# Migratory dynamics of cyathostomin larvae in a Bermuda grass pasture in South America

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**ABSTRACT**: Studies of the migratory dynamics of cyathostomin infective larvae ( $L_3$ ) in different seasons and with two types of irrigation were carried out over 12 months (September 2006 to September 2007) in the subtropical climate of the Baixada Fluminense region of Rio de Janeiro state, Brazil. Four faecal masses weighing 500 g each from naturally infected horses were placed in a Bermuda grass ( $Cynodon\ dactylon$ ) pasture in the beginning of each season. Samples of faeces and grass were collected every 15 days until the end of each season. The highest recovery in faeces occurred in Autumn (491 910  $L_3$ /kg dried herbage) and in pasture was achieved in Winter (9 963  $L_3$ /kg dried herbage). The lowest number of infective larvae recovered from faeces (55 100  $L_3$ /kg dried herbage) and pastures (2 188  $L_3$ /kg dried herbage) were achieved in Spring. The nonparametric Kruskal-Wallis test showed a significant difference in infective larvae recovery between the seasons. The collection time of the samples did not affect the larva recovery. The results suggest that in the conditions of the region studied, animals maintained in pasture are at permanent risk of infection.

Keywords: Cyathostominae; infective larvae; irrigation; seasons

Strongylid nematodes belonging to the Cyathostominae subfamily, known also as small strongyles, are the main parasitic pathogens of grazing horses (Love et al., 1999). Studies of the migration of the free-living stages of strongyles have been carried out in many regions of the world (Baudena et al., 2000; Langrova et al., 2003; Ramsey et al., 2004; Bezerra et al., 2007). However, most studies were performed in areas of temperate climate. Because of the small number of studies in subtropical climates, there is a lack of information about the epidemiology of these helminthes in such climates. Environmental factors have a major impact on parasite populations, especially on the free-living stages that occur in pastures (Stromberg, 1997). Temperature and rainfall are considered the main environmental factors influencing the ecology of cyathostomin larvae in the development of these helminthes from eggs into infective larvae (Ramsey et al., 2004).

Moisture is required for infective larvae to migrate (Langrova et al., 2003). Thus the introduction of irrigation may change the epidemiology of parasitism (Gruner et al., 1989).

The aim of this study was to evaluate the effect of irrigation on the migration of cyathostomin larvae during the different seasons of the year in a subtropical climate.

#### **MATERIAL AND METHODS**

The experimental area was located at the W.O. Neitz Parasitology Research Station of the Department of Animal Parasitology (DAP) of the Institute of Veterinary Science at the Rural Federal University of Rio de Janeiro (UFRRJ), located at 22°41' South latitude and 43°41' West longitude, at an altitude of 33 m. The experiments were performed in the university's helminthology labora-

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tory. The pasture consisted of only Bermuda grass (*Cynodon dactylon*).

## The experiment

Faecal samples were obtained from horses kept at DAP and naturally infected by cyathostomins. Aliquots of fresh faeces were collected monthly from the rectum of the animals to monitor the infection, which was measured using faecal egg count s (FEC) according to the McMaster technique (Gordon and Whitlock, 1939). Two experimental plots of Bermuda grass of 5.50 m<sup>2</sup> each were used, only one was under irrigation. Two fresh faecal aliquots of approximately 500 g, with FEC varying between 1000 and 3050, were deposited in the plots on the first day of spring, summer, autumn and winter, from September 2006 to September 2007. After one week, faecal and grass samples were collected at regular intervals of fifteen days until the end of each season. Sampling was performed twice a day (8 a.m. and 5 p.m.). The grass samples were collected from a region of the sward 2 to 20 cm tall (to mimic the grazing of horses) and the faecal samples weighed approximately 2 g. The faecal and grass samples were processed using the Baermann technique, according to Quinelato et al. (2008). Infective larvae were counted and identified according to Bevilaqua et al. (1993). The plot was uniformly irrigated five days a week with 0.55 mm (3 l) of water, between 1 p.m. and 2 p.m. This irrigation time was chosen to evaluate the influence of moisture on the recovery of infective larvae (L<sub>3</sub>) at 5 p.m.

The weather data (rainfall and air temperature) were provided by the Seropedica Agricultural Weather Station (INMET/PESAGRO – RJ) and soil temperature was measured weekly in the experimental plots.

All data were tabulated every fortnight using an Excel spreadsheet.

## Statistical analysis

The number of  $L_3$  recovered from the grass and faecal samples in each season at different times and under both irrigation regimes was analyzed by the nonparametric Kruskal-Wallis test (P < 0.05) (Sampaio, 1998; Zar, 1999). Statistical analysis was performed with the BioEstat program (Ayres et al., 2005).

#### **RESULTS**

#### Weather data

The mean values of rainfall, air and soil temperatures are shown in Figure 1. The mean temperatures and rainfall were higher in summer (air temperature of 28.4 °C, soil temperature of 26.5 °C and rainfall of 471.8 mm).

## **Spring**

The average  $L_3$  recovery was 55 100  $L_3$ /kg dried herbage (dh) to faeces and 2 188  $L_3$ /kg dh to grass (Figure 2). There was a higher percentage of  $L_3$  recovery on faecal samples from the non-irrigated plot (P > 0.05) and on grass samples from the irrigated plot (P < 0.05) (Figure 3). There was no significant difference between the collection times, but a greater recovery of  $L_3$  at 8 a.m. in grass samples and at 5 p.m. in faecal samples, both in the irrigated plot, was detected.

### **Summer**

The average  $L_3$  recovery was 199 745  $L_3$ /kg dh and 2 268  $L_3$ /kg dh in faecal and grass samples, respec-

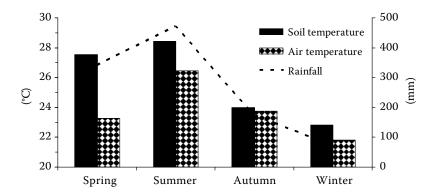


Figure 1. Rainfall and average air and soil temperature from September 2006 to September 2007

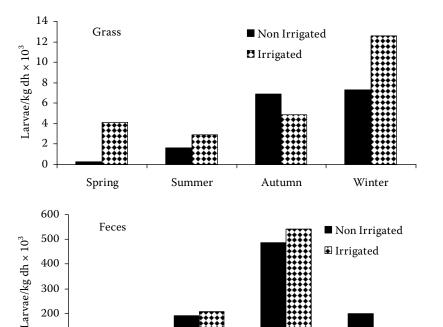


Figure 2. Infective larvae recovered from faeces and grass from September 2006 to September 2007

tively (Figure 2). There was no significant difference in recovery of infective larvae, but a higher percentage of  $L_3$  recovery was observed in the faecal and grass samples from the irrigated plot (Figure 3).

Summer

Spring

At 8 a.m. a higher number of cyathostomin infective larvae were recovered from faecal samples in the non-irrigated plot, and in grass samples this number was higher in the irrigated plot, but the differences were not statistically significant.

#### Autumn

100

The highest average number of infective larvae was recovered in this season in the faeces (491 910  $\rm L_3/kg$  dh). In grass samples, the average recovery was 5 890  $\rm L_3/kg$  dh (Figure 2). There were more  $\rm L_3$  in the irrigated plot in faeces and in the non-irrigated plot in grass (Figure 3), but also without a significant difference.

At 5 p. m. there was higher recovery of  $L_3$  in feces (irrigated plot) and in grass (non-irrigated plot). Again, the difference was not significant.

#### Winter

The mean number of  $\rm L_3$  recovered from the faecal samples was 146 580  $\rm L_3/kg$  dh. The highest recov-

ery of  $L_3$  in grass samples (9 963  $L_3$ /kg dh) was in this season (Figure 2). The greatest percentage of infective larvae recovery was in faeces from the non-irrigated plot and in grass from the irrigated plot, but without significant difference (Figure 3).

There was also no significant difference between the two collection times for the faeces and grass, although the greatest recovery of  $L_3$  was at 8 a.m. in grass from the non-irrigated plot and in faeces from the irrigated plot.

#### Comparison of all seasons

Winter

Autumn

Figure 2 shows a comparison of the number of cyathostomin infective larvae recovered from faeces and grass, over all seasons, with significant differences. The greatest number of  $L_3$  were recovered from faeces in autumn in comparison to spring and summer (P < 0.05). In the sward, there was significant differences in infective larvae recovered between autumn and spring, winter and spring and winter and summer.

#### **DISCUSSION**

The influence of the handling of the sward climatic and of variables over different seasons of the

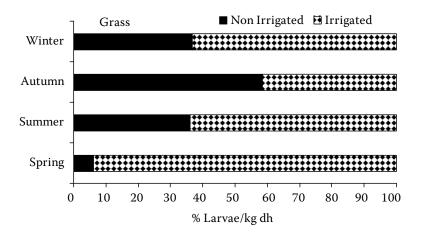
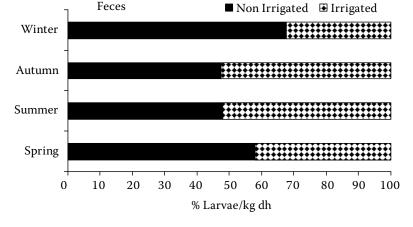


Figure 3. Percentage of infective larvae recovered from faeces and grass in non-irrigated and irrigated plots



year on the migratory behavior of cyathostomin infective larvae were evaluated for 12 months.

The results of this study show the importance of faeces as a reservoir, since periodic migration of infective larvae from faecal plots to herbage was observed. These results agree with those of previous studies performed in different climate conditions (English, 1979a,b; Langrova et al., 2003). In the climate conditions of the Baixada Fluminense, the presence of  $L_3$  in faeces was observed throughout the year regardless of season. This does not occur in temperate climate zones of the Northern hemisphere, where the winter is very cold (Kuzmina et al., 2006).

Infective larvae of cyathostomins were detected on herbage throughout the experimental period due to their periodic migration from the faeces to the grass, especially in response to rainfall. These findings agree with those of studies conducted in areas of similar climate conditions (English, 1979a,b).

Moisture is an important variable for the migration of infective larvae from faeces to grass. Therefore, irrigation may help  $L_3$  migration, especially during the autumn and winter when the amount of rainfall is lower. In addition to the moisture requirement for larval migration, even in small quantities, irri-

gation may reduce the temperature of the existing microclimate, contributing to the development of infective larvae (Gruner et al., 1989; Bezerra et al., 2007). This effect was observed in spring, when the soil temperature caused a reduction in  $L_3$  recovery from non-irrigated herbage. These findings suggest there was a decrease in soil temperature in the irrigated plot due to watering, which may have favored recovery of the infective larvae from the grass samples. The influence of soil temperature was demonstrated in previous studies in the same experimental area (Bezerra et al., 2007; Quinelato et al., 2008; Couto et al., 2008, 2009).

In the present study, the collection times (8 a.m. and 5 p. m) did not influence recovery. This was also observed in other studies conducted in the same region using Tifton 85 grass (Quinelato et al., 2008) and Bermuda grass (Couto et al., 2008).

The results of the present study suggest that grazing animals, in irrigated and non-irrigated pastures in the subtropical climate of the Baixada Fluminense region can be infected by cyathostomins throughout the year, but especially during autumn and winter. Thus, these findings may be used as a model for subtropical and tropical climates.

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