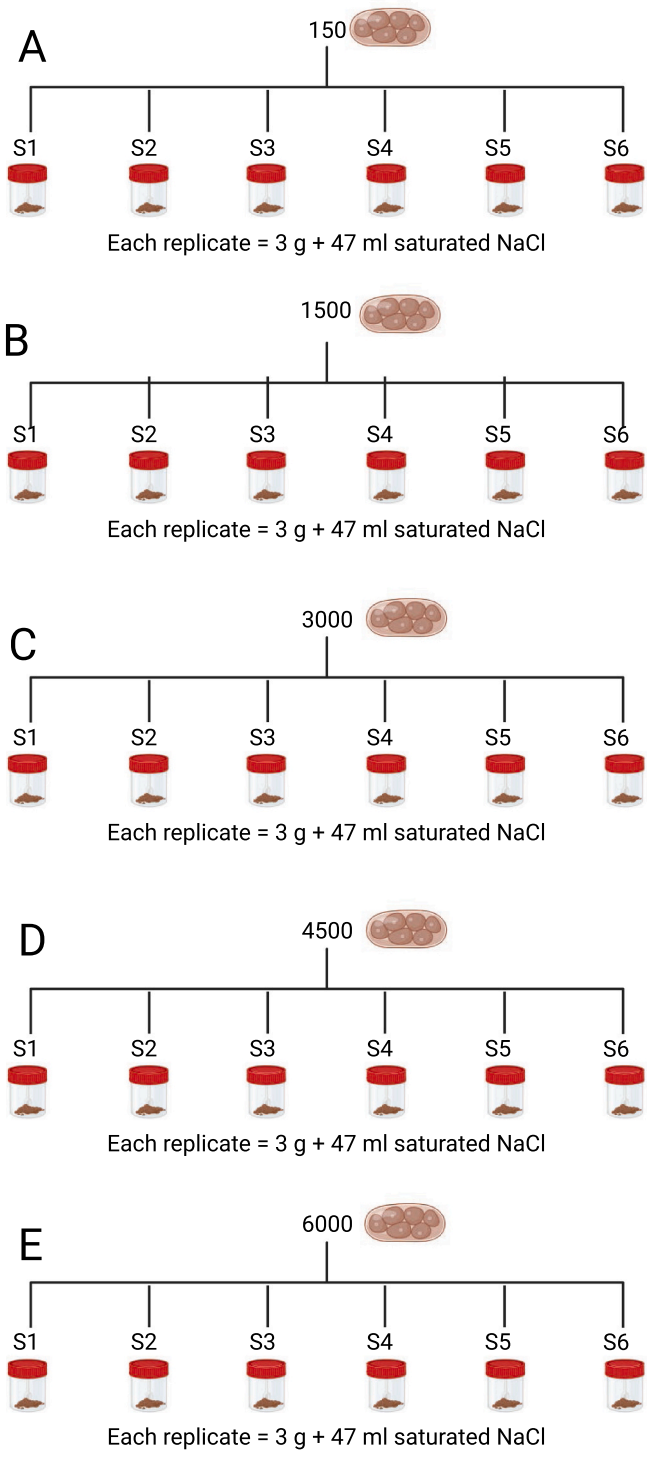

Fig. 2. Illustration of the setup for experiment B.

(Experiment B) were performed using the Mann Whitney test in the Group Comparision platform. Accuracy was calculated as follows: (observed value / expected value) x 100. If the observed count was higher than the expected count, accuracy was: 1-((observed value – expected value)/expected value) x 100.The samples were then compared using the Mann Whitney test as described above. The pairwise FEC measurements of the AI-based and manual counting methods were compared using Spearman rank correlation analysis. Results are reported at 95 % confidence intervals (CI) with normal approximation, and p-values below 0.05 were considered statistically significant.

3. Results

3.1. Experiment A

The measurements were performed twice with the same infected faeces across 3 sample dilutions (1:1, 1:10, and 1:100). Using the extended OC mode (experiment A1), the mean OC values were 12691, 1745 and 170 EPG, respectively. The same results were 13315, 1812 and 121 EPG using the standard OC mode (experiment A2) (Supplementary Table 1). Using MM, the same samples from experiment A1 yielded 14267, 1967 and 333 EPG, respectively, while the samples from experiment A2 yielded 17422, 2433 and 144 EPG, respectively. As shown in Fig. 3, EPG values differed between the biological replicates, but also between the triplicates at each sample dilution. In experiment A1, CVs ranged from 5.6 % to 40 % using OC, while for MM they ranged from 20 % to 60 %. For both methods, the CV decreased with the more concentrated samples. However, overall EPGs were lower with OC than with MM, and in some cases this difference was significant (Supplementary Table 1), with OC measurements being more accurate as they were closer to the expected counts. Accuracy estimates at the sample level showed that MM generally overestimated counts more often than OC (Supplementary Table 2). The average accuracy with OC extended mode (experiment A1) and MM was 69 % and 52 %, respectively. Using the standard mode (experiment A2) the mean accuracy was 76 % for OC and 37 % for MM. The confindence intervals decreased with increasing FEC but was always lower for OC compared to MM. A significant was only observed at the lowest count using the extended mode. The variability (SD values) of accuracy was also lower for OC compared to MM, regardless of reading mode, but especially at the lowest concentration.

3.2. Experiment B

The observed counts were generally lower than the expected values (Fig. 4, Supplementary Table 3). While eggs were found in all samples with OC at the lowest expected concentration (≈150 EPG), no eggs were detected with MM in half of the samples. Although the numerical EPG values were generally slightly higher in the MM counts, no significant differences were found between the two counting methods. There was a strong statistically significant ($P < 0.0001$) positive correlation (Spearman $r = 0.98$, 95 % CI 0.95–0.98) between the results (Fig. 5 A). Similar to experiment A, CVs (OC range 27–93 %, MM range 34–173 %) were lowest at the highest concentration. This was independent of the counting method used. As in experiment A, the mean accuracy for all samples was higher for OC (56 %) than MM (42 %) (Supplementary Table 4). Confidence intervals ranged between 9–25 %, with the exception of MM at the lowest FEC (42 %). Although major deviations in accuracy were observed between OC and MM at a sample level, no significant diffrences were found.

3.3. Field study

As shown in Fig. 5B, the strongyle egg counts of the field samples analysed with both counting methods showed a strong, highly significant positive correlation (Spearman $r = 0.93$ CI 95 % 0.90–0.95, $p \leq 0.0001$). Thanks to the previous screening approximately the same number of samples for each EPG class were included: i) Low: ≤ 50–200 EPG ($n = 53$), ii) Medium: 250–1000 EPG ($n = 52$), iii) and High: ≥ 1000 EPG ($n = 51$). As shown in Table 1, higher EPG values tended to produce results where MM counts exceed OC counts. Conversely, MM counts tended to be lower than or equal to OC counts at lower EPG values. In the low interval, 16 of 22 (72 %) samples that showed no parasite eggs with MM (≤ 50 EPG) were positive with OC. This indicates that MM deviates more at higher FEC values (in the Medium and High EPG intervals) than in the low range. In addition, the difference between the methods appeared to increase with increasing EPG values. Despite

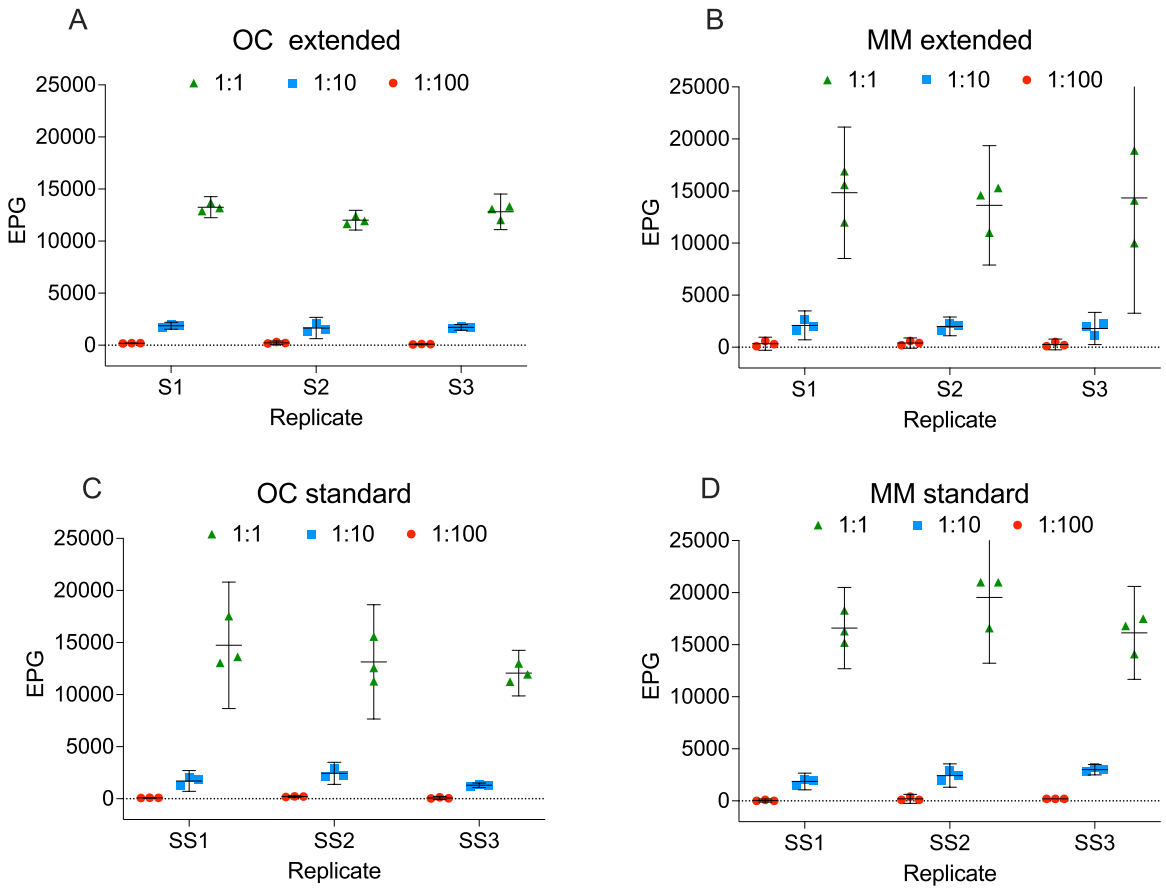

Fig. 3. Scatter plot with mean and 95 % confidence interval (crosses) for experiment A, in which the faeces of a sheep containing *Haemonchus contortus* eggs were serially diluted. The number of eggs per gram (EPG) in aliquots of the same filtrate were counted in parallel using MM=McMaster and OC=OvaCyte™. S1 to S3 were measured in extended OC mode and SS1 to SS3 in standard OC mode. "MM extended" and "MM standard" are counted in the same way but are corresponding to the suspensions analysed with OC using the extended and standard mode, respectively. The expected EPGs were 150 (red), 1500 (blue) and 15000 (green). Each point represents one measurement.

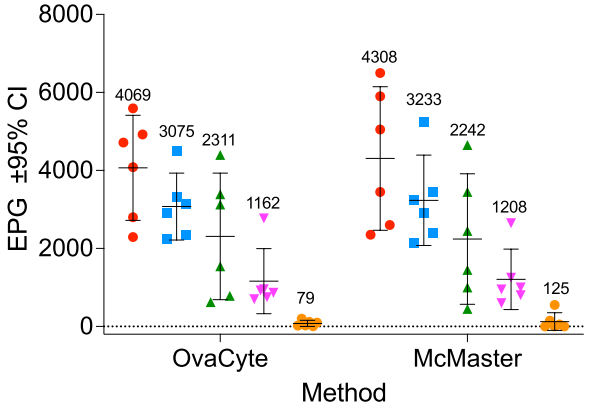

Fig. 4. Scatter plot with mean value and 95 % confidence interval (crosses) for experiment B in which the faeces of a sheep containing *Haemonchus contortus* eggs were serially diluted. The values in the figure are the mean for 6 biological replicates. The estimated number of eggs added to 20 g faecal slurry was 3000 (yellow), 30000 (purple), 60000 (green), 90000 (blue) and 120000 (red). Accordingly, the expected EPGs were 150, 1500, 3000, 4500 and 6000. OC mode used: standard.

these differences, the line representing no difference between the methods remained within the 95 % interquartile range across all three EPG intervals (Fig. 6).

4. Discussion

This study presents a comparative analysis between two methods for counting nematode eggs in ovine faecal samples. We used an automated AI-based method (OvaCyte™) and compared it with a traditional method (McMaster counting technique), which is still the most widely used in livestock parasitology (Sabatini et al., 2023). Overall, the results showed consistency between different experimental set-ups and field samples from Swedish farms. Moreover, as the correlations between the methods are strong over the whole dynamic range considered, OC is reliable for counting nematode eggs in sheep and can be used as a tool integrated with traditional methods like MM, depending on the required application context.

When counting nematode eggs in faecal samples, several factors can influence the accuracy (the closeness of the measurement to the true value), precision (the consistency of repeated measurements on the same sample), and sensitivity (reflected in the number of egg-positive samples in this study) of the diagnostic method used (Slusarewicz et al., 2016). Even though most egg counting methods rely on the same principles (dilution of the sample, followed by flotation of parasitic elements), comparing methods can be challenging as none of the available techniques can provide the correct, "true" numbers. Nevertheless, in the present study, accuracy was assessed as the deviation between the expected values in experiment A and B and the obtained FEC. According to our observations, deviations from the expected values were observed in both methods, but in general the values seem to be slightly more consistent with OC compared to MM. Although the presence of strongyle

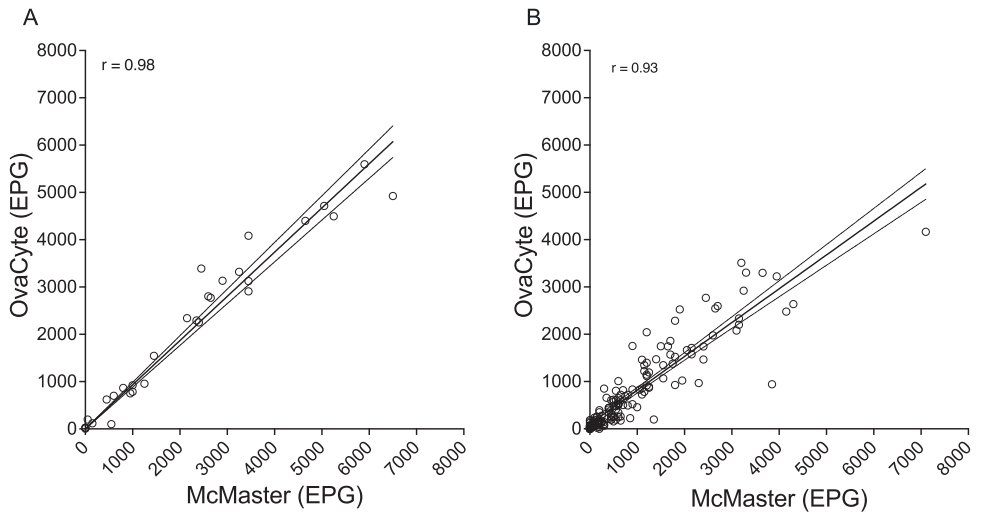

Fig. 5. (A) Scatterplot of all individual counts for OvaCyte™ (standard mode) and McMaster performed on samples originating from Experiment B. (B) Scatter plot of all individual counts for OvaCyte™ (standard mode) and McMaster obtained from faecal samples of sheep naturally infected on pasture (N = 156). Each point represents the measurements of aliquots of the same sample preparation (filtrate) analysed in parallel.

Table 1
The distribution of the measured values in relation to the two egg counting methods applied to the field samples (n = 156). When McMaster (MM) and OvaCyte™ (OC) measurements were the same (MM=OC), while when MM was lower than OC (MM<OC) and if MM was higher than OC (MM>OC).

Category	EPG - interval	n	MM=OC	MM<OC	MM>OC
Low	0–200	53	6 (11 %)	30 (57 %)	17 (32 %)
Medium	250–1000	52	0 (0 %)	16 (31 %)	36 (69 %)
High	> 1000	51	0 (0 %)	14 (27 %)	37 (73 %)

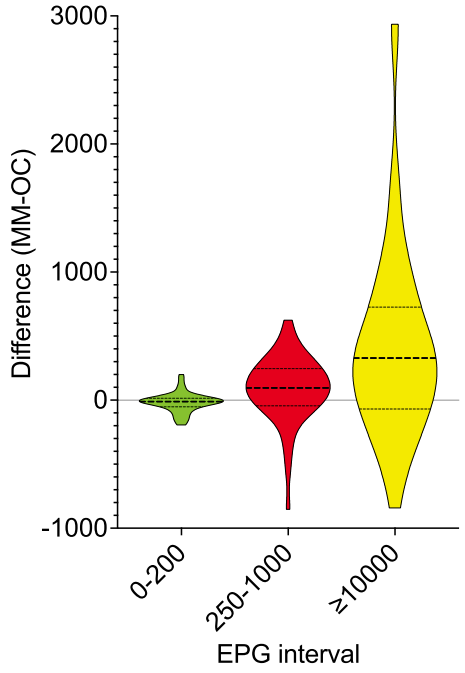

Fig. 6. Differences between McMaster and OvaCyte™ measurements based on samples from naturally infected sheep. Values are shown as truncated violin plots with median values and 95 % interquartile ranges; green = 0–200 EPG (n = 53), red = 250–1000 EPG (n = 52), and yellow = ≥ 1000 EPG (n = 51). OC mode used: standard.

eggs in ovine faeces has been previously investigated using an older version of this technique (McEvoy et al., 2024), our study is the first to evaluate the performance of the latest version of the OC model by combining experimental results with stratified field data. This, to determine the reliability of the OC device compared to MM in terms of egg recovery rates, in contrast to previous investigations focusing on AI-detection of parasite eggs (e.g., Cure-Bolt et al., 2024).

In line with similar studies (e.g. Bosco et al., 2018; Bucki et al., 2023), the manual calculations were performed by an experienced technician. This minimises observer's bias in manual MM counts. It is also important to note that the distribution of nematode eggs in faeces is inherently heterogeneous. So when aliquots are taken from different portions of the faecal sample, they may contain different amounts of eggs due to natural variation. This is something that has been observed in several independent studies (e.g. Amadesi et al., 2020; Daş et al., 2020; Vadlejch et al., 2011). In contrast to previous studies, all measurements in our study were performed in parallel with both methods on aliquots from the same sample preparation (i.e. faecal suspension in flotation solution). This approach minimises the potential bias that arises when using different sample preparations (i.e. the aforementioned uneven distribution of eggs in different portions of the faecal material and when analysing the same material at different time points) and allowed us to focus more precisely on the variability caused by methodological differences. Overall, this approach provided valuable insights into the evaluation of the AI-based method carried out in this study.

In experiment A, we compared egg counts in serially diluted faecal samples to assess precision and accuracy. As expected both methods showed a progressive decrease in egg counts as a function of dilution factor. The precision (CV) between replicates increased with concentrations in favour of OC, especially when using the extended mode. Interestingly, significant differences in FEC between methods were observed with the extended OC read mode, but only at the highest dilution (1:100). In addition, higher egg counts were observed with MM in the two more concentrated samples (1:10 and 1:1) when using the standard mode. This is consistent with McEvoy et al. (2024), who also reported significantly lower FEC values with OC than with MM. In addition, accuracy decreased with sample dilution for both methods, and with less variation using OC. Our data also suggest that MM values overestimated expected counts more often than OC, but this remains speculative and requires further investigation.

In experiment B, we compared the performance of the two diagnostic methods (OC standard and MM) using the estimated concentrations of eggs in spiked samples. The results showed a strong positive correlation

between the methods (r = 0.98). In contrast to experiment A, we generally observed a lower FEC than the expected values. The precision was overall lower than in experiment A and was in most cases also higher for MM than for OC. For similar estimated FEC (≈1500 EPG), CVs in experiment A ranged from 14 % to 32 %, while in experiment B they were 61–68 %. The same pattern was observed at the lowest concentration (≈150 EPG). One possible explanation for the lower precision in experiment B is the lack of technical replicates. In experiment A at total of 9 samples per dilution (egg concentration) were analysed, whereas in experiment B only 6 samples were analysed. These results reflect the natural variability between biological replicates at all egg concentrations and are consistent with previous findings. For example, Torgerson et al. (2012) have shown that egg counts from different subsamples vary naturally, even in a well-homogenised sample. As in experiment A, we analysed aliquots of the same faecal suspensions (faeces + flotation solution) using both counting methods to minimise the potential variability introduced by using different sample preparation methods. Despite the experimental design, we observed systematic differences in accuracy and precision both within and between the experiments, which seemed to be associated with the different FEC levels. Interestingly, the measurements with OC in experiment B did not yield significantly higher values than the MM method. From a practical point of view, this is positive, as it has been speculated that overestimation of EPG values by AI-based measurements could lead to unnecessary anthelmintic treatments (Stear et al., 2024). This should be investigated in future studies.

When analysing field samples, we aimed to compare the performance of OC and MM on a wide range of FECs. The results showed a strong correlation between the methods (r = 0.93), which is consistent with the experimental data but also with previous studies that have used the OC device in cattle, horses or sheep (Elghryani et al., 2020; 2023; McEvoy et al., 2024). In agreement with the observations at the lowest expected count (≈150 EPG) in experiment B, the OC method detected more positive samples at low EPG values than the MM method, indicating that the OC method has a higher sensitivity than MM. This can be attributed to the larger volume analysed with the OC device, which is approximately 7 times larger than that of the MM when using the standard read time (on average 2.17 ± 0.16 mL for the OC device compared to 0.3 mL for the MM device). This result is consistent, for example with the mini-FLOTAC method, which analyses 1 mL of sample and therefore has a lower detection limit than the MM methods (Amadesi et al., 2020; Cringoli et al., 2010). Accordingly, more egg-positive samples were detected by using the OC extended read mode (where images are captured from 4.4 to 4.6 mL of suspension), but at the expense of scan time. To test the device under conditions closer to the time constraints of the routine, we limited the use of extended mode to experiment A1. Although both methods generally agreed at all EPG intervals within the 95 % interquartile range, the MM FEC values at higher egg concentrations showed a larger deviation from the values obtained with OC.

To summarise, the strong positive correlation between OC and MM in both experimental and field set-ups indicates that the two methods are largely in agreement with each other. This was the case despite inherited differences between sample preparation steps used in the experiments and in the field study. As no major differences in analytical performance were found, the AI-based OC method proves to be a promising, reliable and efficient alternative to manual counting. Although variability was observed with both OC and MM, the correlations remained strong, suggesting that both methods are suitable for nematode egg counting, depending on the desired precision and application context. According to our experience, the major strength of OC is the independence from trained personnel and also the higher egg recovery rate at low concentrations (<150 EPG), while the major strength of MM is that the sample examination is quicker compared to the AI-based technique. These aspects must be taken in account when choosing one technique versus the other. However, it remains unclear whether the OC method underestimates FEC due to AI processing or whether MM overestimates egg counts at higher concentrations. Further investigation is required to determine the cause of this discrepancy and to improve the performance of this AI based method.

CRediT authorship contribution statement

Johan Höglund: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Giulio Grandi:** Writing – review & editing, Project administration, Methodology, Conceptualization. **Jaroslav Vadlejch:** Writing – review & editing, Resources.

Ethics approval

The experimental infection of sheep with *Haemonchus contortus* was approved by both the institutional ethics and animal welfare committee of the Czech University of Life Sciences Prague and The Ministry of Education, Youth and Sports of the Czech Republic (MSMT-15857/2022–5).

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.2025.110533.

Data availability

All data is available upon request from the corresponding author.

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