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OVERBERG RESEARCH PROJECTS. X. FAECAL EGG COUNTS IN THE INTERPRETATION OF NEMATODE WORM BURDENS IN SHEEP

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ABSTRACT

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Worm egg counts were compared with nematode worm burdens from data collected from >400 sheep killed in experiments on the epidemiology of nematode parasites in the Overberg, in the winter rainfall area of the southern Cape Province. Data were analysed in several ways but no method could be found to accurately estimate the number of nematodes present from the faecal egg count in respect of individual sheep. However, the mean natural log egg count (epg) can roughly predict the mean natural log nematode count in groups of sheep.

INTRODUCTION

The most widely used method of diagnosing nematode parasites in the live sheep is the faecal worm egg count. Gordon (1948, 1958) based his classical studies on the epidemiology of nematode parasites in summer and winter rainfall areas in Australia on this technique. Gordon (1981) states that a high egg count indicates the presence of numerous adult worms and a low count only a few worms but he expresses some difficulty in the interpretation of medium egg counts. More recently, the tests for detecting resistance by nematode parasites to anthelmintics described by Presidente (1985) resulted in the development of the faecal egg count reduction test (FECRT) which has been perfected by Anderson (1989), Martin (1988) and others.

A more accurate method is to slaughter sheep, recover, count and identify nematode larvae and adults microscopically but this is an expensive, laborious and time-consuming method.

We have been studying the epidemiology of the common nematodes of sheep on improved dry-land pastures at Boontjieskraal (Fig. 1: 3) in the winter rainfall region of the southern Cape Province (Reinecke & Louw, 1989; Louw, 1989a; Louw & Reinecke, 1990).

Reinecke & Louw (unpublished observations, 1988) carried out similar studies during 1987 and 1988 with suckling lambs and hoggets on spray-irrigated grass legume pastures at Elandskloof and Tygerhoek, 30 an 70 km from Boontjieskraal respectively (Fig. 1: 5 & 6).

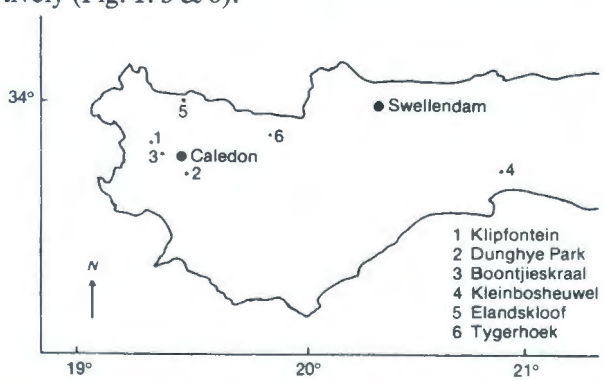

FIG. 1. The Overberg, situated in the winter rainfall area of the southern Cape Province. 3. Boontjieskraal; 5. Elandskloof; and 6. Tygerhoek; the 3 farms referred to in the text (from Louw, 1989b)

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Faecal egg counts were compared with worm counts post mortem, the data analysed by various statistical methods and the findings are presented in this paper.

MATERIALS AND METHODS

Parasites

In our studies on the epidemiology of nematode parasites 2 parallel flocks of control and treated sheep grazed adjacent pastures. The controls were only treated to prevent mortalities and the treated flock was dosed with anthelmintics as prescribed by Dr Herbst, General Practitioner, Caledon.

Six sheep per group were slaughtered every 6 weeks, for periods of 12–18 months. All ingesta and digests from the abomasum, as well as the small intestinal ingesta, were washed on sieves with 38 µm apertures and the washings fixed and preserved in formalin. Total and differential larval and adult nematode counts were carried out microscopically (Reinecke & Louw, 1989).

Faecal egg counts

Faeces were collected from the rectum of each sheep at necropsy and egg counts (epg) done, using a modification of the McMaster technique (Reinecke, 1983).

Analysis of the data

A new approach was used for the interpretation of faecal egg counts (McKenna, 1987). Correlation coefficients (Steel & Torrie, 1960); regression coefficients and intercepts; the mean egg and mean worm counts for each group of 6 sheep that were slaughtered (excluding any incomplete groups) were calculted. The same correlation and regression analysis were also carried out on the means. McKenna's (1987) method was also applied. Two-way tables were constructed and interpreted.

RESULTS

Data

Reinecke & Louw (1989), Louw (1989a), Louw & Reinecke (1990) and Reinecke & Louw (unpublished observations, 1989) included all 3rd stage larvae (L_3), 4th stage larvae (L_4) and adults of all genera in the nematode worm counts post mortem. From July–October the proportions of L_3 and L_4 of the total worm burdens were:

Haemonchus: 58–98 % in hoggets,

Nematodirus: 58–74 % in ewes, and

Teladorsagia: 50–64 % in ewes, suckling lambs and hoggets.

Only for *Trichostrongylus* were adult worms dominant throughout the year.

Statistical analyses

A. McKenna (1987) drew up 2-way tables using the following categories for egg and nematode counts.

For egg counts:

1	≤ 500 epg	(low)
2	500–2 000 epg	(moderate)
3	> 2 000 epg	(high)

For nematode counts:

1	≤ 4 000 worms	(low)
2	4 000–10 000 worms	(moderate)
3	> 10 000 worms	(high)

TABLE 1 Two-way tables of McKenna (1987) for all data, using the categories for egg counts (epg) and nematode counts described above

epg	Nematodes			
	1	2	3	
1	196	18	24	Misclassified = 35 %
2	36	33	17	Underestimated = 14 %
3	14	26	44	

TABLE 2 Data of young animals using 2-way tables of McKenna (1987). Categories as described in Table 1

epg	Nematodes			
	1	2	3	
1	119	10	8	Misclassified = 36 %
2	31	22	10	Underestimated = 11 %
3	11	20	22	

TABLE 3 Data of old animals, using 2-way tables of McKenna (1987). Categories as described in Table 1

epg	Nematodes			
	1	2	3	
1	78	8	16	Misclassified = 28 %
2	5	11	7	Underestimated = 22 %
3	3	6	22	

TABLE 4 Data of treated animals, using 2-way tables of McKenna (1987). Categories as described in Table 1

epg	Nematodes			
	1	2	3	
1	57	10	16	Misclassified = 43 %
2	22	24	15	Underestimated = 20 %
3	9	16	35	

TABLE 5 Data of control animals, using 2-way tables of McKenna (1987). Categories as described in Table 1

epg	Nematodes			
	1	2	3	
1	139	8	8	Misclassified = 23 %
2	14	9	2	Underestimated = 9 %
3	5	10	9	

Definition of terms in Tables 1–5:

The "misclassified" percentage reflects the sheep percentage in the egg count categories 1, 2 and 3 which do not correspond with those appearing in the nematode count categories 1, 2 and 3. These are too high to be satisfactory.

The "underestimated" percentage classification indicates those sheep that have more nematodes than is indicated in their egg count category. These are sheep with a low egg count but a moderate or high nematode count, and those with a moderate egg count in the high nematode category. These percentages are also unacceptably high in some tables.

In our data even the rough prediction of nematode categories from egg count categories is unsatisfactory for individual sheep. It is interesting to note that in McKenna's (1987) example, misclassified was 19 % and underestimated was only 5 %. Hence in his data the egg count category was a much better predictor of the nematode category than in our data.

B. Correlation coefficients were calculated to measure the extent of the association between egg counts and nematode counts. After both variables were transformed to natural logs +1, Pearson's product moment coefficient (Steel & Torrie 1960, p. 183) and for these type of data, the more appropriate Spearman's correlation coefficient (Steel & Torrie 1960, p. 409), were calculated. (Spearman's correlation is based on ranks derived from the data and is therefore not influenced by abnormally high or low counts). These coefficients are given in Table 6.

TABLE 6 Pearson's and Spearman's coefficients described in B above

	Pearson on raw data	Pearson on natural logs of data	Spearman
All data	0,386	0,683	0,700
Young animals	0,492	0,733	0,751
Old animals	0,553	0,651	0,672
Treated animals	0,323	0,649	0,575
Controls	0,446	0,618	0,667

A Pearson correlation of 0,7 implies that only $0{,}7^2 \times 100 = 49$ % of the variation of nematode counts is explained by egg counts. It is clear from Table 6 that egg counts will not give a reliable estimate of nematode counts. Additional analyses are described in the following section.

C. Regression analyses were done in order to determine whether the relationship between natural log egg counts and natural log nematode counts changed over tables of data, or over age, or over treated and control animals. From these analyses it became clear that:

1. The regression coefficients over tables of data differ highly significantly (P=0,0001). These coefficients range from 0,21 for Table 5 to 0,63 for Table 3—a threefold difference! The intercepts for these 2 tables were also quite different—4,15 for Table 3 and 6,66 for Table 5. These facts imply that for an egg count of 1000 epg, for example, one would estimate a nematode count of 3330 for Table 5 and 4924 for Table 3. For 10 000 epg the estimated worm counts are 5 400 for Table 5 and 21 000 for Table 3! This illustrates that egg counts and the overall regression equation cannot be used to predict nematode counts accurately. There must be factor(s) other than egg counts and random (sampling) variation which determine nematode counts.

2. The regression coefficients for young (Table 2) and old sheep (Table 3) do not differ significantly. The intercepts, however, do so (P=0,0001). For 1 000 epg the estimates of nematode counts for young and old sheep are 3 184 and 6 902 respectively, and for 10 000 epg the estimates are 9 636 and 18 303—again demonstrating the futility of using an overall regression equation to estimate nematode counts from egg counts.

3. Different regression equations for young and old sheep might be considered.

The regression equations fitted to the data are:

All data: log (nematode) = 5,2494 + 0,4500 log (epg)

Young sheep: log (nematode) = 4,7433 + 0,4810 log (epg)

Old sheep: log (nematode) = 5,9136 + 0,4236 log (epg)

TABLE 7 The residuals from regression lines in terms of nematode counts

Residual size categories	Percentages residuals in size categories		
	All data	Young sheep	Old sheep
0– 100	52,5	52,8	53,4
100– 500	6,6	10,7	3,9
500– 1 000	6,9	4,4	1,5
1 000– 5 000	15,7	17,1	13,7
5 000–10 000	6,4	6,3	10,8
10 000–20 000	7,8	7,9	10,3
>20 000	4,2	0,8	6,4

Since the residuals are the errors that would have been made if the nematode counts were estimated from egg counts, it is clear from Table 7 that although the fit of regression lines is not too bad (R^2-value of 47 %, 54 % and 42 % respectively) the percentages of large errors are too high. (An R^2-value indicates the percentage variation in log nematode counts which is explained by log egg counts).

4. It was postulated that there might be differences in the regression relationships for treated and control sheep. The old and young groups were kept separate and within each of these groups separate regression equations were fitted for the treated and control sheep. The equations plus the R^2-values are given in Table 8.

TABLE 8 Differences in the regression equations for young and old sheep and R^2-values

	Fitted regression equation	R^2
Young controls	log (nem)= 4,962 + 0,434 log (epg)	52 %
Young treated	log (nem) = 4,490 + 0,533 log (epg)	56 %
Old controls	log (nem) = 5,994 + 0,413 log (epg)	43 %
Old treated	log (nem) = 5,789 + 0,440 log (epg)	41 %

In young sheep this illustrates a fairly large and significant difference (P=0,0003) between regression coefficients for treated and control animals.

For the old sheep the difference is much smaller and marginally significant (P=0,043).

D. Another attempt to achieve a satisfactory prediction of nematode counts was to consider groups of 6 sheep as a single observation. This implies that the mean egg and mean nematode counts were calculated for each group of 6 sheep. (Note that a few incomplete groups fall away). Correlations and regression equations were calculated from these means and are presented in Table 9. (The rationale behind this attempt was that since each group of 6 sheep came from the same background, the calculation of means would get rid of some unwanted variation).

TABLE 9 Correlations and regression equations for mean faecal egg and nematode counts for groups of 6 sheep

	Pearson correlation on logs	Spearman correlation on raw data
All data	0,816	0,779
Young animals	0,893	0,885
Old animals	0,753	0,641
Treated animals	0,714	0,734
Control animals	0,805	0,813

	Regression equation	R-square
All data	*mnem = 4,807 + 0,552 mepg**	0,666
Old animals	mnem = 5,340 + 0,568 mepg	0,567
Young animals	mnem = 4,343 + 0,571 mepg	0,798
Treated animals	mnem = 5,085 + 0,469 mepg	0,510
Control animals	mnem = 4,838 + 0,540 mepg	0,648

* mnem = mean of log nematode count
** mepg = mean of log egg count

The above results are more promising, especially for young animals and, to a lesser extent, for all animals and control animals, regardless of age. Hence further studies were done on these 3 groups.

Firstly, 2-way tables described by McKenna (1987) were drawn up.

TABLE 10 Two-way tables described by McKenna (1987) applied to groups of 6 sheep each

Young animals

	1	2	3	
1	15	1	0	Misclassified = 33 %
2	9	7	0	Underestimated = 2 %
3	1	3	6	

Control animals

	1	2	3	
1	9	1	0	Misclassified = 38 %
2	5	6	2	Underestimated = 9 %
3	1	4	6	

All animals

	1	2	3	
1	25	2	0	Misclassified = 31 %
2	9	10	2	Underestimated = 6 %
3	1	7	12	

TABLE 11 Correlations and regression equations for faecal egg and nematode counts

Experiments	Pearson correlation on logs	Spearman correlation on raw data
1	0,710	0,720
2	0,639	0,663
3	0,783	0,837
5	0,501	0,513
10	0,574	0,597

Experiments	Regression equation	R-square
1	log (nematode) = 5,166 + 0,551 log (epg)	0,505
2	log (nematode) = 4,965 + 0,448 log (epg)	0,408
3	log (nematode) = 4,152 + 0,625 log (epg)	0,613
5	log (nematode) = 6,661 + 0,213 log (epg)	0,251
10	log (nematode) = 6,592 + 0,335 log (epg)	0,330

TABLE 12 Probabilities of the first half of the egg counts falling into various categories of nematode counts

epg	Nematode count							
	0–500	500–2 000	2 000–4 000	4 000–6 000	6 000–8 000	8 000–10 000	10 000–20 000	>20 000
<100	0,51	0,26	0,09	0,03	0,03	0,00	0,05	0,02
100–500	0,26	0,17	0,13	0,22	0,04	0,00	0,17	0,00
500–2 000	0,03	0,16	0,21	0,24	0,08	0,13	0,13	0,03
2 000–5 000	0,00	0,08	0,17	0,25	0,00	0,00	0,42	0,08
>5 000	0,00	0,00	0,03	0,09	0,17	0,11	0,26	0,34

McKenna-type tables still indicate a large percentage of misclassification. The underestimated percentage, however, has decreased substantially if Table 10 is compared with Tables 1, 2 and 5. From this we may conclude that there is an indication that, if the mean natural log + 1 egg counts (epg) are calculated, there will only be a small number of cases where the nematode population is underestimated. If > 6 sheep per group are used, results would probably improve. This is applicable to a greater extent for young sheep.

E. The same correlation and regression analyses were also conducted separately for each experiment. The results are shown in Table 11.

There are large differences between regression equations and correlations in these results, emphasizing the absence of a universal method of accurately predicting the number of nematodes in sheep from faecal egg counts. Moreover, the R^2-values tend to be low, a further indication of the inability of egg counts to predict the number of nematodes fairly accurately. There must be other factors pertaining to specific experiments which influence the relationship.

F. Finally, McKenna's (1987, p. 95) new approach to the interpretation of faecal egg counts was applied to the data. The dataset was divided in two halves; every alternative sheep in one half and the other in the second half. The first half was used to draw up McKenna's table of probalities and the second half was used to evaluate the method (Table 12).

Comparing this table with McKenna (1987, Table II, p. 95) it immediately becomes clear that we have much more 'spread' in our probabilities. This means that our relationship between egg counts and nematode counts is weaker than McKenna's.

Applying the above probabilities to the egg counts of the second half, we get the estimated nematode distribution for this half shown in Table 13: The first column gives the distribution estimated from the egg counts, while the second column gives the observed distribution.

TABLE 13 The estimated distribution compared with the observed distribution on the 2nd half of the egg counts falling into the various categories of nematode counts

Nematodes	Estimated distribution	Observed distribution
0– 500	26	26
500– 2 000	18	20
2 000– 4 000	13	20
4 000– 6 000	13	7
6 000– 8 000	6	7
8 000– 10 000	4	0
10 000– 20 000	14	11
>20 000	6	10

If we compare the 2 columns of the above table it is clear that there is fair agreement. The nematode distribution as estimated from egg counts using McKenna's method, gives a fairly accurate picture of the actual observed distribution. It is an indication that McKenna's method is usable. It should be noted that the probability table used may only be applicable to sheep from the same region and extensive studies should be done to confirm this. Moreover, a large number of egg counts (> 100?) are necessary to apply McKenna's method.

DISCUSSION

The new approach to the interpretation of faecal egg counts by McKenna (1987) seems to be the most promising for estimation. Comparisons between groups of sheep (n = 6) using the mean natural log + 1 egg count (epg) with the mean natural log + 1 nematode count, also seems promising. It was concluded that McKenna's methods would be valid only if large groups (possibly 100 or more sheep) were used. Some of our results showed a wider spread and a higher percentage of egg counts which underestimated the nematode worm burdens, compared with the results of McKenna (1987) and McKenna & Simpson (1987).

One possible explanation is that we included L_3 and L_4 in the total worm burden of all genera, including *Nematodirus,* which McKenna (1987) excluded. Moreover, with reference to larvae other than *Nematodirus* McKenna (1981) stated, "In addition, although at times large numbers of such stages, usually in the form of inhibited larvae are known to occur in New Zealand, *it is unlikely that they are exerting any pathogenic effects on the host*" (our emphasis). However, these stages were included in our calculations because the larvae of *Teladorsagia* (syn. *Ostertagia*) *circumcincta* cause sufficient pressure necrosis on the glandular epithelium to destroy the parietal and zymogen cells. Also, hypoalbuminaemia sets in at 10–13 days and by the 14th day after infection, the pH rises and pepsin concentration falls (Horak & Clark, 1965).

McKenna (1981) also states, ". . . early fourth stage larvae, which are not recovered in routine counts". The worm recoveries to which he refers were done according to the technique described by Robertson & Elliot (1966) who used 60 mesh/linear inch sieves (apertures 350 µm) to sieve the abomasal and small intestinal ingesta. Few larvae would be retained by this equipment. Neither did they digest the abomasal wall, which is known to contain large numbers of L_3 and L_4 of *Teladorsagia.* We used 400 mesh sieves (38 µm apertures) for the ingesta and included the abomasal wall digests. In our experiments L_3 + L_4 of *Teladorsagia* exceeded adults in ewes, suckling lambs and hoggets from July–November (see results above) (Reinecke & Louw, 1989; Louw, 1989a).

In our opinion, larvae of all the genera present cannot be ignored for sheep grazing on improved pastures in the winter-rainfall areas, particularly those of *Teladorsagia* which form > 50 % of the total worm burdens of all sheep in winter and spring.

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