Modifications of the mini-FLOTAC technique used on cattle and sheep feces to facilitate its use in field routines

Modificações na técnica de mini-FLOTAC para amostras de fezes bovinas e ovinas para facilitar a rotina de campo

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Abstract

The purpose of this study is to compare the Gordon and Whitlock (GW) and mini-FLOTAC (MF) egg counting techniques in order to propose modifications of the latter, which is a current and more sensitive egg counting technique. The idea was to read only one compartment of the MF chamber, and also to propose a model to enable the use of the fill-FLOTAC (FF) device for sheep feces. To this end, strongylid eggs per gram of feces (EPG) were counted using a pool of cattle and sheep feces. The results indicated that there was no significant difference between the proposed methods, except for the use of the FF device for sheep fecal pellets without first weighing the sample or the use of a correction factor of 2.6. Reading only one compartment of the MF device, using the fill-FLOTAC device to perform the test renders the mini-FLOTAC technique more efficient in counting eggs per gram of feces than the Gordon and Whitlock method.

Keywords: Coproparasitology. Diagnosis. FEC. Intestinal parasites.

Resumo

Este estudo teve como objetivo comparar o método de Gordon e Whitlock (GW) com o mini-FLOTAC (MF) para contagem de ovos de helmintos em amostras de fezes, a fim de propor algumas modificações neste último método, que é considerado a técnica mais sensível. Foi proposta a leitura de apenas um dos dois compartimentos da câmara de MF, e também um modelo para ajustar o uso

do dispositivo fill-FLOTAC (FF) com fezes de ovinos. Para isso, realizou-se a contagem de ovos de estrongilídeos em pool *de fezes bovinas e ovinas com cada variação de técnica proposta. Os resultados demonstraram não haver diferença significativa entre os métodos propostos, exceto para o uso do dispositivo FF para os cíbalos fecais de ovinos sem antes realizar a pesagem da amostra ou utilizar um fator de correção de 2.6. A leitura de apenas um compartimento do dispositivo MF, usando o dispositivo fill-FLOTAC para realizar o teste, torna a técnica mini-FLOTAC mais eficiente na contagem de ovos por grama de fezes do que o método de Gordon e Whitlock.*

Palavras-chave: *Coproparasitologia. Diagnóstico. OPG. Parasitas intestinais.*

Introduction

Endoparasitic diseases are known to cause economic losses in ruminant production worldwide. The increasingly frequent reports of anthelmintic resistance in sheep and cattle has led to advances in laboratory diagnostic testing with more accurate and efficient techniques (Holsback et al., 2015; Kenyon et al., 2016; Molento et al., 2016; Salgado e Santos, 2016). Such techniques enable their users to more accurately ascertain the efficacy of drugs in *in vivo* tests, and to identify which individuals in the herd could be selectively treated for worms (Coles et al., 2006; Edith et al., 2018).

The most widely used technique for counting eggs per gram of feces (EPG) is the Gordon and Whitlock (GW) method (Gordon and Whitlock, 1939). However, the way this technique is employed in the routine of different diagnostic laboratories varies significantly. Moreover, the analytical sensitivity of this technique is low, considering that the results of counts are multiplied by 50 (Cringoli et al., 2010).

To improve the accuracy and sensitivity of fecal egg counts, Cringoli et al. (2010) developed the FLOTAC technique. Given that this technique is laborious and requires laboratory facilities, Barda et al. (2013) then devised a modification of this method, which they called the mini-FLOTAC (MF) technique. The technique involves placing feces in a fill-FLOTAC (FF) device, which has a cavity that serves as a measure of volume. This device was developed to dispense with the use of scales and sieves, and to render the test more practical, making it an interesting alternative for use in the field. However, the longer time spent in reading the MF chamber than that required by the GW technique may make it difficult to use in routine testing when large numbers of samples have to be processed.

This study aimed to devise modifications to simplify the strongylid egg counting process without impairing its accuracy, ensuring that its quality is the same as or better than the methods widely used in these routine laboratory tests.

Material and methods

Fresh fecal samples available in the laboratory were used to prepare pools of about 60 g, which were hand mixed for 10 minutes. A total of four pools of cow dung and four pools of sheep fecal pellets were used. Figure 1 illustrates the tests performed on each pool of cow dung, while Figure 2 illustrates those performed on the pools of sheep fecal pellets. The fecal samples of both species used in these tests had the consistency expected for healthy animals. For sheep, only samples with humid but consistent fecal pellets, related to score "zero" of diarrhea described by Rosalinski-Moraes et al. (2012) were used. For cattle, the normal consistency assumed was soft, firm but not hard (Ireland-Perry and Stallings, 1993).

For the GW technique, 2 g of feces diluted in 28 ml of supersaturated NaCl solution (specific gravity = 1.2) were weighed. The feces were diluted, filtered, and an aliquot was placed in a McMaster counting chamber. After 10 minutes, reading was performed in an optical microscope under 100x magnification, counting the Strongylid eggs and then multiplying the result by 50, which corresponded to the EPG.

The mini-FLOTAC (MF) technique was performed according to the manufacturer's instructions for herbivores, which are also described by Castro et al. (2017). The FF device has a cavity into which fecal matter is deposited, with a capacity of approximately 5g, and a container into which 45 ml of supersaturated NaCl solution is added. After depositing the feces and solution in the FF device, the device was closed, the material was homogenized and then deposited in the mini-FLOTAC chamber.

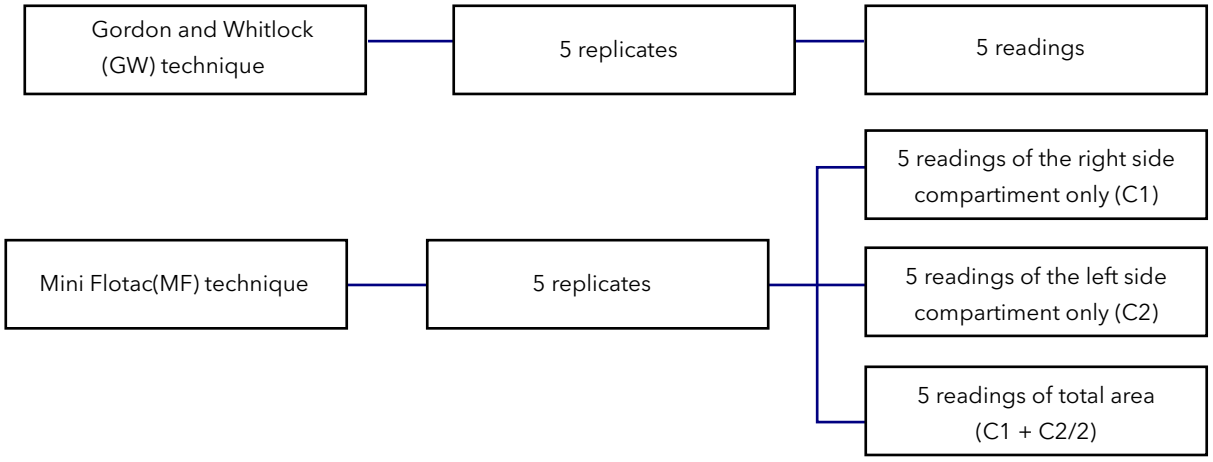

Figure 1 - Explanatory diagram of the tests performed on each of the four pools of cow dung.

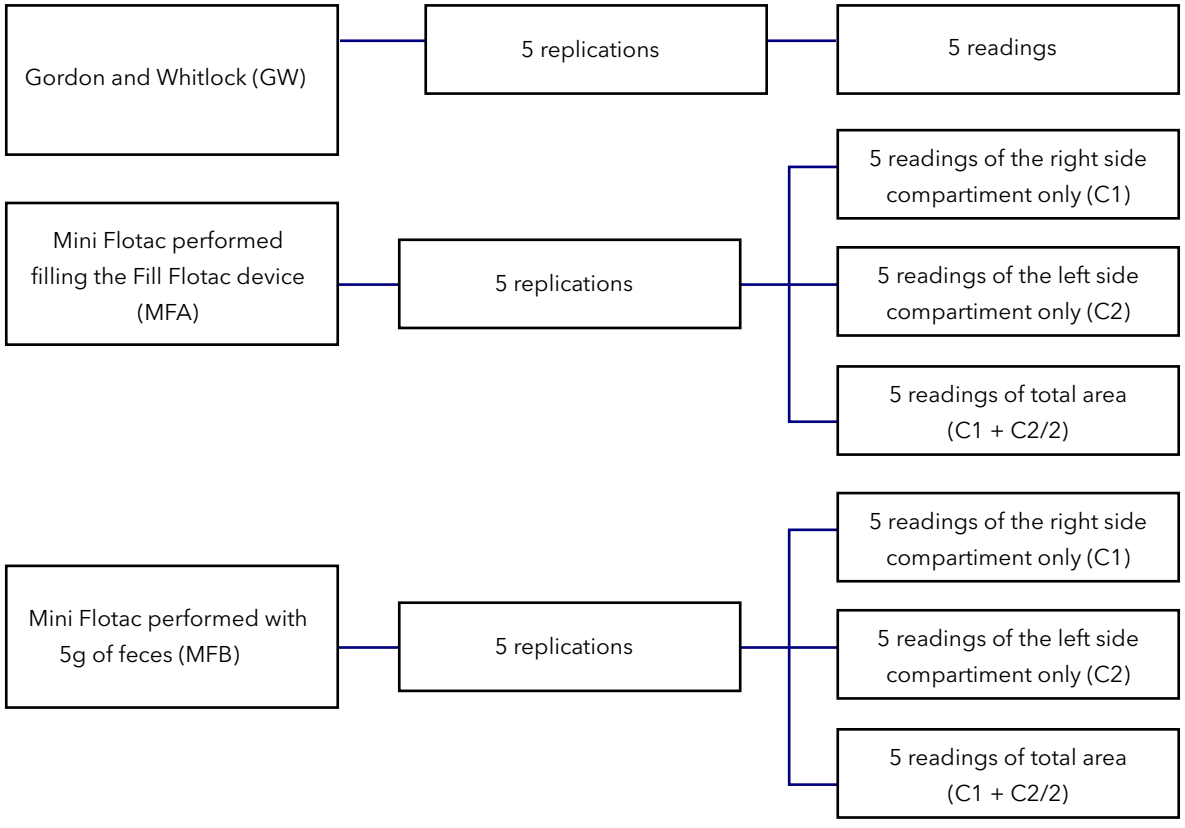

Figure 2 - Explanatory diagram of the tests performed on each of the four pools of sheep fecal pellets.

To evaluate the accuracy of the device using the MF technique on bovine feces, the amount of fecal matter deposited in the cavity was checked to ensure that it weighed 5g. On sheep feces, the MF exam was performed in two ways, called MFa and MFb. In the MFa test, 5g of the fecal sample were weighed on a precision balance and placed in the FF device. In the MFb test, the cavity of the FF device was filled with feces, whose weight was also checked on a precision scale, without corrections.

After a 10 minute wait, reading was performed in an optical microscope under 100x magnification. The counts obtained in compartments 1 and 2 were recorded separately. To determine the EPG value, the counts were multiplied by 10, considering only the strongylid egg counts. To determine the total count according to the manufacturer's instructions, the counts of the two compartments were added and then divided by two. The purpose of this procedure was to evaluate the quality of the methodology when performed in less time, by reading of only one compartment of the MF chamber.

Data were obtained based on total egg count determined by the GW method and by the count of the compartments of the MF chamber. The experiment was statistically analyzed by analysis of variance (ANOVA), using a randomized block design (RBD), and the means were compared using Tukey's test at a 5% level of significance. Each treatment was repeated five times (readings of tests) per block (fecal pool), making a total of 20 results per segment of readings.

Results and discussion

The average results of strongylid egg counts (EPG) in the four bovine fecal pools by the Gordon and Whitlock (GW) method were compared with the results obtained by the three different methodological variations in the reading of the mini-FLOTAC (MF), showing no statistical difference (Table 1). Likewise, no significant difference was found in the mean EPG of the fecal sheep pools as a function of the methodology used for quantification (Tables 2 and 3).

Table 1 - Mean, standard deviation (SD) and range of variation (RV) of eggs per gram of feces (EPG) in four pools of cow dung, determined by the Gordon and Whitlock (GW) method and the mini-FLOTAC (MF) method: counting only compartment 1 (C1) and only compartment 2 (C2), and average count of the two compartments (T)

	GW			MF-C1			MF-C2			MF-T		
	Mean	SD	RV	Mean	SD	RV	Mean	SD	RV	Mean	SD	RV
Pool1	160	129	250	110	56	130	96	17	40	103	28	75
Pool2	1340	338	850	1480	90	220	1552	78	190	1516	79	205
Pool3	600	262	700	800	110	240	800	96	230	800	87	230
Pool4	890	343	950	794	63	160	886	87	240	840	52	130
Total	2990	1072	2750	3184	319	750	3334	278	700	3259	247	640
Mean*	747[a]	268	687	796[a]	80	187	833[a]	70	175	814[a]	62	160

Note: *Results with different letters are significantly different by Tukey's test ($p < 0.05$).

Table 2 - Mean, standard deviation (SD) and range of variation (RV) of strongylid eggs per gram of feces (EPG) in four pools of sheep fecal pellets, determined by the Gordon and Whitlock (GW) and mini-FLOTAC (MF) methods, using 5 g of feces (MFa): counting only compartment 1 (C1) and only compartment 2 (C2), and average count of the two compartments (T)

	GW			MFa-C1			MFa-C2			MFa-T		
	Mean	SD	RV	Mean	SD	RV	Mean	SD	RV	Mean	SD	RV
Pool1	670	189	450	708	127	330	683	165	420	695,5	139	245
Pool2	240	108	250	428	76	190	416	83	210	422	75	200
Pool3	1180	404	950	774	88	260	748	78	180	761	85	195
Pool4	1820	732	1850	1298	128	290	1278	152	360	1288	138	215
Total	3910	1433	3500	3208	419	1070	3125	478	1170	3166,5	437	855
Mean*	977[a]	358	875	802[a]	104	267	781[a]	119	292	791[a]	109	213

Note: *Results with different letters are significantly different by Tukey's test ($p < 0.05$).

Table3 - Mean, standard deviation (SD) and range of variation (RV) of strongylid eggs per gram of feces (EPG) in four pools of sheep fecal pellets, determined by the Gordon and Whitlock (GW) and mini-FLOTAC (MF) methods, using the fill-FLOTAC (FF) feces collector and employing the correction factor of 2.6 to obtain the final result (MFb): counting only compartment 1 (C1) and only compartment 2 (C2), and average count of the two compartments (T)

	GW			MFb-C1			MFb-C2			MFb-T		
	Mean	SD	RV	Mean	SD	RV	Mean	SD	RV	Mean	SD	RV
Pool1	670	189	450	811	210	468	821	429	702	816,4	239	484
Pool2	240	108	250	540	109	286	514	85,8	208	527,8	93	131
Pool3	1180	404	950	97	533	1248	1014	351	806	995,8	426	934
Pool4	1820	732	1850	1383	241	572	1450	335,4	910	1417	278	620
Total	3910	1433	3500	3712	1094	257	3801	1201	2626	3757	1037	2169
Mean*	977[a]	358	875	928[a]	273	643	950[a]	300	656	939[a]	259	542

Note: *Results with different letters are significantly different by Tukey's test ($p < 0.05$).

The equivalence between the mean EPG counts indicates that the use of the GW method is feasible, with the advantage of speedy sample processing due to fast reading, since the chamber has a smaller counting area. Alowanou et al. (2021) spent 15 min average to prepare and count three replicates of sheep feces of 202.01 ± 99.25 epg. For performing MF technique, the average time spent was 30 minutes.

However, the results obtained with GW technique varied widely and showed large standard deviations. Several studies reported that the GW technique produced significantly larger standard deviations than the MF in different laboratories (Castro et al., 2017; Noel et al., 2017; Alowanou et al., 2021). Both Castro et al. (2017) and Noel et al. (2017) suggested that this difference in the range of variation is probably due to the fact that the multiplication factor applied to the EPG by the GW techniques is 5 to 10 times higher than that employed in the MF. This difference in the range of variation may mean that the MF is more sensitive and accurate than the GW, but reading takes longer because the MF counting chamber is larger.

Although only one compartment of the MF chamber was read, and the multiplication factor was increased from 5 to 10 to determine the EPG, the standard deviation of this technique remained lower than that of the GW (Tables 1, 2 and 3) and also enabled faster processing. Therefore, when multiple samples need to be processed rapidly in the field, the MF technique with this modification may be more advantageous and accurate than the GW method.

A single measurement per individual is normally used when selective treatment based on egg counts or even *in vivo* EPG reduction testing is required. Thus, it is important to employ techniques whose results do not show wide variations, providing counts closer to the real EPG count. According to Castro et al. (2017), in order to avoid false negatives, this is even more important in the case of herbivores that shed few helminth eggs in their feces.

It was found that the sheep fecal mass deposited in the collector of the fill-FLOTAC (FF) device was lower than expected. The mass of the 20 fecal samples collected in the device (FF) using the MF methodology showed an average weight of approximately 1.97 g and a standard deviation of 0.21 g. As can be seen in Table 4, the result of the counts by this MF technique showed statistically significant differences compared to the GW method and to the other variations of the mini-FLOTAC technique. In the search for a solution that would allow for the use of the device, a correction factor of 2.6 was proposed. This factor was determined based on the average of the divisions of the expected fecal mass (5 g) by the mass obtained at each weighing of fecal matter, thus constituting the MFb technique. The implementation of the technique resulted in the equivalence of the averages of EPG counts (Tables 2 and 3). However, the fact that this technique does not involve the use of a correction factor prevented it from being compared with the other techniques, since it presented a significant difference (Table 4).

Table 4 - Mean EPG in four pools of sheep fecal pellets, determined by the Gordon and Whitlock (GW) and mini-FLOTAC (MF) methods, using the fill-FLOTAC (FF) feces collector, the precision balance (MFa), and the correction factor of 2.6 to obtain the final result (MFb): counting only compartment 1 (C1) and only compartment 2 (C2), and average count of the two compartments (T)

	GW	MF			MFa			MFb		
		C1	C2	T	C1	C2	T	C1	C2	T
Mean (EPG)*	977[a]	357[b]	365[b]	361[b]	802[a]	781[a]	791[a]	928[a]	950[a]	939[a]

Note: *Results with different letters are significantly different by Tukey's test ($p < 0.05$).

To use the MF technique, Rinaldi et al. (2014) and Alowanou et al. (2021) weighed sheep feces, since it is very difficult to collect this amount of feces in the form of pellets using the FF collector. However, the proposed modification for filling the device and using the correction factor for counting not only dispenses with the use of scales but also makes the technique safer and simpler to perform in the field.

When only one compartment of the MF device was counted, the procedure became even faster while still presenting a smaller range of variation and lower standard deviation than the GW technique (Tables 2 and 3). Thus, MF is a technique that can be used for fast evaluations of the strongylid parasitic load in sheep herds in the field, provided the correction factor is used.

All fecal pools that were sampled for this study ranged from 100 to 1800 Strongylid eggs per gram, and no other parasitological findings were considered. Results obtained by Amadesi et al. (2020) showed a sensitivity of 100% for both GW and MF techniques in samples with 100 epg or more. Below 100 epg, the sensitivity of GW ranged from zero to 66%. This can impact diagnose of low infection intensities or eggs and oocysts that are usually excreted in low output. Alowanou et al. (2021) found significant higher prevalence of *Strongyloides* sp., *Nematodirus* spp., *Marshallagia* sp. in sheep samples analyzed by MF technique than those analyzed by GW. These findings are important to show that the adaptions proposed in the current work are limited to quantify Strongylid eggs in cattle or sheep to determine the EPG cut-off value for anthelmintic treatment or to access treatment efficacy in fecal egg counts reduction testing.

Conclusion

Reading only one compartment of the MF device using the fill-FLOTAC device to perform the test renders the mini-FLOTAC technique more efficient in counting eggs per gram of feces than the Gordon and Whitlock method. The correction factor of 2.6 must be used when performing the mini-FLOTAC using the FF device to process sheep fecal pellets.

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