EVALUATION OF THE EFFECTIVENESS OF THE METHOD OF CENTRIFUGATION-FLUCTUATION FOR THE DIAGNOSIS OF *ANOPLOCEPHALA PERFOLIATA* (GOEZE, 1782) IN EQUINES

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SUMMARY: The porpouse of this study was to evaluate the performance of a coproparasitological technique of centrifugation-fluctuation for the detection of *Anoplocephala perfoliata* eggs in equine cecum-colon fecal samples. Three hundred and ten fecal samples were taken from equines abated in a slaughter-house in Apucarana county, Paraná state, Brazil. The results of the fecal exames were compared to intestinal inspection. From 66 infected animals the fecal analyses detected 15 positive and 7 false positive, 41 false negative and 237 negative results. The sensitivity was 23% indicating that this techinique is not appropriate for the detection of *Anoplocephala perfoliata* eggs in equine cecum-colon fecal samples.

KEY WORDS: *Anoplocephala perfoliata*, centrifugation-flexcmation, diagnosis, equines.

INTRODUCTION

Horse breeding in Brazil has been stimulated and has technologically advanced in recent years, with the intention to improve performance and to minimize the economic damages. Helmintoses in the equines and the problems associated with it diminish performance and production. Literature regarding cestodes (*Anoplocephala magna*, *Anoplocephala perfoliata* and *Paranoplocephala mamillana*), as causing gastrointestinal disturbances in equines, have been intensified in the last years, and some epidemiological studies have been carried out (PROUDMAN *et alii*, 1998).

A. perfoliata is the cestode most commonly found in equines (URQUHART, 1996).

Presently, coproparasitological examinations are used as the method of diagnosis, and epidemiological studies are performed based on these methods (NILSSON *et alii*, 1995). GOMES *et alii*, (1997) in an investigation at the Jockey Club of São Paulo from 1992 to 1995, observed an increasing incidence of cestodes by the positive results of the coproparasitological examinations (WILLIS method). In 1992, of the 558 samples analyzed, 1 (0.2%) sample presented positive results for *Anoplocephala spp*, which subsequently increased to 6%, 26% and 33% in the years 1993, 1994 and 1995, respectively.

NILSSON *et alii*, (1995) had verified that 53% of the animals that had been submitted the coproparasitological examinations were egg-positive; and that the animals did not exhibit clinical signs of gastrointestinal tract disturbances for a long period of time. BARCLAY, (1982) in a study performed with animals submitted to laparotomy with verified infections of *Anoplocephala perfoliata*, removed some fecal samples, which were analyzed (centrifugal-fluctuation) and found no evidence of eggs in the feces.

The necessity of the development of more sensitive techniques for egg detection in feces through coproparasitological examination is desirable since the method of centrifugal-fluctuation commonly used for the egg detention of cestodes in feces, exhibits questionable sensitivity (PROUDMAN and EDWARDS, 1993). However, a modified version of the centrifugal-fluctuation method was developed to validate the diagnosis of cestode infections in equines, in the attempt to increase the sensitivity of the examination, reaching an overall 61% sensitivity, which still does not fully validate the procedure. The technique is however easy to execute and it does not require equipment or expensive reagents (PROUDMAN and EDWARDS, 1992).

Serological examinations (ELISA), possess greater sensitivity and specificity. This test allows the detection of

parasitic antigens in the animal, before or during the clinical manifestation of the illness and facilitates determination of the infection intensity (PROUDMAN *et alii*, 1998, HöGLUND *et alii*, 1995). However it is an examination that demands expensive equipment and reagents.

The objective of this work was to evaluate the effectiveness of the method of centrifugal-fluctuation for the coproparasitological diagnosis of cestodes in equines.

MATERIALS AND METHODS

Animals and Sampling

The study was carried out in a refrigerated slaughter house in Apucarana, located in the state of the Paraná, which processes abated equines. The establishment abates on average 2500 animals per month and the sample consisted of 310 intestinal tracts, calculated following the technique of proballistic sampling of finite populations, COCHRAN (1977). The animals originated from the states of São Paulo, Mato Grosso du Sul, Paraná and Santa Caterina. The experiment was carried out between the months of January and December of 1998. Twenty to 30 samples were examined each month, harvested fortnightly on random days of the week.

Material Harvest

The intestines were inspected in the line of abatement, where the anatomical parts were randomly selected 15 minutes after the slaughter, then washed in tap water and placed in ventral position where the incisions were made in the thin intestine in the distal position of the ileum, approximately fifty centimeters of the ileo-cecal valve , in the ileo-cecal valve, the cecum and colon more than one hundred centimeters of the ceco-colical valve following the technique of GREINER *et alii*, 1994.

Soon after, the fecal content not adherent to the intestinal wall was inspected and removed, thereby collecting all of the units of joined cestodes. The cestodes were counted and classified according to the described morphological descriptions of LICHTENFELS, 1975; FREITAS, 1980. The degree of parasitism was estimated according to the criteria established by FOGARTY *et alii*, 1994: up to 20 parasites, from 21 to 100 parasites and more than 100 parasites. The samples of fecal content harvested from the cecum and colon were processed in the Laboratory of Parasitology at the State University of Londrina. The samples were kept in plastic bags under refrigeration until the processing.

Coproparasitologicals Examinations

The examinations were carried out following the technique of PROUDMAN and EDWARDS, (1992). Forty g of fecal content was placed in a becker and vigorously mixed with 5 to 10 ml warm distilled water to form a consistent paste. The mixture was then filtered through gaze and the resultant solution was harvested in two 10 ml centrifuge tubes. The tubes were then centrifuged at 1200 G's for 10 minutes. The supernatant was discarded and the pellet was resuspended in warm distilled water. The same procedure was repeated and the pellet was resuspended in a solution of saturated sucrose, (450 g granulated sugar in 350 ml warm water), the tubes again were centrifuged at 1200 G's for 10 minutes. The supernatants were removed from the tubes and placed in 8 ml wide mouth containers and covered by slides. After two hours the slides were examined to verify the presence or absence of eggs adhered to the inferior surface of the slides.

Statistical Analysis

A calculation of sensitivity and specificity was used for the examination, in accordance with the proballistic model for the comparison of disgnostic tests considered by GART and BUCK (1966). The Kappa agreement was used to determine the efficiency of the test through the agreement of the factors considered by LANDIS and KOCH, 1977. We calculated the dependence of the degree of infection and the coproparasitological examination through the X^2 test for independence or association.

RESULTS AND DISCUSSION

The centrifugation-fluctuation technique is relatively easy to execute and does not require expensive equipment or reagents, however the main disadvantage is the expense of time of more than two hours (PROUDMAN and EDWARDS, 1992). The results of the coproparasitological examinations (centrifugation-fluctuation) and their relation to the prevalence of the examinations *post-mortem* are described in Table 1. There was observed 15 examinations with positive results from the 66 parasitized animals and seven false positive results. Sensitivity was of 23% and the specificity was 97%, according to the calculation considered by GART and BUCK (1966). The number of false negative examinations was relatively high (51), as consequence, the test exhibited low sensitivity (Table 1). The examination proved to be of low effectiveness through the agreement of the factors (k = 0.25) according to the calculation of the kappa coefficient (LANDIS and KOCH, 1977). This index is inferior as compared with the findings of PROUDMAN and EDWARDS, (1992). The number of false positive examinations found can be attributed to the presence of a reduced number of cestodes in the intestinal tract not adhered to the mucosa and not identified in the fecal mass.

Table 1 - Results of examinations by the technique of centrifugation-fluctuation relative to degree of infection of *Anoplocephala perfoliata*, in equines abated in a refrigerated slaughter-house in Apucarana - PR.

Coproparasitological	Positive	Negative	Total
Positive	15*	7	22
Negative	51	237	288
Total	66	244	310

*number of animals

The mature segments of the *Anoplocephala perfoliata* are eliminated in the feces and if they disintegrate during intestinal transit, they liberate eggs in the environment. The observed discrepant sensitivity can result from sporadic and irregular separation of the proglote, provoking a discontinous distribution of eggs in the fecal mass. The sensitivity of the test depends on the deposition of these eggs in the feces, consequently this phenomenon reduced the probability of finding the results, being therefore, an inadequate test for the positive diagnosis by not exhibiting high sensitivity (NILSSON *et alii*, 1995).

The degrees of infection for the post mortem examination and the results of the coproparasitological examination are in Table 2. The employed technique is qualitative, and could not distinguish an animal with two cestodes from animals that possessed two hundred cestodes. Many of the observed false negative examinations were obtained from animals that exhibited a reduced number of parasites. When we exclude the animals that present low infection (animals with up to 20 parasites) we observe a greater sensitivity of the test (39%), comparable to the study of PROUDMAN and EDWARDS, (1992) and if one consider animal with high levels of parasitism (more than 100 parasites), sensitivity is still greater (70%). We can conclude that the sensitivity of the examination is greater for animals that exhibit high parasitic loads. It was observed that the result of the tested examination depends upon the concurrent degree of infection. This hypothesis was tested through X^2 for independence, which demonstrated the dependence of the tested variables.

Table 2 - Distribution of the number of equines abated in a slaughter-house in Apucarana - PR, between the months of January to December of 1998, infected with *Anoplocephala perfoliata*, according to the degree of infection and the results obtained by coproparisitological analysis.

DEGREE OF INFECTION	ANIMALS		
	NUMBER EXAMINED *post-mortem*	PRESENCE OF EGGS IN FECES	
	Nº	Nº	%
Absence of parasites	244	7	2.9
Up to 20 parasites	38	5	13.1
From 21 to 100 parasites	18	4	22.2
> 100 parasites	10	7	70

The results of the coproparasitological examinations distributed monthly are shown in Table 3 and present a greater percentage of positive results in the month of December. Also they were distributed by degree of infection during the various months and are demonstrated in Table 4.

The greatest index of parasitized animals was in the month of April, however the result of the coproparasitological examinations suggested a low index of positive diagnoses (7.7%), which is a function of the elevated number of animals infected with low levels of parasitism (with up to 20 parasites), however in December, there was recorded a greater index of the number of parasitized animals, being that the result of the

Table 3 - Frequency of the monthly occurrence of *Anoplocephala perfoliata* equines abated in a slaughter-house in Apucarana - PR, and results of coproparisitological analysis obtained between the months of January to December of 1998.

ANIMALS	MONTHS											
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AGU	SEP	OCT	NOV	DEC
POSITIVES	4* (15,3)	8 (32)	2 (10)	11 (42.3)	8 (28.6)	4 (14.3)	4 (16.7)	3 (13)	3 (12)	5 (20.8)	2 (7.7)	12 (36.3)
COPRO-PARASITOLOGICAL	1 (4)**	1 (4)	2 (10)	2 (7.7)	2 (7.1)	2 (7.1)	– 0	2 (7.7)	3 (12)	1 (4.2)	2 (7.7)	5 (15.5)
TOTAL	25	25	20	26	28	28	24	26	25	24	26	33

*Number of animals; **Percentage

Table 4 - Distribution of the number of equines abated in a slaughter-house in Apucarana - PR, infected with *Anoplocephala perfoliata*, examined *post mortem*, according to the degree of infection, from January to December of 1998.

DEGREE OF INFECTION	MONTHS											
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AGU	SEP	OCT	NOV	DEC
Absence of parasites	22* (88)**	17 (68)	18 (90)	15 (57.7)	20 (71.4)	24 (85.7)	20 (83.3)	23 (87)	22 (88)	19 (79.2)	24 (92.3)	21 (63.7)
Up to 20 parasites	1 (4)	4 (16)	0	9 (34.6)	4 (14.3)	3 (10.7)	4 (16.6)	3 (3.8)	2 (8)	3 (12.5)	1 (3.8)	4 (12.1)
From 21 to 100 parasites	2 (8)	3 (12)	2 (10)	1 (3.8)	2 (7.1)	0	0	0	0	1 (4.2)	0	7 (21.2)
>100 parasites	1 (4)	1 (4)	0	1 (3.8)	2 (7.1)	1 (3.5)	0	0	1 (4)	1 (4.2)	1 (3.8)	1 (21.2)
Total	25	25	20	26	28	28	24	26	25	24	26	33

* Number of animals; **Percentage

coproparasitological examination revealed a larger index of positive diagnoses (15.5%), which is a function of a greater number of animals with elevated infection levels (more than 100 parasites) as demonstrated in Table 4.

Of the samples of analyzed fecal content, 87% presented positive results for other parasites (*Strongylus spp, Parascaris sp, Strongyloides spp, Dictiocaulus spp, Eimeria spp, Oxyuris sp*); 6.4% exhibited positive results of multiple infections with *A. perfoliata* and other nematodes, 1.6% had presented positive results only for *A. perfoliata*, 2.8% had presented negative results (exempt of parasites), and 2.2% of the results were not observed.

More recent serological techniques are indicated as an alternative means of diagnosis as well as the need for more consistent epidemiological studies. The serological diagnosis through ELISA immunoenzymatic assays detects antigens of the parasite in the animal before any symptom of the illness is manifest, as such, these studies are waranted.

SUMÁRIO

O objetivo deste estudo foi o de avaliar a performance de uma técnica coproparasitológica de centrífugo-flutuação para detecção de ovos *do Anoplocephala perfoliata* em amostras fecais de ceco e colon de equinos. Foram colhidas 310 amostras fecais de equines abatidos no abatedouro de Apucarana, PR, Brasil. Os resultados das amostras fecais foram comparados com o nível de infecção detectados em exame *post mortem*. De 66 animais infectados, as análises fecais detectaram 15 positivos, 7 falsos positivos, 41 falsos negativos e 237 negativos. A sensibilidade foi de 23% e a especificidade de 97% indicando que esta técnica não é apropriada para a detecção de ovos de *Anoplocephala perfoliata* em conteúdo fecal de ceco e colon.
PALAVRAS-CHAVE: *Anoplocephala perfoliata*, centrifugo-flutuação, diagnóstico, equinos.

REFERENCES

BARCLAY, P.W.; PHILIPS, N.T.; FOERNER, J.J. Intussuception associated with *Anoplocephala perfoliata* infection in five horses. *Journal of American Veterinary Medical Assotiation*, v 180, n 7, p 752-753, 1982.

COCHRAN, W.C. *Sample techiniques*. 3 ed. New York: John Willey, Cap. 4: the estimation of sample size, p 93-97, 1977.

FOGARTY, U.; DEL PIERO F. ; PURNELL, R.E. ; MOSURSKI, K.R. Incidence of *Anoplocephala perfoliata* in horses examined at an Irish abattoir. *Veterinary Record,* v 34, p 515-518, 1994.

FREITAS, G.M. *Helmintologia Veterinária*, 4ª ed., Rabelo, Belo Horizonte, 1980.

GART, J.J.; BUCK, A.A. Comparison of a screening test and a reference test in epidemiologic studies. *American Journal of Epidemiology,* v.83, n.1, p. 593 - 602, 1966.

GOMES, T.L.S.; HUSSNE, M.; TUNALA, V.; TAMURA, E.Y. Pesquisa sobre a incidência de tênias em equídeos . *A Hora Veterinária* , n 95, p 11-12, 1997.

GREINER, E.C. ; LANE, T.J. ; Effects of the daily feeding of pyrantel tartrate on *Anoplocephala perfoliata* infection in three horses: A pilot Stud. *Journal of Equine Sciense* , v 14 , n 1 , p 43-44 , 1994.

HöGLUND, J. ; LJUNGSTRöM, B.L. ; NILSSON, O. ; UGGLA, A. Enzyme-linked immunosorbent assay (ELISA) for the detecction of antibodies of *Anoplocephala perfoliata* in horse sera. *Veterinary Parasitology*, v 59 , n 2 , p 97-106 , 1995.

LANDIS, J.R.; KOCH, G.G. The measurement of observer agreement for categorical data. *Biometrics,* v.33, p.159, 1977.

LICHTENFELS, J.R. Cestoda parasitic in domestic equids. *Proceedings of Helmintological Society of Washington*, v 42, p 71-73, 1975.

NILSSON, O. ; LJUNGSTRöM, B.L. ; HöGLUND, J. ; LUNDQUIST, H. *Anoplocephala perfoliata* in horses in Sweden: Prevalence, infection levels and intestinal lesions. *Acta Veterinary Scandinavia* , v 36, p 319-328 , 1995.

PROUDMAN,J.C.; EDWARDS,G.B. Validation of a centrifugation/flotation technique for the diagnosis of equine cestodiasis. *Veterinary Record*, v 131, p 71-72, 1992.

PROUDMAN,J.C. ; EDWARDS,G.B. Are tapeworms associated with equine colic? A case control study. *Equine Veterinary Journal*, v 25, n 3, p 224-226, 1993.

PROUDMAN, J.C.; FRENCH, N.P.; TREES, A.J. Tapeworm infection is a significant risk for spasmodic colic and ileal impaction colic in the horse. *Equine Veterinary Journal*, v 30, n 3, p 194-199, 1998.

URQUHART, G.M.; ARMOUR, J.; DUNCAN, J.L.; DUNN, A.M.; JENNINGS, F.W. *Parasitologia Veterinária*, 2ª ed., Guanabara Koogan, Rio de Janeiro, 1996.

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