Comparing McMaster and Mini-FLOTAC for endoparasites diagnostic in goats

Comparação das técnicas de McMaster e Mini-FLOTAC para diagnóstico de endoparasitos de caprinos

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ABSTRACT

The Brazil Northeast region has the higher goat national herd with the endoparasitosis as one of the main factors interfering in these productive chain. Aiming to compare diagnostic techniques to quantify goats endoparasites eggs and oocysts, eggs and oocysts recovered from feces were identified from 45 goats, Capra aegagrus hircus, by counting eggs per gram of feces (EPG) McMaster (conversion factors 100x and 25x) and Mini-FLOTAC (conversion factors 5x and 10x). The statistical analysis were performed in the SPSS program version 21.0 and the statistical differences and accordance between the techniques, by Friedman (p<0.05) and kappa (p-value), respectively. Eggs from Strongyloides, Strongyloides sp., Trichuris sp. and Eimeria sp were identified. The coproparasitological methods applied were efficient in quantifying and identifying recovered eggs and oocysts. Although the similarity and accordance among techniques regarding the Eimeria sp. oocysts, the McMaster 25x; Mini-FLOTAC 5x and Mini-FLOTAC 10x were the methods that better recovered such oocysts. The Strongyloides eggs were equally recovered by all the techniques. The Strongyloides sp. eggs were better recovered by the McMaster 25x; McMaster 100x and Mini-FLOTAC 5x techniques; and the Trichuris sp. by McMaster 100x. Considering that both techniques used in this work were capable on recovering every eggs and oocysts, both can be adopted for coproparasitological diagnostic in goats.

RESUMO

A região Nordeste do Brasil possui o maior rebanho nacional de caprinos, sendo as endoparasitoses um dos principais fatores que interferem nessa cadeia produtiva. Objetivou-se comparar técnicas de diagnóstico para quantificar ovos e oocistos de endoparasitos de caprinos. Identificou-se ovos e oocistos recuperados das fezes de 45 caprinos, Capra aegagrus hircus, por contagem de ovos por grama de fezes (EPG) McMaster (fatores de conversão 100x e 25x) e Mini-FLOTAC (fatores de conversão 5x e 10x). As análises estatísticas foram realizadas no programa SPSS versão 21.0 e as diferenças estatísticas e concordância entre as técnicas, por Friedman (p<0.05) e kappa (p-value), respectivamente. Identificou-se ovos do tipo Strongyloides, Strongyloides sp., Trichuris sp. e oocistos de Eimeria sp. Os métodos coproparasitológicos empregados foram eficientes na quantificação e identificação dos ovos e oocistos recuperados. Apesar da similaridade e concordância entre as técnicas em relação aos oocistos de Eimeria sp., o McMaster 25x; Mini-FLOTAC 5x e Mini-FLOTAC 10x foram os métodos que melhor recuperaram estes oocistos. Os ovos do tipo Strongyloides foram igualmente recuperados por todas as técnicas. Os ovos do tipo Strongyloides sp. foram melhores recuperados por meio das técnicas de McMaster 25x; McMaster 100x e Mini-FLOTAC 5x; e os ovos Trichuris sp., por McMaster 100x. Considerando que ambas as técnicas empregadas, no presente trabalho, apresentaram a capacidade de recuperar todos os ovos e oocistos encontrados, ambas podem ser adotadas para diagnóstico coproparasitológico em caprinos.

Keywords: Helminths, Small ruminants, Protozoa, Flotation techniques, Faeces

Palavras-chave: Helminhos, Pequenos ruminantes, Protozoários, Técnicas de Flutuação, Fezes

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INTRODUCTION

The goats herd is an economic activity that has been standing out in the northeastern Brazil, due to its rusticity and adaptability to the semiarid conditions. However, the gastrointestinal endoparasites has generated high economic losses, which has intensified especially because of the parasite resistance development in herds, condition that may culminate in increasing animal deaths. Factors such as drugs misuse, added to endoparasites inefficient exams, have increased these serious problems in the productive sector of sheep and goats (AHID et al., 2008; AHID et al., 2009; PEREIRA et al., 2017; MAGALHÃES, 2018).

Aiming to prevent serious problems in this productive scope it is indispensable the sensitive and exclusive diagnostic for the small ruminants gastrointestinal endoparasites. The small ruminants coproparasitology can work as good pointer of the infection level by gastrointestinal parasites in sheep and goats, helping in implementing appropriate management measures enabling the parasite control in these herds (RINALDI et al., 2014; RAHAL et al., 2020).

In the laboratorial routine, it must always be choosing an eggs and oocysts detection method with high sensibility and with simple and fast execution, besides of having a low operational value. In this context, some studies have evaluated the adaptation of such existent techniques in order to obtain an animal parasitosis diagnostic improvement, such as the small ruminants (CHAGAS et al., 2011; RINALDI et al., 2014; CRINGOLI et al., 2017).

Several analysis techniques are used in the coproparasitological practice in goats. However, it is known that although there are techniques able to detect by themselves the animal real parasitism, most of the times, using only a single technique doesn’t provide the host real parasitic charge, especially when co-infections are observed. However, in a laboratorial routine, it is not always possible performing many diagnostic techniques for a single fecal sample, in order to the economic viability and the time, as well (VIEIRA et al., 2018).

Among the available coproparasitological techniques that enable observing protozoa oocysts and nematode eggs, there are those by McMaster flotation (CHAGAS et al., 2011; GORDON; WHITLOCK, 1939) and Mini-FLOTAC (CRINGOLI et al., 2017). However, it’s been observed differences in eggs and oocysts accounting among these techniques and even in the found parasites diversity.

Thus, it turns indispensable an evaluation study of analysis techniques for fecal eggs and oocysts accounting, aiming to identify the most adequate for the coproparasitological diagnostic routine in such hosts.

MATERIAL AND METHODS

The experiments followed the Ethics Commission for Animals Used in Research from the Universidade Federal do Semi-Árido (UFERSA). Project approved under opinion CEUA-UFERSA 11/2018, under process number 23091.004479/2018-28. It was used 45 goats, Capra aegagrus hircus (Linnaeus, 1758), regardless age and sex, from the city of Mossoró, RN, naturally infected and not treated with anti-helminthic for a minimum period of ninety days previous the sample collection.

Fecal samples were daily collected from the animals rectal ampoule for the coproparasitology in the Animal Parasitology Laboratory (LPA), UFERSA. The eggs per gram (EPG) accounting technique was used (Gordon e Whitlock,1939) and modified from Chagas et al. (2011), with conversion factors 1:25 and 1:100, named respectively McMaster 25x and McMaster 100x. Fecal analysis were made according Rinaldi et al. (2014), Cringoli et al. (2017), for each Mini-FLOTAC collected sample. For this last technique, the conversion factors were 1:5 and 1:10, respectively, which were named as Mini-FLOTAC 5x and Mini-FLOTAC 10x.

Eggs and oocysts were identified by morphology (HOFMANN, 1987; FOREYT, 2005). The obtained data were analyzed by the statistical program SPSS, version 21.0. statistical differences among the groups were verified by the Friedman test and then, the concordances after applying the Kappa test were analyzed. Values of p<0.05 were considered as significant.

RESULTS AND DISCUSSION

The recovered eggs analysis by the samples simple frequency (%) showed that the techniques McMaster 100x and McMaster 25x presented positivity for Eimeria sp. oocysts of 97.8% and 96.6%, respectively. And, the techniques Mini-FLOTAC 5x and Mini-FLOTAC 10x recovered 100% showing higher frequency among the other recovered parasites (Table 1).

The technique McMaster 100x recovered 91.1% of Strongyloidea eggs and 93.3% with Mini-FLOTAC 10x, while in McMaster 25x and Mini-FLOTAC 5x, a similar positivity of 95.6% was observed. For Strongyloides sp., similar results of 26.7% were observed for the techniques McMaster 100x and McMaster 25x and, 33.3% for Mini-FLOTAC 5x and Mini-FLOTAC 10x. While for Trichuris sp., the McMaster 100x presented positivity of 17.8% and 11.1% for Mini-FLOTAC 10x, being equally recovered in the techniques McMaster 25x and Mini-FLOTAC 5x (Table 1).

Cardoso et al. (2017) and Dias et al. (2017) highlighted that the coccidia gender Eimeria are those who most affect the goats production in the world. Regarding the helminths found in the present study, it is noted that other previous works indicated the presence of Oesophagostomum sp., Haemoncus sp., and Strongyloides, as the parasites that most affect sheep and goats in the
western Rio Grande do Norte, Brazil (AHID et al., 2008; FONSECA et al., 2011).

Table 1. Single frequency of 45 goats analyzed samples.

| Groups   | McMaster (100x) | McMaster (25x) | Mini-FLOTAC (5x) | Mini-FLOTAC (10x) |
|----------|-----------------|----------------|------------------|------------------|
|          | POS  | NEG  | POS  | NEG  | POS  | NEG  | POS  | NEG  |
| Eimeria  | (%)  |      |      |      |      |      |      |      |
|          | 44   | 01   | 43   | 02   | 45   | 0    | 45   | 0    |
| (97.8)  | (2.2)| (95.6)| (4.4)| (100)| (0)  | (100)| (0)  |
| Strongylida | (%)  |      |      |      |      |      |      |      |
|          | 41   | 04   | 43   | 02   | 43   | 02   | 42   | 03   |
| (91.1)  | (8.9)| (95.6)| (4.4)| (95.6)| (4.4)| (93.3)| (6.7)|
| Strongyloides | (%)  |      |      |      |      |      |      |      |
|          | 12   | 33   | 12   | 33   | 15   | 30   | 15   | 30   |
| (26.7)  | (73.3)| (26.7)| (73.3)| (33.3)| (66.7)| (33.3)| (66.7)|
| Trichuris | (%)  |      |      |      |      |      |      |      |
|          | 08   | 37   | 04   | 41   | 04   | 41   | 5    | 40   |
| (17.8)  | (82.2)| (8.9)| (91.1)| (8.9)| (91.1)| (11.1)| (88.9)|

% = Percentage; POS = Positive; NEG = Negative.

Considering the same technique, McMaster 100x did not show to be statistically different in accounting eggs from Strongylida, Trichuris sp., and Strongyloides sp., however provided lower recovering average for Eimeria spp. (Table 2).

Silva et al. (2013) obtained higher averages in diagnosing such oocysts by the OOPG technique using Mini-FLOTAC 10x. In the present work, McMaster 25x and Mini-FLOTAC 10x showed statistical difference for lower average obtained for Trichuris sp. eggs, and Mini-FLOTAC 10x showed significant difference only for Strongyloides sp. eggs, being lower regarding all the recovered eggs and oocysts (42.15 ± 10.29) (Table 2).

Comparing every technique and considering the Strongyloides eggs recovering capability, as well as Eimeria sp. oocysts, it was observed that the McMaster 100x technique presents lower sensibility. For Strongyloides sp. eggs, the Mini-FLOTAC 10x technique presented lower sensibility. Considering Trichuris sp. eggs, the Mini-FLOTAC 10x technique was diverse from McMaster 25x; McMaster 100x and Mini-FLOTAC 10x (Table 2).

Table 2. Average values ± standard error for eggs per gram (EPG) accounting and Mini-FLOTAC in 45 goats.

| Groups   | McMaster (100x) | McMaster (25x) | Mini-FLOTAC (5x) | Mini-FLOTAC (10x) |
|----------|-----------------|----------------|------------------|------------------|
|          | Eimeria sp.     | Strongylida    | Strongyloides sp.| Trichuris sp.    |
|          | 82.8 ± 29.1aB   | 107.95 ± 32.89bA| 169.08 ± 70.57aA| 251.03 ± 111.48aA|
|          | 272.62 ± 48.04bA| 278.5 ± 47.58aA| 142.41 ± 74.7aA  | 69.04 ± 52.29bB  |
|          | 212.37 ± 43.68bA| 291.56 ± 44.76aA| 151.25 ± 37.72aA| 150.0 ± 141.69aB |
|          | 176.15 ± 42.84bA| 215.47 ± 40.43aA| 42.15 ± 10.29bB | 110.0 ± 95.03abA |
|          | (p<0.05 – Friedman); A,B = Different capital letters in columns mean statistical difference (p<0.05 – Friedman). |

Regarding the Kappa concordance values, among the studied techniques, it was observed that there was a moderated to strong concordance, therefore considered as similar (Table 3).

Table 3. Kappa concordance values (p-value) among the EPG and Mini-FLOTAC techniques for every found parasite.

| Tests       | McMaster (100x) | McMaster (25x) | Mini-FLOTAC (5x) |
|-------------|-----------------|----------------|------------------|
| McMaster (25x) | 0.812 *         | -              | -                |
| Mini-FLOTAC (5x) | 0.786 *         | 0.812 *        | -                |
| Mini-FLOTAC (10x) | 0.732 *        | 0.759 *        | 0.857 *          |

* = Statistical significance (p<0.001).
In the present study, despite the concordance and similarity among the techniques, it was found that *Eimeria* sp. oocysts were better recovered by McMaster 25x; Mini-FLOTAC 5x and Mini-FLOTAC 10x techniques. The Strongylida eggs were similarly detected by every technique. The *Strongyloides* sp. eggs were better detected by McMaster 25x; McMaster 100x and Mini-FLOTAC 5x techniques; and the *Trichuris* sp. eggs by McMaster 100x.

CONCLUSIONS

Considering that both flotation techniques used in the present study resulted in equal capability on detecting the presence of Strongylida eggs and *Eimeria* sp. oocysts, both the McMaster technique and using the Mini-FLOTAC, with their respective conversion factors, can be adopted in the coproparasitological diagnostic in goats.

REFERENCES

AHID, S. M. M. et al. Espécies do gênero *Eimeria Schneider*, 1875 (Apicomplexa: Eimeriidae) em pequenos ruminantes na mesorregião oeste do estado do Rio Grande do Norte, Brasil. Revista Ciência Animal Brasileira, v. 10, n. 3, p. 984-989, 2009.

AHID, S. M. M. et al. Parasitos gastrintestinais em caprinos e ovinos da região oeste do Rio Grande do Norte, Brasil. Revista Ciência Animal Brasileira, v. 9, n. 1, p. 212-218. 2008.

CARDOSO, I. F. et al. Biologia e epidemiologia da eimeriose em ruminantes. Revista de Ciência Veterinária e Saúde Pública, v. 4, Suplem. 2, 2017.

CHAGAS, A. C. S.; NICIURA, S. C. M.; MOLENTO, M.B. Manual Prático: metodologias de diagnóstico da resistência e de detecção de substâncias ativas em parasitas de ruminantes. Brasília: Embrapa Informação Tecnológica, 2011, 153 p.

CRINGOLI, G. et al. The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. Nature Protocols, v.12, p.1723-1732, 2017.

DIAS, L. M. P. et al. Diagnóstico e controle da coccidiose em ruminantes. Revista de Ciência Veterinária e Saúde Pública, v. 4, Suplem. 2, 2017.

FOREYT, W. J. Parasitologia Veterinária: Manual de referência. ed. São Paulo: Editora Roca Ltda, 2015.

FONSECA, Z. A. A. D. et al. Relação sexual do parasitismo por *Haemonchus contortus* em Caprinos (Capra hircus). PUBVET, v. 5, N. 31, Ed. 178, Art. 1200, 2011.

GORDON, H. M., WHITLOCK, H. V., A new technique for counting nematode eggs in sheep faeces. Journal of the Council for Scientific and Industrial Research, v. 12, p. 50-52. 1939.

HOEFFMANN, R. P. Diagnóstico de parasitismo veterinário. Sulina, Porto Alegre, 1987, 15.

MAGALHÃES, K. A. et al. Boletim do Centro de Inteligência e Mercado de Caprinos e Ovinos: Pesquisa Pecuária Municipal 2017: efetivo dos rebanhos caprinos e ovinos. Sobral: Embrapa Caprinos e Ovinos, 2018, 05-08p.

SILVA, L. M. R. et al. Mini-FLOTAC for the diagnosis of *Eimeria* infection in goats: an alternative to McMaster. Revista Small Ruminant Research, v. 114, n. 2-3, p. 280-283, 2013.

PEREIRA, J. S. et al. *Eimeria intricata* Spiegel, 1925 em ovinos de Mossoró, Rio Grande do Norte, Brasil. Revista Brasileira de Ciência Veterinária, v. 5, p. 69-71, 2017.

RAHAL, N. M. et al. Ocorrência de espécies do gênero *Eimeria Schneider*, 1875, em cordeiros confinados. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, vol. 72, n. 2, 2020.

RINALDI, L. et al. Comparison of individual and pooled faecal samples in sheep for the assessment of gastrointestinal strongyle infection intensity and anthelmintic drug efficacy using McMaster and Mini-FLOTAC. Veterinary parasitology, v. 205, n. 1-2, p. 216-223, 2014.

VIEIRA, F. P. R. et al. Uso de métodos de flutuação na rotina laboratorial para diagnóstico das principais helmintoses de animais domésticos. Tópicos especiais em ciência animal, p. 87, 2016.