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Gastrointestinal nematode infections in sheep raised in Botucatu, state of São Paulo, Brazil

Infecções por nematódeos gastrintestinais em ovinos criados em Botucatu, estado de São Paulo, Brasil

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Abstract

Gastrointestinal nematode infections were evaluated in sheep raised in Botucatu, state of São Paulo, Brazil between April 2008 and March 2011. Every month, two tracer lambs grazing with a flock of sheep were exposed to natural infection with gastrointestinal nematodes for 28 consecutive days. At the end of this period, the lambs were sacrificed for worm counts. *Haemonchus contortus* presented 100% of prevalence. The seasons exerted no significant influence on the mean intensity of *H. contortus*, which ranged from 315 worms in November 2010 to 2,5205 worms in January 2011. The prevalence of *Trichostrongylus colubriformis* was also 100%, with the lowest mean intensity (15 worms) recorded in February 2011 and the highest (9,760 worms) in October 2009. In the case of *T. colubriformis*, a significant correlation coefficient was found between worm counts vs. rainfall (r = –0.32; P < 0.05). Three other nematodes species were found in tracer lambs, albeit in small numbers. Their prevalence and mean intensity (in parenthesis) were as follows: *Oesophagostomum columbianum* 28% (25.2), *Cooperia curticei* 7% (4.5) and *Trichuris* spp. 2% (1). In conclusion, the environmental conditions of the area proved to be highly favorable for the year-round transmission of *H. contortus* and *T. colubriformis*.

Keywords: *Haemonchus contortus, Trichostrongylus colubriformis,* tracer lamb, nematode burden, seasonal variation.

Resumo

A ocorrência de infecções por nematódeos gastrintestinais foi avaliada de abril de 2008 até março de 2011em ovinos criados em Botucatu, estado de São Paulo. Todos os meses, dois cordeiros traçadores foram expostos à infecção natural por nematódeos gastrintestinais, durante 28 dias consecutivos, ao pastejar junto com um rebanho de ovelhas. Ao final desse período, os animais foram sacrificados para a identificação e quantificação dos helmintos. *Haemonchus contortus* apresentou prevalência de 100%. Não houve influência significativa das estações do ano na intensidade média de *H. contortus,* que variou de 315 vermes em novembro/2010 a 25.205 vermes em janeiro/2011. *Trichostrongylus colubriformis* também apresentou prevalência de 100% com a menor intensidade média (15 vermes) em fevereiro/2011 e a maior (9.760 vermes) em outubro/2009. No caso de *T. colubriformis*, houve correlação significativa entre as contagens de vermes x precipitação (r = –0,32; P < 0,05). Outras três espécies de nematódeos foram encontradas nos cordeiros traçadores, no entanto em pequenas quantidades, com as seguintes prevalências e intensidades médias (entre parênteses): *Oesophagostomum columbianum* 28% (25,2), *Cooperia curticei* 7% (4,5) e *Trichuris* spp. 2 % (1). Em conclusão, as condições ambientais da área foram muito favoráveis durante todo o ano para a transmissão de *H. contortus* e *T. colubriformis*.

Palavras-chave: *Haemonchus contortus, Trichostrongylus colubriformis,* cordeiro traçador, carga parasitária, variação sazonal.

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Introduction

Gastrointestinal nematode infections are the major constraint to sheep production worldwide. The distribution of different nematode species and the risk of massive infections are greatly influenced by environmental conditions. For instance, *Teladorsagia circumcincta* is an important parasite of sheep in locations with long cold winters (MEDEROS et al., 2010), while its presence is not observed in sheep raised in regions with higher temperatures, where other species, particularly *Haemonchus contortus* and *Trichostrongylus colubriformis*, predominate among the parasitic fauna (AMARANTE et al., 2004; SILVA et al., 2012a). The occurrence of *H. contortus*, however, is not restricted only to high temperature regions, for it has been reported in sheep flocks raised even at latitudes near the polar circle (LINDQVIST et al., 2001). *H. contortus* demonstrates an impressive capacity to adapt and survive in adverse environmental conditions. It has been demonstrated that the worm population can survive inside the host for more than a year, producing fertile eggs that ensure its preservation in a flock (SANTOS et al., 2014).

Several factors influence the epidemiology of parasitic diseases. The degree of environmental contamination by the free living stages of nematodes is greatly influenced by the interaction between two major factors: first, the presence of susceptible animals shedding large numbers of eggs in feces, and second, environmental conditions suitable for the development and survival of the free living stages of nematodes. Other factors are also important, such as the number of animals per area and the frequency and efficacy of anthelmintic treatments.

In this study, gastrointestinal nematode infections in sheep were evaluated monthly from April 2008 to March 2011 in Botucatu, state of São Paulo, in a region with a humid subtropical climate characterized by warm and wet conditions in summer and low rainfall and mild temperatures in winter.

Materials and Methods

Study location

The experiment, which was approved by the Ethics Committee for Animal Research of UNESP – 86/07 – CEEA, was conducted on a sheep farm belonging to the São Paulo State University – UNESP, located at 22° 82' latitude South and 48° 41' longitude West, at an altitude of 613 m a.s.l., in Botucatu, state of São Paulo, Brazil. The climate type is Cwa, according to the Köppen classification, and the data on average temperature, relative air humidity and rainfall during the period of this study were obtained from the Department of Environmental Sciences, College of Agricultural Sciences, UNESP (Table 1).

Management of tracer lambs

The animals used in this experiment were the same as those used in a previously published study about *Oestrus ovis* epidemiology (SILVA et al., 2012b). Briefly, during the 36 months of observations, 72 Ile de France weathered male lambs less than a year old were used. The tracer animals, which were purchased from a commercial sheep farm, were naturally infected with gastrointestinal nematodes upon their arrival at the University facilities. For this reason, they were housed and orally drenched once a day for three consecutive days with levamisole phosphate (10 mg/kg, Ripercol® L 150 F, Fort Dodge) and albendazole (10 mg/kg, Valbazen® 10 Cobalt, Pfizer). A week later, the same protocol was carried out with trichlorfon (100 mg/kg, Neguvon®, Bayer S.A.) to eliminate any remaining gastrointestinal nematode infection. A series of fecal examinations were performed to confirm the elimination of infection by nematodes. While indoors, the animals were fed with an amount of concentrate (Tech Ovin Unique, Socil®) corresponding to 1% of their mean live weight and were given free access to Tifton hay and tap water.

Every month from April 2008 to March 2011, two tracer lambs were exposed to natural infection with gastrointestinal nematodes for 28 consecutive days, while grazing with a sheep flock. The sheep farm where the tracer lambs were placed had 156 Bergamasca sheep when the study began and 202 animals when it ended. The adult ewes were kept permanently in rotational grazing on *Panicum maximum* cv Tanzania grass on an area of 8 hectares. Lambing occurred in winter and, after weaning at two months of age, the young animals were kept indoors until they were over one year old. This procedure was adopted on the farm to prevent the mortality of young animals due to haemonchosis. Due to the low amount of forage during winter and early spring (from June to October), the animals received additional corn silage feed daily.

On the day the tracer animals were placed with the flock, fecal samples were collected randomly from 20 ewes on the farm. Each sample was subjected to nematode fecal egg counts (FEC) and a composite fecal culture was prepared for the production of infective larvae, which were classified to the level of genus, as described by Ueno and Gonçalves (1998).

Animals of the flock were subjected to selective anthelmintic treatment based on FEC, which was performed by the people responsible for worm control on the farm. During the study, the authors of this paper did not interfere in this worm control procedure.

Worm counts

After grazing with a sheep flock for 28 consecutive days, two tracer lambs were sacrificed monthly for worm counts. The abomasum was opened along its greater curvature and the contents placed in a container. An aliquot of 10% of the abomasal contents was preserved in 5% formalin. The mucosal layers of all the abomasa were soaked in saline solution at 38 °C for 6 h. The entire content of digested material was collected and preserved in 5% formalin. A similar procedure was employed to process the small intestine, from which an aliquot of 10% of digested material was collected. The large intestine was opened and an aliquot of 10% of the contents was collected and preserved. Worm identification and counting procedures were performed on the preserved material, as described by Ueno and Gonçalves (1998). The identification of *Cooperia* and *Trichostrongylus* down

Table 1. Monthly rainfall and averages of temperature and relative air humidity from April 2008 to March 2011.

Month /Year	Rainfall (mm)	Maximum temperature (°C)	Minimum temperature (°C)	Relative air humidity (%)
April/2008	102.8	25.6	17.4	68
May	115.7	22.8	13.8	54.7
June	30.8	24.5	13.5	60
July	0	24.6	13.8	40.5
August	104.1	25.5	15.1	53.3
September	29.9	25.5	15.1	63.4
October	153.8	26.8	18.2	72.1
November	69.3	27.3	16.6	73.2
December	136.5	28.4	17.1	70.5
January 2009	331.6	26.6	18.2	84.4
February	141.6	28.4	19.5	78.3
March	111.5	28.8	19.2	73.9
April	86.8	26.7	16.5	72.5
May	62.7	24.9	15.0	73.9
June	102.7	20.8	11.3	73.2
July	143.8	22.6	13.0	79.6
August	89.1	24.1	13.7	67.6
September	150.5	25.5	15.8	76.1
October	141.8	26.7	15.1	77.6
November	289	29.5	19.8	76.1
December	327.1	26.9	19.1	81.2
January 2010	350.5	27.5	19.5	69.7
February	134.6	29.4	20.1	53.6
March	134.6	28.4	18.7	57.3
April	71.7	26.4	17.1	56.4
May	39.5	24.4	14.1	46.3
June	22.8	25.5	12.4	48.2
July	55.3	26.3	13.7	39.8
August	0	27.6	12.3	49.5
September	63.1	27.6	13.6	52.1
October	56.1	27.7	13.4	48.2
November	139.4	28.4	16.3	56.1
December	243.2	28.7	18.3	78
January 2011	712.3	29.6	19.6	85
February	189.6	29.8	19.4	77
March	162	25.8	18.3	78

Source: Department of Environmental Sciences, College of Agricultural Sciences, São Paulo State University – UNESP, Botucatu, SP, Brazil.

to species level was based on the morphology of spicules of male specimens (UENO; GONÇALVES, 1998).

In addition, the presence and number of longitudinal ridges (synlophe) was evaluated at the esophageal-intestinal junction, at the mid-body and 1-2 mm after the mid-body of 10 male and 10 female *Haemonchus* specimens obtained from each animal, to properly determine the *Haemonchus* species present (LICHTENFELS et al., 1994). Worm processing for synlophe analysis, as well as determination of the number of ridges, were performed according to Silva et al. (2014).

Statistical analyses

An analysis of variance was performed to compare the degree of infection throughout the year. Data were transformed using log10(x + 1) prior to analysis and grouped according to the season of the year: autumn (March-April-May), winter (June-July-August), spring (September-October-November) and summer (December-January-February). Significant differences between season means were determined by Tukey's test at 5%. Spearman's rank correlation coefficients between worm burden and climatic variables (temperature, rainfall and air relative humidity) were estimated. All the analyses were performed using SAS version 9.2.

Descriptive statistical analyses were used to summarize the data, as proposed by Bush et al. (1997), using the following terms:

Prevalence: the number of hosts infected with each nematode species divided by the number of hosts examined;

Intensity (of infection): the number of each nematode species in a single infected host;

Mean intensity: the total number of each nematode species found divided by the number of hosts infected with that parasite.

Results

The mean intensity of *H. contortus* ranged from 315 worms in November 2010 to 25,205 worms in January 2011 (Figure 1). In other words, *H. contortus* presented 100% of prevalence. There was no significant influence of the seasons on the mean intensity of *H. contortus* (Table 2). No significant correlation coefficients were recorded between *H. contortus* worm counts and climatic variables. *T. colubriformis* also presented 100% prevalence, with the lowest worm burdens during the summer months (Figure 1; Table 2). In the case of *T. colubriformis*, the lowest mean intensity (15 worms) was recorded in February 2011 and the highest (9,760 worms) in October 2009. The only significant correlation coefficient was recorded between *T. colubriformis* worm counts vs. precipitation ($r = -0.32$; $P < 0.05$).

Three other nematode species were found in the tracer lambs, albeit in small numbers. Their prevalence and mean intensity (in parenthesis) were as follows: *Oesophagostomum columbianum* 28% (25.2), *Cooperia curticei* 7% (4.5) and *Trichuris* spp. 2% (1).

Each month, fecal samples were collected randomly from 20 ewes on the farm. The FEC values showed the typical aggregate distribution, with most of the sheep showing low FEC and a few with high FEC. In 29 of the 36 months of sampling, medians of FEC were lower than 1000 eggs per gram (EPG). However, several animals shed large number of eggs in feces, with values exceeding 5000 EPG on 32 occasions, and one ewe showing a maximum of 55,200 EPG in December 2009 (Table 3). *Haemonchus* and *Trichostrongylus* infective larvae presented an overall average of 93.1% and 6.5%, respectively, in fecal cultures from ewes. A few *Oesophagostomum* larvae were also detected on five occasions, with a maximum of 8% in July 2010 (Table 3).

The synlophe analysis confirmed only the presence of *H. contortus* infecting the experimental sheep, with no evidence of the presence of *Haemonchus placei*.

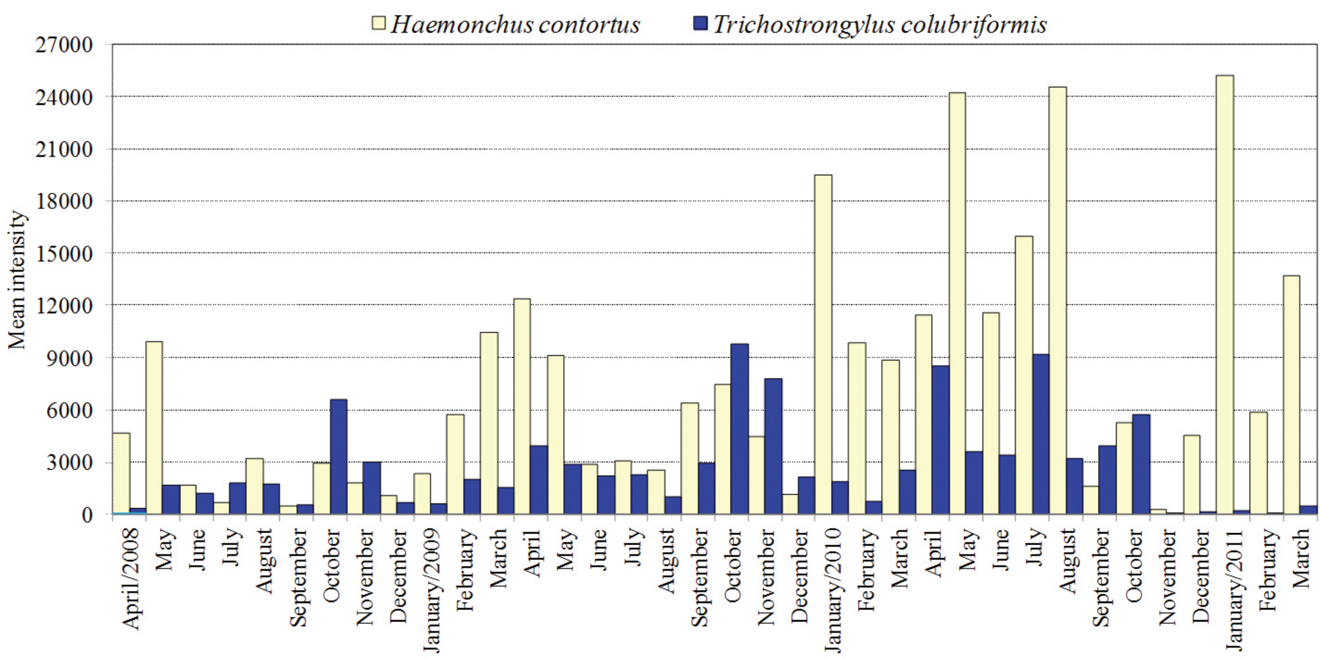

Figure 1. Mean intensity of *Haemonchus contortus* and *Trichostrongylus colubriformis* in tracer sheep from April 2008 to March 2011, in Botucatu, state of São Paulo, Brazil.

Table 2. Mean intensity and prevalence of *Haemonchus contortus* and *Trichostrongylus colubriformis* in tracer lambs that grazed on a contaminated pasture during 28 days in Botucatu, state of São Paulo, Brazil. Two lambs were evaluated per month from April 2008 to March 2011 (18 per season, with a total of 72 animals).

Species	Stage of development	Autumn	Winter	Spring	Summer
H. contortus	Early L4	1834 (0 – 11691)	3448 (0 – 21260)	550 (0 – 5581)	568 (0 – 3050)
	Late L4	888 (0 – 2590)	438 (0 – 2480)	513 (0 – 5332)	1049 (0 – 8640)
	Early L5	400 (0 – 3320)	108 (0 – 540)	786 (0 – 9619)	450 (0 – 2940)
	Late L5 and adults	6487 (333 – 15760)	3316 (186 – 12000)	4258 (40 – 12399)	5683 (5 – 18305)
	Total burden	9614 (4392 – 19186)	7316 (186 – 30350)	6109 (123 – 32942)	7755 (68 – 30421)
	Prevalence	100%	100%	100%	100%
T. colubriformis	Total burden	2448 ab (10 – 9180)	2936 a (395 – 15830)	4830 a (70 – 12840)	956 b (10 – 2620)
	Prevalence	100%	100%	100%	100%

Arithmetic means with different letters in row are significantly different. Minimum and maximum values are in parenthesis. L: larvae.

Table 3. Median (range in parenthesis) of nematode fecal egg counts (FEC) and third stage larvae in fecal cultures from samples collected randomly from 20 ewes on the experimental sheep farm.

Month /Year	FEC	Third stage larvae (%)		
		H. contortus	*T. colubriformis*	*O. columbianum*
April/2008	700 (0 – 11000)	94	6	0
May	1100 (0 – 38100)	100	0	0
June	350 (0 – 6500)	91	9	0
July	2000 (0 – 26400)	96	4	0
August	2300 (0 – 34000)	90	10	0
September	800 (0 – 8300)	96	4	0
October	400 (0 – 15600)	95	5	0
November	150 (0 – 9000)	74	26	0
December	100 (0 – 8500)	100	0	0
January 2009	1400 (0 – 22400)	99	1	0
February	250 (0 – 22600)	93	7	0
March	950 (0 – 7500)	96	4	0
April	550 (0 – 11400)	95	5	0
May	100 (0 – 2200)	91	9	0
June	800 (0 – 20100)	93	7	0
July	950 (0 – 45700)	98	2	0
August	800 (0 – 4300)	96	4	0
September	0 (0 – 9900)	86	14	0
October	100 (0 – 17000)	93	7	0
November	0 (0 – 9800)	95	5	0
December	100 (0 – 55200)	81	18	1
January 2010	100 (0 – 7100)	99	1	0
February	300 (0 – 5900)	94	4	2
March	800 (0 – 27300)	100	0	0
April	350 (0 – 69100)	96	4	0
May	550 (0 – 18800)	76	22	2
June	550 (0 – 31900)	97	3	0
July	550 (0 – 8800)	72	20	8
August	0 (0 – 1300)	87	13	0
September	0 (0 – 5800)	95	5	0
October	1950 (0 – 7400)	100	0	0
November	300 (0 – 3900)	96	4	0
December	2150 (0 – 25300)	97	1	2
January 2011	100 (0 – 7400)	98	2	0
February	200 (0 – 12700)	98	2	0
March	1700 (0 – 9600)	93	7	0

Discussion

During this trial, the researchers did not interfere in the protocol of anthelmintic treatments that has been adopted for several years by the people responsible for worm control of the sheep flock where the tracer lambs were placed monthly. However, we were aware of the severe anthelmintic resistance was on that farm. In a study conducted in the late 80s, ivermectin, oxfendazole and levamisole showed very poor efficacy in that flock (AMARANTE et al., 1992). Later, moxidectin and closantel were used intensively, but also resulted in anthelmintic resistance (information not published). Therefore, we can assume that the protocol of anthelmintic treatments employed during the trial had a very limited effect (possibly none) on the epidemiology of *H. contortus* and *T. colubriformis*, the major parasites detected in this study.

With regard to *H. contortus* epidemiology, a "*Haemonchus* season" is usually observed in Brazil's most important sheep production regions, such as the south. This season occurs especially during warm moist summer months, with a reduction in the prevalence of *H. contortus* during cold winter months (SANTIAGO et al., 1976; RAMOS et al., 2004). In contrast, in regions with year round high temperatures, the limiting factor for *H. contortus* transmission is the lack of moisture during prolonged periods of drought, such as those observed in Brazil's semiarid northeast (CHARLES, 1995; SOUZA et al., 2013). This seasonal trend in the prevalence and/or intensity of infection was not observed in our experimental conditions, in which *H. contortus* transmission, with high worm burdens, occurred year round. These observations are consistent with those of other studies conducted in the same area, which demonstrated the year-round presence of *H. contortus* third stage larvae on pastures grazed by sheep (AMARANTE; BARBOSA, 1995; CARNEIRO; AMARANTE, 2008).

In the region of this trial, occasional rainfall occurs during the so-called "dry season." For instance, during the 36 months of the trial, no rainfall was recorded only in two months, July 2009 and August 2010. Coincidentally, in August 2010, tracer lambs acquired a massive worm infection, with an average of more than 24,000 *H. contortus* specimens. This finding clearly indicates that the free living stages of *H. contortus* were able to survive during the winter, despite the absence of rains. Although first and second stage larvae are considered highly vulnerable to desiccation, once their development to the L3 stage is complete, all the major trichostrongylidae species are considerably less susceptible to unfavorable climate conditions (O'CONNOR et al., 2007). It has been reported that, in the laboratory and in the field, infective larvae are able to survive several cycles of desiccation/rehydration, in a process called anhydrobiosis, in which metabolic activity is decreased and larval survival is extended (LETTINI; SUKHEDEO, 2006).

Trichostrongylus colubriformis was the second most important parasite. *T. colubriformis* presented a lower worm burden than *H. contortus* in most of the months of this trial. In part, this was because the permanent sheep of the flock shed on average 14.3 times more *Haemonchus* eggs than *Trichostrongylus* eggs, based on the FEC and on the identification of third stage larvae from cultures. However, on average, the tracer lambs presented only 2.76 times more *H. contortus* (overall mean of 7,694 specimens) than *T. colubriformis* (overall mean of 2,792 specimens). Therefore, we can infer that, in fact, the free living stages of *T. colubriformis* presented a higher rate of development and survival than *H. contortus* in the environment. There are indications that the free-living stages of *T. colubriformis* are more tolerant to dry conditions and low temperatures than *H. contortus* (reviewed by O'CONNOR et al., 2006).

Trichostrongylus colubriformis showed averages exceeding 4,000 specimens on six occasions (October 2008, 2009 and 2010; November 2009; April and July 2010). This draws attention to the high mean intensity of infection that always occurs in October. During the trial, the average temperatures in October varied from 20.6 to 22.5 °C and the rainfall ranged from 56.1 to 153.8 mm, i.e., there was association of mild temperatures with moderate rainfalls. These are possibly the best environmental conditions for the transmission of *T. colubriformis*. In contrast, high temperatures associated with heavy rainfalls that occurred during summer caused a decline in *T. colubriformis* transmission.

In conclusion, the results of this trial indicate that the environmental conditions of this region are extremely favorable for the year-round transmission of *H. contortus* and *T. colubriformis*.

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