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Seasonal distribution of gastrointestinal nematode infections in sheep in a semiarid region, northeastern Brazil

Distribuição sazonal de infecção por nematóides gastrintestinais em ovinos em área semiárida, Nordeste do Brasil

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Abstract

The objective of this study was to determine the seasonal distribution and gastrointestinal nematode parasite load in crossbred Santa Inês tracer lambs, and to correlate the rainfall during the study period with occurrences of parasitic infections. Sixty-four male tracer lambs between the ages of four and eight months were used in the study. Two tracer lambs were inserted into the herd every 28 days to determine the pattern of infective larvae available in the environment. Variation in the fecal egg count (FEC) of nematodes was observed at the study site, with many samples containing undetectable parasite loads during the dry season. The larvae identified in coprocultures were *Haemonchus* sp., *Trichostrongylus* sp., *Cooperia* sp., *Strongyloides* sp. and *Oesophagostomum* sp. The nematodes recovered at necropsy were *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Cooperia punctata*, *C. pectinata*, *Trichuris* sp., *Oesophagostomum* sp. and *Skrajabinema ovis*. The total number of larvae and the total number of immature and adult forms recovered from the tracers showed seasonal distributions that significantly correlated with the amount of rainfall received that month (p value ≤ 0.000 in all cases). The species *H. contortus* was predominant in the herd and should be considered to be main pathogenic nematode species in these hosts under these conditions.

Keywords: FEC, tracer animals, *Haemonchus contortus*, *Trichostrongylus* spp., *Cooperia* spp.

Resumo

O objetivo deste estudo foi determinar a distribuição sazonal e a carga parasitária de nematóides gastrintestinais em cordeiros traçadores, mestiços da raça Santa Inês, e relacionar a ocorrência dessas infecções com a variável chuva no mês. Foram utilizados 64 animais, machos, com idade variando entre quatro e oito meses. Em intervalos de 28 dias, dois traçadores foram inseridos no rebanho para determinar o padrão de disponibilidade de larvas infectantes no ambiente. Variação na contagem de ovos por grama (OPG) de nematóides foi observada no local do estudo, com alta frequência de amostras com carga parasitária indetectável no período seco. Das coproculturas foram identificadas larvas de *Haemonchus* sp., *Trichostrongylus* sp., *Cooperia* sp., *Strongyloides* sp. e *Oesophagostomum* sp. Os nematóides recuperados à necropsia foram *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Cooperia punctata*, *C. pectinata*, *Trichuris* sp., *Oesophagostomum* sp. e *Skrajabinema ovis*. O total de larvas, de formas imaturas e de adultos recuperados dos traçadores, mostraram distribuição sazonal significativamente relacionada com a ocorrência de chuva no mês (valor- $p \leq 0,000$, para todos os casos). A espécie *H. contortus* foi predominante no rebanho e deve ser considerada a espécie de nematóide mais importante nesses hospedeiros, sob as condições do estudo.

Palavras-chave: OPG, animais traçadores, *Haemonchus contortus*, *Trichostrongylus* spp., *Cooperia* spp.

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Introduction

The sheep industry is a traditional activity comprising small farms and subsistence animal-rearing in the northeastern region of Brazil (JESUS-JUNIOR et al., 2010; SIMPLÍCIO et al., 2003), and 56.7% of the national sheep flock is found there (IBGE, 2010).

This activity provides an alternative supply of meat and dairy products, thus contributing towards improving the food quality of the rural population and keeping people settled on the land (LEITE, 2004). Nevertheless, it has not ensured availability of these animals for industrial production. The reasons range from climatic conditions to inappropriate herd handling.

With regard to sanitary management, one of the most important issues in connection with the sheep industry is infection by parasites of the sheep's gastrointestinal tract (VIEIRA et al., 1997; SILVA et al., 1998), since these can cause metabolic and systemic changes that affect animal growth and milk and wool production (DARGIE; ALLONBY, 1975; HOLMES, 1987; FOX, 1997).

In animals on pasture, the majority of these infections are mixed (SOUZA et al., 2012), even if the impact depends on the intensity of the infection and the host's immune and physiological status.

Implementation of appropriate management practices for promoting animal health is a real challenge for breeders worldwide. Use of anthelmintic treatment has been questioned because of the emergence of parasite strains that are resistant to the drugs commonly used (MELO et al., 2003). Moreover, growing numbers of consumers are demanding animal products that are free of pathogens and any chemical residues (KRAMER; THAMSBORG, 2007). In addition, climate change will probably cause changes to both the epidemiology and the intensity of parasite infections (WALTHER et al., 2002).

To support management practices, epidemiological knowledge about the more prevalent and more pathogenic parasite species in each region is needed. Therefore, the objectives of this study

were to determine the seasonal distribution and parasite load of gastrointestinal nematodes in tracer lambs and to correlate the rainfall during the study period with occurrences of parasitic infections.

Materials and Methods

Study area and herd management

The field study was conducted on the São Vicente farm in the municipality of Lajes (5° 42' 00" south and 36° 14' 41" west), Rio Grande do Norte, Brazil (Figure 1), from March 2005 to August 2007.

The field study area has a semiarid climate with low precipitation that is irregular and occurs mainly between January and June (Figure 2). The predominant vegetation is hyperxerophilous caatinga with an abundance of cacti and shrubs. The pasture area comprised 897 hectares, divided into four plots, on which sheep of different age groups were allowed to graze. The native vegetation constituted the staple food for the flock. The farm also had a small number of cattle that did not graze in the same locations as the sheep.

The farm had a herd of approximately 1,200 predominantly crossbred Santa Inês sheep (*Ovis aries*), of which around 750 were breeding females and 20 were breeding males. These males comprised one Dorper, one Morada Nova and 18 Santa Inês sheep.

The animals that were being finished were provided with water and mineral salt *ad libitum*. Twice a day, these animals received a commercial concentrate specific for fattening sheep (186 g/animal). Furthermore, their diet was supplemented with a mixture of salt and sugar cane molasses.

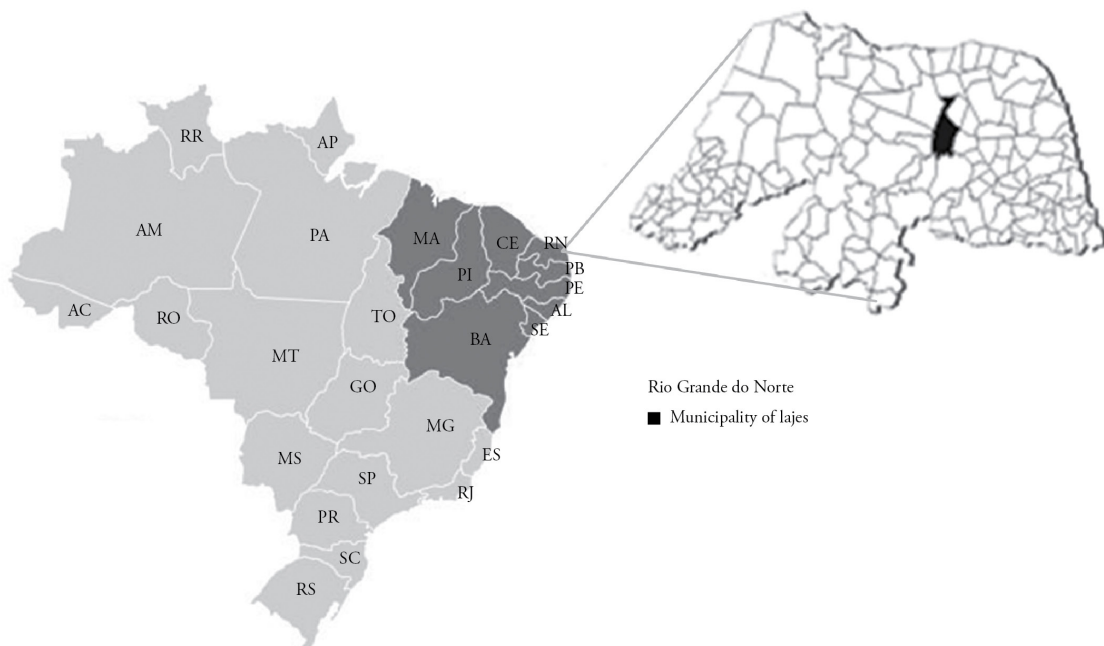


Figure 1. Northeastern region of Brazil, state of Rio Grande do Norte and municipality of Lajes, where the samples were collected.

Anthelmintic treatment was administered to the lambs before confinement and to the pregnant sheep, when deemed necessary. For the remainder of the herd, this was done at the beginning of the rainy season and when there were clinical signs of helminthiasis. The drugs used were albendazole and Ripercol L.

The breeding scheme was continuous, but with a greater number of births during the rainy season. After birth, the lambs were identified and allowed to feed at the udder for two months.

Experimental animals and collection and processing of fecal samples

Two helminth-free tracer lambs were introduced into the herd every 28 days. These animals were crossbred Santa Inês males (four to eight months of age). Samples were collected from the tracer lambs on day 1 (before anthelmintic treatment: B-ttt), day 14 (after anthelmintic treatment: A-ttt) and day 44 (before autopsy: Aut).

Feces were collected directly from the rectum, placed individually in polypropylene collectors and transported to the lab in coolers containing ice cubes. The fecal egg count (FEC) of nematodes was determined by means of the modified McMaster technique (UENO; GONÇALVES, 1988). Each sample was coprocultured, and 100 larvae were identified per sample (WYK et al., 2004).

Tracer animals and procedure

The tracer lambs were treated with albendazole (Albendazol, Labovet) at a dose of 3.8 mg/kg of live weight and levamisole hydrochloride (Ripercol L, Fort Dodge, Brazil): 5.0-7.5 mg/kg for animals that weighed 10-15 kg and 7.5-10.0 mg/kg for animals that weighed 16-20 kg. The animals were held in quarantine for 14 days to confirm that they were free from nematodes. The tracers were introduced into the herd and allowed to graze for 28 days. They were then put into stalls for two days, and on the 44th day of the experiment, the animals were weighed, slaughtered and autopsied.

This work scheme led to occurrences of two autopsies in months with 31 days (August 2005 and August 2006). Also, there were two autopsies in February 2006, but in this case it was due to adjustments to the farm's work schedule.

The gastrointestinal tract was removed and separated into three sections: abomasum, small intestine and large intestine. The abomasum was opened longitudinally, along the greater curvature, using scissors. The contents of the abomasum were collected in a plastic receptacle and the mucosal surface was immediately washed with warm (about 30 °C) mammalian Ringer's solution using moderate pressure. The contents and the wash solution were packed in an aluminum container at room temperature for 30 minutes prior to fixation. Each section of the intestine was opened longitudinally using an enterotome and then washed.

The abomasum and small intestine were distended across stainless steel screens and the mucosal surfaces were brought into contact with mammalian Ringer's solution at 37 °C for two hours in a stainless steel container. The liquid was then passed through a metal sieve with 38 µm pores and the material retained in the sieve was added to the contents of the corresponding organ (UENO; GONÇALVES, 1988).

The final volume from each segment of the digestive tract was hot-fixed (60 °C) at a ratio of one part formaldehyde for every ten parts of intestinal contents, as described by Anderson and Verster (1971).

The fixed material from the abomasum and small intestine was passed through a metal sieve with 38 µm pores, and the fixed material from the large intestine was passed through a metal sieve with 150 µm pores under a strong stream of water. All of the material retained in the sieves (including nematodes) was stained with an aqueous iodine solution and examined under a stereomicroscope. The nematodes of the gastrointestinal tract were separated and quantified (EYSKER; KOOYMAN, 1993), and were then clarified in a lactophenol solution for species identification.

The data were expressed as prevalences and mean intensities. The prevalence was the number of hosts infected with one or more individuals of a particular parasite species, divided by the number of hosts examined for that parasite species. The mean intensity was the total number of parasites of a particular species found in a sample, divided by the number of hosts infected with that parasite (BUSH et al., 1997).

Weather data

Climatological reference values for maximum temperature, minimum temperature and relative humidity were obtained from EMPARN (Empresa de Pesquisa Agropecuária do Rio Grande do Norte).

The monthly amounts of rain over the course of the study period were obtained from the EMPARN weather station located in the urban center of the municipality.

Statistical analysis

The statistical analysis was conducted using the R software, version 3.0.1, with the statistical significance level set at $p < 0.05$. The variable of rainfall during the month was defined as the total rainfall (mm) during the thirty days prior to fecal sample collection. The relationships between the independent variable of rainfall during the month and other dependent variables were analyzed using a Poisson regression. The dependent variables examined were the FEC of nematodes, the number of *Haemonchus* sp. larvae in the coprocultures and the total number of nematodes recovered from the autopsy. The *Haemonchus* sp. larvae were taken into consideration in the statistical analysis because they presented greater prevalence and intensity in the coprocultures.

The relationship between the variable of the animals' weights (continuous variable) and the variables of rainfall during the month and total number of nematodes recovered at autopsy were analyzed by means of linear regression.

Results

Fecal egg count and coprocultures

A total of 64 animals were examined prior to receiving anthelmintic treatment; 58 of these animals were slaughtered and

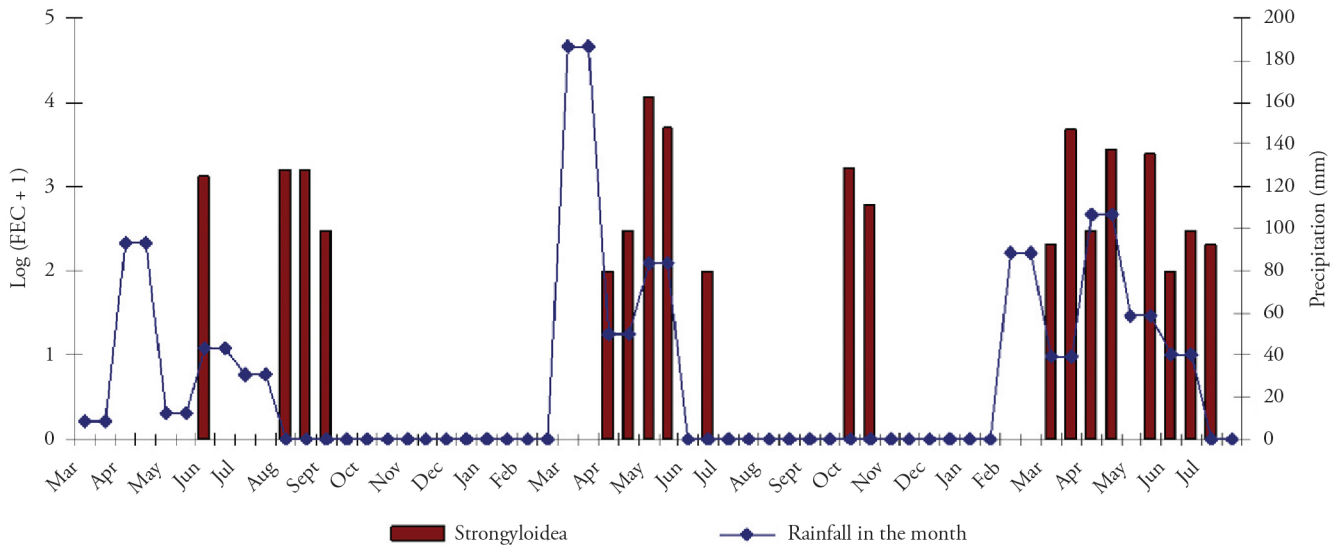


Figure 2. Temporal distribution of eggs from the feces of tracer lambs in samples collected before anthelmintic treatment, in relation to the rainfall during that month; samples were collected between March 2005 and July 2007 in the municipality of Lajes, Rio Grande do Norte, Brazil.

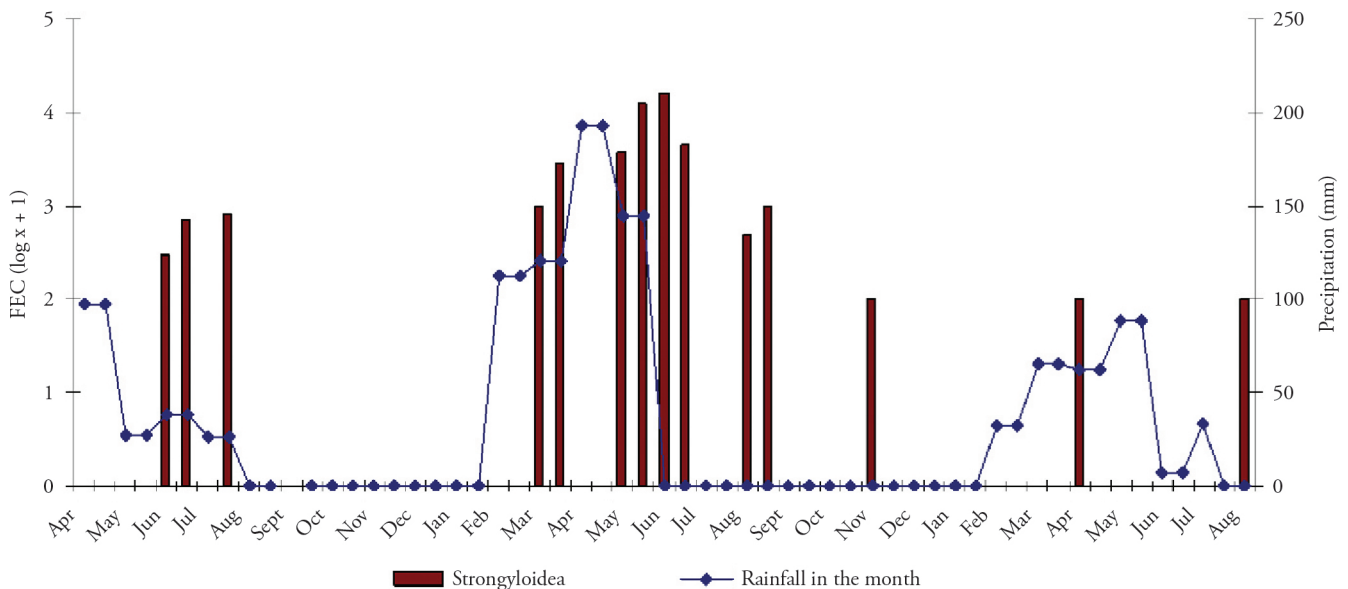


Figure 3. Temporal distribution of eggs from the feces of tracer lambs in samples collected at autopsy, in relation to the rainfall during that month; samples were collected between April 2005 and August 2007 in the municipality of Lajes, Rio Grande do Norte, Brazil.

autopsied. One animal (no. 32) that should have been autopsied in September 2005 died before the scheduled date for autopsy. Another animal (no. 165) disappeared from the herd in July 2007, and was never found.

The FEC from the animals sampled before treatment ranged from 100 to 11,800 and the FEC from the animals autopsied ranged from 100 to 16,000. In both cases, the FEC of nematodes did not show any significant relationship with variation in rainfall.

In order to have a better overall view of the data, we chose to transform the data to a more suitable scale. Thus, the FEC data were transformed into $\log_{10}(x + 1)$ form, where x represented

the FEC for each animal. Moreover, to avoid occurrences of $\log_{10}(0)$, we added one unit to all the FEC values (Figures 2 and 3).

The coprocultures from the 64 animals sampled before treatment were positive for *Haemonchus* sp. (25.0%), *Trichostrongylus* sp. (4.7%) and *Oesophagostomum* sp. (9.4%). The coprocultures from the 58 animals autopsied were positive for *Haemonchus* sp. (44.4%), *Trichostrongylus* sp. (22.2%), *Cooperia* sp. (3.7%), *Strongyloides* sp. and *Oesophagostomum* sp. (1.9%). The variable of rainfall showed significant relationships with the presence of *Haemonchus* sp. larvae in the coprocultures of material collected before treatment (p value ≤ 0.000) and at autopsy (p value ≤ 0.000).

The animals whose coprocultures were positive in samples collected after anthelmintic treatment were excluded from necropsies. These animals were no. 33, which should have been autopsied in September 2005 (coproculture with larvae of *Trichostrongylus*); no. 54 and no. 55A, which should have been autopsied in July 2006; and no. 67, which should have been autopsied in November 2006 (in both cases with positive coprocultures for *Haemonchus*).

Helminths present in tracers

A total of 14,646 nematode specimens were recovered during the study, including both adult and immature forms (Table 1). *H. contortus* represented 84.2% of the nematodes collected, with

a total of 12,332 specimens, a range from 1 to 4,809 nematodes isolated from each animal and an average of 212.6 nematodes/animal (Figure 4). The prevalence of immature *Haemonchus* (L₄ final) was 20.7%.
Trichostrongylus sp. represented 12.9%, *Cooperia* sp. 1.4%, *Trichuris* sp. 0.6% and *Oesophagostomum* sp. 0.04% (Figure 5 and Figure 6). *S. ovis* represented 0.007%, with only one specimen (Table 1).
The number of nematodes isolated from each tracer animal ranged from 1 to 5,588, with the greatest variation found in *Haemonchus* sp. The variation in rainfall showed a significant relationship with the total number of nematodes recovered during the autopsy (p value \cong 0.000).

Table 1. Prevalence and mean intensity of nematodes recovered from autopsied tracer lambs; samples were collected between April 2005 and August 2007 in the municipality of Lajes, Rio Grande do Norte, Brazil.

Nematodes Genus/Specie	Prevalence %	Mean Intensity											
		Jan (4)	Feb (6)	Mar (4)	Apr (6)	May (6)	June (6)	July (3)	Aug (10)	Sept (2)	Oct (4)	Nov (3)	Dec (4)
<i>H. contortus</i>	62.9	0	0	363.5	208.5	475	1026.8	40.6	7.3	0	0.8	22.3	0
<i>Haemonchus</i> sp. (L ₄ last)	19.4	0	0	33.0	0.5	16.7	0	0	0.7	0	0	0	0
<i>Cooperia</i> sp.	6.5	0	0	0	0	0.2	0	0	0.4	0	0	0	0
<i>Cooperia pectinata</i>	6.5	0	0	30.0	0.2	6.5	0.2	0	0	0	0	0	0
<i>Cooperia punctata</i>	11.3	0	0	0.5	2.0	0.5	3.0	0	0	0	0	0	0
<i>Trichostrongylus</i> sp.	11.3	0	0	0.5	0.3	0	0.3	0	1.3	0	0	0	0
<i>T. colubriformis</i>	21.0	0	0	2.3	3.8	122.3	126.8	0	34.7	0	0	0	0
<i>Oesophagostomum</i> sp. (L ₄ initial)	1.6	0	0	0	0.5	0	0	0	0	0	0	0	0
<i>Oesophagostomum</i> sp. (L ₄ last)	8.1	0	0	0	2.8	0.5	0	0	1.9	0	0	0	0
<i>S. ovis</i>	1.6	0	0	0.3	0	0	0	0	0	0	0	0	0
<i>Trichuris</i> sp.	27.4	0.3	0.7	1.3	0.8	0	0	0	2.4	0.5	0	4.8	6.5

() Total number of autopsied animals.

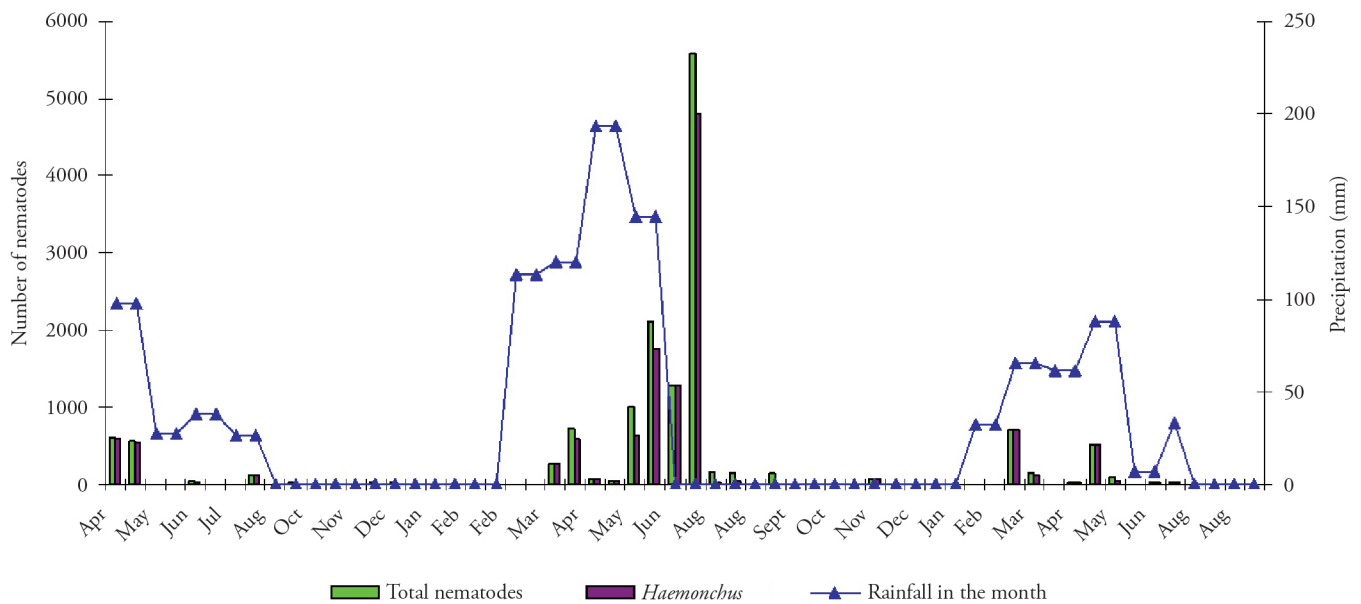


Figure 4. Temporal distribution of the numbers of gastrointestinal nematodes and *H. contortus* recovered from autopsied tracer lambs, in relation to the rainfall during that month; samples were collected between April 2005 and August 2007 in the municipality of Lajes, Rio Grande do Norte, Brazil.

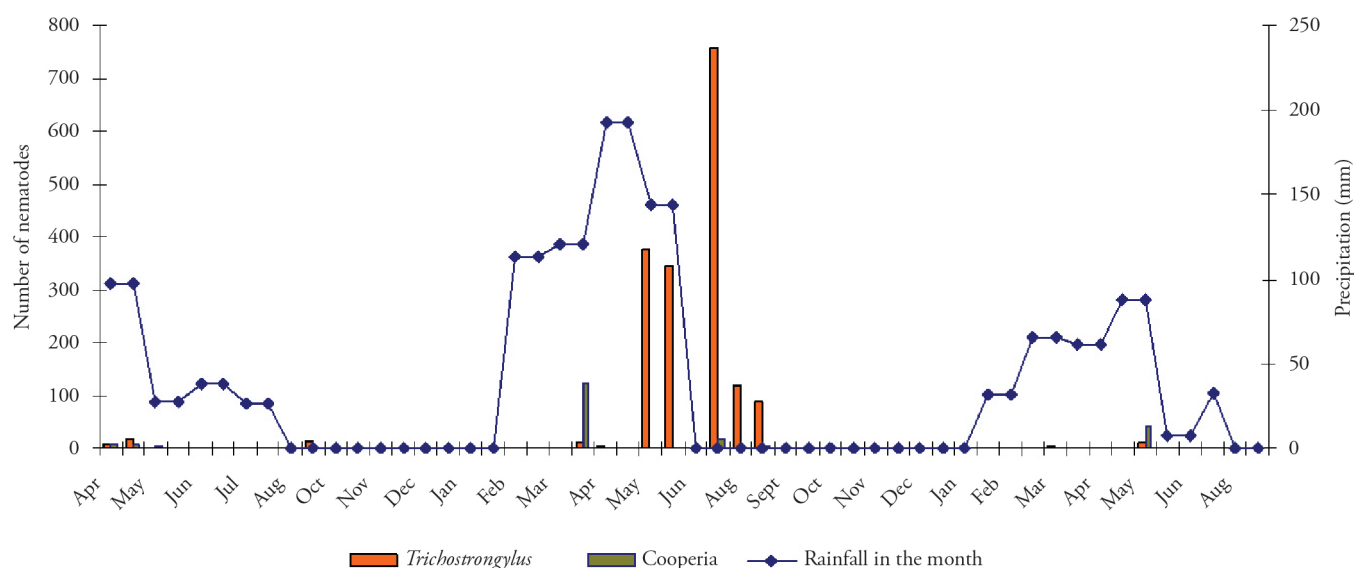


Figure 5. Temporal distribution of *Trichostrongylus* sp. and *Cooperia* sp. recovered from autopsied tracer lambs, in relation to the rainfall during that month; samples were collected between April 2005 and August 2007 in the municipality of Lajes, Rio Grande do Norte, Brazil.

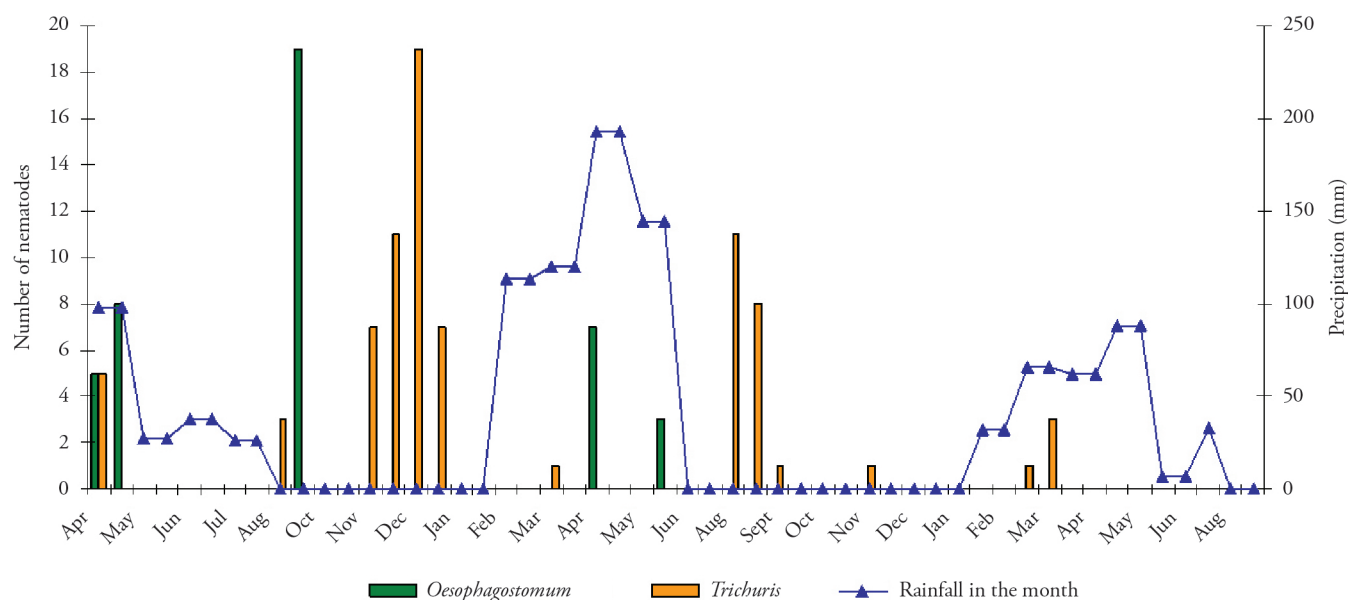


Figure 6. Temporal distribution of *Oesophagostomum* sp. and *Trichuris* sp. recovered from autopsied tracer lambs, in relation to the rainfall during that month; samples were collected between April 2005 and August 2007 in the municipality of Lajes, Rio Grande do Norte, Brazil.

The weights of the animals autopsied ranged from 10 to 26 kg, with a mean weight of 18.0 kg ($s = 4.17$) and a median of 19 kg. The variable of the animals' weights was unrelated to the variations in rainfall and to the total number of nematodes recovered during the autopsy.

The climatological data are shown in Figure 7. The temperature maxima ranged from 29.2 to 32.2 °C (July and January, respectively). The minimum temperature ranged from 23.6 to 26.2 °C (July and February, respectively). Relative humidity ranged from 60.0% to 81.0% (September and April, respectively).

Discussion

The great variation in the FEC in tracer lambs was due to the occurrence of a few cases with high numbers of parasites (UENO; GONÇALVES, 1988) and the high frequency of samples in which parasite loads were not detected. A previous study on Santa Inês lambs in São Paulo, Brazil, showed a FEC that remained below 1,000 during an experiment that lasted for more than ten months (AMARANTE et al., 2004).

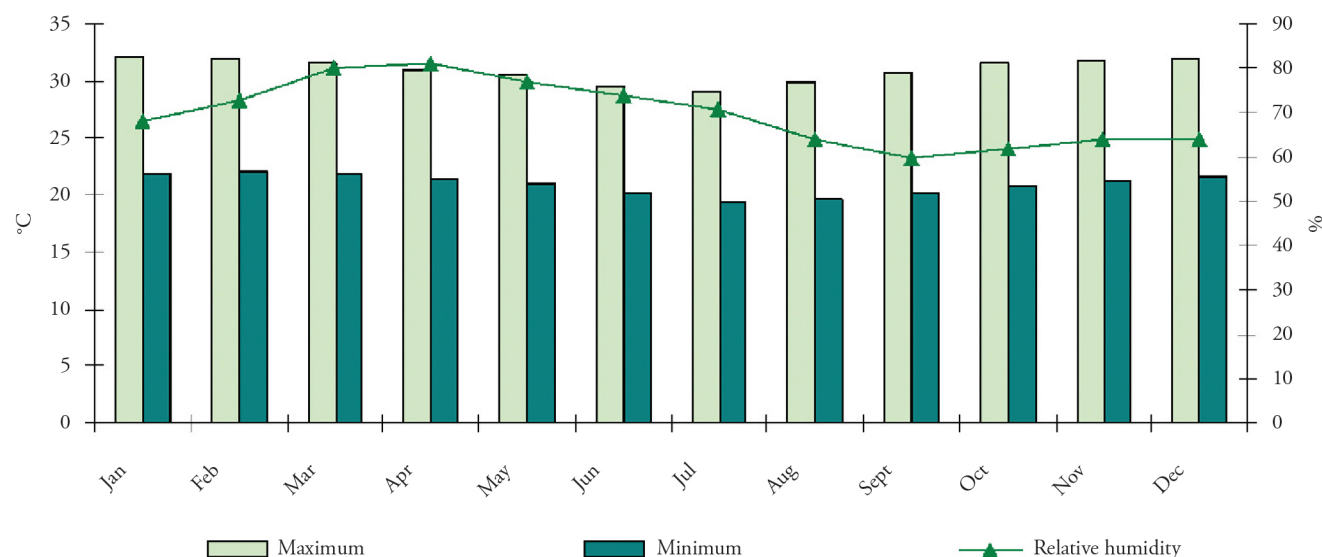


Figure 7. Estimated temperature (°C) and relative humidity (%) data between January and December 2006, in the municipality of Lajes, Rio do Norte, Brazil.

The greatest parasite load detected by the FEC came from samples obtained during the rainy season (the period from the onset of the rains until about two months after the end). This pattern agrees with the findings from other studies, even those conducted under different climatic conditions (LIMA, 1998; AGYEI et al., 2004).

Haemonchus sp. larvae were the predominant larvae identified in the coprocultures, followed by *Trichostrongylus* sp., *Oesophagostomum* sp., *Cooperia* sp. and *Strongyloides* sp. *Oesophagostomum* sp. larvae were only found in samples collected before anthelmintic treatment, because of the peculiar traits of this parasite's life cycle (DASH, 1973; BRITO et al., 1996; PIMENTEL-NETO et al., 1999).

Coprocultures on some of the samples collected after treatment were positive. This indicates that these nematodes may have been resistant to both anthelmintics used in this experiment.

The occurrences of *Haemonchus* sp. larvae were discontinuous and statistically related to variations in monthly rainfall. *Strongyloides* sp. larvae were seen only in one sample, which was collected during the autopsy (no. 56). This animal was four months old at the beginning of the experiment and six months old at the time of the autopsy and also had a low weight (11 kg). In this case, young age and nutritional deficiencies may have been factors contributing towards establishment of this infection. *S. papillosus* has been found in goats aged five to six months (SILVA et al., 1998).

The degree of infection due to *Haemonchus* spp. in sheep can be considered light when the total number of nematodes recovered is less than 500, moderate when it is between 500 and 1,500, heavy when it is greater than 1,500 and fatal when it is more than 3,000 (UENO; GONÇALVES, 1988). According to these parameters, the parasite loads in this study were light for most of the animals.

Interpreting the significance of the parasitic load is a challenge. In naturally infected Corriedale lambs, between 12 and 8,943 specimens of *Haemonchus* were recovered per lamb, but the secretory

epithelium was intact in all animals (OLIVEIRA-SEQUEIRA et al., 2000; REINECKE; GROENEVELD, 1991). However, it seems that low parasite loads have pathogenic significance in arid and semiarid regions (MORALES et al., 2001).

H. contortus was the species that was most prevalent and also the species that reached the greatest parasite load in the host. In the semiarid climatic conditions of Brazil, this species has also frequently been found in tracer goats, with a prevalence that varies among studies (CHARLES, 1989; VIEIRA et al., 1997). This indicates that *H. contortus* is able to survive at high temperatures and low relative humidity.

Immature forms of *H. contortus* were found only as L₄ last, thus indicating that this study found no evidence of hypobiosis. The source of infection for the tracer lambs must have been the animals of the herd.

The second most frequently identified and abundant nematode found in autopsied animals was *T. colubriformis*, which is in agreement with other studies conducted on sheep (VIEIRA et al., 1997) and goats (SILVA et al., 1998) in this region.

Two species of *Cooperia* were observed: *C. punctata* was the most prevalent and *C. pectinata* had greater intensity in infections. Almost one third of the tracer lambs were infected with *Trichuris* sp., but the mean intensity of these infections was low. Infections with *Oesophagostomum* sp. occurred at low frequency and with low intensity. Studies on the prevalence of the species *O. columbianum* in goats in northeastern Brazil have reported a range from 22.6% (VIEIRA et al., 1997) to 87.0% (CHARLES, 1989).

Occurrences of nematodes in the tracers, other than *Trichuris*, were observed from around one month after the beginning of the rains until around two months after the end of the rains, when it was still possible to find infecting larvae on the pasture. In November 2006, in the middle of the drought period, light rain occurred and consequently, nematode occurrences in the autopsies were observed.

Infection by *Trichuris* occurs through ingestion of eggs of this nematode. These eggs are eliminated with the feces of the infected animals and are spread on the ground when the fecal matter decomposes. Because the vegetation becomes scarce and the soil is uncovered during the drought period in the semiarid region, the animals seek remnants of food in the soil and thus may come into contact with the *Trichuris* eggs and become infected.

The infection rate due to *Trichuris* is low because, even though the eggs are resistant, the climatic conditions of the semiarid region during the drought months are quite adverse, because of both the lack of rain and the relative low humidity and high temperature.

Santa Inês lambs are more resistant to helminthic infection than Suffolk and Ile de France lambs (AMARANTE et al., 2004). However, despite this resistance and the adverse weather conditions during some parts of the year, the present study revealed that helminthic infections in Santa Inês lambs can be intense during the rainy season.

In conclusion, variation in the FEC of nematodes was observed at the study site, with many samples possessing undetectable parasite loads during the dry season. The rainfall variation between the months showed a significant relationship with the number of nematodes recovered at autopsy. The species *H. contortus* occurred throughout the rainy season. It was the most prevalent and abundant species found in the tracer lambs and varied in intensity among the animals. These results showed that there is a seasonal pattern of gastrointestinal nematode infection prevalence in sheep.

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