Nematode genera diversity in cattle: similarity of between-sire progenies

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Nematode genera diversity in cattle: similarity of between-sire progenies

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Abstract – Breeding cattle for resistance to nematode infection is mostly based on egg excretion. This, however, does not allow for generic identification of the nematodes involved. Unless we know whether the selected resistance is directed against one or several particular genera, a strong bias could be introduced in the selection programs. In order to estimate the likelihood of this potential bias we investigated nematode genera diversity in the progeny of four sires in 1992 and seven sires in 1994. Three of the four Aberdeen Angus sires used in 1992 were related while the seven sires in 1994 were unrelated. Diversity was assessed using at least ten individual faecal cultures for each progeny group during each of the two sampling periods (beginning and end of grazing period, April and September). It was estimated by the relative proportion of each genera (*Ostertagia, Cooperia, Haemonchus, Trichostrongylus* and *Oesophagostomum*) or by either the Shannon (genera diversity) or Pielou (genera evenness) index. The Shannon index was repeatable when measured at 2-week intervals within the same progeny group on ten random faecal samples. No significant difference was recorded between sire genera diversity over the two sampling periods. This indicated that hosts have a limited effect on the nematode genera diversity as assessed by faecal cultures, and that the selection of resistant hosts could probably be achieved using faecal egg counts. © Inra/Elsevier, Paris

nematode / diversity / faecal culture / cattle / selection / resistance

Résumé – Diversité générique des nématodes parasites de bovins : similarité entre les descendances des reproducteurs. La sélection des bovins résistant à l'infestation pour les nématodes est fondée sur l'excrétion des œufs de parasites dans les fèces, ce qui ne permet pas d'assurer une diagnose des genre de nématodes en cause. Un biais fort est introduit dans les opérations de sélection de bovins résistant aux infestations si nous ne savons pas contre quels genres de nématodes est dirigée cette résistance. Afin d'estimer ce biais éventuel, la diversité générique au sein

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de la descendance de quatre (1992) et de sept (1994) reproducteurs. Trois des quatre reproducteurs Aberdeen Angus étaient membres de la même famille en 1994 ; les sept reproducteurs en 1994 n'étaient pas liés génétiquement. La diversité a été estimée sur dix coprocultures individuelles au moins pour chaque descendance à deux périodes d'étude (début et fin de la saison de pâturage, avril et septembre). Cette diversité a été estimée soit par les proportions de chaque genre (*Ostertagia, Cooperia, Haemonchus, Trichostrongylus* et *Oesophagostomum*) ou par les indices de Shannon (diversité générique) ou de Pielou (équitabilité générique). L'indice de Shannon est répétable lorsqu'il est mesuré à deux semaines d'intervalle sur dix coprocultures au sein de la descendance de chaque reproducteur. Aucune différence de diversité générique n'a été observée entre les descendances des différents reproducteurs aux deux périodes d'étude. Cela indique que les hôtes ont un impact limité sur la diversité générique établie au moyen de coprocultures, et la sélection d'hôtes résistants peut raisonnablement s'effectuer sur la simple mesure d'excrétion des œufs de parasites dans les fèces. © Inra/Elsevier, Paris

nématodes / diversité / coproculture / bovin / sélection / résistance

1. INTRODUCTION

Due to the increase in worm resistance to anthelmintics and consumer concern about drug residues in animal products, much attention has been paid to developing alternative means of controlling helminth infections in ruminants. Most of the investigations have focused on breeding small ruminants for resistance to nematodes [8], although some have concentrated on cattle resistance to helminths [1, 4, 10, 11, 18, 19, 22]. Very different values of heritabilities were recorded for the different nematode genera found in cattle (from 0.04 *(Cooperia)* to 0.29 *(Haemonchus)* [4] and from 0.13 *(Cooperia)* to 0.40 *(Haemonchus)* [15]) and selection for reduced faecal egg counts might change the relative proportion of the nematode genera. This point is very important as selection might be directed against 'all strongyles' mostly based on egg output, or against 'one or several particular genera' based on data established from larval differentiation.

According to the aspect of genera diversity considered, the various measures can be classified into three groups: the number of genera (genera richness), diversity and evenness (equitability) measures. Most of the diversity indices incorporate two independent factors: the number of genera and evenness [13], the latter measuring the way the individual worms are partitioned among the different genera. Among the various indices, two, Shannon for diversity, and Pielou for evenness, are among the best adapted to characterize diversity and evenness in the digestive-tract strongyle communities of ruminants [7]. In the present work we will focus on nematode generic assemblage variability between the progenies of different sires, based on faecal cultures, and using diversity and equitability indices, in order to determine whether selection directed against 'all strongyles' would result in a reduction of worm burden for all worm species or not. To our knowledge, this has never been studied.

2. MATERIALS AND METHODS

2.1. Animals and protocols *(table I)*

The investigations were performed at the INTA-Anguil (Argentina) experimental station in 1992 and 1994, under a temperate climate in the Pampas. The yearly rainfall was 600 mm; rainfall was scarce during the colder months of June and July (monthly mean temperature: 10 °C). In the two experiments, 6-month-old cattle were weaned in April, males

Table I. Characteristics of sampled calves and nematode infection (eggs per gram and infective third-stage (L3) per gram of faeces) of the progeny of four (s1, s2, s3 and s4 in 1992) and 7 (s5 to s11 in 1994) sires.

No. of calves (sire)	No. of male/ No. of female	No. of samples Apr/Sept	Eggs per gram (range) (April then September)	L3 per gram (range) (April then September)
1992	42/36	42/36	350	126
20 (s1), 22 (s2),			(217–516)	(86–203)
17 (s3), 19 (s4)			119	40
			(103–135)	(21–51)
1994	75/44	66/53	97	34
18 (s5), 22 (s6),			(32–139)	(11–42)
30 (s7), 9 (s8),			163	57
12 (s9), 13 (s10),			(92–355)	(41–112)
15 (s11)				

were castrated, and then grazed on pastures until September. In 1992, the progeny (160 animals) of four Aberdeen Angus bulls mated with Aberdeen Angus females were investigated (sires s2 and s4 had the same father, s3 was the son of s4, and s1 was unrelated to the others). The calves were treated with oxfendazole and then put to graze on an alfalfa pasture (2/hectare) previously used by sheep. In 1994, the progeny (105 animals) of six unrelated Aberdeen Angus (s5 to s10) and one Santa-Gertrudis (crossbred Zebu and *Bos taurus*: s11) bulls mated with Aberdeen Angus females were studied. The calves were put to graze (1.8/ha) without any treatment on a pasture (oats and alfalfa) previously grazed by cattle.

2.2. Parasitological procedures

Faecal samples from at least ten randomly selected animals in each sire progeny group were taken in April and in September for both experiments. The sample characteristics are shown in *table I*. In the 1992 experiment, two calves were necropsied in June and additional faecal samples were also taken in August. The faecal egg counts (modified McMaster technique: Roberts and O'Sullivan [14]) and percentages of the different genera in the individual faecal cultures were measured (identification of at least 50 third-stage larvae, based on Keith [9]).

2.3. Analysis of data

Genera diversity was established by means of the Shannon index, coded as Ish (ranging between 0 when only one genus was found and the natural log of the number of genera recorded when there was more than one genus) or Pielou equitability coded as Eq (0 when only one genus was found to 1 when all genera were equally represented in the community of worms) using the Biodiv program [2]. The confidence interval of the Shannon index and Pielou equitability were assessed using the resampling bootstrap procedure (2 000 repeats with Simstat software: Péladeau and Lacouture [12]), as the statistical distribution of such indices is not known [6]. Multivariate variance analysis (Manova) was used to assess between-sire differences with respect to the whole genera assemblage. Significance was tested by the Roy, Pillai and Hotelling tests using the statistical software Stat-Itcf [17]. The number of L3 of the different nematode genera were analysed for differences among sampling periods. Multiple correspondence analysis was also used to determine the respective importance of calf sex, month of sampling and sire on proportions of the nematode genera. The total number of calf-season samples was 78 in 1992 and 119 in 1994. This multivariate analysis investigated relations between categorical variables of different characteristics (i.e. sire progeny versus proportions of the different nematode genera). *Trichostrongylus* and

Oesophagostomum were distributed into three classes while *Cooperia*, *Haemonchus*, *Ostertagia*, Shannon index of diversity and Pielou equitability were distributed into four classes with equal numbers of calves in order to obtain the same weight in each class. The active variables were the proportions of nematode genera. The Shannon index of diversity, Pielou equitability, calf sex, month and sire were used as supplementary variables, i.e. they did not take part in the construction of the axes but were only added in relation to the active variables afterwards. The relative importance of each axis was determined by the percentage of variance it accounted for.

3. RESULTS

3.1. The confidence interval of the Shannon index of nematode diversity

The distribution of Ish is not known and thus its confidence interval can only be assessed using resampling methods. The results of at least ten faecal cultures per sire progeny from each sampling period (April or September) were used. Eleven (four in 1992 and seven in 1994 sire progeny groups) progeny were tested for April data and the same progeny were tested again for September data. The upper and lower limits of the confidence interval ($P = 0.05$) were established using the bootstrap procedure and are shown in *figure 1*. The confidence intervals were of the same magnitude for both small and large Shannon index values. Similar results were found for equitability (data not shown). This indicated that the small Shannon indices had a relatively low accuracy, whereas higher indices were determined more accurately. It also meant that the Shannon indices, when established on an average of ten individual faecal cultures from ten calves, presented a range between a minimum and maximum value of approximately 0.25.

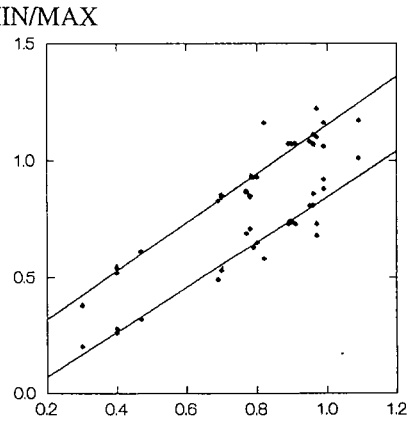

Figure 1. Shannon index of diversity (Ish) and confidence interval (at $P = 0.05$, Max and Min correspond to the upper and lower limits, respectively) established by means of a bootstrap resampling procedure on the results obtained from the progeny of four (1992) and seven sires (1994) in the April and September faecal samplings.

3.2. Nematode diversity in the progeny of four sires based on faecal samples 2 weeks apart (August and September 1992)

There was no large variation between the two periods as shown in *figure 2*. The average differences (data not shown) between the two sampling periods were: 6 % (*Ostertagia*), 11 % (*Haemonchus*), 13 % (*Cooperia*), 3 % (*Trichostrongylus*) and 0.14 for the Shannon index. This showed that over a short period the results derived from the faecal cultures of ten calves were similar.

3.3. Nematode diversity in relation to intensity of infection

The intensity of infection was lower in 1994 than in 1992 *(table I)*. The Ish diver-

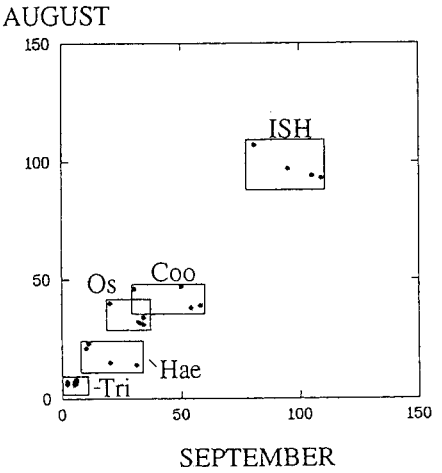

Figure 2. Nematode diversity in the progeny of four sires sampled at 2-week intervals (August and September 1992) as assessed from faecal cultures. (The estimates are repeatable when they are located near the diagonal line). The generic data are expressed in percent of the total community – Coo: *Cooperia*; Hae: *Haemonchus*; Os: *Ostertagia*; Tri: *Trichostrongylus*. The Ish (Shannon index of diversity) are multiplied by 100.

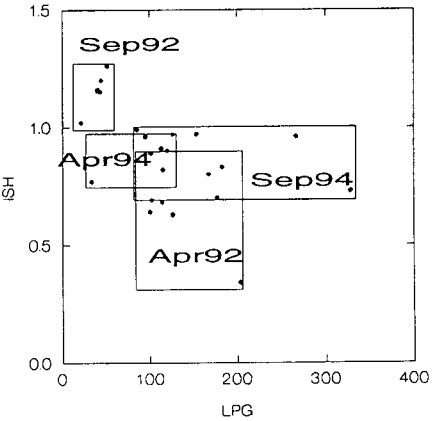

Figure 3. Shannon index of diversity (ISh) and intensity of infection estimated by the number of infective larvae per gram of faeces (LPG). The individual values for all sires are presented (small empty dot). The rectangles correspond to the range of values within periods (April or September) of a given year (1992 and 1994).

sity index was independent of year (F = 0.31; P = 0.59) but depended on period of study (F = 16.5; P = 0.01) as shown by a two-factor ANOVA. The reverse was observed for the intensity of infection estimated from larval cultures (larvae/g/faeces: LPG). It depended on the year (F = 5.3; P = 0.03) but not on the period (F = 0.06; P = 0.93). There was no significant relationship between Ish and LPG within each sampling period *(figure 3)*.

3.4. Nematode diversity in the progeny of four sires grazed on a pasture previously used by sheep (1992)

The following species were found in the two necropsies undertaken in June: *Haemonchus placei* (0, 225 worms), *Cooperia oncophora* (840, 1 400), *Ostertagia ostertagi* (587, 3 500) and *Trichostrongylus colubriformis* (222, 2 700). In faecal cultures, the dominant genera were *Haemonchus* (over 70 % of larvae) in April and *Cooperia* in September (40–50 % of larvae) (see *figure 4*: 1992). The Manova of the April and September results did not indicate any significant difference between sires based on faecal cultures. The necropsies in June should represent an intermediate fauna level between April and September: *Haemonchus* and *Cooperia* were largely over-represented in faecal cultures, whereas *Ostertagia* and to a larger extent, *Trichostrongylus*, were strongly under-represented. The Shannon index was 0.39 in April and 0.95 in September *(table II)*. Equitability increased

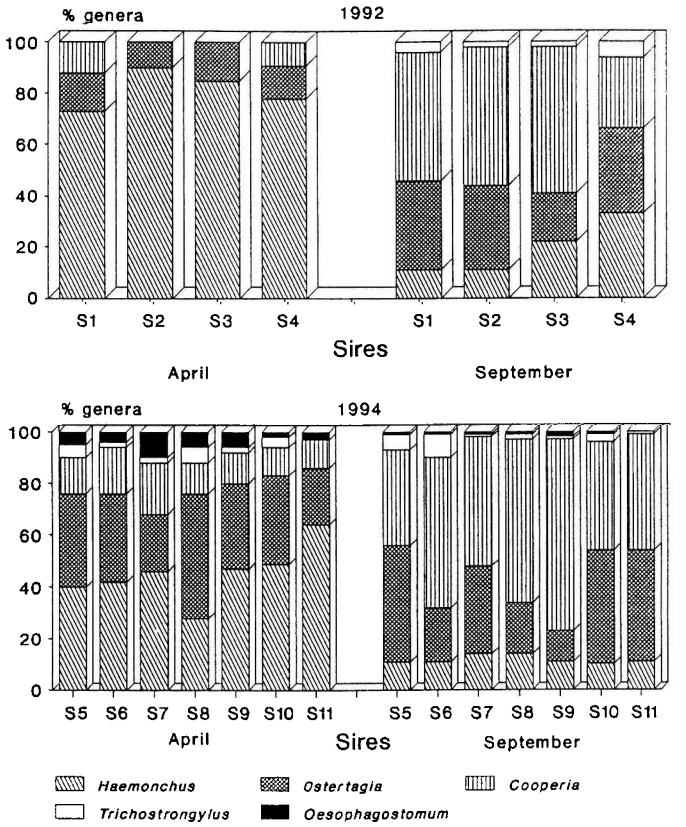

Figure 4. Proportions of the trichostrongyle genera in relation to sire and month of sampling. Pastures previously grazed by sheep (1992) or cattle (1994).

as well from 0.45 (April) to 0.84 (September). Those two indices were less variable within sire progeny in September than in April.

From the multiple correspondence analysis (*figure 5*: 1992), the following may be concluded.

When the cultures were predominantly *H. placei* (April) there was a low index of diversity. In September, when several genera occurred in high proportions, the indices of diversity were high.

The four progeny groups were very similar (their positions on the graph are located near the origin), and were not characterized by any particular assemblage of nematode genera. A slight difference was seen between the s1 progeny on one side and the related s2, s3 and s4 on the other.

3.5. Nematode diversity in the progeny of seven sires grazed on a pasture previously used by cattle (1994)

The proportions of genera in the assemblages are shown in *figure 4* (1994).

Table II. Shannon index and confidence interval ($P = 0.05$; established by the bootstrap resampling procedure) within the progeny of each sire at the beginning (April) and end of the grazing period (September) in 1992 and 1994.

	Sires	Shannon index	Confidence interval
1992	April		
	1	0.47	0.32–0.61
	2	0.30	0.20–0.38
	3	0.40	0.26–0.54
	4	0.40	0.28–0.52
	September		
	1	0.99	0.32–0.61
	2	0.78	0.20–0.30
	3	0.95	0.77–1.00
	4	1.09	1.01–1.17
1994	April		
	5	0.91	0.73–1.07
	6	0.90	0.74–1.07
	7	0.96	0.86–1.07
	8	0.97	0.73–1.10
	9	0.89	0.73–1.07
	10	0.82	0.58–1.16
	11	0.77	0.69–0.87
	September		
	5	0.70	0.53–0.85
	6	0.96	0.81–1.11
	7	0.80	0.65–0.93
	8	0.97	0.68–1.22
	9	0.79	0.63–0.93
	10	0.99	0.88–1.16
	11	0.69	0.49–0.83

Haemonchus was dominant (40–60 %) in April and *Cooperia* (40–80 %) in September. The Manova of the April and September results did not reveal any significant difference between sires based on faecal cultures. The Shannon index *(table II)* and equitability in April and September were 0.89 versus 0.84, and 0.80 versus 0.66, respectively; the within-sire progeny variability did not change much between the two sampling periods, in contrast to that recorded in 1992.

From the correspondence analysis shown on *figure 5* (1994) the following may be concluded.

When the cultures were predominantly *H. placei* (April) there was a high index of diversity. In September, when *Cooperia* were predominant the indices of diversity were low.

The seven progeny groups in 1994 were not as similar as those studied in 1992: the s5, s8 and s11 groups were not char-

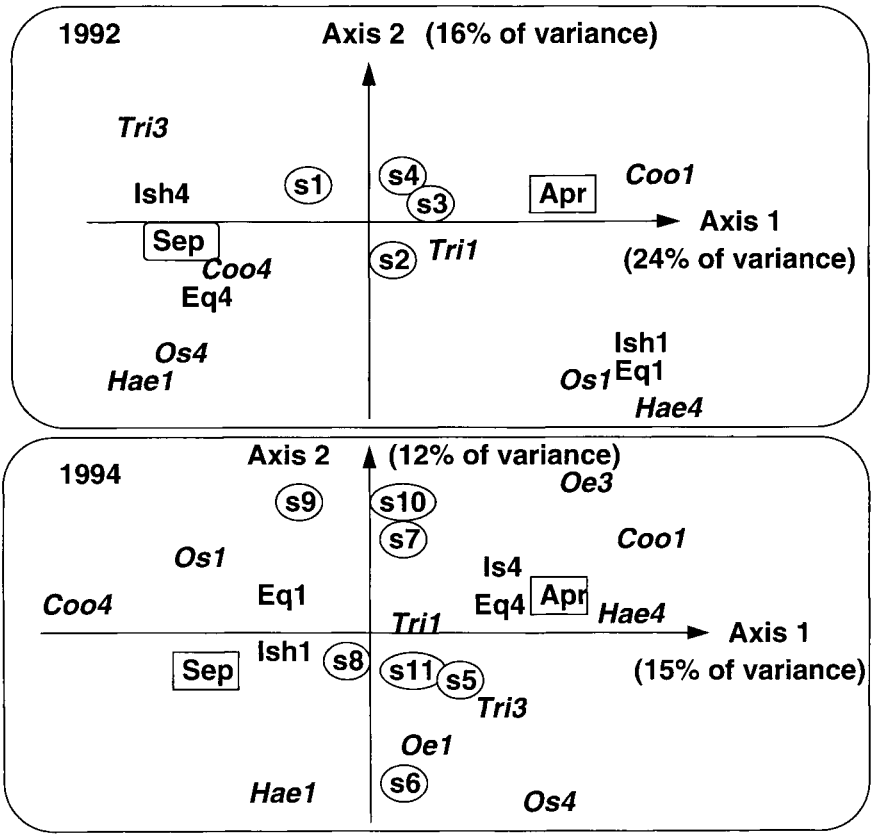

Figure 5. Diversity and proportions of the trichostrongyle genera as assessed by faecal cultures in relation to sire and month of sampling: multiple correspondence analyses. The numerical data were arranged into four classes (three for *Trichostrongylus* and *Oesophagostomum*) including an equal number of hosts, coded from one to three or four on increasing values of the following measured variables in 1992 and 1994 experiments: Coo stands for *Cooperia* percentage in population (Coo1: absence, Coo4 > 50), Haem for *Haemonchus* (Hae1: < 10, Hae4 > 55 in 1994 and > 88 in 1992), Os for *Ostertagia* (Os1: < 10, Os4 > 31 in 1992 and > 49 in 1994), Tri for *Trichostrongylus* (Tri1: absence, Tri3 > 8), Oe for *Oesophagostomum* (Oe1: absence, Oe3 > 12 in 1994). The Shannon index (Ish) and equitability (Eq) were also coded from one to four based on increasing values. The categorical variables were month (Apr, April and Sep, September) and sires (s1 to s4 in 1992 and s5 to s11 in 1994). The categorical variables, Shannon index and Equitability were used as supplementary variables in the analysis, i.e. they did not participate in the analysis, and were only located afterwards on the projection of active variables (proportions of trichostrongyle nematode genera) onto the plane. The variables located at the periphery of the graph are the most important descriptors of the genera community: see in 1992, Tri3, Hae1, Coo1 and Hae 4. The variables located near the origin (Tr1 and sires 1 to 4 in 1992) are not important descriptors of the studied situation. The proximity of variables located at the periphery of the figure indicates that these variables are closely related. For example, in the 1992 data, a cluster including Ish1, Eq1, Os1, Hae4 is observed, which indicates that lower values of Ish and Eq are found in a similar parasitic situation, e.g. associated low *Ostertagia* and high *Haemonchus* percentages. Conversely, variables found at the two extremes of one axis are negatively correlated (see September and April, or low and high percentage of *Haemonchus*).

acterized by any particular assemblage, the s9, s10 and s7 groups with a high intensity of *Oesophagostomum* and/or low infection with *Trichostrongylus,* and the s6 progeny had a lower intensity of *Oesophagostomum* and/or high *Trichostrongylus* infection. The differences were slight, however, as axis 2 represented only 12 % of variance.

4. DISCUSSION

The advantage of estimating diversity from L3 in faecal cultures is obvious. It does not require much time, it is not destructive, and as such, can be repeated on several occasions. It is also a fairly good indicator of risk [21]. Conversely, the percentage of genera established by means of faecal cultures is not an exact reflection of the assemblage of worm communities in the animal as was shown by the two necropsies performed in 1992. The correlation between percentage of genera established either by faecal cultures or adult worm numerations at necropsy are relatively high (from 0.4 to 0.7 depending on genera) according to Bryan and Kerr [5]. Further investigations are needed to relate the groupings based on necropsy data and the larval faecal cultures. Some species, *Haemonchus placei* and to a lesser extent *Cooperia* sp. [1] are known to shed large numbers of eggs, and as a result they will be over-represented in faecal cultures. Whereas others, such as *Trichostrongylus* sp. and *Ostertagia* [22], shed fewer eggs and will be under-represented in faecal cultures. This was apparently the case in our study if we compare the results from the June 1992 necropsies and April faecal cultures. The low intensity of *Haemonchus* at necropsy is in contrast with its high frequency in faecal cultures. The L3 are a very poor indicator of helminthic fauna during periods of larval inhibition. In the Pampas, the most inhibited worms are *O. ostertagi* [20]. Although faecal cultures offer a distorted picture of generic worm burden, they appear to be a repeatable instrument (our data from August and September 1992) which might be of value for comparing calf infection levels. This agreed with Barlow and Piper [4], who found repeatabilities of, respectively, 0.30, 0.31 and 0.25 for *Haemonchus*, *Cooperia* and *Oesophagostomum* larval counts in cattle, which were reasonably high and similar to those recorded for *Eimeria* oocysts (0.25 [23]) or egg counts (0.56 [3]) in lambs. Information derived from Shannon indices or equitabilities is similar to that derived from the description of assemblages by the proportion of the different genera using a correspondence analysis. These indices might be useful summaries of diversity.

The variability of between-sire progeny nematode genera diversity was small. Thus in 1992, the progeny of related sires were very similar and in the 1994 experiment, the progeny of unrelated sires were also similar with respect to the major genera. This agreed with the findings of Barlow and Piper [4] or Seifert [16]. Estimates of egg count heritability were not much different for genera which were largely represented in the assemblage (*Haemonchus*, *Cooperia* or *Oesophagostomum*). This fact indicated that resistance to nematodes was probably an 'all strongyles' one, rather than resistance directed toward a particular genus or species. This statement was supported in our data by the absence of relationship between the Shannon index of diversity and an estimate of intensity of infection based on the number of larvae per gram of faeces. Differences in genera assemblages appear to be the consequence of environmental factors such as climate or previous contamination of pasture, as previously shown in calves of the same region [21], rather than individual host responses.

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