Animal Health

Original

Performance of McMaster and Mini-FLOTAC Techniques in the diagnostic of *Paramphistomum* spp. in Bovines

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Received: February 9, 2020; Accepted: February, 2020; Published: March, 2020.

ABSTRACT

Background: Paramphistomosis is an emerging, hard to diagnose, parasitic disease, which causes enormous economic losses. The aim of this study was to evaluate the performance of coprological techniques, like McMaster and Mini-FLOTAC to diagnose *Paramphistomum* spp. from bovine samples.

Methods: The recovery of *Paramphistomum* spp.eggs was analyzed in bovine fecal samples, using McMaster and Mini-FLOTAC with known quantities of epg. Different flotation solutions of $ZnCl_2$ and $ZnSO_4$ were used, with densities of 1.45 and 1.50 mg/mL. Then, the performance of McMaster and Mini-FLOTAC was determined in fecal samples from sacrifice bovines (n=40), as well as samples from dairy herds (n=155).

Results: Both techniques yielded similar egg recovery accuracies, for known epg in different flotation solutions (59-77 %). Accuracy was similar in both McMaster (16.93-25.83%) and Mini-FLOTAC (17.83-25.05%). Linearity was observed in epg counts between McMaster and Mini-FLOTAC, in fecal samples from slaoughter bovines (R^2=0.93) and dairy herds (R^2=0.71). The prevalences of *Paramphistomum* spp. in dairy herds were 42.9% (McMaster), and 43.5% (Mini-FLOTAC).

Conclusions: The two coprological techniques are highly accurate, with significant recovery of *Paramphistomum* spp. eggs in bovine feces, thus indicating the feasibility of these parasitological diagnostic tools.

Key words: diagnostic, accuracies, *Paramphistomum*, prevalence (*Source:* CAB)

INTRODUCTION

Como citar (APA)
Casado Simón, E., González Salotén, M., Díaz Fernández, A., Gutiérrez Lastra, Z., Madera Quintana, J., & Arenal Cruz, A. (2020). Desempeño de McMaster y Mini-Flotac en el diagnóstico de Paramphistomum spp. en bovinos. *Revista de Producción Animal*, *32*(1).

Paramphistomosis is a parasitic disease caused by trematodes that affect ruminants and several other wild species, especially in tropical regions (Rojas *et al*., 2015). It can lead to acute gastroenteritis and anemia with high morbidity, particularly in young animals (Kifleyohannes *et al*., 2015). This trematode can cause enormous economic losses that impact on wool, meat, and milk productions.

Rangel-Ruiz, Albores-Brahms and Gamboa-Aguilar (2003), and Silva (2006) have said it is an emerging disease, since its extension is known. This rise can be caused by an overestimation in the prevalence of *Fasciola hepatica* in regions where the two trematodes co-exist simultaneously, in addition to the lack of effective drugs to treat paramphistomosis locally (Pinedo *et al*., 2010).

The clinical diagnostic of *Paramphistomum* is difficult due to the absence of signs or because the existing signs are similar to other parasitosis. The immunological methods and detection of antibodies in the serum are not conclusive to diagnose the parasite (Rieu *et al*., 2007). Likewise, the diagnostic of this parasite is done routinely, and consists in demonstrating the existence of eggs in infested cattle, using the coprological sedimentation technique (Piña, 2013; Silva, 2006).

McMaster is a commonly used technique in veterinary laboratories, because it can vary according to the volume of feces to be examined (Bosco, 2014). Mini-FLOTAC is a new technique belonging to the family of flotac (Godber *et al*, 2015), and was introduced recently as an alternative to McMaster (Cringoli *et al*., 2010). So far, no reports have been published on the use of Mini-FLOTAC in the diagnostic of *Paramphistomum* spp.

The diagnostic of digenean trematodes in Cuba is poor. In addition to it, there are few specific studies for treatment and control of this trematodiasis in bovine herds affected by this parasite in Cuba (Vázquez *et al*., 2015). Also, considering that the diagnostic method used in Cuba for detection of trematode eggs is simple sedimentation, which is a little reliable qualitative coprological method, it is important to search for other dependable methods.

Therefore, the aim of this paper is to compare the performance of coprological techniques McMaster and Mini-FLOTAC to diagnose *Paramphistomum* spp. from bovine samples.

MATERIALS AND METHODS

Two experiments were done to fulfill the purpose of this study. In the first, *Paramphistomum* spp. egg recovery from bovine fecal samples with known quantities of eggs per grams of feces was evaluated using different flotation solutions. In the second experiment, fecal samples from slaughter bovines, and samples from dairy herds were collected to compare the performance of McMaster and Mini-FLOTAC in the diagnosis of *Paramphistomum* spp.

Sample collection

A total of 40 fecal samples from sacrifice bovines were collected directly from the rectum of animals at the slaughterhouse in Guanamaquilla, municipality of Camagüey, Cuba. Additionally, other 155 fecal samples were collected from milking cow herds, at the Triangulo 3 Livestock Company, in the municipality of Camagüey. Randomly, the feces from 20 milking cows were collected, and same-weight quantities were prepared to make up the herd sample.

All the samples were sealed in polyethylene bags, which were tagged, and stored in cold temperature. Then, they were carried to the Laboratory of Parasitology at the Faculty of Agricultural Sciences, University of Camagüey.

Egg collection and preparation of known samples

Approximately, 15 live adult *Paramphistomum* spp. specimens were collected directly from the rumen of slaughtered bovines. They were washed with 0.9% NaCl, and placed in 400 mL glass Erlenmeyers, with 1X PBS solution (1.8 mmol/L KH_2PO_4; 8.4 mmol/L Na_2HPO_4; 12.6 mmol/L KCl, and 136.8 mmol/L NaCl, pH 7.2).

The trematodes were carried to the Laboratory of Parasitology, University of Camagüey, at room temperature. Later, one last wash was performed with 1X PBS to remove the remains of blood and ruminal contents. To stimulate oviposition, it was incubated in a 100 mL glass Erlenmeyer containing 1X PBS, at 37 ºC, for 4 hours. Then, the individuals were removed, and the eggs were collected by removing the supernatant to a final volume of 10 mL of 1X PBS solution. Egg count had five replicas of 100 µL each.

Preparation of the fecal samples with known quantities of trematode eggs

The *Paraphistomum* spp. egg content in the Erlenmeyer was decanted up to 5 mL, and another egg count was performed. The egg concentration was adjusted to 1000 epg in feces (previously sterilized in autoclave at 121 ºC, and 1 atm, for 15 minutes), free from the parasite eggs. Different dilutions of egg-free feces were made at known concentrations of 5, 25, 125, 250, 500, and 1000 epg.

Comparison of flotation solutions for detection of *Paraphistomum* spp. eggs

A volume of 27 mL (1:10 dilution) from one of the flotation dilutions below was added to three grams of feces from each known concentration of epg: 1.45 g/mL $ZnSO_4$, 1.45 g/mL $ZnCl_2$, 1.5 g/mL $ZnCl_2$, and 1.5 g/mL $ZnCl_2$ + Sucrose. Then, they were mixed and sieved through a 1 mm mesh. The suspension was homogenized and the Mini-FLOTAC and McMaster chambers were filled. The contents were left to rest for at least 10 minutes. Then the eggs were counted using a microscope (10X). The dilution factors were 5 (Mini-FLOTAC) and 10 (McMaster). A count was considered positive when at least one egg of *Paramphistomum* spp. was observed.

Determination of *Paramphistomum* spp. by epg count in sacrifice bovines and dairy herds

Each coprological technique was performed to the 40 fecal samples from sacrifice bovines and the 155 samples from the milking cows, by triplicate, for a total of six counts per sample. Three grams of feces from each group per unit or replicate of the feces from the slaughtered animals were diluted in 27 mL of sucrose-saturated solution containing $ZnCl_2$, with a 1.52 g/mL density. Later, the procedure described in the previous section was developed.

Statistical analysis

Graphpad Prism 7.0 (2016) was used for regression analyses between McMaster and Mini-FLOTAC and the known and observed epg. Bifactorial ANOVA was conducted for comparison of VC (%), the factors studied were the particular technique used and density. The accuracy value was dependent on the recovery value of the linear regression curves between the known and observed epg. The precision per cent of each technique was calculated by subtracting 100 per cent of the VC (%). Sensitivity, specificity, the negative predictive value (NPV), positive predictive value (PPV), and prevalence, were determined online, using the MedCalc Software bvba, Ostend, Belgium.

RESULTS

Analysis of *Paramphistomum* egg recovery from fecal samples

Coprological techniques like McMaster (McM) and Mini-FLOTAC (MF) showed a wide range of possibilities for diagnostic of *Paramphistomum* spp. in cattle, at different concentrations of epg (5, 25, 125, 250, 500, and 1000), and densities (1.45 and 1,5 g/mL), as shown in figure 1.

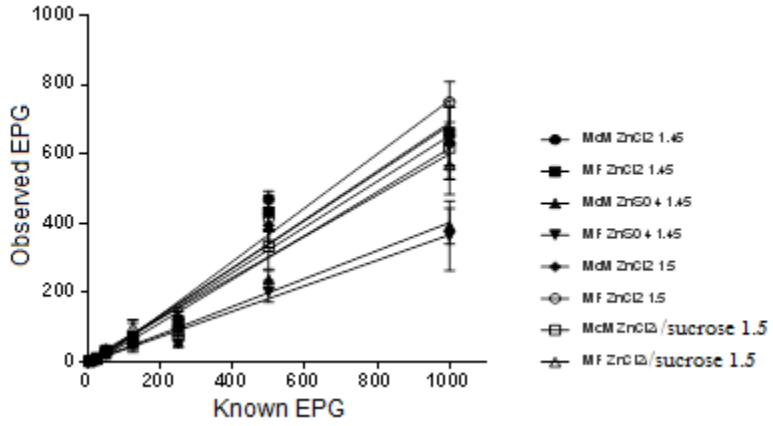

Figure 1. *Determination of Paramphistomum* spp. eggs in fecal samples with known quantities of eggs from the parasite (known epg, ranging 5--1000). McM (McMaster), MF (Mini-FLOTAC).

The curves indicate similar recovery values in McMaster and Mini-FLOTAC. However, the $ZnSO_4$ solutions in the 1.45 g/mL density had lower recovery values in both chambers than in the other solutions. The recovery per cents of the $ZnCl_2$ solutions were between 59 and 70% (Table 1).

Table 1. Equations that describe the detection of *Paramphistomum* spp. eggs in feces with known quantities of eggs from the parasite

Flotation solution	Density (g/mL)	Equation	R^2
McM $ZnCl_2$	1.45	Y= 0.69*X - 3.81	0.94
MF $ZnCl_2$	1.45	Y= 0.70*X – 8.15	0.97
McM$ZnSO_4$	1.45	Y= 0.40*X – 1.43	0.97
MF $ZnSO_4$	1.45	Y= 0.37*X – 0.74	0.98
McM $ZnCl_2$	1.50	Y= 0.66*X – 4.98	0.97
MF $ZnCl_2$	1.50	Y= 0.77*X – 20.15	0.98
McM $ZnSO_4$	1.50	Y= 0.69*X – 12.68	0.96
MF $ZnSO_4$	1.50	Y = 0.59*x – 7.19	0.98

MF: Mini-FLOTAC, McM: McMaster Y= Number of eggs per grams of feces observed, X= Number of eggs per grams of known feces.

The variation coefficients (VC) were similar in the two techniques (Table 2).

Table 2. Variation coefficient (VC) and accuracy of McMaster and Mini-FLOTAC using different flotation solutions

Solution	Density (mg/mL)	VC (%)	Accuracy (%)
McM $ZnCl_2$	1.45	21.32	78.68
MF $ZnCl_2$	1.45	22.02	77.98
McM$ZnSO_4$	1.45	16.93	83.07
MF $ZnSO_4$	1.45	25.05	74.95
McM $ZnCl_2$	1.50	21.72	78.28
MF $ZnCl_2$	1.50	17.83	82.17
McM $ZnSO_4$	1.50	25.83	74.62
MF $ZnSO_4$	1.50	19.01	80.99

MF: Mini-FLOTAC, McM: McMaster.

Comparison

The epg values detected by both techniques had a linear ratio (R^2 = 0.93). The regression curve was 1.36, indicating that Mini-FLOTAC was able to detect 36% more eggs than McMaster (Figure 3).

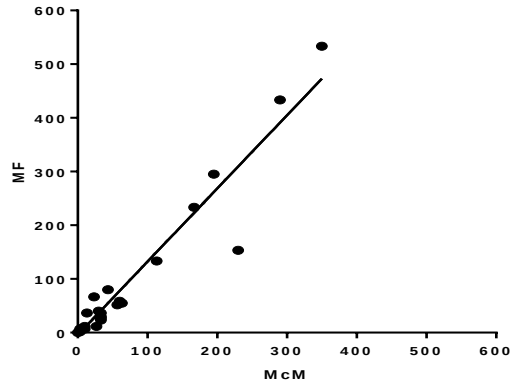

Figure 3. Detection of *Paramphistomum* spp. eggs per grams of feces, using McMaster (McMM-X) and Mini-FLOTAC (MD-Y) in the flotation solution: $ZnCl_2$ (1.45 g/mL), MF = 1.36*McM - 4.11. R^2 =0.93 (n=40).

In the $ZnCl_2$ flotation solution with the 1.5 density, similar values of sensitivity, specificity, and negative and positive predictive values were found when detecting *Paramphistomum* spp. eggs using the two methods (Table 3).

Table 3. Sensitivity, specificity, and predictive values in egg counts using McMaster and Mini-FLOTAC.

	McMaster $ZnCl_2$ 1.50 g/mL	**Mini-FLOTAC $ZnCl_2$ 1.50 g/mL**
Sensitivity	77.38% [66.95% -85.80%]	77.38% [66.95%- 85.80%]
Specificity	9167% [77.53%- 98.25%]	91.67% [77.53- 98.25%]
Negative Predictive Value	63.46% [53.60%- 72.31%]	63.46% [53.60%- 73.61%]
Positive Predictive Value	95.59% [53.60%- 98.47%]	95.59% [¨87.93%-98.47%]

[Confidence interval 95 %]

Figure 4 represents the linear regression analysis of the dairy herd samples, the equation that described is, MF = 0.76*McM + 1.38 (R^2= 0.71). The curve indicates that Mini-FLOTAC detected 24% less than McMaster.

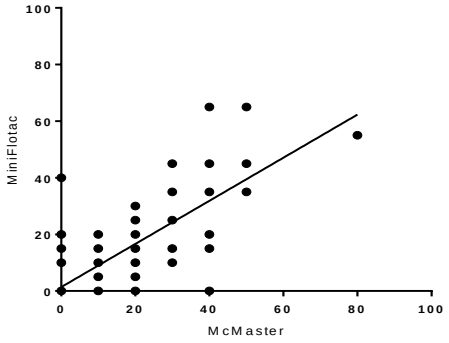

Figure 4. Detection of *Paramphistomum* spp. per grams of bovine feces (samples from dairy herds) McMaster (McM) and Mini-FLOTAC (MF), flotation solution (1.50 g/mL), MF = 0.76*McM + 1.38 (R^2= 0.71) (n=155).

The prevalence values in the detection of *Paramphistomum* spp. in dairy herds was 42.9 using McMaster, and 43.5 using Mini-FLOTAC. Both coprological techniques showed high sensitivity, though Mini-FLOTAC´s percent was higher (91.1%) than McMaster´s (87 %). Likewise, the negative predictive value was higher with Mini-FLOTAC (91.9 %) than with McMaster (88.7 %). No false positives were detected in the analysis of samples (Table 5).

Table 5. Sensitivity, specificity, and predictive detection values through egg counts with McMaster and Mini-FLOTAC to detect *Paramphistomum* spp. in bovine fecal samples from dairy herds.

	MacMaster	Mini-FLOTAC
Sensitivity	86.99% [80.57% -91.51%]	91.1% [85.36%- 92.74%]
Specificity	100% [97.49%- 100%]	100% [97.49- 100%]
Negative Predictive Value	88.69% [83.01%- 92.64%]	91.98% [86.76%- 95.25%]
Positive Predictive Value	100% [97.06%- 100%]	100% [¨97.19%-100%]

[Confidence interval at 95 %]

DISCUSSION

The studies to evaluate accuracy and precision in the detection of parasite eggs in animals are based on the fecal samples collected from individuals infested naturally or experimentally (Ojeda-Robertos *et al*., 2014). Therefore, the actual number of eggs might be incorrect. In this study, the samples were prepared with a known number of eggs from the parasite, which facilitated the determination of the real precision and accuracy of the techniques used to diagnose *Paramphistomum* spp. in cattle.

The accuracy of egg counts is essential when evaluating the issue of parasite resistance against antihelminthic (Eysker and Ploeger, 2000). Increased accuracy also contributes to positive prediction of samples with few eggs and less variability (Barda *et al*., 2013; De Castro *et al*., 2017). More accurate and precise egg count methods ensure more reliable diagnostic and better evaluation of the effectiveness of antiparasitic treatments.

In this study, both techniques showed similar accuracy and precision, with known samples of epg, regardless of the flotation solution. The low VC and high precision of McMaster possibly occurred because the dilution factor used was 10, and the readouts included all the areas in the chambers. The accuracy and precision of McMaster are known to be directly related to the readout area (Cringoli *et al*., 2004).

There is evidence from other studies comparing Mini-FLOTAC and McMaster for the diagnostic of nematodes eggs in cattle, and helminths in humans (Bosco, 2014; Glinz *et al*., 2010). Silva *et al*. (2013) detected that the accuracy of McMaster and Mini-FLOTAC are similar. However, there are reports of lower variation coefficients in Mini-FLOTAC than in McMaster, when detecting gastrointestinal parasites in horses (Noel *et al*., 2017).

In this study, the recovery of *Paramphistomum* spp. eggs in cattle, using McMaster and Mini-FLOTAC was similar to the reports made by Rinaldi *et al*. (2014). The authors evaluated these techniques in pooled fecal samples from cattle to detect gastrointestinal strongylus eggs. Likewise, the results of parasite egg recovery reported in this study, using the two techniques, are higher than the reports published in other studies (Noel *et al*., 2017). Similar studies in ovine demonstrated that Mini-FLOTAC was better to detect gastrointestinal parasite eggs in that species (Rinaldi *et al*., 2014; Silva *et al*., 2013).

McMaster is the most widely used coprological diagnostic method in veterinary parasitology (Vadlejch *et al*., 2011). Comparative experiments between McMaster and Mini-FLOTAC to diagnose *Eimeria* spp. eggs in naturally infested sheep, demonstrated that Mini-FLOTAC´s values were higher ($P<0.05$) than McMaster´s (Silva *et al*., 2013). In this study, the epg values achieved with Mini-FLOTAC seemed somewhat contradictory; the values of slaughter cattle were greater using Mini-FLOTAC; however, in the pooled samples from dairy herds analyzed with McMaster, the counts were higher. The parasitological diagnostic may be influenced by the density of the flotation solution used, and the consistency of feces (Cringoli *et al*., 2010). The parasitic burden in samples from the dairy herds was lower, since they had been pooled, which might reduce the precision of the diagnostic, and the similarity of the two techniques.

Comparative experiments between fecal egg counts of gastrointestinal nematodes using McMaster and Mini-FLOTAC in horses and cattle showed similar epg results. Nonetheless, the VC were significantly lower in Mini-FLOTAC, so the authors recommended its use in animals with low fecal counts (De Castro *et al*., 2017). The difficulties using coprological methods demand the need to include more sensitive methods that produce a more dependable diagnostic of *Paramphistomum* infestation in cows, and its economic impact (Rieu *et al*., 2007).

There is an increase in the number of studies of *Paramphistomum* spp. prevalence due to the drop observed in cattle productivity caused by the parasite. In the district of Moyabamba, Perú, the prevalence of the parasite was 55% in dairy herds (Rojas *et al*., 2015). Likewise, in the Netherlands, prevalences between 82.6 and 83.3% were detected (Ploeger *et al*., 2015), higher than the values achieved in this study. The high prevalence of *Paramphistomum* spp. found in this study may be associated to the presence of biotopes in the grazing areas. Additionally, all the animal categories graze in groups, which calls for adequate risk assessment studies that contribute with effective measures to control paramphistomosis.

CONCLUSIONS

Both coprological techniques are highly precise, with elevated recovery values in the detection of *Paramphistomum* spp. eggs from bovine fecal samples, which enables their applicability to conduct parasitological diagnostic.

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AUTHOR CONTRIBUTION

Author participation was as follows: Conception and design of research: EC, MG and AA. Data analysis and interpretation: EC, ZG, JM, and AA. Redaction of the manuscript: EC, AD, and AA

CONFLICT OF INTERESTS

The authors declare no conflict of interests.