Mini-FLOTAC and McMaster egg counting method for detection of gastrointestinal parasites in small ruminants: a comparison study

[*Mini-FLOTAC e Método de contagem de ovos McMaster para detecção de parasitos gastrointestinais em pequenos ruminantes: um estudo comparativo*]

"Scientific Article/Artigo Científico"

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Abstract

The proper diagnosis of gastrointestinal parasites of small ruminants requires the development of multivalent techniques characterized by high sensitivity, specificity, precision, reproducibility, and the ability to quickly detect and monitor infections that pose risks to animal health. The aim of this study was to evaluate the performance of Mini-FLOTAC technique and McMaster egg counting (gold standard) in the detection of gastrointestinal parasites of goats and sheep. A total of 789 fecal samples were analyzed (401 of sheep and 388 of goats). From those, nematode eggs were detected in 80.86% (638/789), being 72.57% (291/401) from sheep and 89.43% (347/388) from goats. The Mini-FLOTAC technique presented a better performance to detect helminth eggs in both goats (88.40%; 343/388) and sheep (71.57%; 287/401) ($\chi 2 = 10.358$; $p < 0.0001$). *Kappa* analysis revealed a weak concordance between techniques for goats ($k = 0.342$; $p < 0.001$) and sheep ($k = 0.281$; $p < 0.001$). Data herein reported suggests that the Mini-FLOTAC is a technique more sensitive than the McMaster egg counting, therefore its use might be adopted for the diagnosis of gastrointestinal infection in small ruminants.

Keywords: parasitological methods; goats; sheep; diagnosis.

Resumo

O diagnóstico adequado de parasitos gastrointestinais em pequenos ruminantes requer o desenvolvimento de técnicas multivalentes, caracterizadas por alta sensibilidade, especificidade, precisão, reprodutibilidade, capacidade de detectar e monitorar rapidamente infecções que apresentam riscos à saúde animal. O objetivo deste estudo foi avaliar o desempenho da técnica Mini-FLOTAC e da contagem de ovos McMaster (padrão-ouro) na detecção de parasitos gastrointestinais de caprinos e ovinos. Foram analisadas 789 amostras fecais (401 de ovinos e 388 de caprinos). Ovos de nematódeos foram detectados em 80,86% (638/789), sendo 72,57% (291/401) em amostras de ovinos e 89,43% (347/388) de caprinos. A técnica de Mini-FLOTAC apresentou melhor desempenho para detectar ovos de helmintos tanto em caprinos (88,40%; 343/388) quanto em ovinos (71,57%; 287/ 401) ($\chi 2 = 10,358$; $p < 0,0001$). A análise *Kappa* revelou uma fraca concordância entre as técnicas para caprinos ($k = 0,342$; $p < 0,001$) e ovinos ($k = 0,281$; $p < 0,001$). Os dados aqui relatados sugerem que o Mini-FLOTAC é uma técnica mais sensível que a contagem de ovos de McMaster, portanto, seu uso pode ser adotado para o diagnóstico de infecção gastrointestinal em pequenos ruminantes.

Palavras-chave: métodos parasitológicos; caprinos; ovino; diagnóstico.

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Introduction

Gastrointestinal nematodes (GIN) are important parasites of goats and sheep (Selemon, 2018; Squire et al., 2019), causing several economic losses. In fact, in Brazil the annual cost associated with parasitic diseases in ruminants has been estimated at 7.11 billion dollars (Grisi et al., 2014). Different species of nematodes (e.g., *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Strongyloides papillosus* and *Oesophagostomum colubianum*) and cestodes (e.g., *Moniezia expansa*) have been described parasitizing small ruminants worldwide (Diop et al., 2015; Dey et al., 2020). It is known that animals with intense parasitism usually demonstrate weakness, severe weight loss and diarrhea (Jesse et al., 2019). In many cases, these hosts may present a subclinical infection, with typical clinical signs characterized by a delay in growth, decrease in weight gain and reduction of milk production (Ibrahim et al., 2014; Yusof and Isa, 2016; Sargison, 2020).

The correct diagnosis of these infections has been a challenge and depends on the use of combinations the different techniques characterized by sensitivity and specificity variable (Banoo, 2006). For a long time, the detection of immature forms of helminths in feces of ruminants has been performed through classical copromicroscopic methods (Vadlejch et al., 2011). Undoubtedly, one of the most important tools is the McMaster egg counting method that was developed by Gordon and Whitlock in 1939 and until now has been widely employed throughout the world (Paras et al., 2018; Went et al., 2018). This technique is important for individual diagnostic, as well as for epidemiological investigations, tests of anthelminthic drug effectiveness and monitoring of parasite resistance to chemicals (Kenyon et al., 2009). Although it had been extensively used, this technique presents a sensitivity of detection of 50 eggs per gram of feces, which may favor the occurrence of false-negative results (Kaplan and Vidyashankar, 2012).

Over the last years, the search of more sensitivity methods has stimulated several studies worldwide. One of the most recent tools developed for this purpose was the Mini-FLOTAC technique that has been used for the detection of parasites of medical and veterinary concern. In addition, this method has been widely applied in laboratories with limited resources, as well as in the field (Barda et al., 2013). Previous studies have demonstrated that the sensitivity of the Mini-FLOTAC is higher than other methods such as Kato-Katz and McMaster method (Barda et al., 2014; Paras et al., 2018). Currently, it is considered one of the best copromicroscopic tools, presenting low operational cost, easy handling and a sensitivity to detect up to five eggs per gram of feces (Cringoli et al., 2013; Silva et al., 2013; Rinaldi et al., 2014). The search for the best technique has been the reason of inquiry and incentive to the creation of new methodologies or even the modification and improvement of techniques that already exist. Therefore, the aim of this study was to evaluate the performance of the Mini-FLOTAC and McMaster egg counting method in the detection of immature forms of gastrointestinal parasites of small ruminants.

Material and Methods

Study area and ethical aspects

A transversal study was performed in 36 small ruminant farms located in the microregion of Garanhuns (8°53′25″South and 36°29′34″West), State of Pernambuco, Northeastern Brazil. This area is featured by a semi-arid climate with an annual average temperature of 22°C (from 17°C to 30°C), rainfall mean of 147 mm (from 25 mm to 295mm), and relative humidity of 90%.

All animal owners provided written consent for feces sampling and laboratory analysis performed in this study.

Animals

The minimum sample size (n = 384) was estimated based on the goat population (n = 35,770) and the sheep population (n = 99,606) of the study area (IBGE, 2016). In addition, an estimated prevalence of 50%, confidence level of 95% and statistical error of 5% were considered (Thrusfield, 2004). The farms were randomly selected by convenience (Reis, 2003). There were no exclusion criteria in relation to breed, gender, age and rearing systems of the animals.

Sampling and laboratorial procedures

From March 2017 to May 2018, fecal samples (n = 789) were collected from the rectum of goats (n = 388) and sheep (n = 401) using plastic gloves. After collection, samples were stored in isothermal boxes (4°C) until laboratory processing.

Each sample was individually processed by the McMaster egg counting method (Gordon and

Whitlock, 1939), using sugar-saturated solution (density = 1.25s.g.). On the other hand, the modified Mini-FLOTAC technique was performed using the saturated solutions of Zinc Sulfate ($ZnSO_4$) (density = 1.35s.g.) and Sodium Chloride (NaCl) (density = 1.20s.g.) (Barda et al., 2013). Microscopic analysis was performed at 10X and 40X magnifications in order to detect eggs of gastrointestinal parasites.

Data analysis

Descriptive statistics was initially performed to obtain relative and absolute frequencies. After that, the distribution analyses of the values (normality) by the Kolmogorov-Smirnov (K-S) test followed by a comparison among the values by the non-parametric test of Mann-Whitney were performed (Sampaio, 1998). The Chi-square test with Yates correction ($\chi 2$) was used to compare the occurrence of parasites infecting goats and sheep. A 5% significance level was considered. The BioEstat software version 5.3 was used for statistical evaluation (Ayres et al., 2007). For the comparison between the diagnostic tests (McMaster method and Mini-FLOTAC), the *Kappa* concordance index was used (Landis and Kock, 1977), and values of sensitivity, specificity, predictive positive value, predictive negative value and accuracy were obtained considering the McMaster method technique as the gold standard. The program IBM SPSS *statistics* 23.0 was used and the significance level adopted was of 5%.

Results

Out of all samples analyzed, 80.86% (638/789) scored positive for the presence of eggs of gastrointestinal parasites in at least one technique employed, being 89.43% (347/388) of goats and 72.57% (291/401) of sheep ($\chi 2 = 35.159$; $p<0.0001$). The Mini-FLOTAC technique (goats 88.40%; 343/388 and sheep 71.57%; 287/401) presented a better performance for detection of helminth eggs than the McMaster technique (goats 65.21%; 253/388 and sheep 34.16%; 137/401) ($\chi 2 = 10.358$; $p < 0.0001$) (Table 1).

Table 1. Positivity for gastrointestinal helminths infecting goat and sheep in Microregion of Garanhuns, Northeastern, Brazil.

Parasite	**McMaster method % (n/N)**		**Mini-FLOTAC % (n/N)**	
	Goat	**Sheep**	**Goat**	**Sheep**
Positivity Total (%)	65.21 (253/388)	34.16 (137/401)	88.40 (343/388)	71.57 (287/401)
Strongyloidea	79.84 (202/253)	81.75 (112/137)	69.68 (239/343)	76.31 (219/287)
Trichuris ssp.	2.37 (6/253)	5.11 (7/137)	1.46 (5/343)	0.70 (2/287)
Strongyloides spp.	0.79 (2/253)	2.92 (4/137)	-	-
Moniezia spp.	-	1.46 (2/137)	0.58 (2/343)	0.35 (1/287)
Strongyloidea + *Trichuris* spp.	5.53 (14/253)	4.38 (6/137)	7.87 (27/343)	7.67 (22/287)
Strongyloidea + *Strongyloides* spp.	7.11 (18/253)	2.92 (4/137)	10.50 (36/343)	6.62 (19/287)
Strongyloidea + *Moniezia* spp.	3.16 (8/253)	-	4.66 (16/343)	6.27 (18/287)
Strongyloidea + *Strongyloides* spp.+ *Moniezia* spp.	0.40 (1/253)	1.46 (2/137)	2.04 (7/343)	-
Strongyloidea + *Trichuris* spp. + *Strongyloides* spp.	0.40 (1/253)	-	2.33 (8/343)	-
Strongyloidea + *Trichuris* spp. + *Moniezia* spp.	0.40 (1/253)	-	0.29% (1/343)	-
Strongyloidea + *Trichuris* spp.+ *Strongyloides* spp. + *Moniezia* spp.	-	-	0.29% (1/343)	-
Strongyloides spp. + *Trichuris* spp.	-	-	0.29% (1/343)	0.70% (2/287)
Strongyloides spp. + *Moniezia* ssp.	-	-	-	1.39% (4/287)

The *Kappa* analysis revealed a weak concordance between techniques employed for both goats ($k=0.342$; $p<0.001$) and sheep ($k=0.281$; $p<0.001$). In particular, the Mini-FLOTAC showed high sensitivity for detection of Strongyloidea eggs in both species (goats 98.37% and sheep 96.77%). The values of sensitivity, specificity, predictive positive and negative values, and accuracy are shown in Table 2.

Table 2. Evaluation of Mini-FLOTAC and McMaster (gold standard) techniques for the detection of gastrointestinal helminths of goats and sheep from Microregion of Garanhuns, Pernambuco, Brazil.

	Goat		Sheep	
	Strongyloidea	***Trichuris* ssp.**	**Strongyloidea**	***Trichuris* ssp.**
Sensitivity (%)	98.37	59.09	96.77	61.54
Specificity (%)	34.27	91.80	41.52	95.88
Predictive Positive Value (%)	71.94	30.23	42.55	33.33
Predictive Negative Value (%)	92.45	97.39	96.64	98.67
Accuracy (%)	74.74	74.74	58.60	58.60

Discussion

This study evaluated two diagnostic techniques and observed that the Mini-FLOTAC presented higher performance in the detection of eggs of gastrointestinal helminths than the McMaster method for both goats (88.40%; 343/388) and sheep (71.57%; 287/401) species (Table 1). The overall results here in obtained is similar to those previously reported in Italy, where this method (i.e., Mini-FLOTAC technique) presented a better performance than McMaster technique (Cringoli et al., 2017). Previous studies had already demonstrated the superiority of the Mini-FLOTAC to other parasitological diagnostic methods (Rinaldi et al., 2014; Paras et al., 2018). For example, this tool produces less false negative results than other techniques widely employed (e.g., McMaster, Modified-Wisconsin) (Cringoli et al., 2017; Paras et al., 2018). In fact, the Mini-FLOTAC is accurate and easier to perform, without the need of scales and filtering debris; in addition, it can be used for fecal analysis in the laboratory or in the field (Noel et al., 2017; Bosco et al., 2018). Conversely, the McMaster method is used to make simple and quick treatment decisions, but it is not recommended for the use of selective therapy and evaluation of anthelmintic efficacy, with a smaller standard deviation (Castro et al., 2017).

Although the *Kappa* analysis presented a weak concordance between techniques, in both methods here in employed, a high percentage of eggs of the Superfamily Strongyloidea were detected in goats (86.34%) and sheep (70.32%). It is important to highlight that after culture of feces most of eggs hatching in larvae belonging to the genus *Haemonchus* in goats (93.1%) and sheep (96.9%). This genus comprises important species of great pathogenic relevance for ruminant species (Jesse et al., 2019). In addition, helminths of the Superfamily Strongyloidea include a great number of nematodes that parasitize livestock animals, being implicated as a cause of significant losses in the production of small ruminants (Souza et al., 2012).

In the present study, goats were the most parasitized species when compared to sheep (89.43%; $\chi 2 = 35.159$; $p < 0.0001$). Most likely this is related to the sanitary management used in each farm, in which those of goats are deficient when compared with sheep. In the area of study, the goat meat represents an important source of protein to the local inhabitants. However, in most of the cases this kind of culture is still performed with low levels of sanitary care since are represented for little producers. Additionally, the feeding behavior of each species may be an important factor. It is known that sheep obtain food close to the ground, and are exposed since early ages to parasitic infections, developing a good immunological response and making them more resistant to parasitic diseases when adults (Torres-Acosta and Hoste, 2008; Sykes, 2010).

For the goat species, the Mini-FLOTAC presented a better performance in the detection of any type of egg. One of the advantages compared to other methods is the use of different flotation solutions, increasing the egg recovery percentages in all samples (Capasso et al., 2019). The Mini-FLOTAC showed higher sensitivity for detection of Strongyloidea eggs (98.37% and 96.77%) for goats and sheep, respectively. This data indicates that this technique is able to reveal a more realist scenario regarding the quantity of eggs, which is useful in studies to assess the efficacy of treatments through determination of parasite loads.

Finally, this study demonstrated that the Mini-FLOTAC technique might be considered most precise and reliable than the McMaster method for detection of gastrointestinal parasites of small ruminants. Therefore, its use on the laboratorial routine should be stimulated, especially considering features as low cost, feasibility and reliability.

Conflict of Interest

The authors declare no conflict of interest.

Ethics Committee

All procedures here in performed were approved by the Ethical Committee for Animal Use (ECAU) of the *Universidade Federal Rural de Pernambuco* (approval number 06/2017).

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