THE INTERPRETATION OF THE POPULATION DYNAMICS OF BOVINE GASTROINTESTINAL NEMATODES WITH THE USE OF TRACER ANIMALS.

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SUMMARY: The comparative results are given for tracer and permanent beef cattle during a two-year epidemiological experiment. Two animals in each category were slaughtered monthly from June 1987 to May 1989. During the first year, faecal samples were taken from the animals when they were stabled for 30 days, and again before slaughter ("initial" and "final" samples). During the second year, faecal samples were taken weekly during the stabling period ("sequential" samples). Larval cultures and recalculated, proportional egg-counts were compared with pasture contamination levels (Weybridge technique) and *post mortem* helminth burdens. The relatively high oviposition rates of the genera *Haemonchus* and *Oesophagostomum* are reflected in modifications in the proportions of the larval cultures of the sequential samples. The initial samples from tracer animals were closely related to the pasture contamination levels as recorded by the Weybridge technique.

KEY WORDS: Gastrointestinal nematodes, pasture contamination, tracers, egg counts, larval cultures, *post mortem* counts, beef cattle.

INTRODUCTION

Three techniques have commonly been employed to estimate the population dynamics of gastrointestinal helminths in ruminants namely, pasture larval counts, faecal egg-counts and the use of tracer animals. In the course of a two-year epidemiological experiment using tracer and permanent beef cattle on improved pastures in the Cerrado region of central Brazil, carried out at the National Centre for Beef Cattle Research (CNPGC, part of the Brazilian Corporation for Agricultural Research - EMBRAPA), it was noted during the first year that changes occurred in the relative proportions of the genera of gastrointestinal nematodes during the 30-day stabling period before slaughter, as evidenced by faecal samples taken at the start and end of this period to determine egg counts per gram of faeces and make larval cultures. In the second year of the experiment, therefore, faecal samples were taken weekly during the stabling period and changes in the egg counts and larval cultures recorded, and these observations related to pasture larval counts and *post mortem* helminth counts. The results of this experiment, which have a bearing on the use of tracer animals in epidemiological experimentation, are presented here and discussed.

MATERIALS AND METHODS

A description of the materials and methods used was given by GUERRERO & LEANING (1990) and BIANCHIN, HONER & NASCIMENTO (1990). Specifically, two tracer and two permanent animals of the same age and origin were slaughtered each month from June 1987 to May 1989. The worm-free tracers remained on pasture for 30 days and were then stabled for a further 30 days to facilitate post mortem nematode counts. During the first year of the experiment (June 1987 to May 1988), faecal samples were taken from tracers and permanent animals at the beginning ("initial samples") and at the end ("final samples") of the stabling period. During the second year (June 1988 to May 1989), faecal samples were taken weekly ("sequential samples") from both groups of animals. Grazing densities were maintained throughout the experiment by the presence of non-experimental animals of the same age and origin.

The modified MacMaster technique (GORDON & WHITLOCK, 1939) was used to obtain egg-counts per gram of faeces (EPGs) and that of ROBERTS & O'SULLIVAN (1950) for larval cultures (LCs). Pasture samples were taken following the Weybridge technique (Anonymous, 1971), the counts of infective larvae (L_3) being converted to L_3/kg dry matter (DM). Larvae were identified using the key developed by KEITH (1953). The *post mortem* techniques used were detailed by BIANCHIN (1991). Data was normalized by square root transformations and analyzed by regression methods (least squares). Meteorological data was obtained from the CNPGC synoptic station, located about 2km from the experimental pasture.

RESULTS AND DISCUSSION

Four genera of gastrointestinal nematodes predominated in the tracer and permanent animals, namely, *Cooperia*,

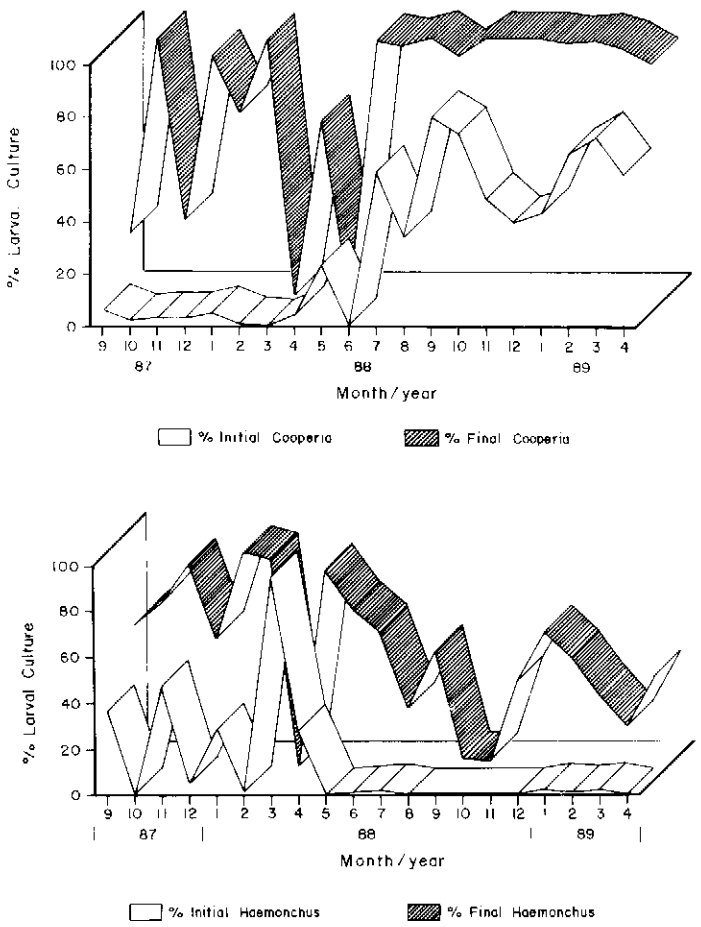

Fig. 1 - Percentage occurrence of the genera *Cooperia* (1A) and *Haemonchus* (1B) in initial and final larval cultures (LCs) of tracer animal faecal samples from September 1987 to April 1989.

Haemonchus, *Trichostrongylus* and *Oesophagostomum*.

In Fig. 1A and 1B the initial and final LC counts are shown for *Cooperia* and *Haemonchus* in the tracer animals during the entire experiment. Two changes were seen in the proportions of these LCs: one during the course of the entire experiment (along the X-axis) and the other between the initial and final counts (Y-axis). Although during the second year, for example, the tracers were stabled with very low counts of *Haemonchus* larvae and high ones for *Cooperia*, some compensating mechanism was evidently functioning to change the final proportions, as measured by LC percentages at the final count. Similar observations were available for the permanent animals, other helminth genera and for EPG counts during the first year of the experiment. Fig. 2 presents the sequential mean EPGs for tracer and permanent animals during the second year of the experiment. It can be seen that these follow a similar pattern (permanent animals r =0.8632, P =0.060; tracers r =0.8849, P =0.046), although the mean EPGs of the tracers were less than half of those of the permanent animals.

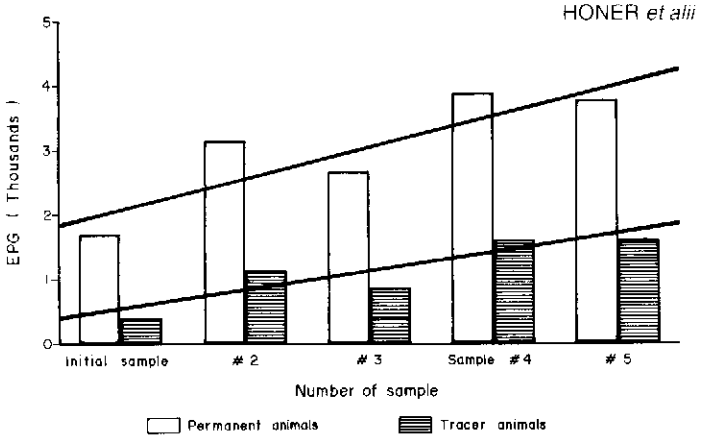

Fig. 2 - Mean sequential counts of eggs per gram of faeces (EPGs) for permanent and tracer animals (48 animals in each category).

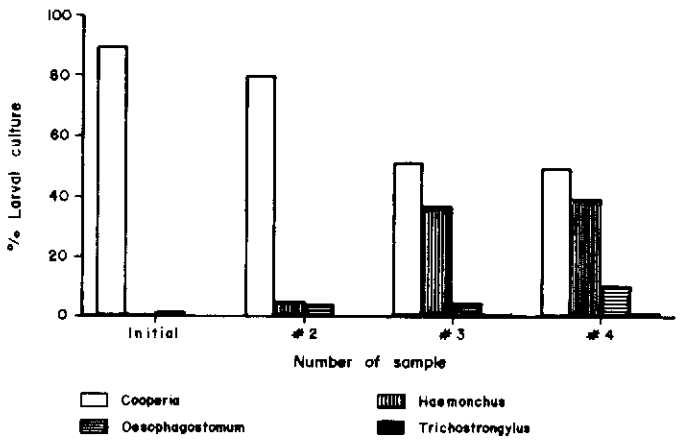

Fig. 3 - Mean proportions of larvae L_3 of the four genera of gastrointestinal nematodes in the sequential faecal samples of tracer animals.

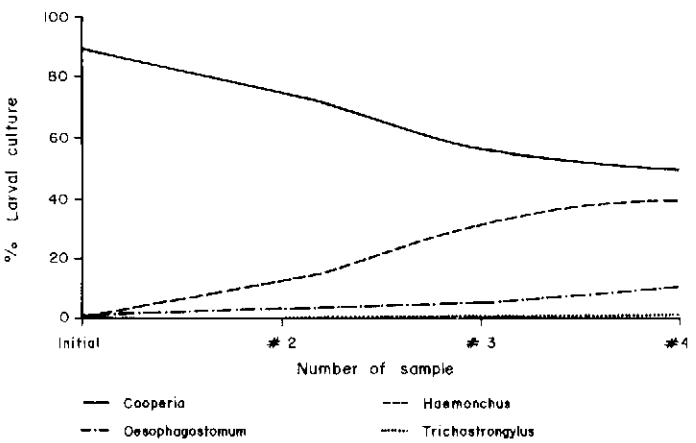

Fig. 4 - Smoothed best-fit curves derived from Fig. 3, representing the mean proportions of the four genera of gastrointestinal nematodes in the larval cultures of tracer animals.

As explained previously, weekly larval cultures were made during the second year and these are presented in Fig. 3 in terms of mean values. The summary of the regression analysis (least squares of transformed LCs * sequential samples) is given in Table 1, where it can be seen that

Table I - Summarized regression analysis of the sequential samples (EPG counts and larval cultures) taken from stabled tracer (t) and permanent (p) beef cattle, 48 animals in each category, samples 1 to 4.

Sample type	t/p	r	P	a	b
EPG	t	0.8849	0.0461	234.10	291.70
EPG	p	0.8632	0.0595	1572.70	486.10
Larval cultures Genera					
Cooperia	t	-0.9473	0.0527	104.80	-14.97
Cooperia	p	-0.9776	0.0224	51.75	-4.08
Haemonchus	t	0.9326	0.0674	-16.67	14.83
Haemonchus	p	0.8889	0.1111	18.90	8.13
Trichostrongylus	t	0.9444	0.0556	-0.42	0.35
Trichostrongylus	p	0.8569	0.1431	2.25	0.72
Oesophagostomum	t	0.9303	0.0697	-1.95	2.76
Oesophagostomum	p	0.0611	0.9389	13.00	0.12

r = regression coefficient; P = probability value; a = intercept and b = slope, of regression line.

Table II - Population parameters of the four principal species of gastrointestinal nematodes in tracer (t) and permanent (p) animals, 48 in each category

Helminth* species	t/p	Mean	CV%	Max	Min	Normal	Chi^2
Hcon	t	772.5	78.9	2197	0	+	8.67
Hcon	p	1309.5	103.0	5887	20	+	10.67
Cpun	t	4503.4	102.8	17003	102	-	12.00
Cpun	p	9528.5	91.8	38035	121	-	12.67
Tax	t	122.5	110.9	457	5	-	17.33
Tax	p	924.9	60.6	2266	240	+	6.00
Orad	t	183.7	80.9	600	0	+	5.33
Orad	p	347.2	120.5	1844	6	-	13.33

Hcon=*Haemonchus contortus*; Cpun=*Cooperia punctata*; Tax=**Trichostrongylus axei**; Orad=*Oesophagostomum radiatum*; CV%=coefficient of variation; Normal=normality of the count distribution, tested by Chi^2, value given in the last column.

there was a significant decrease in the proportion of *Cooperia* larvae (r = -0.9473) and significant increases in those of larvae of *Haemonchus* (r = 0.9326), *Trichostrongylus* (r = 0.9444) and *Oesophagostomum* (r = 0.9303). This situation is summarized in Fig. 4, where smoothed, best-fit curves represent the relative proportions of larvae of these four genera in the faeces of the tracer animals during the stabling period. Similar results were obtained for the permanent animals, Fig. 5, but their exposure to pasture contamination over longer periods of time resulted in some diferences in their helminth population structures. This can be most clearly seen in Table 1, where, with the exception of *Cooperia*, the values of P for the permanent animals are lower than those of the tracers. The most striking difference was in the case of *Oesophagostomum*, the animals coming off pastures with high proportions of these larvae in the cultures. For this genus, high initial counts declined in the second sample and subsequently increased again linearly; for this reason the value of r is only 0.0611. A graphical representation of the proportions of the four genera in the permanent animals, represented with smoothed best-fit curves is given in Fig. 6, which should be compared to Fig. 4 for the tracer animals. When pasture contamination is being measured, the interpretation of tracer animal data depends on the complex relationship between animal behaviour, the immunological status of the host, the relationship between larval cultures and true helminth burdens, relative oviposition rates and competition between the genera present. EPG counts are frequently criticized because they do not truly reflect the helminth population in the host, giving information only of the presence of adult female helminths. For this reason the monitoring of helminth population dynamics is imprecise, when compared to that of ectoparasites (HONER & GOMES, 1990).In the present case, where *post mortem* helminth counts were available for all animals in the experiment, it was feasible to examine the possibility of estimating the proportions of helminths of the four genera being considered, weighting their participation on the basis of their approximate oviposition rates.

Female *Haemonchus* and *Oesophagostomum* are the most prolific egg-layers (2000 - 10000 eggs/day), while *Cooperia* and *Trichostrongylus* produce relatively few eggs (200 eggs/day). The maturing of females of the first two genera during the stabling period of tracer animals could lead to a "dilution" of the eggs of the latter genera, thus giving the appearance of a shift in the helminth population as already noted. The stabling period of 30 days is close to, or less than, the prepatent period of many helminths, with the exception of *Cooperia spp*, where the stabling period is about twice that of prepatency. Table 2 presents the *post mortem* mean burdens per species of the two categories of animals and Table 3 the overall proportions (%) of the principal genera in both categories. The proportions of the four genera in the two groups taken as entities are not significantly different (P>0.01), but Table 2 shows that the populations of the principal species are different in size (permanent helminth burdens being between twice and five times the size of those of the tracers), and also in their frequency distributions, for example, Fig. 7 for *T. axei* and *Oe. radiatum*. These differences reflect the longer exposure of the permanent animals to pasture contamination when compared to the naive tracers. Within the limits of the present experiment it would appear that the tracers reflected satisfactorily the relative proportions of the principal genera of helminths present in the permanent animals (goodness of fit between entire groups P = 0,78).

When EPG counts were converted into proportional egg-counts per genus, Fig. 8 was obtained, for final samples. The recalculated EPG counts reflect more closely the *post mortem* helminth burdens of *Cooperia* and *Trichostrongylus*, i.e. genera with a low egg-laying rate, than the other two genera present. In these, the EPGs overestimate worm counts

Table III - Proportions (%) of the principal genera in tracer and permanent animals, 48 animals in each category.

Helminth genus	Category: Tracer	Permanent
Cooperia	80.7	78.7
Haemonchus	13.8	10.8
Trichostrongylus	2.2	7.6
Oesophagostomum	3.3	2.9

Kolmogorov-Smirnoff two-group test: $D_{max} = 0.0500$; Critical value at 0.5 level = 0.1923. Chi^2 0.5000. P = 0.7788.

Table IV - Relative proportions genera of gastrointestinal nematodes found by larval cultures and *post mortem* worm counts, in two permanent animals that died shortly after entering the stable.

Animal nº	Days stabled	EPG	Larval culture %				Post-mortem counts			
			C	H	T	O	C	H	C/H	C
266p	3	6300	65	19	4	12	77456	8529	9.1	90.1
257p	5	4100	32	30	0	38	26975	10270	2.6	72.4

C = *Cooperia*; H = *Haemonchus*; T = *Trichostrongylus*; O = *Oesophagostomum*; p = permanent animal.

and especially those of the permanent animals. The comparison of sequential LCs with true helminth burdens yields Fig. 9, which illustrates the mechanism of the proportional shifts shown in Figs. 4 and 6, where smoothed best-fit curves were given. The initial and second LCs approximate the burdens of *Haemonchus* and *Trichostrongylus*; in the case of *Cooperia* they grossly overestimate tracer burdens and equally underestimate those of the permanent animals. The third and final LCs overestimate *Haemonchus* in both categories of animals, approximate the tracers burdens of *Cooperia* but continue to underestimate the permanent burdens, reflect the true burdens of *Trichostrongylus* and overestimate to a certain degree those of *Oesophagostomum*. In Fig. 2, where the progression of the sequential "raw" EPGs was given, there is fall in total EPG between the second and third count and this is reflected in the separation of (initial + second) and (third + fourth) LCs in Fig. 9.The aim of using tracers animals in epidemiological experimentation is to measure the pasture contamination rates to which permanent animals may be exposed during specified periods of time. BRYAN & KERR (1988) found that tracers and pasture sampling were similarly effective in representing pasture contamination, within the limits of their experiment. Throughout the present experiment, the Weybridge technique was applied as an independent measure of contamination. Figs. 10 and 11 present the results obtained by this technique in terms of L_3/kg DM versus initial LCs for *Haemonchus* and *Cooperia*. In the first year of the experiment, both techniques identified fluctuations in the pasture contamination levels, but without exact correlation.

During the second year of the experiment both the tracer and the pasture LCs identified a low level of contamination for *Haemonchus* and a high level for *Cooperia*. Both techniques identified definitive, synchronized changes in the ratio of L_3s after May 1988, Fig. 10 and 11, and this could be related principally to the lack of rainfall during the exceptionally dry season, Fig. 12. During the course of this experiment two permanent animals died shortly (three and five days) after being stabled. Their initial LCs and *post mortem* helminth burdens are shown in Table IV, where the opposed trends between *Cooperia* (C) and *Haemonchus* (H) can be seen in the ratio of the two genera C/H. The relatively large proportion of *Oe. radiatum* can also be seen, with *post mortem* counts of 146 and 181 adults, respectively.The loss of helminth burdens in stabled, non-treated ruminants has been known for some time (GIBSON, 1964; TURTON & CLARK, 1974). The latter authors calculated this loss as 7% per day in stabled, untreated sheep infected artificially with *Haemonchus contortus*. Observations of this nature were responsible for the development of the controlled test for anthelmintics in naive ruminants, i.e those maintained helminth-free before exposure to artificial infections (HONER, 1980). The tracer animals in this experiment were maintained free of infection until entering on pasture and can, therefore, be considered as naive. If these had been slaughtered directly after their removal from pasture, the impression obtained as to the composition of the helminth burden might be very different to that obtained if the animals were slaughtered after three to four weeks of stabling. In the first case, it might be possible to conclude that the genus *Cooperia* was dominant, but accompanied by large numbers of *Haemonchus* larvae. This may explain earlier records of the possible occurrence of hypobiosis in *Haemonchus spp* in various regions of Brazil (PIMENTEL NETO, 1976; BIANCHIN, 1978; MELO, 1977, MELO & GOMES, 1979), since in each case animals were slaughtered on removal from pasture or maintained for only a few days in stables. BIANCHIN (1991) and the participants of the I Meeting on the epidemiology of Bovine Nematodes in Brazil (HONER, 1991), found no evidence for hypobiosis in Brazil, with the exception of *Ostertagia spp.*, and then especially in the southern part of the country.

The objections raised by BRYAN & KERR (1988) as to the suitability of tracer animals for measuring pasture contamination are apparently, at least partially, eliminated by the type of tracer animal employed in this experiment, since there is a close relationship between the L_3/kg DM counts and the initial LC proportions. The preparation of the tracers as used in this experiment and others carried out at the CNPGC (BIANCHIN, 1991; BIANCHIN, HONER & NASCIMENTO, 1990) implies that these animals are of the same age and origin, i.e. with a grazing experience similar to that of the permanent animals, so that their bite size and grazing selectivity can be presumed to be similar. It has been shown (CHACON, STOBBS & BALE, 1978) that bite size

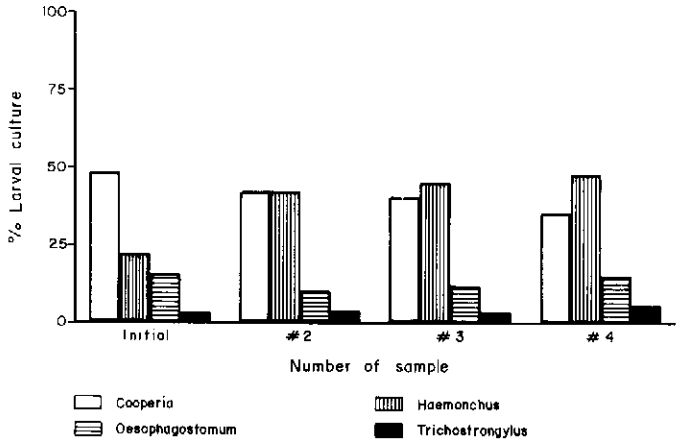

Fig. 5 - Mean proportions of larvae L3 of the four genera of gastrointestinal nematodes in the sequential faecal samples of permanent animals. The levels and shifts in proportions should be compared with Fig. 3.

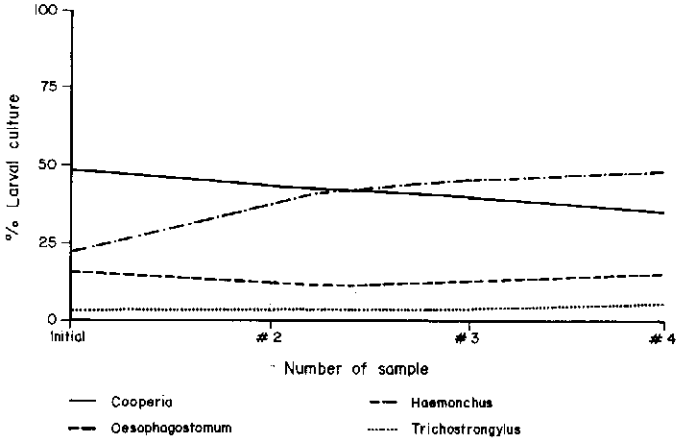

Fig. 6 - Smoothed best-fit curves derived from Fig. 5, representing the mean proportions of the four genera of gastrointestinal nematodes in the larval cultures of permanent animals.

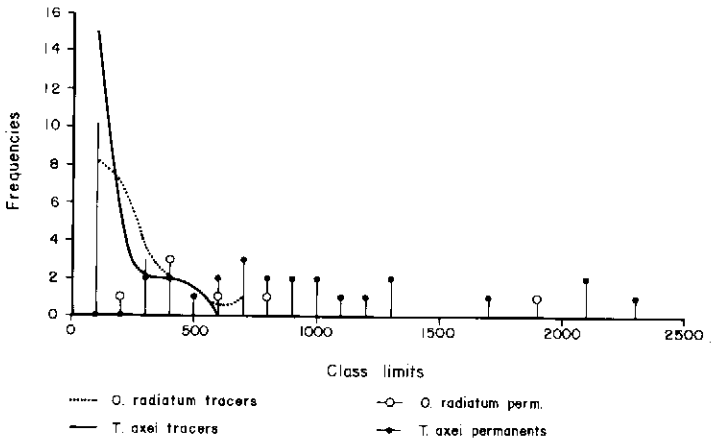

Fig. 7 - Frequency distributions of *Oe. radiatum* and *T. axei* in tracer and permanent animals. The frequencies of the tracer animals are concentrated to the left of the graph, while those of permanent animals have a greater degree of dispersion.

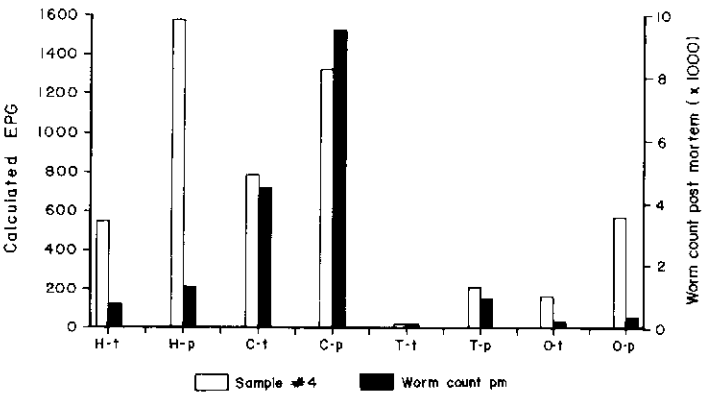

Fig. 8 - Conversion of mean final EPGs into mean proportional generic counts, compared to mean helminth counts *post mortem*, for both categories of animal. Legend: t = tracer animals, p = permanent animals;
H = *Haemonchus*, C = *Cooperia*, T = *Trichostrongylus*,
O= *Oesophagostomum*.

and larval intake are related. If this is accepted, then the larval intake of the tracer animals is closely related both to the pasture sampling technique and the intake of the permanent animals as depicted by initial LCs. The interpretation of the later LC proportions is more indirect, however, since relative oviposition rates modify the final proportions obtained.
This is also true for the relationship between LCs and converted EPG counts. Finally, it should noted that tracer helminth burdens reflect generic proportions only, differing both in size and dispersion from those in the permanent animals.

SUMÁRIO

São apresentados os resultados comparativos de animais traçadores e permanentes durante um experimento epidemiológico com bovinos de corte durante dois anos. Dois animais de ambas as categorias foram sacrificados mensalmente de junho de 1987 a maio de 1989. Durante o primeiro ano, amostras fecais foram coletadas por ocasião do início e da conclusão (imediatamente antes do sacrifício) da estabulação dos animais por um período de 30 dias. No segundo ano, estas amostras foram coletadas semanalmente (amostras "seqüenciais"). Larvaculturas e contagens de ovos por grama de fezes proporcionais por gênero foram comparadas com os níveis de contaminação da pastagem (técnica de Weybridge) e contagens *post mortem* de helmintos. As altas taxas de oviposição dos gêneros *Haemonchus* e *Oesophagostomum* refletiram-se em mudanças nas proporções das larvaculturas das amostras seqüenciais. As amostras iniciais dos animais traçadores mostraram-se relacionadas aos níveis de contaminação da pastagem registrados pela técnica de Weybridge.
PALAVRAS CHAVE: nematódeos gastrintestinais, contaminação da pastagem, traçadores, contagens de ovos, larvaculturas, contagens *post mortem*, gado de corte.

REFERÊNCIAS

ANONYMOUS. (1971) Manual of veterinary parasitological laboratory techniques, Her Majesty's Stationary Office, London, 131p. (MAFF Technical Bulletin, 18).
BIANCHIN, I. (1978) Interação entre *Haemonchus placei*, *Trichostrongylus axei*, *Ostertagia ostertagi* e *Ostertagia*

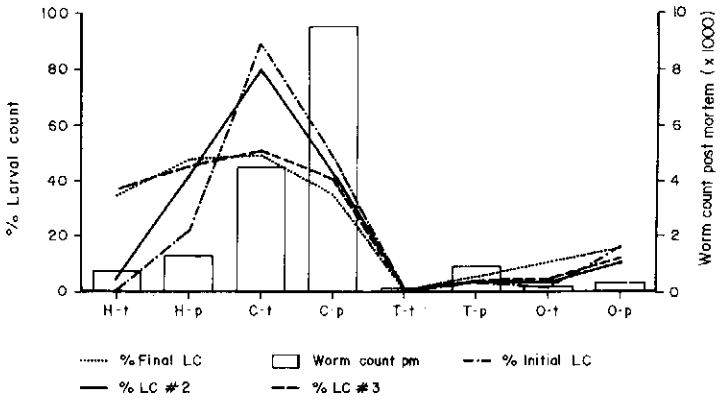

Fig. 9 - Relationship between sequential LCs (1-4) and helminth burdens *post mortem* per category of animal. Legend as in Fig. 8.

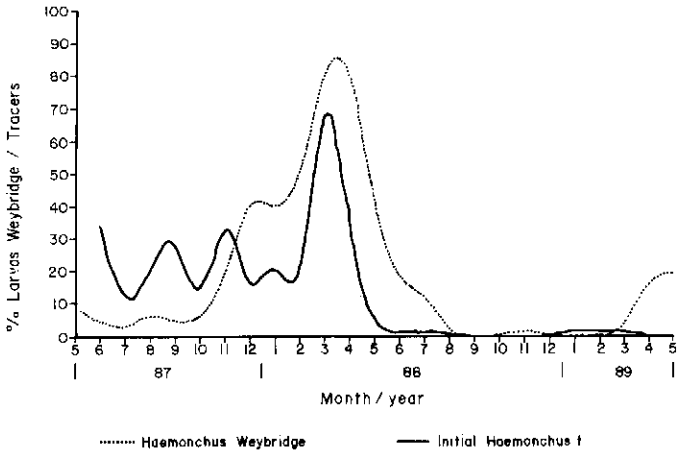

Fig. 10 - Smoothed best-fit curves of the relationship between percentages of *Haemonchus* L_3 as recorded by the Weybridge technique, and the initial LCs in tracer animals.

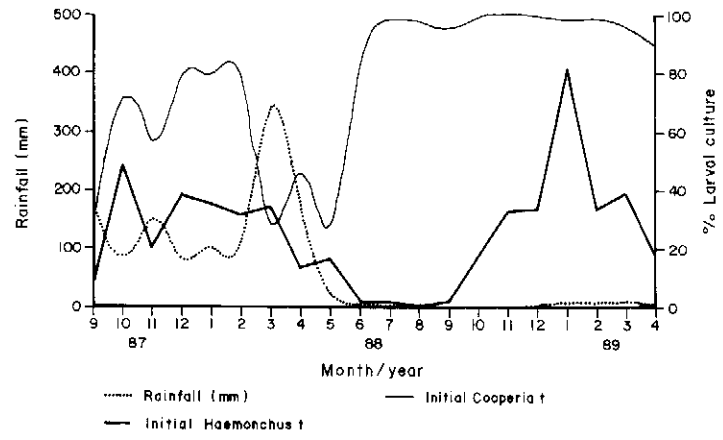

Fig. 11 Smoothed best-fit curves of the relationship between *Cooperia* L_3 as recorded by the Weybridge technique, and the initial LCs in tracer animals.

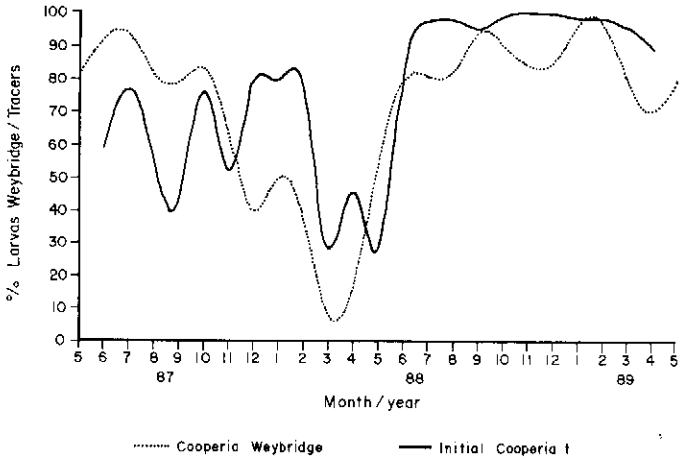

Fig. 12 - Relationship between mean monthly rainfall and mean percentages in tracer LCs for the genera *Cooperia* and *Haemonchus*, during the experimental period.

lyrata (Trichostrongylidae) em bezerros, no Estado do Rio de Janeiro. Tese de Mestrado. UFRRJ, Rio de Janeiro, 94p.

BIANCHIN, I. (1991) Epidemiologia e controle de helmintos gastrintestinais em bezerros a partir da desmama, em pastagem melhorada, em clima tropical do Brasil. Tese de Doutorado. UFFRJ, Rio de Janeiro, 162p.

BIANCHIN, I.; HONER, M.R. & do NASCIMENTO, Y.A. (1990) Epidemiology of helminths in Nellore beef cattle in the cerrados of Brazil. **In**: Proceedings of the symposium: epidemiology of bovine nematode parasites in the Americas, 16th World Buiatrics Congress, 6th Latin American Buiatrics Congress, Salvador, Brazil, 1990. MSDAGVeT, p. 41- 47.

BRYAN,R.P. & KERR, J.D.(1988) The grazing behaviour of cattle in relation to the sampling of infective nematode larvae on pasture. *Vet. Parasitol. 30*: 73 - 82.

CHACON, E.A.; STOBBS,T.H. & DALE, M.B. (1978) Influence of sward characteristics on grazing behaviour and growth of Hereford steers grazing tropical grass pastures. *Austr. J. Agric. Res.29*: 89-102.

GIBSON, T.E. (1964) The evaluation of anthelminthics for the removal of gastro-intestinal nematodes of sheep - an improved form of the controlled test. *Parasitol. 54*: 545-550.

GORDON, H.McL. & WHITLOCK, H.V. (1939) A new technique for counting nematode eggs in sheep faeces. *J. Coun. Sci. and Ind. Res. Austr. 12*: 50-52.

GUERRERO, J. & LEANING, W.H.D. (1990) Strategic nematode parasite control programs in grazing cattle based on epidemiological information. **In**: Proceedings of the symposium: epidemiology of bovine nematode parasites in the Americas, 16th World Buiatrics Congress, 6th Latin American Buiatrics Congress, Salvador, Brazil, 1990. MSDAGVeT, p. 9 -15.

HONER, M.R. (1980) Tipos de testes de anti-helmínticos. **In**: Anais II Seminário Brasileiro de Parasitologia Veterinária, CBPV-EMBRAPA, Forteleza. Brasil, p.235 -250.

HONER, M.R. (1991) Relatório da I Reunião sobre epidemiologia de nematódeos de bovinos no Brasil. *Rev. Bras. Parasitol. Vet., 1*: 5-7.

HONER, M.R & GOMES, A. (1990) O manejo integrado de mosca-dos chifres, berne e carrapato em gado de corte.(Circular Técnica 22), EMBRAPA-CNPGC, Campo Grande, Brasil, 60p.

KEITH, R.K. (1953) The differenciation of the infective larvae of some common nematode parasites of cattle. *Austr. J. Zool.1*: 223-235.

MELO, H.J.H. (1977) Evidência preliminar de "hipobiose" ou "desenvolvimento interrompido" de nematódeos gastrintestinais de bezerros zebu criados extensivamente em zona de cerrado de Mato Grosso. *Pesq. Agrop. Bras.12*: 197-204,

MELO. H.J.H. & GOMES, A. (1979) Inibição do desenvolvimento de *Cooperia* e *Haemonchus* em bezerros Zebú criados extensivamente em ambiente de clima tropical. *Pesq. Agro. Bras. 14*: 29 -35.

PIMENTEL NETO, M. (1976) Epizootiologia da hemoncose em bezerros de gado de leite no Estado do Rio de Janeiro. *Pesq. Agrop. Bras. 11*: 101-114.

ROBERTS, F.H.S. & O'SULLIVAN, P.J.(1950) Methods for egg counts and larval cultures for strongyles infecting the gastro-intestinal tract of cattle. *Austr. J. Agric. Sci.* **1**: 99-102.

TURTON, J.A. & CLARK, C.J. (1974) The effect of natural worm loss on the estimate of anthelminthic activity in an anthelminthic test with *Haemonchus contortus*, *Parasitol.*, **69**: 191-196.

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