Comparison between modified Gordon & Whitlock and Centrifugal Fluctuation techniques for diagnosis of helminths in cattle

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ABSTRACT. Paixão J.L.F., Prata M.C.deA., Furlong J., Tassinari W.deS., Bittencourt V.R.E.P. & Angelo I.daC. **Comparison between modified Gordon & Whitlock and Centrifugal Fluctuation techniques for diagnosis of helminths in cattle.** [Comparação entre as técnicas Gordon & Whitlock modificada e Centrífugo Flutuação para diagnóstico de helmintose em bovinos.] *Revista Brasileira de Medicina Veterinária, 38(Supl. 3):167-174, 2016.* Programa de Pós-Graduação em Ciências Veterinárias, Anexo 1, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR 465 - Km 7, Seropédica, RJ, 23.890-000, Brasil. E-mail: jose.paixao@ifsudestemg.edu.br

The present study aimed to verify the degree of correlation between the modified Gordon & Whitlock (GW) and Centrifugal Fluctuation (CF) techniques in the diagnosis of gastrointestinal endoparasites in cattle, and to compile a table with the degree of helminth infection of the Strongyloidea Superfamily, measured by number of eggs per gram of feces (EPG) using the two techniques. Feces samples were collected directly from the rectal bulb of 12 females, ¾ Dutch / zebu, from six months of age, weighing between 100 and 150 kg, naturally parasitized by gastrointestinal nematodes, at Santa Mônica Dairy Station, Embrapa/Gado de Leite, in the Municipality of Valença, Rio de Janeiro, Brazil. Samples were collected every 21 days in a period of 28 months. EPG counts were performed at the Parasitology Laboratory of Embrapa, in the Municipality of Juiz de Fora, MG, Brasil. The CF technique detected 80.81% of positive samples, against 15.17% of the GW technique, demonstrating that the CF technique is more sensitive than the GW for EPG count in cattle. The GW technique showed a higher Coefficient of Variation (CV = 237%) than CF (CV = 68%). However, there was a significant agreement between them (*rho* = 0.12), confirming the existence of a correlation between them. This confirmation allowed the elaboration of a table of the degree of helminth infection of the Strongyloidea Superfamily based on the two techniques, which will aid in the determination of the treatment threshold. Thus, the absence of parameters in the decision making for the treatment of helminths in cattle, from the degree of infection, considered the great disadvantage of the CF technique, was eliminated.

KEY WORDS**.** Parasitism intensity, treatment threshold, EPG, Bovine Parasites

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RESUMO. O presente estudo objetivou verificar o grau de correlação existente entre as técnicas Gordon & Whitlock modificada (GW) e Centrifugo Flutuação (CF) no diagnóstico de endoparasitoses gastrintestinais em bovinos, e elaborar uma tabela com o grau de infecção de helmintos da Superfamília Strongyloidea, medido pelo número de ovos por grama de fezes (OPG) através das duas técnicas. Foram coletadas amostras de fezes, diretamente da ampola retal, de 12 fêmeas, ¾ holandês/zebu, a partir de seis meses de idade, pesando entre 100 a 150 kg, naturalmente parasitadas por nematóides gastrointestinais, na Fazenda Santa Mônica, da Embrapa Gado de Leite, em Valença, RJ, Brasil. As amostras foram coletadas a cada 21 dias, no período de 28 meses. As contagens de OPG foram realizadas no Laboratório de Parasitologia da Embrapa, em Juiz de Fora, MG. A técnica CF detectou 80.81% de amostras positivas, contra 15.17% da técnica GW, demonstrando que a técnica CF é mais sensível que a GW para contagem de OPG, em bovinos. A técnica GW apresentou maior Coeficiente de Variação (CV = 237%) que a CF (CV = 68%). Entretanto, verificou-se concordância significativa entre elas (*rho* = 0,118), confirmando a existência de uma correlação entre as mesmas. Esta confirmação permitiu a elaboração de uma tabela do grau de infecção por helmintos da Superfamília Strongyloidea baseada nas duas técnicas, que auxiliará na determinação do limiar de tratamento. Dessa forma, a ausência de parâmetros na tomada de decisão para tratamento de helmintose em bovinos, a partir do grau de infecção, considerada a grande desvantagem da técnica CF, foi eliminada.

PALAVRAS-CHAVE. Intensidade do parasitismo, limiar de tratamento, OPG, parasitos de bovinos

INTRODUCTION

In Brazil, helminths can cause annual losses in the order of US $ 7.11 billion (Grisi et al. 2014). The intensity of the parasitism is important in the definition of parameters that determine the treatment threshold (ABIDU et al., 2000; Vercruysse et al. 2001). More precise techniques are ideal in defining parameters (Fortes & Molento 2013). Necropsy would be more accurate, but it is naturally limited by economic, operational, and ethical issues.

Gastrintestinal parasitism is monitored predominantly through egg counts per gram of feces (EPG) (Rinaldi et al. 2014). This technique is indicated as a reliable and representative measure of the magnitude of parasitism (Amarante & Amarante 2016). The EPG count can be an important tool in determining the economic treatment threshold, since the number of eggs eliminated in a grazing season can determine the number of infective larvae in the second half of the season (Vercruysse et al. 2001). The Gordon & Whitlock (GW) technique is considered the standard in the EPG count in sheep (Bosco et al. 2014, Kenyon et al. 2016) and has the advantage of presenting a "*Guide for interpretation of Helminths` egg count in ruminants* "(Ueno & Gonçalves 1998), which indicates the degree of helminth infection, and can be used as a parameter in determining the treatment threshold. However, due to the low sensitivity (Fernandes 2005), there are doubts as to the accuracy of the economic threshold of treatment.

Another common technique is Centrifugal Fluctuation (CF), which, according to Ueno & Gonçalves (1998), allows a smaller deformation of the eggs, of helminths, that float. Another advantage of CF is to be more sensitive when compared to GW (Fernandes 2005). This greater precision related to GW could accredit it for the determination of an economic threshold of treatment, but until this date the degree of gastrointestinal helminth infection in cattle was not known, based on EPG, which made the CF technique devoid of economic significance. The present work aimed to verify the degree of correlation between the modified Gordon & Whitlock and Centrifugal Fluctuation techniques in the diagnosis of helminth in cattle, which allowed, after the use of statistical models, a table with the degree of helminth infection of the Strongyloidea Superfamily, which will assist in the determination of the economic threshold for treatment of helminthes in cattle.

MATERIAL AND METHODS

Study area

The work was carried out at the Santa Mônica Experimental Field Station, EMBRAPA Milk Cattle (CESM-EMBRAPA), located in Valença, RJ and in the Laboratory of Parasitology of EMBRAPA Milk Cattle (CNPGL), in Juiz de Fora, MG.

Study data

Twelve females, ¾ of Dutch / zebu blood, six months old and weighing between 100 and 150 kg, naturally parasitized by gastrointestinal nematodes, participated in the experiment. The animals were kept in paddocks with Brachiaria decumbens grass and water at will. Stool samples were collected directly from the rectum, with the aid of individually separated and labeled plastic bags. They were then packaged in isothermal boxes containing ice and immediately sent to the laboratory. Samples were collected every 21 days, in the period of 28 months, totaling 39 collections / animal.

Diagnostic techniques

In the laboratory, each faecal sample was homogenized, weighed and separated to count the number of eggs per gram of faeces (EPG) using the following techniques: Gordon and modified Whitlock (GW) and Centrifugal Fluctuation (CF) (Sloss et al. 1978, Ueno & Gonçalves 1998).

Modified gordon and whitlock technique (gw)

The preparation of the samples for examination through the GW technique was done by placing 2 g of feces in a sieve, adding 58 ml of hypersaturated NaCl solution. After trituration with a glass stick, the fecal suspension was homogenized with the aid of a pipette. A small amount of the suspension was placed in a McMaster Chamber. After a 2 minutes rest, the eggs were counted in the two areas of the Chamber under an ocular microscope, with a 10x objective. The total EPG was calculated by adding the number of eggs found in the two chambers (right and left) and multiplying the sum by 100 (Ueno & Gonçalves 1998).

Centrifugal fluctuation technique (cf)

For the CF technique, the sugar solution was prepared by mixing 500g of sugar, 360 ml of water and 6.5 ml of phenol. The fecal suspension was prepared by placing 2g of feces in a sieve. The sugar solution was added to half the vessel and homogenized with a glass rod. After the removal of the tami, the suspension was centrifuged for five minutes at 1500 rpm and transferred to a test tube until a meniscus formed on the surface of the tube. After this procedure, a coverslip was placed on the tube for 10 minutes at rest, and then it was abruptly removed, deposited on the slide and taken under a microscope to count the eggs of helminths (Sloss et al. 1978).

Comparison between the techniques

The sensitivity degree of the two techniques was evaluated by calculating the percentage of positive and negative samples for gastrointestinal nematode eggs in each of the test methods. Boxplot type graphs (Martins 2014) were constructed with the purpose of comparing the distribution of responses among the techniques. The coefficient of variation (CV) was calculated to measure the variability between the responses of each of them. In order to deal with the discrepant variability, originally demonstrated by the responses, the logarithmic transformation (log (1 + X)) was used. The normality between the data was verified through the test of Shapiro Wilk (Conover 1980). The agreement between the diagnostic techniques was evaluated by the Spearman correlation test (Lehmann & D'Abrera 2006).

In order to verify the functional relationship between the techniques and the cutoff points between the related levels of infection, a statistical regression model was adjusted, based on the limits of the table presented by Ueno & Gonçalves (1998) as "*Guide for interpretation of the helminth egg count in ruminants*", which determines that the mixed infection in cattle is considered moderate when the EPG number is equal to 200 and heavy when the number equals = 700. In order to measure the

agreement between categorized levels of infection of each technique, the kappa concordance index was calculated and tested statistically (Siegel 1988, Fleiss et al. 2003).

RESULTS

Table 1 shows the absolute and relative frequencies of positive (FP) and negative samples for helminth eggs in each of the methods used, demonstrating that the CF technique presented 80.81% of the positive samples, against 15.17% of the GW technique. These results clearly demonstrate that the CF technique is more sensitive than GW technique for the EPG diagnosis in cattle.

The normalization of the data was not possible, even after the use of the logarithmic function (log (1 + X)). Through the "boxplots" (Figure 1) it was possible to observe that the distribution of the EPG responses obtained through the GW technique (CV = 237%) presented greater variability than the EPG results obtained through the CF technique (CV = 68%). However, after the Spearman correlation test and the dispersion diagram (Figure 2), built to verify the relationship between the two techniques, a concordance between them (*rho* = 0.12) was verified, confirming the existence of a correlation between them. Statistical tests showed significance in this relation (*p-value* <0.05).

From the significant agreement between categorized data (kappa = 13.38% [95% CI: 5.21%; 22.55%]), (*p-value* < 0.05) (Table 2) which showed the degree of gastrointestinal helminth infection of the Strongyloidea Superfamily in cattle based on the EPG count through Gordon e Whitlock modified (GW) and Centrifugal Fluctuation (CF), based on the parameters established by Ueno & Gonçalves (1998).

DISCUSSION

The results observed in the present study allow us to infer that the CF technique is more sensitive and more accurate than the GW technique (CVGW > CVCF). Bosco et al. (2014), in a comparative study between FLOTAC techniques (a variation of the GW technique, which uses a larger faecal aliquot (20g)), FECPAK (a variation of the Centrifugal Fluctuation technique, but easier to read by eliminating debris of the eggs counting area) and GW, also found a much smaller number of positive samples with GW technique (100%, 66.7% and 41.7%, respectively), concluding, also, by their lower sensitivity. These authors concluded that any of these methods is suitable for decision-making regarding the treatment of cattle against gastrointestinal nematodes. However, it should be pointed out that, at the time, only GW technique presented a table with the degree of gastrointestinal infection by helminths in cattle.

These results are divergent from those found by Levine et al. (1960), which demonstrated that the GW technique was more sensitive than the CF technique in a comparative study with bovine and ovine faeces for nematode eggs of the Strongyloidea superfamily. Also Rodrigues et al. (1995), comparing the same techniques for counting eggs of gastrointestinal nematodes in horses, found a greater sensitivity in the GW technique.

Although the relationship found between the two techniques in this study is relatively low (*rho* = 0.12, *p-value* <0.05), there is a statistically significant agreement between them, which confirmed the existence of a correlation between them and motivated the search for the degree of this correlation. Taking into account the practicality and cost of performing the two techniques compared in this study, it is possible to verify that the GW technique is simpler and faster for execution, and can even be performed in the field, since the Mc Master Camera is provided, which cost is relatively low. According to Amarante & Amarante (2016), the ideal methodology must be accurate, but it should also be fast and easy to execute by non-specialists.

The greatest limitation of the methods of analysis for monitoring representative flocks is the time and cost of realization (Kenyon et al. 2016). The greater practicality and agility in the execution of the technique diminish its cost and makes possible the periodic monitoring of the infection. According to Fernandes et al. (2005), the GW technique is more adequate for epidemiological research, where there is a large number of samples or when it is desired to obtain faster results, or when there is limited labor available for the execution of the work. However, when the objective is to

verify the resistance of ruminant parasites to anthelmintics, Fortes & Molento (2013) recommended the use of more sensitive techniques for an accurate and early diagnosis.

The results presented in this study clearly demonstrate the higher sensitivity of the CF technique compared to GW technique. These results corroborate the results found by Fernandes et al. (2005), when they compared these techniques for EPG counting in sheep. Because CF is more sensitive but more complex and time-consuming, it is best suited to cases where there are few samples to be examined and a centrifuge is available, enough time and labor to perform all necessary steps , without prejudice to the results or when more precise results are needed. These studies confer a further advantage to the CF technique, since the table showing the degree of gastrointestinal helminth infection of the Strongyloidea Superfamily, produced in this study (Table 2), ends with the great disadvantage that it presented, that is, the lack of parameters for decision making based on the degree of infection. The production of this table places this technique among the real possibilities for use in studies that have as purpose the establishment of an economic threshold of treatment or not treatment.

CONCLUSION

This study allows concluding that there is a significant agreement between the comparative techniques. Each one with its advantages and disadvantages. The GW technique, although less sensitive, is faster, simpler, and cheaper to perform, making it more suitable for epidemiological surveys and large-scale examinations; while the CF technique is more sensitive, but more complex and time-consuming, therefore, more suitable for small sample exams and laboratory routines requiring greater precision, such as resistance testing for anthelmintics, or studies to define the treatment threshold, for example. The table with the degree of helminth infection in cattle, based on the EPG count, built in the present study, allows both techniques to be used in studies that aim to establish an economic treatment threshold of gastrointestinal parasitic diseases in cattle, eliminating a large disadvantage, previously existing in the use of the CF technique.

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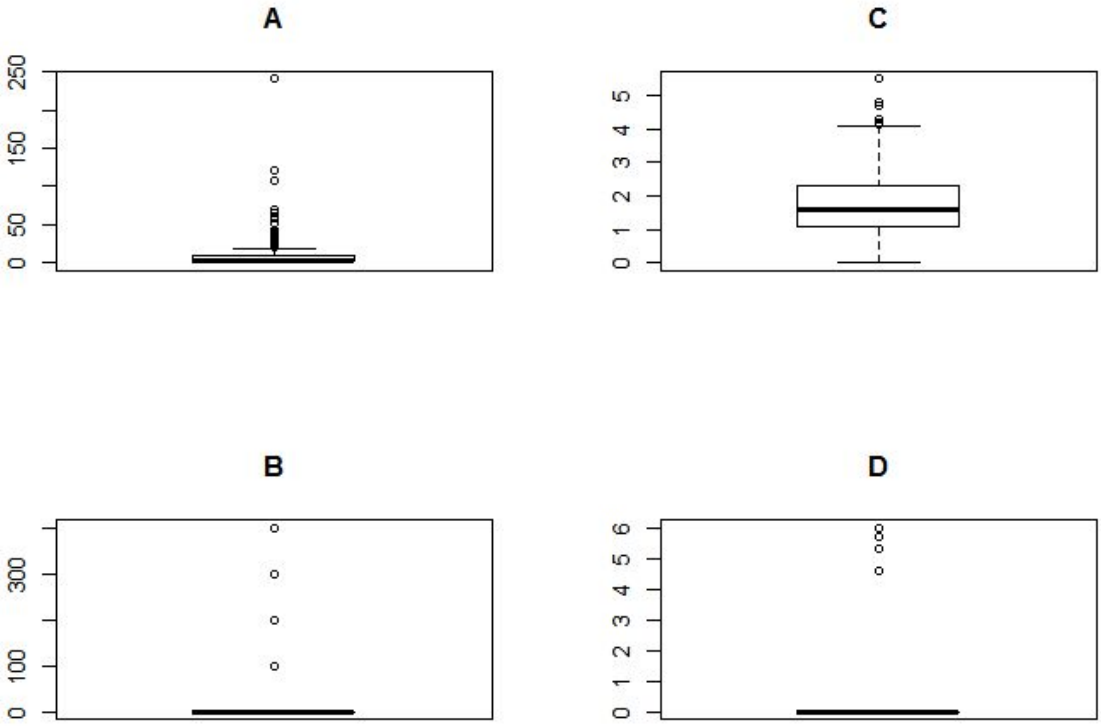

Figure 1 - Distribution of the EPG count obtained through the modified Gordon and Whitlock (GW) and Centrifugal Fluctuation (CF) techniques (UENO & GONÇALVES, 1998), in the original scale (A and B) and logarithmic scale (log(1+x)(C and D) for counting of eggs per gram of feces in cattle (*rho* = 0.118 and p-value <0.05).

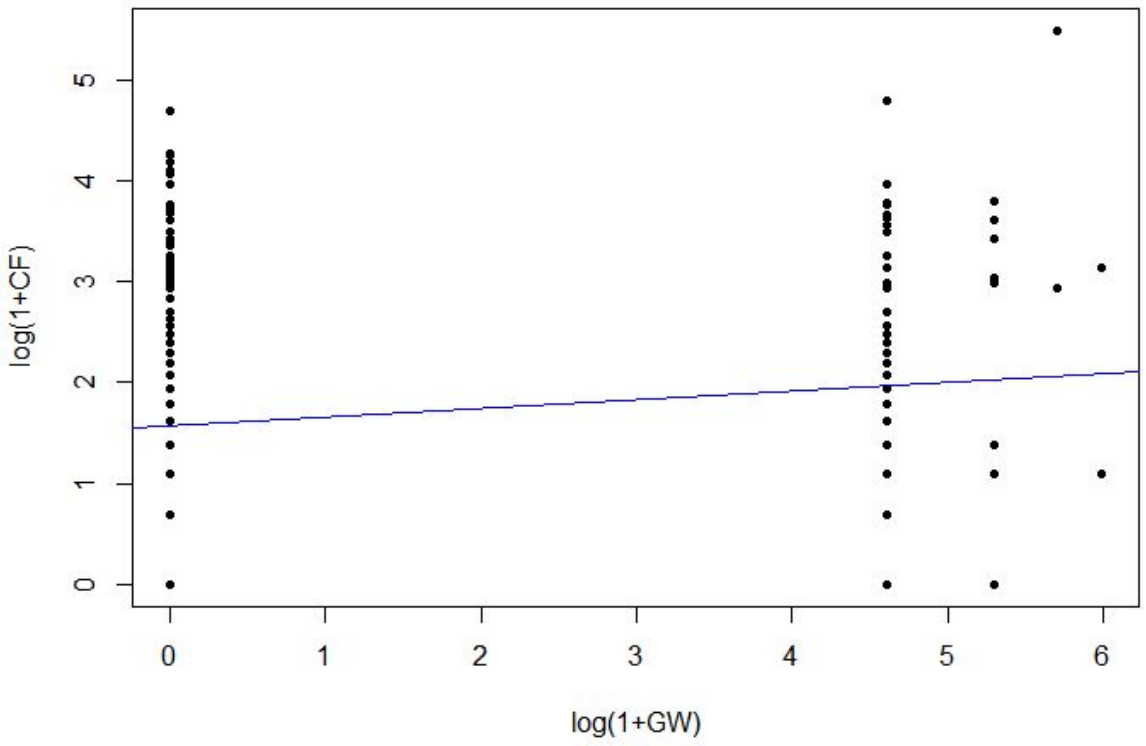

Figure 2 - Relationship between the EPG count obtained through the modified Gordon and Whitlock (GW) and Centrifugal Fluctuation (CF) techniques (UENO & GONÇALVES, 1998) *rho* = 0.118 (p-value <0.05).

Table 1 - Comparison of the frequency of positive faecal samples (FP) for gastrointestinal nematode eggs in cattle through the modified Gordon and Whitlock (GW) and Centrifugal Fluctuation (CF) techniques (UENO & GONÇALVES, 1998).

TECHNIQUE	SAMPLES	FP (%)
Modified Gordon and Whitlock (GW)	435(100%)	15,17 %
Centrifugal Fluctuation (CF)	396(100%)	80,81 %

Table 2 - Degree of helminth infection of the Strongyloidea Superfamily in cattle, established based on the EPG count, through the modified Gordon and Whitlock (GW) and Centrifugal Fluctuation (CF) techniques.

HELMINTHS	TECHNIQUE	DEGREE OF INFECTION		
		mild	moderate	heavy
Superfamily Strongyloidea	Gordon and Whitlock modified (GW)	<200	200-700	>700
	Centrifugal Fluctuation (FC)	<26	26- 71	>71