doi: 10.5216/rpt.v46i4.51012

ORIGINAL ARTICLE

RELIABILITY OF DIFFERENT METHODS OF DIAGNOSING INTESTINAL PARASITES IN HORSES

Lucia Oliveira de Macedo[1], Marcos Antônio Bezerra Santos[1], Leucio Câmara Alves[2], Rafael Antonio Nascimento Ramos[1] and Gílcia Aparecida de Carvalho[1]

ABSTRACT

Parasitism by intestinal nematodes may cause serious injuries to equines affecting their performance, given that the parasites compete for food and cause irritation, internal bleeding and anaemia. The diagnostic methods currently available are not efficient in detecting all the species of parasites simultaneously, hampering reliable diagnosis. Therefore, the purpose of this study was to evaluate four diagnostic methods for detecting equine intestinal parasites in the southern hinterland of Pernambuco, Brazil. Fecal samples (n = 87) were collected and examined through flotation based methods (Willis-Mollay, modified centrifugal flotation and EPG/OPG) and sedimentation (Hoffman). Of the total number of samples examined, 77.0% (67/87) were positive by modified centrifugal flotation; 44.8% (39/87) were positive by the Willis-Mollay method; 34.5% (30/87) by the Hoffman method and 28.7% (25/87) by the EPG/OPG method. The Kappa index indicated moderate concordance between the Willis-Mollay and modified centrifugal flotation techniques (K= 0.477); Willis-Mollay and EPG/OPG (K= 0.466); EPG/OPG and Hoffman (K= 0.425). In conclusion, modified centrifugal flotation presented high sensitivity for detection of parasites of the Strongylida order and *Parascaris* spp. It may, therefore, be used in association with the Willis-Mollay technique as a safe and accurate method of diagnosis.

KEY WORDS: Willis-Mollay; centrifugal flotation; Strongylida; Cestoda.

INTRODUCTION

Brazil has a herd of 5.3 million equines, of which 1.2 million animals are in the northeast of the country (IBGE, 2016). These herbivores are affected by a number of helminths such as small strongyles or cyathostomes (e.g. *Cyathostomum* spp. and *Cylicostephanus* spp.), large strongyles (e.g. *Strongylus vulgaris, S. equinus* and *S. edentatus*), as well as *Parascaris equorum*, *Oxyuris equi, Strongyloides westeri, Trichostrongylus axei, Habronema* spp., *Dictyocaulus arnfield* and *Anoplocephala* spp. (Barbosa et al., 2001; Molento, 2005).

1. Unidade Acadêmica de Garanhuns, Universidade Federal Rural de Pernambuco (UFRPE), Garanhuns, Brazil.
2. Departamento de Medicina Veterinária, UFRPE, Recife, Brazil

Corresponding author: E-mail: gilciasilva@yahoo.com.br

Received: 7/6/2017. Revised: 11/10/2017. Accepted: 19/12/2017.

These helminths are worrying to breeders due to their high incidence affecting growth, weight gain and animal performance (Marbach, 1975; Ogbourne, 1978). There are few studies related to diseases caused by helminths in these animals (Araújo et al., 2008). It is important to highlight that little or no knowledge regarding the correct use of chemical compounds by owners and workers often results in considerable economic losses.

Most of the nematodes mentioned above are pathogenic, due mainly to bloodsucking and injuries caused by migration and inflammatory reaction at the parasitism sites (Jesus, 1963; Reddy et al., 1976; Slocombe, 1985). This damage may vary from injuries to vital organs in the digestive system, to serious disturbances in the enzymatic and hormonal processes in these animals. In addition, there is infection caused by cestodes risking ileum compaction and cramps, mainly *Anoplocephala perfoliata*, which causes inflammatory damage to the ileocecal valve, causing ulcerations of the mucosa (Proudman and Edwards, 1993).

Nowadays, some diagnostic methods used present low sensitivity hampering the correct diagnosis of these parasites. In fact, they are not effective regarding the simultaneous detection of all species of helminths. Therefore, studies are necessary to increase diagnostic methods (Almeida et al., 2008; Forlano et al., 2012; Ferreira et al., 2014; Quadros et al., 2014). For these reasons, the present study aims to evaluate four diagnostic methods (Willis-Mollay flotation; EPG/OPG; modified centrifugal flotation and Hoffman, Pons and Janer spontaneous sedimentation) for detection of eggs and/or oocysts of intestinal parasites in horses.

MATERIAL AND METHODS

Study area

The study was conducted in the southern hinterland of Pernambuco, Brazil, specially in the municipalities of Capoeiras (Latitude 08º44'13" South and Longitude 36º37'36" East); Garanhuns (Latitude 08º50'19" South and Longitude 36º28'12" East)); Jucati (Latitude 08º44'13" South and Longitude 36º25'44" East); Lajedo (Latitude 08º34'33" South and Longitude 36º37'07" East); São João (Latitude 08º52'35" South and Longitude 36º26'55" East); Brejão (Latitude 09º01'48" Southand Longitude 36º34'02" East) and Bom Conselho (Latitude 09º10'11" South and Longitude 36º40'47" East).

Sample collection

From August 2014 to July 2015, equine fecal samples (n = 87) were directly collected from the rectal ampule of the animals. The number of samples per municipality were the following: Capoeiras (n = 11), Garanhuns (n = 15),

Jucati (n = 11), Lajedo (n = 18), São João (n = 11), Brejão (n = 10) and Bom Conselho (n = 11). Samples were placed in plastic bags, identified and stored in isothermal boxes at 4° C for subsequent laboratorial processing. The collection was performed regardless of sex or breed and in animals ranging from two to ten years of age. One collection per animal was performed, which was subdivided in four parts to be used in each of the methods.

Laboratorial processing

The samples were analysed via four different methods for detection of eggs and oocysts: 1) The Willis-Mollay qualitative method of simple flotation (Willis, 1921); 2) flotation and counting of eggs and oocysts per gram of feces (EPG/OPG) in a MacMaster chamber applying the Gordon and Whitlock method (1939) using 2g of feces multiplying the average results by a correction factor of 100; 3) modified centrifugal flotation using a sugar saturated solution according to Martins et al. (2003) and 4) Hoffman, Pons and Janer spontaneous sedimentation (Hoffman et al., 1934). Subsequently, the material was transferred to microscope slides (1 slide for each technique), stained with a lugol solution (2%), covered with coverslips and examined through an optic microscope at 10 and 40X magnifications. The identification of eggs and/or oocysts detected was performed according to Taylor (2010).

Data analysis

A descriptive statistical analysis was performed on the data obtained. The Kappa concordance analysis was applied to compare the diagnostic methods used in this study. The Kappa values were interpreted according to Landis and Kock (1977). The Willis-Mollay method was used as Gold Standard for the calculation of sensitivity, specificity, positive predictive value (PV+) and negative predictive value (PV-). The 5.0 version BioEstat software for microcomputers was used for the statistical calculation (Ayres et al., 2000).

The present study was submitted to the Ethics committee for the use of animals (ECUA) and was registered under number 23082.009734/2013-19 with licence number 028/2014.

RESULTS

79.3% (69/87) of the samples analysed were positive in at least one of the tests. 77.0% (67/87) of the positive samples were detected by the modified centrifugal flotation method; 44.8% (39/87) by Willis-Mollay; 34.5% (30/87) by the Hoffman method, and 28.7% (25/87) by the EPG/OPG method. The overall results regarding simple infections and co-infections noted in each method are presented in Table 1.

Table 1. Simple infections and co-infections detected in equine fecal samples from the Meridional Agreste of Pernambuco using four diagnostic methods.

Method	Parasites	Frequency (%)
Simple flotation (Willis-Mollay, 1921)	Strongylida	43.7 (38/87)
	Parascaris spp.	1.1 (1/87)
EPG/OPG (Gordon and Whitlock, 1939)	Strongylida	27.6 (24/87)
	Parascaris spp.	1.1 (1/87)
Spontaneous sedimentation Hoffman (et al., 1934)	Strongylida	19.6 (17/87)
	Parascaris spp.	4.6 (4/87)
	Eimeria spp.	2.3 (2/87)
	Anoplocephala spp.	1.1 (1/87)
	Strongylida + *Parascaris* spp.	4.6 (4/87)
	Strongylida + *Eimeria* spp.	2.3 (2/87)
Modified centrifugal flotation (Martins et al., 2003)	Strongylida	52.9 (46/87)
	Parascaris spp.	4.6 (4/87)
	Eimeria spp.	1.1 (1/87)
	Oxyuris spp.	1.1 (1/87)
	Strongylida + *Parascaris* spp.	12.6 (11/87)
	Strongylida + *Eimeria* spp.	1.1 (1/87)
	Strongylida + *Parascaris* spp. + *Eimeria* spp.	2.3 (2/87)
	Strongylida+ *Parascaris* spp. + *Anoplocephala* spp.	1.1 (1/87)

Table 2. Absolute and relative frequency, average number of eggs and standard deviation of gastrointestinal parasites in equine fecal samples from the Southern Hinterland, PE.

	Strongylida	*Parascaris* spp.	Total (N)
Willis-Mollay			
AF (n)	1037	5	1042
Mín	0	0	-
Max	187	5	-
$\bar{x}$±SD	11.91±33.0	0.05±0.53	-
RF (%) (n/N)	99.52	0.48	100.00
EPG/OPG			
AF (n)	17100	100	17200
Mín	0	0	-
Max	2200	100	-
$\bar{x}$±SD	196.55±476.04	1.14±10.72	-
RF (%) (n/N)	99.41	0.59	100.00
Hoffmann			
AF (n)	148	25	173
Mín	0	0	-
Max	35	6	-
$\bar{x}$±SD	1.70±4.73	0.28±1.10	-
RF (%) (n/N)	85.54	14.46	100.00
Modified centrifugal flotation			
AF (n)	13971	156	14127
Mín	0	0	-
Max	2200	72	-
$\bar{x}$±SD	160.58±314.15	1.79±8.33	-
RF (%) (n/N)	98.89	1.11	100.00

Legend: n – number of eggs; N – total number of eggs; AF – absolute frequency; RF – relative frequency; $\bar{x}$ - average; SD – Standard deviation.

Strongylida and *Parascaris* spp. parasites were the most frequently detected helminths. The average number of eggs detected through all methods is shown in Table 2.

Kappa analysis yielded the following results: Willis-Mollay against EPG/OPG (K= 0.466; p<0.001): moderate concordance. Willis-Mollay against Hoffman (K= 0.337 p<0.001): considerable concordance. Willis-Mollay against centrifugal flotation (K= 0.447 p<0.001): moderate concordance. EPG/OPG against Hoffman (K= 0.425 p<0.001): moderate concordance. EPG/OPG against centrifugal flotation (K= 0.293 p<0.001): considerable concordance. Hoffman against modified centrifugal flotation (K= 0.240 p<0.001): considerable concordance. The values of sensitivity, specificity, predictive positive value (PV+), predictive negative value (PV-) and accuracy are presented in Table 3.

Table 3. Evaluation of the EPG/OPG, Hoffman and modified centrifugal flotation methods in relation to Willis-Mollay (Gold Standard) for the diagnosis of gastrointestinal parasites found in equine fecal samples, Meridional Agreste, PE.

	EPG/OPG		Hoffman		Modified centrifugal flotation	
	Strongylida	*Parascaris* spp.	Strongylida	*Parascaris* spp.	Strongylida	*Parascaris* spp.
Sensitivity	55.0%	0.0%	47.0%	0.0%	97.0%	100.0%
Specificity	92.0%	99.0%	90.0%	95.0%	45.0%	80.0%
Predictive Value (+)	84.0%	0.0%	78.0%	0.0%	51.0%	6.0%
Predictive Values (-)	73.0%	99.0%	69.0%	99.0%	96.0%	100.0%
Accuracy	76.0%	98.0%	71.0%	94.0%	64.0%	80.0%

DISCUSSION

In this study, four different parasitological diagnostic methods (Willis-Mollay flotation, Gordon and Whitlock (EPG/OPG), Hoffman, Pons and Janer spontaneous sedimentation and modified centrifugal flotation) were evaluated. The results proved distinctive according to each of the techniques, especially considering positivity, namely 77.0% (67/87), 44.8% (39/87), 34.5% (30/87) and 28.7% (25/87) applying the modified centrifugal flotation, Willis-Mollay, Hoffman and EPG/OPG methods respectively.

The differences noted may be related to specific characteristics presented by each method (Table 1). The Strongylida order parasites were the most frequently detected, regardless of the method used, followed by *Parascaris*

spp., *Anoplocephala* spp. and *Oxyuris* spp. Similar results were obtained in a previous study in which a large number of animals infected by parasites of the Strongylida order were observed (Forlano et al., 2012; Quadros et al., 2014).

The diagnosis of *Anoplocephala* spp. and *Eimeria* spp. was only possible by the sedimentation and modified centrifugal flotation methods. On the other hand, *Parascaris* spp. was the most common parasite detected using the Hoffman method (14.5%). A previous study performed in the South of Minas Gerais, detected low parasite frequencies by the EPG/OPG method. The disparity observed in the number of analysed samples in both studies may likely have influenced the final result (Rosa, 2014).

It is important to highlight the high number of eggs detected in some samples. For instance, Strongylida and *Parascaris* spp. eggs (2200 and 100) and (2200 and 72) detected respectively via EPG/OPG and centrifugal flotation, revealed that these animals presented high parasitism, reinforcing the importance of using these methods for diagnosing equine intestinal parasites.

Considering the Willis-Mollay method as Gold Standard, the modified centrifugal flotation method presented high sensitivity (97.0%) to Strongylida and to *Parascaris* spp. (100.0%) indicating that this method is the best in comparison with the Hoffman and EPG/OPG methods which presented low sensitivity for detecting Strongylida eggs (47.0% and 55.0%), respectively. These findings corroborate those described by Martins et al. (2003), where a 96.0% sensitivity was noted. Although the EPG/OPG technique, when compared to Willis-Mollay presented moderate concordance (K=0.466) the sensitivity of this method to Strongylida was low (55.0%).

The results pointed to a higher reliability regarding the identification of infection by intestinal parasites in the modified centrifugal flotation method, which also detected parasitism by *Oxyuris* spp., not noted in the other methods. According to Almeida et al. (2008) the centrifugal flotation method is better regarded than the simple flotation technique, due to its superior sensitivity and specificity.

The variation between the methods according to the Kappa values was 0.24 to 0.46 interpreted as "considerable" to "moderate" concordance respectively, indicating the need for parallel tests, in order to enhance the diagnosis of Strongylida and Parascaris spp. In general, the Willis-Mollay and centrifugal flotation methods proved accurate when used simultaneously, detecting a higher number of eggs of the Strongylida order and *Parascaris* spp.

In this context, the use of more than one method for fecal parasitological diagnosis is recommended, since the use of only one technique might not reveal the true parasitism, especially considering that, in many cases the animals present co-infections.

In conclusion, the modified centrifugal flotation method presented high sensitivity to parasites belonging to the Strongylida order and to *Parascaris* spp. and may be used in association with the Willis-Mollay method for reliable diagnosis.

REFERENCES

1. Almeida JC, Augusto MM, Silva TG, Toledo OS, Souza DF, Antunes J, Molento MB. Prevalência quantitativa de Anoplocephala sp. e uniformidade de ovipostura em equinos. *Rev Bras Parasitol Vet 17*: 158-162, 2008.
2. Araújo NKS, Ahid SMM, Bezerra ACDS, Dias RVC, Ferreira HIP, Neto JCF, Fernandes JAS. Avaliação da eficácia dos anti-helmínticos Ricobendazole® e abamectina gel composto® em Equinos de vaquejada. *Acta Vet Brasilica 2*: 47-49, 2008.
3. Ayres M, Ayres J, Santos AS. *Bioestat 2.0: aplicações estatísticas nas áreas de ciências biológicas e médicas*. Sociedade Civil Mamirauá: Belém, 2000.
4. Barbosa OF, Rocha RF, Silva GS, Soares VE, Veronez VA, Oliveira GP, Landim VJC. Survey on Cyathostomina and nematodes (Strongylidea, Strongylidae) in pasture bred horses from São Paulo State, Brazil. *Semina: Ci Agr 22*: 21-26. 2001.
5. Ferreira GMS, Dutra FAF, Filho EFA, Santos ACG. Parasitismo gastrintestinal e hematologia em equinos e asininos da mesorregião da aglomeração urbana, São Luís, Maranhão. *Arch Vet Sci 19:* 22-30, 2014.
6. Forlano M, Canelón J, Mujica F, Álvares E, Concepción J, Granda F. Prevalencia de endoparásitos en caballo criollo venezolano en dos hatos del estado Apure - Venezuela. *Gaceta Ci Vet 17*: 11-17, 2012.
7. Gordon HM, Whitlock HV. A new technique for counting nematode eggs in sheep faeces. *J Council Sci 12*: 50-52, 1939.
8. Hoffman WA, Pons JA, Janer JL. The sedimentation concentration method in Schistosomiasis mansoni. *P R J Public Health 9*: 281-298, 1934.
9. Instituto Brasileiro de Geografia e Estatística – IBGE. Banco de dados 2016.
10. Jesus Z. Observations on habronemosis in horse. *Phillipp J Vet Med 2*: 133-152, 1963.
11. Landis JR, Kock GG. The measurement of observer agreement for categorical date. *Biometrics 33*: 159-174, 1977.
12. Marbach W. Common internal parasites of horses. *Animal Dis Bulletin 15*: 1-7, 1975.
13. Martins IVF, Verocal GG, Melo RMS, Freitas IF, Correia TR, Pereira MJS, Scott FB. Validação de uma modificação da técnica de centrífugo-flutuação (Beroza et al., 1986) para o diagnóstico de cestóides em equídeos. *Rev Bras Parasitol Vet 12:* 99-102, 2003.
14. Molento MB. Resistência parasitária em helmintos de equídeos e propostas de manejo. *C Rural 35*: 1469-1477, 2005.
15. Ogbourne CP. Pathogenesis of cyathostome (Trichonema) infections of the horse. A review. Commonwealth Institute of Helminthology. *Miscellaneous Public 5*: 1-25, 1978.
16. Proudman CJ, Edwards GB. Are tape worms associated with equine colic a case control study. *Equine Vet J 25*: 224-226, 1993.
17. Quadros RM, Marques SMT, Lisboa BR, Bunn S, Silva MO. Helmintos intestinais em cavalos de raça da região serrana catarinense. *Vet Foco 12*: 10-19, 2014.
18. Reddy AB, Gaur NSN, Sharma UK. Pathological changes due to *Habronema muscae* and *H. megastoma* (Drashiamegastoma) infection in equines. *Indian J Anim Sci 46:* 207-210, 1976.
19. Rosa MHF. *Caracterização das propriedades de mangalarga marchador no sul de Minas Gerias, quanto a prevalência e resistência das helmintoses*. Dissertação (mestrado) – Universidade Federal de Lavras, 2014.
20. Slocombe JOD. Pathogenesis of helminths in equines. *Vet Parasitol 18*: 139-153, 1985.
21. Taylor MA, Coop RL, Wall RL. *Parasitologia veterinária*. 3ª ed., Rio de Janeiro: Guanabara Koogan. 2010. 786p.
22. Willis HH. A simple levitation method for the detection of hookworm ova. *Med J Aust 8*: 375-378, 1921.