RELATIONSHIP BETWEEN FAECAL EGG COUNTS AND TOTAL WORM COUNTS IN SHEEP INFECTED WITH GASTROINTESTINAL NEMATODES

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SUMMARY: In a first experiment, faecal samples were taken from 19 lambs slaughtered for worm counting. Faecal egg counts (FEC) and cultures for the production of infective larvae were carried out on each of these fecal samples. In a second experiment, 46 lambs, randomly allocated in four groups, were slaughtered with the same purpose. However, in this last experiment only one composite culture was set up for each group of lambs. Based on FEC and larvae identification, it was estimated the FEC for each nematode genus. In the first experiment, correlation coefficients between FEC x total worm counting (TWC) and between *Haemonchus contortus* data (FEC x worm counting) were $r = 0.46$ and $r = 0.54$, respectively. In both cases, a considerable increase in the coefficients occurred when data were log transformed ($r = 0.91$). The correlation coefficient for *Trichostrongylus colubriformis* was close to zero using data not transformed, but with the transformation became higher ($r = 0.52$). Different results were obtained with *Cooperia* spp.: the correlation coefficient was 0.88 with data not transformed and became lower when data were transformed ($r = 0.67$). In the second experiment, the correlation coefficients ranged from 0.37 (group 2) to 0.79 (group 3) for FEC x TWB (not transformed data). Log transformation produced higher coefficients, except in one group. Correlation coefficients with *H. contortus* data (not transformed) ranged from 0.40 to 0.78. In all groups, transformation of *H. contortus* data caused a small reduction in the coefficients. The highest correlation coefficient for *T. colubriformis* was obtained with data not transformed in group 1 ($r = 0.73$). In the other groups, they were lower and not significant statistically ($p > 0.05$) not mattering if the data were transformed or not. As preparing faecal cultures and identifying larvae are time consuming procedures, it is quite common in laboratory routine to make a composite culture mixing fecal samples of several animals. The present results show that this procedure causes a reduction in the sensibility of the fecal examination to predict the number of nematodes of each genus in a group of animals. In experiments requiring high precision, the cultures should be made separately for each animal.

KEY WORDS: nematode, sheep, infective larvae, composite culture, *Haemonchus*, *Trichostrongylus*, *Cooperia*.

INTRODUCTION

Faecal examination of sheep, which consist of faecal egg counts (FEC) and identification of infective larvae of nematodes produced in cultures, has been extensively used as parameters for the control of sheep nematodes. Based on fecal examination, it is possible to predict the nematode burden in a flock (UENO & GONÇALVES, 1994). With these results available, the best strategy of control can be recommended, as for example, if it is convenient or not to treat a flock with anthelmintic. It is also possible to choose the most appropriate anthelmintic for each case and checking its efficacy. Faecal examination has been used in surveys of anthelmintic resistance in sheep flocks (AMARANTE *et alii*,1992; ECHEVARRIA *et alii*, 1996) and also to select flocks of sheep with resistance against nematode infections (BISSET *et alii*, 1996)

Some limitations of the fecal egg counts were pointed out by DOUCH *et alii* (1996). According to these authors FEC is influenced by factors such as the level of larval challenge, the species composition, worm burden, and the degree to which worm establishment and adult fecundity is affected by the sheep's immune response. Other influences include: dietary factors, including quality of pasture especially protein levels, pasture species composition, and selective grazing habits. The variability of egg distribution within the fecal sample, the diurnal patterns of egg laying, food transit times and faecal throughout and consistency also affect the measured FEC.

However, a strong relationship has been found between egg counts and worm counts in sheep naturally (ROBERTS & SWAN, 1981) or artificially (AMARANTE *et alii*, 1999a) infected with *Haemonchus contortus*. Correlation coefficients between pre-slaughter FEC and total trichostrongyle burdens in lambs, following periods of natural challenge, proved also to be high (BISSET *et alii*, 1996).

This study was conduct to evaluate the relationship between faecal examination (faecal egg counts and identification of infective larvae produced in cultures) and post-mortem results in sheep infected with gastrointestinal nematodes.

MATERIALS AND METHODS

Experiment I: The animals and paddock used in this study were previously described by AMARANTE *et alii* (1998). Briefly, the animals were Corriedale tracer lambs, five to ten months old. They were placed in a paddock for 14 days and then housed for 28 days. This paddock had been grazed by ewes. The animals were fed with a ration free of contamination by infective nematode larvae while housed. At the end of this period, all animals were slaughtered for worm identification and counting.

Experiment II: Four groups of Corriedale lambs, nine to ten months old, were studied in this trial. The animals were housed just after weaning where they stayed until the end of the experiment. When the experiment started, the animals were harboring natural infection by gastrointestinal nematodes (mean FEC = 1670; 86% of *Trichostrongylus* and 14% of *Haemonchus* larvae in culture). In addition to this natural infection, the animals were artificially infected orally with 5000 *H. contortus* infective larvae. This *H. contortus* strain was resistant to oxfendazole, levamisole and ivermectin (AMARANTE *et alii*, 1992). The groups of lambs were treated with different anthelmintics 28 days after the artificial infection. Seven days after treatment, all animals were slaughtered and faecal examination, worm counting and larvae identification were carried out.

Worm identification and counting: Necropsy, worm identification and counting procedures were performed as described by (UENO & GONÇALVES, 1994).

Faecal examination: A faecal sample was taken from each animal slaughtered. FEC were carried out on samples by a modified McMaster technique (GORDON & WHITLOCK, 1939), where each egg counted represented 100 eggs/g. Cultures for the production of infective larvae of gastrointestinal nematodes (ROBERTS & O'SULLIVAN, 1950) were performed separately for each lamb in experiment I. In experiment II, one composite culture was made for each of the four groups of lambs. One hundred larvae were identified per culture according to descriptions of KEITH (1953).

Statistical analysis: Pearson's correlation and linear regression analysis of FEC and total worm counting (TWC) were performed using the Minitab Version 11 software. Based on FEC and larvae identification, it was estimated the FEC for each nematode genus. Then, the analysis was also performed with the FEC of each nematode genus and its respective number of worms. The data was analyzed with or without logarithmic transformation. FEC was transformed on log (x + 1.5) and worm counting on log (x).

Table 1 - Faecal egg counts (FEC), identification of infective larvae produced in cultures and worm counting in 19 lambs of experiment I.

Animal	FEC	Culture			Worm counting		
		Hc	Tc	Coop	Hc	Tc	Coop
1	0	100%	0%	0%	2	0	0
2	300	98%	2%	0%	297	30	0
3	400	98%	2%	0%	473	81	40
4	500	76%	24%	0%	386	425	0
5	600	41%	44%	15%	86	172	25
6	1500	100%	0%	0%	1041	33	0
7	2000	100%	0%	0%	174	1	0
8	2400	95%	5%	0%	3664	2317	20
9	3000	83%	1%	16%	268	99	336
10	3900	100%	0%	0%	1880	31	0
11	4100	100%	0%	0%	3422	30	0
12	7700	98%	2%	0%	2572	151	0
13	7800	100%	0%	0%	5212	377	0
14	8200	70%	0%	30%	937	58	748
15	9300	100%	0%	0%	1775	112	10
16	10200	99%	1%	0%	2136	1194	0
17	13700	89%	8%	3%	1689	147	21
18	15000	93%	2%	5%	2626	313	35
19	15200	97%	0%	3%	2591	381	326

Hc = *H. contortus*; Tc = *T. colubriformis*; Coop = *Cooperia* spp.

Table 2 - Pearson's correlation (*r*) and linear regression analysis of faecal egg counts (FEC) and number of nematodes in lambs of experiment I.

Data analyzed	n	Log transformed	r	Regression equation**	s	Level of significance
FEC x total worm counting	19	no	0.46	Y = 2755 + 1.38X	4738	P < 0.05
		yes	0.91	Y = 0.08 + 1.08X	0.41	P < 0.001
FEC* x *H. contortus* counting	19	no	0.54	Y = 2208 + 1,83X	4261	P < 0.05
		yes	0.91	Y = 0,17 + 1,08X	0.41	P < 0.001
FEC* x *T. colubriformis* counting	18	no	0.02	Y = 119 + 0.01X	269	P > 0.05
		yes	0.52	Y = -0.32 + 0.72X	0.92	P < 0.05
FEC* x *Cooperia* spp. counting	9	no	0.88	Y = 49 + 2.70X	398	P < 0.001
		yes	0.67	Y = -0.48 + 1.31X	1.05	P = 0.05

* FEC = total FEC x percentage of each genera identified in faecal culture/100.
** In the equation: X = FEC and Y = worm counting
s = standard deviation

Table 3 - Faecal egg counts (FEC), identification of infective larvae produced in cultures and worm counting in four groups of lambs of experiment II.

Group	Number of animals	FEC*	Composite Culture		Worm counting*	
			H. contortus	*T. colubriformis*	*H. contortus*	*T. colubriformis*
1	9	7222 (1500-17900)	81%	19%	2906 (170-7710)	1213 (530-3480)
2	9	5933 (2400-13000)	69%	31%	1684 (990-3340)	1026 (440-2200)
3	9	9667 (200-21800)	94%	6%	1870 (550-3320)	90 (0-600)
4	10	11267 (1200-23700)	94%	6%	3381 (970-6100)	1107 (50-2990)

*Arithmetic means followed by minimum and maximum values between brackets.

RESULTS

Table 1 shows the results of the faecal examination (FEC and larvae identification) and worm counting in lambs of experiment I. *H. contortus* was the most abundant nematode found followed in decreasing order by *T. colubriformis* and *Cooperia* spp. Egg counting ranged from 0 to 15200 FEC.

In experiment I, correlation coefficients between FEC x TWC and between *H. contortus* data (FEC x worm counting) were $r = 0.46$ and $r = 0.54$, respectively (Table 2). In both cases, a considerable increase in the coefficients occurred when data were log transformed ($r = 0.91$). The correlation coefficient of *T. colubriformis* was close to zero ($r = 0.02$) using data not transformed, but with the transformation became higher ($r = 0.52$). Different results were obtained with *Cooperia* data. The correlation coefficient was 0.88 with data not transformed and became lower when data were transformed ($r = 0.67$).

Lambs in experiment II were infected with *H. contortus* and *T. colubriformis* (Table 3). The highest mean FEC (11267 FEC) was found in group 4 corresponding to the highest mean TWC (4488 nematodes). The lowest mean FEC (5933 FEC) corresponded to a mean of 2710 nematodes (the third highest TWC).

T. colubriformis was not found in two animals of group 3. For this reason, the analysis for *T. colubriformis* was performed only with data from seven animals in group 3 (Table 4). In general, lower correlation coefficients were obtained with data of experiment II when compared with experiment I, and most of them were not statistically significant ($p > 0.05$).

The correlation coefficients ranged from 0.37 (group 2) to 0.79 (group 3) for FEC x TWB (not transformed data). Log transformation produced higher coefficients, except in group 3. Correlation coefficients of *H. contortus* data (not transformed) ranged from 0.40 to 0.78. In all groups, transformation of *H. contortus* data caused a small reduction in the coefficients. The highest correlation coefficient for *T. colubriformis* was obtained with data not transformed in group 1 ($r = 0.73$). In the other groups, they were lower and not significant statistically ($p > 0.05$) not mattering if the data were transformed or not.

DISCUSSION

As the egg production of a *Haemonchus* female is estimated to be 25 times higher than the egg production of a *Trichostrongylus* or of a *Cooperia* female (UENO & GONÇALVES, 1994), the FEC alone do not reflect the TWC in a mix infection. This parameter has to be analyzed in relation to the percentage of larvae found in culture. For instance, in group 4 (Table 3, experiment II), 94% of the infective larvae were *H. contortus* and 6% was *T. colubriformis*, but these two species represented, respectively, 75.3% and 24.7% of the TWC. In animal 8 (Table 1, experiment I), there was 5% of *Trichostrongylus* larvae in faecal culture, but in reality 39% of the nematodes of the gastrointestinal tract were *Trichostrongylus*.

In experiment I, in which results of FEC and cultures were available for each animal, a high correlation coefficient was

Table 4 - Pearson's correlation (*r*) and linear regression analysis of faecal egg counts (FEC) and number of nematodes in four groups of lambs of experiment II.

Group	Data Analyzed	n	Log transformed	r	Regression Equation**	s	Level of significance
1	FEC x TWC	9	no	0.53	Y = 3846 + 0.82X	4587	P > 0.05
			yes	0.31	Y = 2.74 + 0.29X	0.34	P > 0.05
	FEC* x Hc	9	no	0.40	Y = 4044 + 0.62X	4002	P > 0.05
			yes	0.21	Y = 3.23 + 0.14X	0.34	P > 0.05
	FEC* x Tc	9	no	0.73	Y = 404 + 0.80X	695	P < 0.05
			yes	0.42	Y = 1.25 + 0.59X	0.31	P > 0.05
2	FEC x TWC	9	no	0.37	Y = 2747 + 1.18X	3640	P > 0.05
			yes	0.44	Y = 1.66 + 0.60X	0.23	P > 0.05
	FEC* x Hc	9	no	0.68	Y = 519 + 2.12X	1979	P < 0.05
			yes	0.65	Y = 0.84 + 0.85X	0.20	P > 0.05
	FEC* x Tc	9	no	-0.21	Y = 2253 - 0.40X	1188	P > 0.05
			yes	-0.13	Y = 3.62 - 0.14X	0.26	P > 0.05
3	FEC x TWC	9	no	0.79	Y = -1053 + 5.47X	4599	P < 0.05
			yes	0.73	Y = -1.68 + 1.69X	0.45	P < 0.05
	FEC* x Hc	9	no	0.78	Y = -1495 + 5.66X	4341	P < 0.05
			yes	0.72	Y = -1.77 + 1.72X	0.46	P < 0.05
	FEC* x Tc	7	no	0.39	Y = 504 + 0.84X	410	P > 0.05
			yes	0.61	Y = 1.39 + 0.69X	0.60	P > 0.05
4	FEC x TWC	10	no	0.68	Y = -1356 + 2.85X	6373	P < 0.05
			yes	0.71	Y = -0.82 + 1.31X	0.34	P < 0.05
	FEC* x Hc	10	no	0.75	Y = -1126 + 3.47X	5319	P < 0.05
			yes	0.71	Y = -0.32 + 1.21X	0.34	P < 0.05
	FEC* x Tc	10	no	0.10	Y = 644 + 0.07X	606	P > 0.05
			yes	0.28	Y = 2.02 + 0.24X	0.46	P > 0.05

* FEC = total FEC x percentage of each genera identified in fecal culture/100
TWC = total worm counting; Hc = *H. contortus* counting; Tc = *T. colubriformis* counting
** In the equation: X = FEC and Y = worm counting.
s = standard deviation
Fecal examination for estimating worm burdens in sheep.

recorded between FEC x TWC and between *H. contortus* data ($r = 0.91$, data log transformed). BISSET *et alii* (1996) obtained similar result in lambs naturally infected by gastrointestinal nematodes (Spearman's rank correlation coefficients of 0.91 and 0.81, respectively, for two years studied). A strong relationship was also present between egg counts and worm counts of *H. contortus* ($r = 0.83$; data log transformed) in Merino sheep from flocks where outbreaks of haemonchosis had occurred (ROBERTS & SWAN, 1981). AMARANTE *et alii* (1999a) obtained a high correlation coefficient ($r = 0.70$, data not transformed) between FEC x worm counting in lambs artificially infected with *H. contortus* and LE JAMBRE *et alii* (1971) also found a high correlation coefficient between the egg counts and the total number of female of *H. contortus* ($r = 0.75$; data square root transformed).

In experiment II, in which results of FEC were available for each animal, but the results of larvae identification were from composite cultures (one culture per group of lambs), the correlation coefficients were usually lower than that found in experiment I and most of them were not statistically significant ($p > 0.05$).

The correlation coefficients were generally low for *Trichostrongylus* and *Cooperia* data. The high *Haemonchus* burden associated with the high egg production of this nematode can have concealed the burdens of those genera in the fecal examinations. However, experiments with pure infection will have to be carried out to study the relationship between egg counts and worm counts of *Trichostrongylus* and *Cooperia*.

The level of resistance of sheep against nematode infections may also have influence on the composition of nematode burden in a sheep flock. AMARANTE *et alii* (1999b) found higher proportions of *Haemonchus* larvae in cultures made with faecal samples of ewes with high FEC than in samples of animal with low FEC. Similarly, AMARANTE *et alii,* (1999c) found in two breeds of ewes, grazing as a single flock, much higher proportions of *Haemonchus* larvae in Rambouillet sheep (susceptible breed) than in Florida Native sheep (resistant breed).

As preparing faecal cultures and identifying larvae are time consuming procedures, it is quite common in laboratory routine to make a composite culture mixing faecal samples of several animals. The present results show that this procedure causes a reduction in the sensibility of the faecal examination to predict

the number of nematodes of each genus in a group of animals. However, if the goal is just to monitor the parasitological status of a flock, they seem adequate because they at least indicate the degree of infection (FEC) and the nematode genera present (larvae identification). In experiments requiring a higher precision, the cultures should be made separately for each animal.

SUMÁRIO

Em um primeiro experimento, amostras fecais foram colhidas de 19 cordeiros abatidos para a realização da contagem de nematódeos no trato gastrintestinal. Contagens de ovos por grama de fezes (OPG) e coproculturas para a produção de larvas infectantes foram realizadas para cada uma das amostras. Em um segundo experimento, 46 cordeiros, aleatoriamente distribuídos em quatro grupos, foram abatidos com a mesma finalidade. Neste último experimento os procedimentos foram similares aos do primeiro com diferença nas coproculturas que, neste caso, foram realizadas a partir da mistura de amostras fecais dos animais de cada grupo de cordeiros. Com base nas contagens de OPG e na identificação de larvas infectantes, foi estimada a contagem de OPG de cada gênero de nematódeo. No primeiro experimento, o coeficiente de correlação entre OPG x contagens totais de nematódeos no trato gastrintestinal (CTN) foi de $r = 0,46$ e entre os dados de *Haemonchus contortus* (OPG x número de *Haemonchus*) foi de $r = 0,54$. Em ambos os casos, um aumento considerável no valor dos coeficientes ocorreu quando os dados foram submetidos a transformação logarítmica ($r = 0.91$). O coeficiente de correlação de *Trichostrongylus colubriformis* foi próximo a zero usando dados não transformados, porém com dados transformados tornaram-se mais elevados ($r = 0.52$). Com os dados de *Cooperia* os resultados foram diferentes: o coeficiente de correlação foi de 0,88 com os dados não transformados e tornou-se mais baixo quando os dados foram transformados ($r = 0.67$). No experimento II, os coeficientes de correlação variaram de 0,37 (grupo 2) a 0,79 (grupo 3) para OPG x CTN (dados não transformados). A transformação logarítmica propiciou elevação no valor dos coeficientes, com exceção de um dos grupos. Os coeficientes de correlação dos dados de *H. contortus* (não transformados) variaram de 0,40 a 0,78. Em todos os grupos, a transformação dos dados de *H. contortus* causou uma pequena redução dos coeficientes. O coeficiente de correlação de *T. colubriformis* mais elevado foi obtido com dados não transformados no grupo 1 ($r = 0.73$). Nos outros grupos, eles foram mais baixos e não foram estatisticamente significativos ($p > 0.05$) não importando se os dados eram ou não transformados. O procedimento de realizar coproculturas a partir da mistura de amostras fecais, como no segundo experimento, é bastante utilizado em laboratórios de parasitologia, pois esta técnica, bem como a identificação de larvas, são procedimentos laboriosos. Os resultados do presente trabalho demonstraram que este procedimento causa redução na sensibilidade dos exames de fezes para estimar o número de nematódeos de cada gênero em um grupo de animais. Em experimentos que requeiram uma maior precisão, as coproculturas devem ser realizadas separadamente para cada animal.

PALAVRAS-CHAVE: nematódeo, ovinos, larva infectante, coprocultura, *Haemonchus*, *Trichostrongylus*, *Cooperia*.

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