COMPARISON BETWEEN PASTURE SAMPLING AND TRACER LAMBS TO EVALUATE CONTAMINATION OF SHEEP PASTURES BY NEMATODE INFECTIVE LARVAE.

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SUMMARY:Over a 1-year period, two techniques were compared to estimate the contamination by infective larvae of sheep gastrointestinal nematodes on pasture. In the first technique, the number of infective larvae was estimated in samples of pasture collected fortnightly from a paddock (one hectare) continuously grazed by 16 sheep. In the second technique, two tracer lambs were placed at every month on the same paddock, where they grazed for two weeks. They were removed from pasture, and after four weeks, sacrificed for nematode counts. Correlation coefficients between the mean monthly number of infective larvae per kilogram of dry matter and the mean monthly number of nematodes in tracer lambs was 0.55 for *Haemonchus* spp., 0.20 for *Cooperia* spp. and 0.10 for *Trichostrongylus* spp.. Sometimes, changes in the infective larval prevalence, followed similar trends in both techniques. Since both techniques showed some limitations, it is suggested that in epidemiological studies, more consistent results can be obtained by using simultaneously these two techniques.

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

INTRODUCTION

Most studies dealing with measurement of pasture contamination by nematode infective larvae are based upon two techniques: direct sampling of pasture (TAYLOR, 1939; VLASSOFF, 1973; BOAG & THOMAS, 1975; AMARANTE & BARBOSA, 1995), or indirect measurement based upon helminth counts in tracer animals (KERBOEUF, 1985; BRAGA & GIRARDI, 1991; SUAREZ & BUSETTI, 1995).

Larval counts on pasture allows to estimate the mean concentration of infective larvae in a given area, at a given time. The nematode counting on tracer animals represents the number of larvae ingested by animals during their stay on pasture, less the number of larvae and/or adults naturally eliminated. The two techniques deal with different parameters in different ways. Notwithstanding, both have been used along the time to estimate pasture contamination or to verify the influence of handling procedures on pasture contamination (WALLER *et alii*, 1981).

Hence, this work was conducted to evaluate these two techniques used to estimate pasture contamination by nematode infective larvae from sheep.

MATERIALS AND METHODS

A paddock of one hectare, from the Sheep sector of FMVZ/ UNESP, Botucatu Campus, São Paulo State, was used in this experiment. The pasture was covered with Coast-cross grass (*Cynodon dactylon*), and 16 sheep naturally infected by gastrointestinal nematodes were placed on it. The animals were left on a continuos grazing system over the 12 months experimental period. Sheep that died during that time were replaced by other ones, in order to keep the same stock. The majority of sheep delivered in July/ August and lambs were weaned with two months-old.

All sheep were treated with ivermectin (200 µg/ kg, Sheep Injectable IVOMEC®, Merck Sharp & Dohme) three months after the beginning of trial. Other antihelminthic treatments were individually done whenever it was necessary, because of high fecal egg counts or clinical signs of helminth infection.

Two Corriedale tracer lambs were placed every month in the paddock. These animals were previously kept indoors, where they were treated with anthelminthics of different groups in order to eliminate naturally acquired infections. They stayed on the paddock for 14 days, being then removed and kept

once again indoors for more 28 days. Afterwards they were slaughtered for worm counting. These animals received a contamination-free diet during the indoor period.

After necropsy, 10 % of abomasal and small intestine contents were collected and kept in plastic vials with 5% formalin. Furthermore, abomasum and small intestine were digested with 1% hidrocloridric acid (UENO & GONÇALVES, 1994). Nematodes obtained from these gastrointestinal samples were counted and identified according to descriptions by REINECKE (1983) and UENO & GONÇALVES (1994). The number of nematodes in the 10 % sample was multiplied by ten and added with the number of adults recovered at digestion.

Grass samples were collected from the paddock following a previously settled "W" shaped trace at every two weeks (TAYLOR, 1939). The grass sampler followed this trace and harvested by hand one sample at every four steps (a distance of approximately 3.5 m). Such methodology was used throughout the experiment. The samples were processed according to AMARANTE & BARBOSA(1995).

Fecal samples for EPG counts (GORDON & WHITLOCK, 1939) were collected on the same day of pasture sampling. Fecal cultures were done to obtain infective nematode larvae (ROBERTS & O'SULLIVAN, 1950). Larvae harvested from cultures or directly from grass were identified according to KEITH (1953).

Monthly data regarding to arithmetic means of number of infective larvae per kilogram of dry matter (L_3/ kg DM) and number of nematodes in the tracer animals were submitted to the Spearmen correlation (SIEGEL, 1956). Separate analysis were conducted for each genera: *Haemonchus*, *Trichostrongylus* and *Cooperia*.

RESULTS

The highest L_3/ kg DM counts for *Haemonchus* spp. on pasture (Figure 1) were found during May, June, July, October and December, being the last month the one with the highest value (524 L_3/ kg DM). No larvae were found in January nor in March. The highest counts in tracer lambs were also found at the beginning of trial, from May to July (Figure 2). A new peak count was observed from December to February, but the levels were lower than the observed at the beginning of trial. The Spearmen correlation coefficient between the monthly mean of L_3/ kg DM and the monthly mean number of nematodes found in tracer lambs was 0.55 ($p<0.07$).

Cooperia spp. larvae were found on pasture in five of the 12-months trial period (August-1992, November-1993, March and April). The highest count was found in April-1993 (312

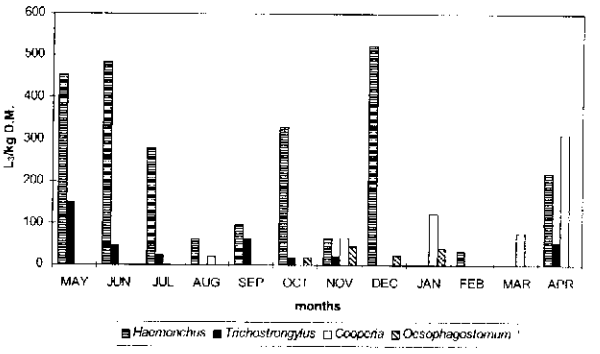

Fig. 1 - Mean monthly counts of *Haemonchus* spp., *Trichostrongylus* spp. and *Cooperia* spp. and *Oesophagostomum* spp. infective larvae per kilogram of dry matter (L_3/ kg DM) obtained from pasture sample collected fortnightly from May 1992 to April 1993.

L_3/ kg DM) together with a rise on the number of *Cooperia* spp. eggs output in feces. *Cooperia* spp. was found in tracer lambs only in December, February and April (Figure 2). *Cooperia curticei* was the species widely found. Some specimens of *Cooperia punctata* were also found in one of the lambs placed in the paddock at April. The Spearmen correlation coefficient between the monthly mean of L_3/ kg DM and the monthly mean number of nematodes found in tracer lambs was 0.20 ($p>0.1$). Although this is a low number, it should be noticed that the highest counts on grass coincided with the highest counts in tracer lambs.

Pasture contamination by *Trichostrongylus* spp. was low throughout the trial (Figure 1). The highest number of larvae was recorded at the beginning of the experimental period (149 L_3/ kg MS in May) coinciding with the highest number of *Trichostrongylus colubriformis* recovered from tracer lambs (Figure 2). The correlation coefficient was 0.20 ($p>0.1$) for this genus.

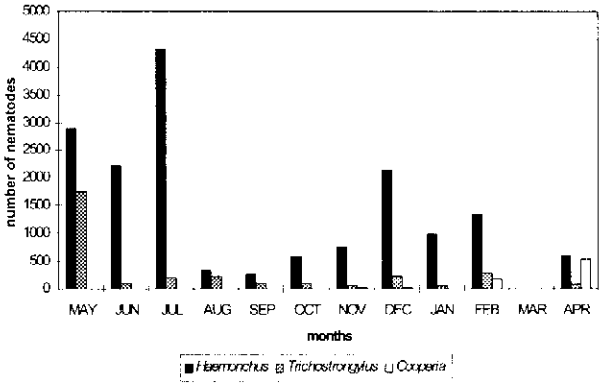

Figure 2 - Mean monthly counts of nematodes of *Haemonchus* spp., *Trichostrongylus* spp., *Cooperia* spp. and *Oesophagostomum* spp. genera recovered from tracer lambs introduced every month on the experimental paddock from May 1992 to April 1993.

Small amount of *Oesophagostomum* spp. larvae were also recorded on pasture from October to January (Figure 1). Such parasite was not found in tracer lambs. The results obtained for this genus are probably due to the low infection levels of ewes, that led to reduced pasture contamination.

A negligible number of immature nematodes was recovered during digestion of abomasum and small intestine. This shows that hypobiosis does not occur in sheep from the area were this study was conducted.

DISCUSSION

In the present experiment, the association between the values obtained with the tracer lambs and direct sampling techniques ranged from low to medium (r=0.10 to 0.55). These relatively low statistical parameters show some inconsistency between the results obtained from the two techniques. Some limitations from both techniques may have determined such differences. In the tracer lamb technique for instance, huge individual variation in the susceptibility to nematodes were observed, as for example the case of lambs introduced in the paddock in February: one showed 2591 specimens of *H. contortus*, while the other had only 86.

Regarding to the tracer animals used on this trial, it should be noticed, that all of them were naturally infected by gastrointestinal nematodes. Thus, it is possible that many of them had the opportunity to develop immunity to parasites. It is known that susceptibility to nematodes is not uniform in groups of sheep infected either naturally (SRÉTER *et alii*, 1994) or artificially (BARGER & DASH, 1987). Hence, different levels of immunological response should have occurred, what led to wide variation in the results obtained with some of the lamb replicates.

Misinterpretations of pasture contamination could also occur if the two tracers had similar levels of immunological responses. For instance, in a month with high levels of contamination, it could be possible that the tracers presented low numbers of parasites due to their resistance. The opposite could also occur if animals are very susceptible.

The use of worm-free tracers would reduce this problem, although it would increase the cost of experiments. But it is possible that some of these animals develop an immune response within the period between their introduction in the pasture and slaughter, avoiding in this way the establishment or expelling the parasites. Such phenomena was observed by RADHAKRISHNAN *et alii* (1972) working with lambs of Florida Native (resistant) and Rambouillet (susceptible) breeds. The authors observed that 28 days after the single *H. contortus* experimental infection, the worm-free Florida Native lambs presented a low number of nematodes, when compared to Rambouillet lambs raised under the same conditions. Conversely, GILL (1991) in a study conducted to study the genetic control of acquired resistance to *H. contortus* infections in Merino sheep, found that the resistant sheep presented higher EPG counts at prime infections than sheep not breed for this trait. On the other side, the opposite occurred in a challenge infection, where sheep breed for resistance presented lower EPG counts. This shows that genetic controlled resistance only appears on a second contact with parasites.

These works shed a light on the complexity of immune responses to gastrointestinal nematodes, which could explain the huge variations obtained with some of tracer replicates. Similar results were presented by WALLER *et alii* (1981), who used worm-free tracers in Australia.

Despite these limitations, the use of tracer animals present some advantages when compared to sampling from pasture. This technique allows for example, the species identification, while in the direct sampling technique only genera can be known. The possibility of studying hypobiosis is another advantage of tracer animals.

The technique of direct sampling from pastures has some limitations. The grass collected will never correspond exactly to the grass ingested by the animals. The simple observation of a paddock shows that grazing is not uniform, and it is common to find intensively grazed areas close to other areas with a surplus of grass. We could confirm such findings at the present experiment, in the same manner as BRYAN & KERR (1988) in a work with cattle in Australia.

It is also very difficult with such technique, to recover the total number of larvae from grass samples. The pasture contamination is hence, greater than the value accessed, and should be kept in mind that if the methodology was kept untouched during all trial, we will demonstrate a seasonal variation, and not the real number of infective larvae present on pasture. This kind of problem is also found in other parasitological techniques, as for example the McMaster technique which records only 16.5 % from the total number of eggs in feces (ROSSANIGO & GRUNER, 1991).

On the other side, the pasture sampling technique has a low cost and is quicker to accomplish than the tracer animals technique.

Analyzing figures 1 and 2, we can verify that, despite the limitations pointed out for both techniques, the results regarding to pasture contamination alongside the time are quite similar. BRYAN & KERR (1988) obtained similar results when compared five methods for assessment of pasture contamination in cattle grazing areas: three methods of pasture sampling by hand, one method with rumen fistulated animals and other with tracers. The authors verified

that all methods were sensible enough to estimate the number of larvae on pasture, excluding only the method with tracer animals under intensive grazing conditions. WALLER *et alii* (1981) presented slightly different results, finding higher variability for the tracer lamb technique when compared to the sampling from pastures, which the authors considered as the most indicated for assessment of pasture contamination.

The results presented in this work indicated that, for studies intending to evaluate the seasonal variation of the number of infective larvae on pasture, the best results are achieved when both techniques are used together. Whenever the use of both techniques can not be accomplished, the choice of the technique to be employed should be based upon the amount of available resources and the project targets, since both the tracer animals and the direct sampling techniques presented similar results.

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SUMÁRIO

Este trabalho foi realizado ao longo de 12 meses com o objetivo de comparar duas técnicas utilizadas para estimar a contaminação da pastagem de ovinos por larvas infectantes de nematódeos gastrintestinais. Em uma das técnicas, a quantidade de larvas infectantes foi estimada diretamente em amostras de capim que foram colhidas quinzenalmente de um piquete, com um hectare de área, pastejado continuamente por 16 ovelhas. Na outra técnica, a contaminação da pastagem do mesmo piquete foi estimada indiretamente com base na contagem de nematódeos em cordeiros traçadores. Mensalmente, dois traçadores foram colocados no piquete onde permaneceram durante duas semanas. Após, foram estabulados por 28 dias, sendo necropsiados ao final desse período para que fosse realizada a contagem de nematódeos parasitas do trato gastrintestinal. O coeficiente de correlação entre o número médio mensal de larvas infectantes por quilograma de matéria seca e o número médio mensal de nematódeos nos traçadores foi de 0,55 para *Haemonchus* spp., 0,20 para *Cooperia* spp. e 0,10 para *Trichostrongylus* spp.. Em relação à tendência da contaminação da pastagem ao longo do tempo, foram obtidos resultados similares com as duas técnicas em algumas ocasiões. Devido ao fato das técnicas terem apresentado algumas limitações, sugere-se que, em estudos epidemiológicos destinados a estimar a variação sazonal do número de larvas na pastagem, resultados mais consistentes podem ser obtidos se as duas técnicas foram empregadas simultaneamente.

PALAVRAS-CHAVE: nematódeos, ovinos, larvas infectantes, pastagem, *Haemonchus, Trichostrongylus, Cooperia*

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