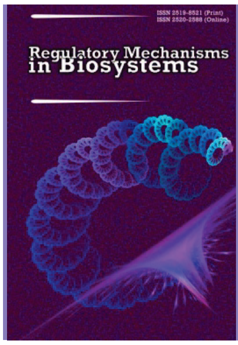

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst., 2024, 15(4), 776–781
doi: 10.15421/0224112

Three different faecal egg counting techniques in ruminants

I. L. Bondarevskyi, O. V. Kruchynenko, O. O. Peredera, R. V. Peredera

Poltava State Agrarian University, Poltava, Ukraine

Article info

Received 05.08.2024
Received in revised form 14.09.2024
Accepted 11.10.2024

Poltava State Agrarian University, Skovorody st., 1/3, Poltava, 36003, Ukraine.
Tel.: +38-099-062-64-96.
E-mail: oleg.kruchynenko@pdau.edu.ua

Bondarevskyi, I. L., Kruchynenko, O. V., Peredera, O. O., & Peredera, R. V. (2024). Three different faecal egg counting techniques in ruminants. Regulatory Mechanisms in Biosystems, 15(4), 776–781. doi:10.15421/0224112

Diagnostics of the gastrointestinal strongiloidosises in ruminants requires methods that are characterized by high analytical sensitivity and accuracy, are easy to replicate, and quickly detect parasite eggs. At the first stage of the study, we tested the analytical sensitivity, accuracy, and precision of the method of quantitative helminthocoproscopic examination, modified McMaster technique, and Mini-FLOTAC (combined with Fill-Flotac). The faecal samples were collected from animals in Kirovohrad and Dnipropetrovsk oblasts. The known numbers of eggs isolated from bovine or sheep faeces were added to the egg-free bovine and sheep cattle stool samples to obtain the values of 10, 50, 200, and 500 eggs per gram of faeces. The objective of the study was assessment of the performances of the coprological diagnostic methods for the egg-spiked samples and samples from naturally-infested ruminants. The method of quantitative helminthocoproscopic examination proved effective in the case of a low concentration of eggs (10–50 EPG), exerting 100% analytical sensitivity. The number of eggs in 1 g of faeces detected using Mini-FLOTAC was not significantly different from the expected estimates at any level of egg density. With increase in the number of eggs to over 200 per 1 g of faeces from cows and sheep, we observed a statistical difference (Tukey's Test) among the three methods. Using a new method of quantitative helminthocoproscopic examination, we were able to detect the highest number of naturally infested animals (54.8% of the cattle, 59.2% of the sheep, and 68.7% of the goats). Lin's concordance correlation coefficient (CCC) was the highest for sheep between the method of Quantitative Helminthocoproscopic Examination and the modified McMasters technique (CCC = 0.93). The provided data indicate the possibility of using the method we proposed in the case of low intensity of animal infestation (3-5 EPG). The prospects for future studies lie in testing the efficiency of Quantitative Helminthocoproscopic Examination on other species of animals and also in cases of their infestations with protozoa, cestodes, and trematodes.

Keywords: method of Quantitative Helminthocoproscopic Examination; modified McMasters technique; Mini-FLOTAC; Strongylida; cattle; sheep; goats.

Introduction

Monitoring the spread of helminths, along with identifying their species composition, age, and seasonal dynamics in a specific natural-climatic zone, represents important goals. Gastronintestinal nematodes are harmful parasites in ruminants (Income et al., 2021; Tachack et al., 2022). In the territory of Ukraine, the infestation of ruminants with gastrointestinal helminths accounted for 56.7% (Kruchynenko et al., 2021). Over the past five years, on animal farms of the central region of Ukraine, gastrointestinal-nematode infestation has become widespread (Prima & Dmytrenko, 2021; Korchan et al., 2023; Bondarevskyi, 2024). It was found that nematodes of the suborder Strongylida (Railliet et Henry, 1913) are some of the commonest in the central and southeast regions of Ukraine, the infestation of sheep reaching 44.1% (Melnychuk, 2019). Experimental studies confirmed that gastrointestinal helminths lead to changes in live weight of the animals, thus entailing substantial economic losses (Boyko et al., 2016).

As is known, veterinary practice uses the generally accepted methods that enumerate eggs (invasive elements) in 1 g of faeces (Cringoli et al., 2013). Despite a broad array of proposed methods, the "gold standard" is still the McMaster technique, developed in the laboratory of McMaster at the Sidney University and whch is extensively used worldwide (Paras et al., 2018; Went et al., 2018). It is the most universal technique of counting eggs in veterinary parasitlogy. The World Association for the Advancement of Veterinary Parasitology (WAAVP) recommends it for evaluating the efficacy of antihelminth drugs in animals (Wood et al., 1995), and also for identification of the resistance of parasites to medical drugs (Coles et al., 1992). Unfortunately, many laboratories are not equipped with the latest equipment, and therefore are often unable to use a centrifuge for diagnostical studies, for example, for the Cornell-Wisconsin methods (Ballweber et al., 2014). For this purpose, quantitative coproscopic diagnostic methods that utilize a counting chamber continue to be relevant (Cringoli et al., 2010; Vadlejch et al., 2011; Cringoli et al., 2017).

Later, in Italy, an alternative to the McMaster technique was developped – FLOTAC, which, according to the data of Cringoli (2006) is more effective. However, a disadvantage of this method is the necessity of centrifuging faecal samples. Therefore, there was developed a simplified apparatus that is highly sensitive to eggs (5 EPG) called Mini-FLOTAC (Barda et al., 2013). In 2021, the Kubic FLOTAC microscope (KFM) was brought out – a compact, cheap, adapted, and easily transported digital microscope, developed specifically for analyzing faecal samples using Mini-FLOTAC or FLOTAC, and suitable for using in field conditions, as well as in laboratories. The assessment detected a high level of concordance (the concordance correlation coefficient = 0.999) between the regular and digital microscopes (Cringoli et al., 2021).

As is known, the flotation principle is based on the ascent of helminths eggs up to the superficial layer of liquid while treating the faecal probes with solutions of salts, the specific weight of which is higher than the density of eggs. Therefore, researchers constantly search for flotation solutions. To detect eggs of helminths, scientists have proposed a considerable variety of flotation solutions with different specific weight. The search for the most optimal ones is ongoing (Rashid et al., 2018; Yevstafieva et al., 2023). It was confirmed that in order to detect eggs of nematodes during faecal analysis, the lowest specific weight of flotation solution is enough. Thus, a quite effective solution for diagnostics of Strongylida eggs of the gastrointestinal tract was the flotation solution of table salt with the specific weight of 1.2 (Bosco et al., 2014). The results of the studies revealed that the simple McMaster method was the most accurate method of detecting Strongylida eggs, while Mini-FLOTAC was the most accurate to detect *Ascaris* eggs (Nápravníková et al., 2019). Vieira et al. (2021) reported that Mini-FLOTAC demonstrated better results in detecting helminth eggs in sheep (71.6%; 287/401) and goats (88.4%; 343/388, χ^2 = 10.4; P < 0.0001).

The objective of the study was the assessment of the coprological methods of diagnostics of helminths in spiked samples and samples from naturally-infested ruminants. We compared the following methods: the method of Quantitative Helminthocoproscopic Examination with the minimum detection limit of 2.5 or 5.0 eggs per gram (EPG) of faeces, the modified McMaster technique (25 or 50 EPG), and Mini-FLOTAC (5 or 10 EPG).

Materials and methods

The research was carried out from March to May 2024 in the scientific laboratory of the Department of Parasitology and Veterinary-Sanitary Expertise (the Poltava State Agrarian University). The study was performed in two stages. At the first stage, the faecal samples with detected and undetected eggs were collected from cattle and sheep kept at private farms in Kirovohrad and Dnipropetrovsk oblasts (central Ukraine). Each sample was analyzed 5 times using the Cornell-Wisconsin technique (Egwang & Slocombe, 1982) with an analytical sensitivity of 1 egg per gram of faeces for presence or absence of eggs of gastrointestinal nematodes. Further, the nematode eggs were isolated from the positive samples using the method of mass isolation, i.e. the method using 4 sieves of various sizes (1 mm, 250, 212, and 38 μm) so as to isolate the eggs from faeces. Later, we collected ten aliquots, 0.1 mL of each, and counted the eggs (Godber et al., 2015). Then, we performed a contamination of the negative faecal samples with eggs of gastrointestinal Strongylida. Suspensions with eggs were added to negative faeces (20 g) and thoroughly homogenized in order to obtain four faecal samples (250 g each) for each level of eggs per gram (10, 50, 200, and 500). Each sample was analyzed using a saturated table-salt solution (the specific weight of 1.2) by the three methods of coproscopic diagnostics: Quantitative Helminthocoproscopic Examination (Kruchynenko et al., 2024), modified McMaster (Zajac & Conboy, 2012), and Mini-FLOTAC combined with Fill-FLOTAC (Cringoli et al., 2017). We analyzed a total of 240 samples of faeces. After thorough homogenization of each faecal sample for each level of eggs per gram, 40 g was weighed for each of the the methods, Quantitative Helminthocoproscopic Examination and modified McMaster, and 50 g for Mini-FLOTAC. In general, we used ten repetitions for each of the methods and for each level of eggs per gram (10, 50, 200, and 500) using individual samples of faeces. The mass of faeces used, coefficient of dilution, volume of reading, and analytical sensitivity of each method are given in Table 1.

The method we propose was performed as follows: a test tube rack with 20 compartments for 10 mL test tubes was taken (Fig. 1). To each compartment, a device was placed, made of plastic in the form of a cylinder of a capacity of 1.5 mL, an inner diameter of 16 mm, and a depth of 7.5 mm. One test tube rack is intended for studying 10 to 20 samples at the same time. Two gram (sheep, goats, carnivores, and birds) or four gram (cattle, swine, horses) stool samples were put into a cup, and flotation solution was added until a 30 mL volume was achieved. It was thoroughly mixed and filtered through a metal sieve with 500 cells into another cup. Then, from one cup into another, the content was poured 5–10 times for an even distribution of eggs in the liquid. Then, we collected 1.5 mL using a syringe, and then the device was filled until formation of meniscus. It was covered by a 18 x 18 mm covering glass (reading area = 324 mm^2). To enhance the efficiency of counting invasive elements (eggs or oocysts), the procedure was repeated to fill the second device from the same sample (reading area = 648 mm^2). The analytical sensitivity for 4 g of faeces equaled 2.5 eggs per 1 g of faeces and 5.0 for 2 g. Seven to ten minutes later, the cover slips were taken off and transferred onto pre-prepared microscope slides. To facilitate the counting of eggs, two parallel lines were drawn on the microscope slide using blue or green permanent 1 mm-thick marker, with 1–2 mm interval.

At the second stage of the study, we compared the three said methods for the ruminants naturally infested with gastrointestinal Strongylida. In total, we examined 203 individuals of cattle (n = 73), sheep (n = 98), and goats (n = 32), ranging 4 months to 8 years-old.

Statistical analyses were performed using MedCalc for Windows, version 20.2 (MedCalc Software, Ostend, Belgium, 2022). Boxplots (indicating median, percentiles, and outliers) were used to evaluate the accuracy and precision of each method. Also, for the each method, we estimated the arithmetic mean for eggs in 1 g of faeces (X), standard deviation (SD), and coefficient of variation (CV). The Shapiro-Wilk test was used to assess the normality of the distribution of indicators. The statistical differences among the methods were determined using the Tukey Test. The differences in the parameters among the groups were considered significant at P < 0.05. Lin's correlation concordance coefficient (CCC) was estimated between the method of Quantitative Helminthocoproscopic Examination and modified McMaster technique; and the Quantitative Helminthocoproscopic Examination and Mini-FLOTAC. The interpretation of CCC was carried out according to the report by McBride (2005).

Table 1
Schematic features of the method of Quantitative Helminthocoproscopic Examination, modified McMaster technique, and Mini-FLOTAC

Name of the method.	Amount of faeces used, grams	Dilution ratio	Reading volume, mL	Reading area, mm^2	Analytical sensitivity, eggs per gram
Quantitative Helminthocoproscopic Examination	4	1:7.5	3.0	648	2.5
Modified McMaster technique	4	1:7.5	0.3	200	25.0
Mini-FLOTAC	5	1:10	2.0	648	5.0

Note: the weight of faeces used for each repetition, dilution ratio, reading volume, reading area and analytical sensitivity of the method of Quantitative Helminthocoproscopic Examination, modified McMaster technique, and Mini-FLOTAC.

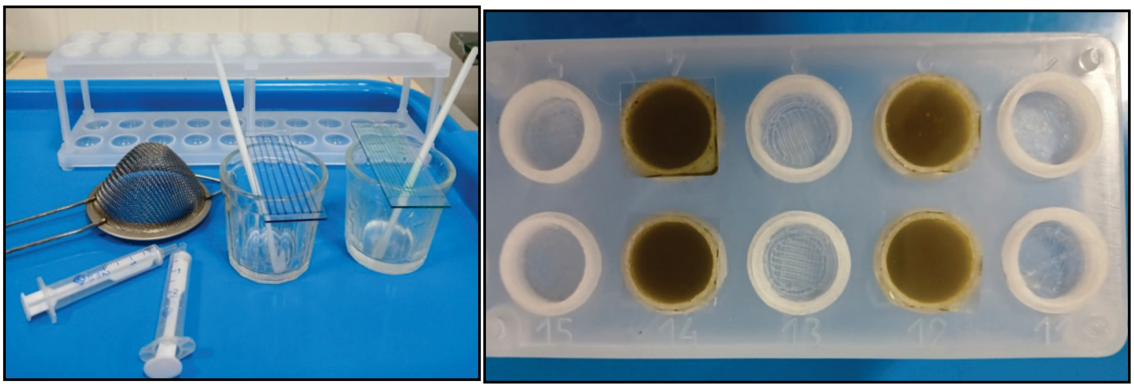

Fig. 1. Kit for the method of Quantitative Helminthocoproscopic Examination

Results

The conducted studies revealed that the method of Quantitative Helminthocoproscopic Examination and Mini-FLOTAC produced 100% analytical sensitivity for all the concentrations of eggs (for faeces of cattle and sheep, contaminated with nematode eggs). On the other hand, the modified McMaster technique exerted 100% analytical sensitivity only for the concentrations of over 200 eggs per gram (20% to 80%, for the concentrations of 10 and 50 EPG). According to Table 2, no statistical difference was observed among the three methods of coproscopic diagnostics for both the 10 and 50 eggs per gram concentrations in cows and sheep. At the same time, with increase in the number of eggs to 200 or 500 in 1 g of faeces, there was a statistically significant difference among the methods ($P < 0.05$). It has to be noted that the variation coefficients were lower for all the concentrations of eggs analyzed using the method of quantitative helminthocoproscopic examination and Mini-FLOTAC, as compared with the modified McMaster technique.

According to our studies, the method of Quantitative Helminthocoproscopic Examination has proved itself effective for detecting low concentrations of nematode eggs, ranging 10 to 50 eggs per gram (Fig. 2a), as indicated by the length of the boxplots. When the level of eggs was lower (10 EPG), the CV% was high, exceeding 100% using the modified McMaster technique (Fig. 2b). Also, this method did not detect all the positive samples when the concentration of parasite eggs in the faecal samples was low. As with Mini-FLOTAC, we should note that the length of the boxplots was very narrow for each level of contamination and for all the variants, indicating high accuracy and precision, compared with the other methods (Fig. 2c).

Table 2
Comparing the three methods of coproscopic diagnostics in cases of adding a known quantity of eggs per 1 g of faeces of cows and sheep ($x \pm SD$, coefficient of variation (CV%), n = 10)

Contamination of negative cattle or sheep faeces, eggs per gram	Method of Quantitative Helminthocoproscopic Examination		Modified McMaster technique		Mini-FLOTAC	
10	7.0 ± 3.1^{a}	43.9	7.5 ± 16.9^{b}	224.9	9.0 ± 3.2^{c}	35.1
	7.5 ± 3.7^{a}	49.7	10.0 ± 21.1^{b}	210.8	9.5 ± 3.7^{c}	38.8
50	38.5 ± 8.0^{a}	20.8	52.5 ± 36.2^{b}	69.0	46.0 ± 5.7^{c}	12.3
	34.2 ± 9.5^{a}	27.7	55.0 ± 40.5^{b}	73.6	48.5 ± 5.3^{c}	10.9
200	143.2 ± 26.5^{bc}	18.5	225.0 ± 60.1^{a}	26.7	190.5 ± 9.3^{a}	4.9
	147.5 ± 23.8^{bc}	16.2	232.5 ± 52.8^{ac}	22.7	193.5 ± 8.5^{ab}	4.4
500	311.0 ± 26.4^{bc}	9.5	530.0 ± 49.7^{ac}	10.4	492.5 ± 7.2^{ab}	2.5
	303.5 ± 24.8^{bc}	9.2	520.0 ± 51.1^{a}	10.8	495.5 ± 7.6^{a}	2.6

Note: different letters indicate values that are reliably different one from another within one line of the table according to the results of comparison using the Tukey Test.

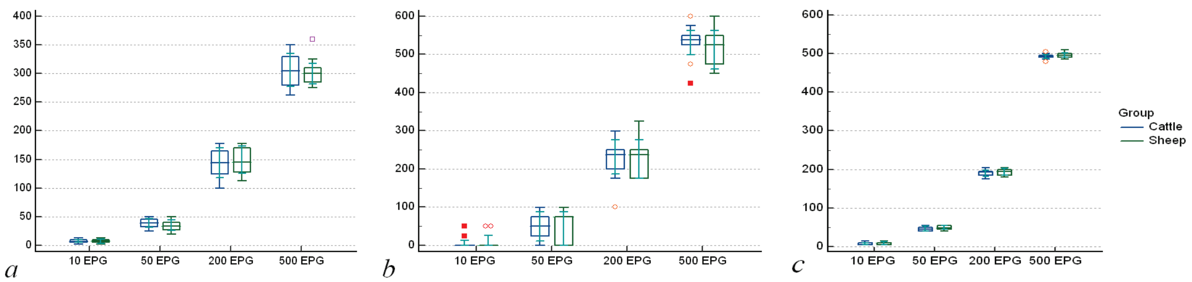

Fig. 2. The numbers of detected eggs of Strongylida in faeces of cows and sheep using the method of Quantitative Helminthocoproscopic Examination (*a*), modified McMaster technique (*b*), and Mini-FLOTAC (*c*): abscissa axis – eggs added to the fecal samples, per gram (EPG); ordinate axis – the numbers of eggs detected using each method; small square – median, upper and lower rectangle borders – 25% and 75% quartiles, vertical line – minimum and maximum values, circles – outliers; n = 10

The second stage was the study of natural nematode infestation of the ruminants. It was found that Quantitative Helminthocoproscopic Examination and Mini-FLOTAC performed better in detecting helminth eggs than the modified McMaster technique. Therefore, using the method of Quantitative Helminthocoproscopic Examination, we detected animals infested with the gastrointestinal Strongylida (54.8% of cattle, 40/73; 59.2% of sheep, 58/98; and 68.7% of goats, 22/32). The Mini-FLOTAC technique detected 52.0%, 58.2%, and 65.6%, respectively. The McMaster technique diagnosed 47.9% of cows, 55.1 % of sheep, and 62.5% of goats. It has to be noted that the highest infestation intensity was found in the sheep, while the lowest was found for the cattle. Quantitative Helminthocoproscopic Examination demonstrated poor correlation concordance (CCC = 0.81) with the McMaster technique and Mini-FLOTAC method (CCC = 0.87) when analyzing faeces from cows that were naturally infested with gastrointestinal Strongylida (Fig. 3a, 3b). For the sheep (Fig. 3c, 3d), the concordance between the method we proposed and the McMaster technique was poor (CCC = 0.89), while it was moderate with the Mini-FLOTAC method (CCC = 0.93). For the goats (Fig. 3e, 3f), there was also the tendency towards poor correlation of our method and the "gold standard", McMaster Grid, (CCC = 0.85), and Mini-FLOTAC method (CCC = 0.90).

Discussion

Comparison of the methods of Quantitative Helminthocoproscopic Examination, Mini-FLOTAC, and McMaster for detecting eggs of gastrointestinal Strongylida in cattle and sheep revealed that Quantitative Helminthocoproscopic Examination and Mini-FLOTAC had greater sensitivity, accuracy, and lower CV than the McMaster's technique. The analysis demonstrated that SD and CV values produced by Mini-Flotac were much lower than those from McMaster in detecting eggs of gastrointestinal nematodes in cattle and horses (Scare et al., 2017). Our data are consistent with the other studies (Bosco et al., 2018) that found that Mini-FLOTAC and Cornell-Wisconsin displayed 100% analytical sensitivity (when examining faeces of sheep of horses contaminated with nematode eggs). On the other hand, the grid and chamber of McMaster demonstrated 100% analytical sensitivity only for the concentrations that exceeded 200 EPG (the analytical sensitivity ranged 8.3% to 75.0% for the lowest concentration of eggs). When analyzing faeces from cattle, Mini-FLOTAC had higher sensitivity (producing 100% efficiency at all the levels of EPG against 0-66.6% efficiency produced by the McMaster chamber and grid at the levels of <100 EPG) and higher accuracy (98.1% of the mean value against 83.2% for McMaster grids and 63.8% for McMaster chambers), and had lower coefficient of variation (10.0% against 47.5% for McMaster grid and 69.4% for McMaster chamber) than McMaster (Amadesi et al., 2020). In our studies, the accuracy of Mini-FLOTAC ranged 90.0–99.1%.

The results of our studies indicate low effectiveness of the McMaster's technique for 10–50 EPG (20% to 80%), suggesting that it is adequate if EPG is above 50, but it is unsatisfactory for lower quantities. Those results are similar to those produced by the study of Vadlejch et al. (2011), who compared the accuracy and precision of different McMaster methods of diagnosing *Teladorsagia circumcincta* in sheep, confirming that this method detects negative samples at lower concentrations. Interestingly, the McMaster grid produced higher FECs than the method of Quantitative Helminthocoproscopic Examination and Mini-FLOTAC for different levels of contamination (50, 100, 200, and 500 EPG). The authors note that when using McMaster (the analytical sensitivity of 50 EPG), the anthelmintic effectiveness was often falsely classified as "normal" or the assessment was impossible due to zero FEC (Kenyon et al., 2016). Therefore, the results of our studies are consistent with other studies that revealed that the McMaster technique produced satisfactory results in cases of low levels of EPG, especially when FECRT is used to assess the efficacy of anthelmintics and detect the resistance to them (Van den Putte et al., 2016; Paras et al., 2017; Bosco et al., 2018).

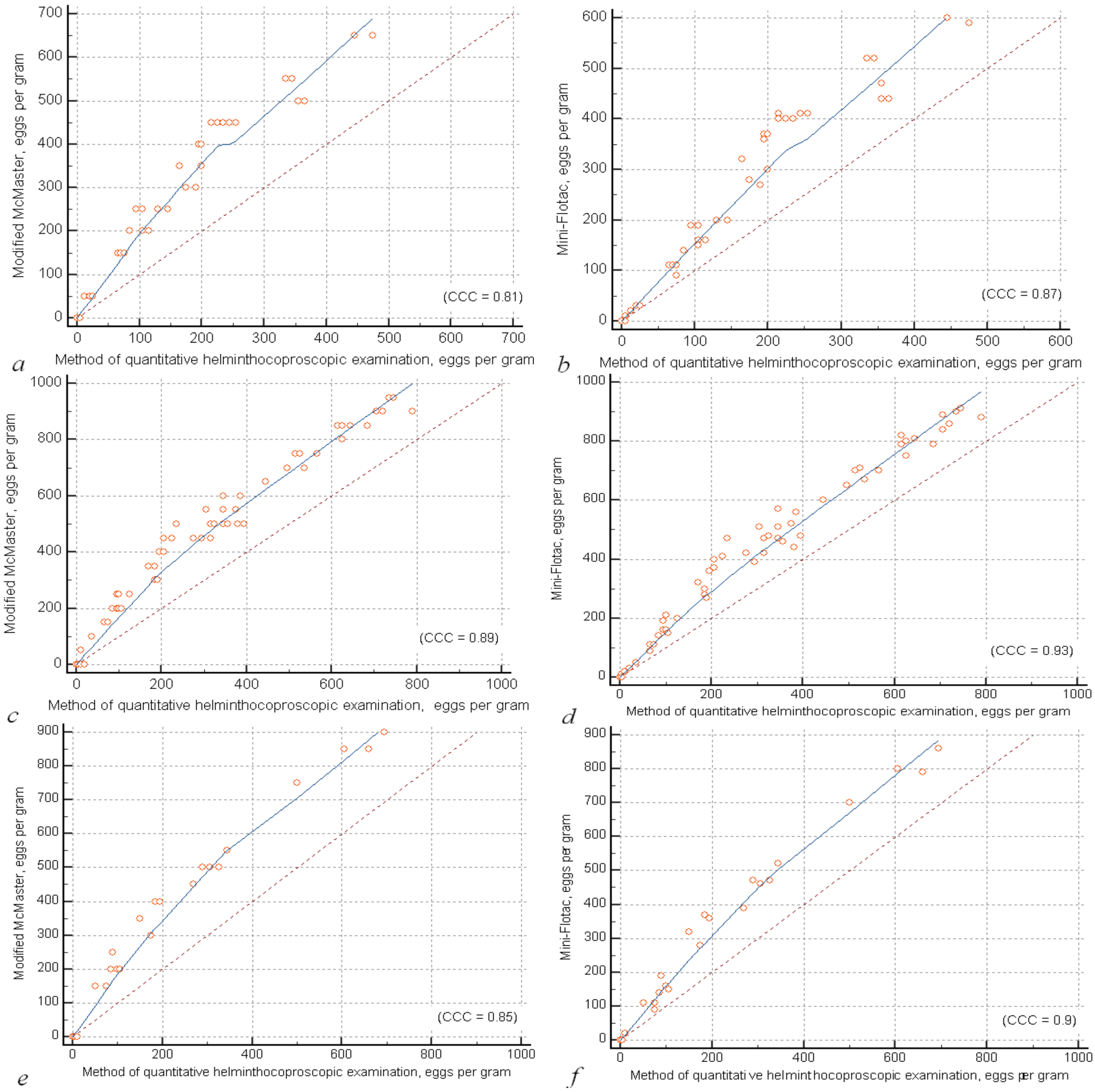

Fig. 3. Lin's concordance correlation coefficient (CCC) plots that compare the estimates of numbers of parasite eggs per 1 g of faeces using the two methods (axes x and y) in cattle (*a*, *b*; n = 73), sheep (*c*, *d*; n = 98), and goats (*e*, *f*; n = 32); red dash line under the angle of 45° indicates line of perfect concordance, and blue line is trend line

As described by Cringoli et al. (2017) and Norris et al. (2019), the procedure of isolating eggs and contamination of faeces, and also selection of a flotation solution can affect the rates of replication of the technique in any experiment with addition of eggs. Therefore, perhaps the solution of table salt (specific weight of 1.2) for the proposed method of Quantitative Helminthocoproscopic Examination is not optimum for the contamination levels of 200–500 EPG and higher. Since in our experiment, Mini-FLOTAC and McMaster recovered a lower number of eggs than was present, further research should be performed using our device with flotation solutions (FS) and the specific weight of SG = 1.27–1.32 so as to augment the efficiency. This opinion is corroborated by the previous studies, which show that different flotation fluids with the same specific weight do not yield the same results for the same parasitic elements (Cringoli et al., 2004).

This study assessed the three diagnostic methods, finding that the novel method of Quantitative Helminthocoproscopic Examination was the most productive, as it was able to detect the highest percentage of animals infested with gastrointestinal Strongylida, particularly, 54.8% of the cattle, 40/73; 59.2% of the sheep, 58/98; and 68.7% of the goats, 22/32). This was achieved by a lower multiplication coefficient (2.5 or 5.0) used in our method, compared to the other ones. In general, the results of our study confirm the opinion that the Mini-FLOTAC technique produces better results than McMaster (Cringoli et al., 2017). The authors came to the conclusion that Mini-FLOTAC is the accepted alternative method to McMaster for quantitative assessment of Strongylida eggs in North American bison (Johnson et al., 2022). At the same time, Mini-FLOTAC is accurate and simple, does not require scales and reduces the impact of filtration debris; and moreover, it can be used to analyze faeces in laboratory and field conditions (Noel et al., 2017; Bosco et al., 2018). By contrast, the McMaster technique is employed to make simple and fast decisions for treatment, but it is not recommended for using selective therapy and assessment of anthelmintic effectiveness with a lower standard deviation (Dias de Castro et al., 2017).

Choice of a diagnostic test for reduction of the number of eggs in faeces was included in the latest WAAVP recommendations (Geurden et al., 2022). The technique of counting eggs in 1 g of faeces with the analytical sensitivity of ≤ 5 EPG is recommended for cattle (Kaplan, 2020). At the same time, to obtain accurate results of FECRT, it is recommended that there are minimum 200 eggs prior to applying the multiplication coefficient (Geurden et al., 2022) with a lower minimum, at least 50 eggs (Kaplan, 2020). The studies conducted in recent years indicate a great interest among scientists in comparing the performances of diagnostic methods not only for ruminants but also for other mammals and birds (Shifaw et al., 2021; Boelow et al., 2022; Class et al., 2023).

The method of Quantitative Helminthocoproscopic Examination was inferior to the other techniques in detecting the numbers of eggs in 1 g of faeces. Our results are different from those obtained by other scientists who compared Mini-FLOTAC (MF) and McMaster (McM) for sheep, goats, and rabbits. Alowanou et al. (2021) reported that they observed a statistically significant difference ($P < 0.001$) in the numbers of Strongylida eggs in small ruminants, in particular, sheep (MF: 202.01 against McM: 174.75) and goats (MF: 147.36 against McM: 143.75). Our data confirmed that the Mini-FLOTAC technique is different from the McMaster technique, more accurate. Moreover, Bosco et al. (2014) reported that the highest coefficient of variation (CV) was found for McMaster, due to the multiplication coefficients that are used to determine eggs per gram based on egg count. The concordance of our method (Lin's concordance coefficient) was the highest when examining sheep (CCC = 0.93).

Conclusion

This study demonstrated that the method of Quantitative Helminthocoproscopic Examination with a minimum detection limit of 2.5 or 5.0 eggs per gram of faeces produced 100% analytical sensitivity for the samples spiked at a low degree of invasion (10–50 EPG). With increase in the number of eggs to 200 or 500 in 1 g of faeces, the difference among the three methods was statistically significant ($P < 0.05$). The method we propose could be used in laboratory settings at low invasion intensity (3.0–5.0 EPG), especially considering such characteristics as low cost, simplicity of use, and reliability. It was found that Quantitative Helminthocoproscopic Examination in naturally infested ruminants is superior to the Mini-FLOTAC technique and the modified McMaster method. The highest level of concordance between Quantitative Helminthocoproscopic Examination and Mini-FLOTAC was observed when analyzing sheep (CCC = 0.93). The prospect of further research is identification of the efficiency of the method of Quantitative Helminthocoproscopic Examination for infestation of animals with protozoans, cestodes, and trematodes.

This research was undertaken within the framework of the scientific topic "Ecological-faunistic monitoring and improvement of methods of diagnostics, control measures and prevention of parasitic diseases in animals" (government registration number 0123U103399).

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.

References

Alowanou, G. G., Adenilé, A. D., Akouèdegni, G. C., Bossou, A. C., Zinsou, F. T., Akakpo, G.-C. A., Kifouly, H. A., Rinaldi, L., von Samson-Himmelstjerna, G., Cringoli, G., & Hounzangbé-Adoté, S. (2021). A comparison of Mini-Flotac and McMaster techniques in detecting gastrointestinal parasites in West Africa Dwarf sheep and goats and crossbreed rabbits. Journal of Applied Animal Research, 49(1), 30–38.

Amadesi, A., Bosco, A., Rinaldi, L., Cringoli, G., Claerebout, E., & Maurelli, M. P. (2020). Cattle gastrointestinal nematode egg-spiked faecal samples: High recovery rates using the Mini-Flotac technique. Parasites and Vectors, 13(1), 230.

Ballweber, L. R., Beugnet, F., Marchiondo, A. A., & Payne, P. A. (2014). American Association of Veterinary Parasitologists' review of veterinary fecal flotation methods and factors influencing their accuracy and use – Is there really one best technique? Veterinary Parasitology, 204(1–2), 73–80.

Barda, B. D., Rinaldi, L., Ianniello, D., Zepherine, H., Salvo, F., Sadutshang, T., Cringoli, G., Clementi, M., & Albonico, M. (2013). Mini-Flotac, an innovative direct diagnostic technique for intestinal parasitic infections: Experience from the field. PLoS Neglected Tropical Diseases, 7(8), e2344.

Boelow, H., Krücken, J., Thomas, E., Mirams, G., & von Samson-Himmelstjerna, G. (2022). Comparison of Fecpakg2, a modified Mini-Flotac technique and combined sedimentation and flotation for the coproscopic examination of helminth eggs in horses. Parasites and Vectors, 15(1), 166.

Bondarevskyi, I. (2024). Poshyrennia parazytoziv shlunkovo-kyshkovoho kanalu zhuinykh u hospodarstvakh Kirovohradskoyi ta Dnipropetrovskoyi oblastey [Spread of parasitoses of the gastrointestinal tract of ruminants on farms in Kirovohrad and Dnipropetrovsk regions]. Scientific Progress and Innovations, 27(1), 139–143 (in Ukrainian).

Bosco, A., Maurelli, M. P., Ianniello, D., Morgoglione, M. E., Amadesi, A., Coles, G. C., Cringoli, G., & Rinaldi, L. (2018). The recovery of added nematode eggs from horse and sheep faeces by three methods. BMC Veterinary Research, 14(1), 7.

Bosco, A., Rinaldi, L., Maurelli, M., Musella, V., Coles, G., & Cringoli, G. (2014). The comparison of Flotac, Fecpak and McMaster techniques for nematode egg counts in cattle. Acta Parasitologica, 59(4), 625–628.

Boyko, O. O., Zazharska, N. M., & Brygadyrenko, V. V. (2016). Vliyanie urovnya zarazheniya gelmintami na izmenenie massi tela ovets v usloviyakh Ukrayini [The influence of the extent of infestation by helminths upon changes in body weight of sheep in Ukraine]. Biosystems Diversity, 24(1), 3–7 (in Russian).

Class, C. S. C., Fialho, P. A., Alves, L. C., Silveira, R. L., Amendoeira, M. R. R., Knackfuss, F. B., & Barbosa, A. D. S. (2023). Comparison of McMaster and Mini-Flotac techniques for the diagnosis of internal parasites in pigs. Revista Brasileira de Parasitologia Veterinaria, 32(2), e013322.

Coles, G. C., Bauer, C., Borgsteede, F. H. M., Geerts, S., Klei, T. R., Taylor, M. A., & Waller, P. J. (1992). World association for the advancement of veterinary parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary Parasitology, 44(1–2), 35–44.

Cringoli, G. (2006). Flotac, a novel apparatus for a multivalent faecal egg count technique. Parassitologia, 48(3), 381–384.

Cringoli, G., Amadesi, A., Maurelli, M. P., Celano, B., Piantadosi, G., Bosco, A., Ciuca, L., Cesarelli, M., Bifulco, P., Montresor, A., & Rinaldi, L. (2021). The Kubic Flotac microscope (KFM): A new compact digital microscope for helminth egg counts. Parasitology, 148(4), 427–434.

Cringoli, G., Maurelli, M. P., Levecke, B., Bosco, A., Vercruysse, J., Utzinger, J., & Rinaldi, L. (2017). The Mini-Flotac technique for the diagnosis of helminth and protozoan infections in humans and animals. Nature Protocols, 12(9), 1723–1732.

Cringoli, G., Rinaldi, L., Albonico, M., Bergquist, R., & Utzinger, J. (2013). Geospatial (s)tools: Integration of advanced epidemiological sampling and novel diagnostics. Geospatial Health, 7(2), 399.

Cringoli, G., Rinaldi, L., Maurelli, M. P., & Utzinger, J. (2010). Flotac: New multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nature Protocols, 5(3), 503–515.

Cringoli, G., Rinaldi, L., Veneziano, V., Capelli, G., & Scala, A. (2004). The influence of flotation solution, sample dilution and the choice of McMaster slide area (volume) on the reliability of the McMaster technique in estimating the faecal egg counts of gastrointestinal strongyles and *Dicrocoelium dendriticum* in sheep. Veterinary Parasitology, 123(1–2), 121–131.

Dias de Castro, L. L., Abrahão, C. L. H., Buzatti, A., Molento, M. B., Bastianetto, E., Rodrigues, D. S., Lopes, L. B., Silva, M. X., de Freitas, M. G., Conde, M. H., & Borges, F. A. (2017). Comparison of McMaster and Mini-Flotac fecal egg counting techniques in cattle and horses. Veterinary Parasitology, Regional Studies and Reports, 10, 132–135.

Egwang, T. G., & Slocombe, J. O. (1982). Evaluation of the Cornell-Wisconsin centrifugal flotation technique for recovering trichostrongylid eggs from bovine feces. Canadian Journal of Comparative Medicine, 46(2), 133–137.

Geurden, T., Smith, E. R., Vercruysse, J., Yazwinski, T., Settje, T., & Nielsen, M. K. (2022). World association for the advancement of veterinary parasitology (WAAVP) guideline for the evaluation of the efficacy of anthelmintics in food-producing and companion animals: General guidelines. Veterinary Parasitology, 304, 109698.

Godber, O. F., Phythian, C. J., Bosco, A., Ianniello, D., Coles, G., Rinaldi, L., & Cringoli, G. (2015). A comparison of the Fecpak and Mini-Flotac faecal egg counting techniques. Veterinary Parasitology, 207(3–4), 342–345.

Income, N., Tongshoob, J., Taksinoros, S., Adisakwattana, P., Rotejanaprasert, C., Maneekan, P., & Kosoltanapiwat, N. (2021). Helminth infections in cattle and goats in Kanchanaburi, Thailand, with focus on strongyle nematode infections. Veterinary Sciences, 8(12), 324.

Johnson, W. L., Reynolds, S., Adkins, C. L., Wehus-Tow, B., Brennan, J., Krus, C. B., Buttke, D., Martin, J. M., & Jesudoss Chelladurai, J. R. J. (2022). A comparison of Mini-Flotac and McMaster techniques, overdispersion and prevalence of parasites in naturally infected North American bison (*Bison bison*) in the USA. Current Research in Parasitology and Vector-Borne Diseases, 2, 100103.

Kaplan R. M. (2020). Biology, epidemiology, diagnosis, and management of anthelmintic resistance in gastrointestinal nematodes of livestock. The Veterinary Clinics of North America. Food Animal Practice, 36(1), 17–30.

Kenyon, F., Rinaldi, L., McBean, D., Pepe, P., Bosco, A., Melville, L., Devin, L., Mitchell, G., Ianniello, D., Charlier, J., Vercruysse, J., Cringoli, G., & Levecke, B. (2016). Pooling sheep faecal samples for the assessment of anthelmintic drug efficacy using McMaster and Mini-Flotac in gastrointestinal strongyle and *Nematodirus* infection. Veterinary Parasitology, 225, 53–60.

Korchan, L., Melnychuk, V., Zamaziy, A., & Prykhodko, Y. (2023). Shlunkovo-kyshkovi parazytozy ovets na terytoriyi hospodarstv Poltavskoyi oblasti [Gastrointestinal parasitosis of sheep on farms in Poltava region]. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies, Series: Veterinary Sciences, 25(109), 84-88 (in Ukrainian).

Kruchynenko, O. V., Bondarevskyi, I. L., & Ivanov, O. M. (2024). Sposib kil'kisnoho hel'mintokoproovoskopichnoho doslidzhennia [Method of quantitative helminthocoproscopic examination]. Patent Ukrayiny No. 156464 (in Ukrainian).

Kruchynenko, O. V., Mykhailiutenko, S. M., Petrenko, M. A., & Kuzmenko, L. M. (2021). Prevalence of gastrointestinal helminths in ruminants in Ukraine: A 5-year meta-analysis. Biosystems Diversity, 29(3), 251–257.

Melnychuk, V. V. (2019). Epizootychna sytuatsiya shchodo nematodoziv travnoho kanalu ovets v umovakh Tsentral'noho ta Pivdenno-Skhidnoho rehioniv Ukrayiny [Epizootic situation of sheep digestive canal nematodoses in the conditions of the Central and South-Eastern regions of Ukraine]. Theoretical and Applied Veterinary Medicine, 7(3), 153–157 (in Ukrainian).

Nápravníková, J., Petrtýl, M., Stupka, R., & Vadlejch, J. (2019). Reliability of three common fecal egg counting techniques for detecting strongylid and ascarid infections in horses. Veterinary Parasitology, 272, 53–57.

Norris, J. K., Slusarewicz, P., & Nielsen, M. K. (2019). Pixel by pixel: Real-time observation and quantification of passive flotation speeds of three common equine endoparasite egg types. International Journal For Parasitology, 49(11), 885–892.

Paras, K. L., George, M. M., Vidyashankar, A. N., & Kaplan, R. M. (2018). Comparison of fecal egg counting methods in four livestock species. Veterinary Parasitology, 257, 21–27.

Prima, O. B., & Dmytrenko, N. I. (2021). Poshyrennia parazytoziv shlunkovo-kyshkovoho kanalu kiz u hospodarstvakh Poltavskoyi oblasti [Spreading parasitoses of goats' gastro-intestinal tract on farms in Poltava region]. Scientific Progress and Innovations, 3, 230–235 (in Ukrainian).

Rashid, M. H., Stevenson, M. A., Waenga, S., Mirams, G., Campbell, A. J. D., Vaughan, J. L., & Jabbar, A. (2018). Comparison of McMaster and Fecpak^{g2} methods for counting nematode eggs in the faeces of alpacas. Parasites and Vectors, 11(1), 278.

Scare, J. A., Slusarewicz, P., Noel, M. L., Wielgus, K. M., & Nielsen, M. K. (2017). Evaluation of accuracy and precision of a smartphone based automated parasite egg counting system in comparison to the McMaster and Mini-Flotac methods. Veterinary Parasitology, 247, 85–92.

Shifaw, A., Feyera, T., Elliott, T., Sharpe, B., Walkden-Brown, S. W., & Ruhnke, I. (2021). Comparison of the Modified McMaster and Mini-Flotac methods for the enumeration of nematode eggs in egg spiked and naturally infected chicken excreta. Veterinary Parasitology, 299, 109582.

Tachack, E. B., Oviedo-Socarrás, T., Pastrana, M. O., Pérez-Cogollo, L. C., Benavides, Y. H., Pinto, C. R., & Garay, O. V. (2022). Status of gastrointestinal nematode infections and associated epidemiological factors in sheep from Córdoba, Colombia. Tropical Animal Health and Production, 54(3), 171.

Vadlejch, J., Petrtýl, M., Zaichenko, I., Čadková, Z., Jankovská, I., Langrová, I., & Moravec, M. (2011). Which McMaster egg counting technique is the most reliable? Parasitology Research, 109(5), 1387–1394.

Van den Putte, N., Claerebout, E., & Levecke, B. (2016). Evaluation of the mini-Flotac technique for detection of gastro-intestinal parasites in large companion animals. Vlaams Diergeneeskundig Tijdschrift, 85(1), 15–22.

Vieira, O. L., Macedo, L. O., Bezerra-Santos, M. A., dos Santos, L. A., de Mendonça, C. L., Alves, L. C., Ramos, R. A. D. N., & de Carvalho, G. A. (2021). Mini-Flotac and McMaster egg counting method for detection of gastrointestinal parasites in small ruminants: A comparison study. Medicina Veterinária, 15(2), 119–124.

Went, H. A., Scare, J. A., Steuer, A. E., & Nielsen, M. K. (2018). Effects of homogenizing methods on accuracy and precision of equine strongylid egg counts. Veterinary Parasitology, 261, 91–95.

Wood, I. B., Amaral, N. K., Bairden, K., Duncan, J. L., Kassai, T., Malone, J. B., Pankavich, J. A., Reinecke, R. K., Slocombe, O., Taylor, S. M., & Vercruysse, J. (1995). World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). Veterinary Parasitology, 58(3), 181–213.

Yevstafieva, V., Melnychuk, V., Budnyk, D., Prykhodko, Y., & Kyrychko, B. (2023). Efektyvnist' flotatsiynykh metodiv za koproskopichnoyi diahnostyky nematodoziv shlunkovo-kyshkovoho traktu velykoyi rohatoyi khudoby [Effectiveness of flotation methods for coproscopic diagnosis of nematodoses of the gastrointestinal tract of cattle]. Veterinary Biotechnology, 43, 24–34 (in Ukrainian).

Zajac, A. M., & Conboy, G. A. (2012). Veterinary clinical parasitology. 8th ed. American Association of Veterinary Parasitologists. Wiley-Blackwell, Iowa.