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VERTICAL MIGRATION OF *Haemonchus sp.* INFECTANT LARVAE ON *Stylosanthes spp.* AND Brachiaria brizantha (*Syn. Urochloa brizantha*) CV. MARANDU FORAGE

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Abstract

The present work aimed to evaluate the influence of *Stylosanthes* spp. (Campo Grande© styles) and *Brachiaria brizantha* cv. Marandu in the vertical migration of *Haemonchus* spp. infective larvae, in the region of Uberlândia, Minas Gerais. Seeds of *Stylosanthes* spp. and *Brachiaria brizantha* cv. Marandu were planted in 50 pots of five liters (20 cm diameter) evenly disposed in an area of the Capim Branco Experimental Farm, Federal University of Uberlândia. When both species reached the minimum height of 20 cm, the pots were experimentally contaminated with sheep feces coprocultures. Samplings were performed, with complete removal of the vegetal material contained in the pots, on days one, three, six, 10 and 14 after contamination. Nematoids were recovered from the plant samples by thermo and hydrotropism, counted under an optical microscope and identified. For statistical analysis, the data were analyzed in a completely randomized design (DIC) in a 2X5 factorial scheme (two fodder species X 5 collection dates). No effect of the forage species was observed on the number of L3 of *Haemonchus* sp. recovered in the feces ($p = 0.7790$), upper stratum ($p = 0.1755$) or lower stratum ($p = 0.7883$) of the forage, however, there was a significant decrease in the number of L3 found with the passing of the days. When comparing the mean L3 recovered in the upper (0.06±0.31 L3) and lower (2.94±0.39) strata, a higher number of larvae was observed in the lower stratum ($p < 0.0001$). Therefore, in both forage species studied, the upper stratum of the plants, preferably pastured by the animals, remained with less contamination of infective larvae and the contamination tended to decrease over the days.

Keywords: *Brachiaria. Haemonchus.* Infectant Larvae. Parasite Control. Trichostrongyloidea.

1. Introduction

Parasitism by gastrointestinal nematodes (GIN) is one of the most important sanitary challenges of grazing ruminants worldwide (Molento et al. 2016). In order to control parasitic diseases, anthelmintics have been used indiscriminately, causing them to gradually lose their effectiveness (Salgado and Santos 2016). Anthelmintic resistance is now a worldwide problem, and there are many tropical flocks where no efficient drug is available (Veríssimo et al. 2012; Salgado and Santos 2016), and even failure to monepantel is reported (Cintra et al. 2016; Albuquerque et al. 2017). Since the control based on drugs is failing, alternative measures based on improve immune response of the host or to lower parasite development and surveillance on environment are being encouraged.

Forages can have an influence on sheep parasitism because of the microclimate for the development and surveillance of non-parasitic stages of GIN (Santos et al. 2012; Troncha et al. 2019). The nutritional support and quality of forage is also important to allow the development of a good immune response in the host (Mendes et al. 2018; Oliveira et al. 2009). Leguminous forages are an option for increasing the diet protein level in grazing livestock systems. *Stylosanthes* spp. is an example of leguminous that can be used associated with grass in a 40% rate (EMBRAPA, 2007).

Estilosantes Campo Grande© is a perennial tropical leguminous, consisting of two different *Stylosanthes* species: 80% of *S. capitata* and 20% of *S. macrocephala*. It was launched by Embrapa in 2000 and was considered high dry matter production mainly in regions with sandier and well-drained soil, in addition to being well adapted to low fertility soils and poor in organic matter (Andrade et al. 2010).

Some experimental studies in the 70s and 80s report the occurrence of lethal/repellent bioactive compounds to the bovine tick *Rhipicephalus microplus* larva in some forage species, and this effect can be the result of volatile compounds or direct contact between the larva and the forage (Barros and Evans 1991). Muro-Castrejón et al. (2003) presented results of the repellent action of *Stylosanthes humilis* and *S. hamata* in *R. microplus* larva *in vitro.*

Little is known about such effect of forage bioactive compounds in trichostrongylid larva. This work aims to compare if Estilosantes Campo Grande has the power to allow the lower presence and vertical migration of *Haemonchus* spp. infectant larvae (L3) than the Marandu palisade grass (*Brachiaria brizantha* Syn. *Urochloa brizantha* cv. Marandu).

2. Material and Methods

The research was held in the Sheep and Goat Production Center, at the Capim Branco experimental farm, which is part of the Federal University of Uberlândia (UFU) School of Veterinary Medicine. The farm is located in the city of Uberlândia, Brazil (18°30'S, 47°50'W) and the Köppen Climate Classification is Aw, tropical savannah with dry winter season. Data collection was performed from July 20 to August 2 2016, when minimum temperatures ranged from 5 ºC to 11 ºC, and maximum temperatures ranged from 25 ºC to 31 ºC. No rainfall was registered during this period.

Stylosanthes spp. (Estilosantes Campo Grande©) and *Brachiaria brizantha* cv. Marandu were planted in pots, and each pot was considered one experimental unit of this study. *Brachiaria* seeds were planted in plastic boxes (9.7 x 30.0 x 49.0 cm) until the emergence of the plantula. These seedlings were then transferred to 25 5L pots (20 cm diameter) in January (water period) 2016. The height of the plants was kept at 20 cm until the beginning of the experiment by manual pruning. *Stylosanthes* spp. was planted directly in 25 other pots in the same period of *Brachiaria brizantha* cv. Marandu transplantation. The plants took about five months to reach the desired height (20 cm), due to the drought.

When all plants reached 20 cm high, each pot was contaminated with a pool of sheep feces with an average of 15 thousand Strongylid eggs per pool. Fecal samples came from sheep naturally infected with GIN remaining from the routine Gordon and Whitlock (1954) technique performed on Lab. The pools had 10 g of feces that were mixed to wood shavings, humidified and kept at room temperature for 7 days before the contamination of the pots, in order to develop the nematode third stage larva (L3). Each pool was placed in the center of the pot, above the soil and at the base of the forage.

The forage sampling was performed on day 1, 3, 6, 10 e 14 post-contamination. All the forage present in the pots was separated vertically at 10 cm high, with pruning shears. The upper portion was packed in paper bags, separated from the lower portion, which was cut close to the ground. The feces from the remaining coproculture were also collected. All samples were kept refrigerated until they were transported to the Laboratory of Parasitic Diseases (LADOP-UFU).

As soon as the samples were received in the laboratory, they were weighed and processed using the Rugai et al. (1954) adapted technique, based on the thermo-hydrotropism of the larva. The method consists on wrapping the different portions (top, bottom and feces) separately in gauze and placing the samples in properly identified sedimentation cups. Water was then added at 42-45 ºC in the cups, until it was in contact with the sample. After 24 hours of sedimentation, the contents from the bottom of the cups were collected with Pasteur pipettes and transferred to labeled tubes. The nematodes found were counted and identified under an optical microscope according to the key proposed by Van Wyk et al. (2004).

Infective larva data were converted by logarithmic transformation (Log L3 + 1). The analysis was performed in the ANOVA procedure, from the S.A.S. statistical package, considering a completely randomized design, in a 2X5 factorial scheme (two forage species and five days of collection), with five replications per treatment. The means were compared by minimum significant deviation (MSD). To compare the number of L3 recovered in the upper and lower strata of the plots, the paired t test was used, using the Graph Pad InStat 3.0 software.

3. Results

6,397 third-stage Strongylid larvae were recovered from forage samples and the feces remaining in the pots. Of these larvae, 80.9% were *Haemonchus* sp., 11.9% *Oesophagostomum* sp., 3.4% *Trichostrongylus* sp., 2.9% *Teladorsagia* sp. and 0.9% *Cooperia* sp. in the *Brachiaria* samples. For *Stylosanthes,* this proportion was 81.41%; 12,6%; 2,5%; 1,5% and 2,0%, respectively. As the results between the genders were similar and the number of larvae was too small for statistics, only data related to *Haemonchus* sp. were presented.

The statistical model showed no effect of the forage species on the number of *Haemonchus* sp. L3 recovered from the feces ($p = 0.7790$), upper ($p = 0.1755$) or lower ($p = 0.7883$) layer of forage, however it demonstrated an effect of the day after contamination. The compared means showed a significant reduction in the number of L3 recovered in the feces and lower stratum over the days (Figure 1).

When comparing the L3 mean recovered in the upper (0.06 ± 0.31 L3) and lower (2.94 ± 0.39) strata, a significant greater number of larvae was observed in the lower stratum ($p < 0.0001$).

4. Discussion

Among Strongylid larvae recovered from forage and feces from animals with natural infection, *Haemonchus* sp. was the most frequent genus by far. This result is in agreement with other studies carried out in southeastern Brazil that indicate that this is the most prevalent genus in sheep and goats (Wilmsen et al. 2014; Melo et al. 2015; Fernandes et al. 2015). In addition, this genus has the greatest biotic potential among trichostrongylids, with an average laying of 5 to 10,000 eggs per day (Ueno and Gonçalves, 1998).

Despite Oliveira et al. (2009) have found lesser number of infective larvae in *Stylosanthes* spp. and *Andropogon gayanus* when compared to *Brachiaria brizantha* cv. Marandu and *B. brizantha* cv. Xaraés, in a study carried out in Botucatu, São Paulo, the same was not verified in the current results. Studies by Carneiro and Amarante (2008) and Oliveira et al. (2009) suggest that the morphology of each plant influences the microclimate, favoring or not the larval development.

According to Cruz-Vasquez and Fernández (2000), the age of the plant has a direct influence on its repellent potential on bovine tick larvae. Potentially toxic substances produced by andropogon grass (*Andropogon gayanus*) have the capacity to reduce the population of tick larvae in the pasture by 20% and 50% when aged 6 months and 1 year, respectively (Cruz-Vasquez and Fernández, 2000). In the present study, the plants used were less than six months old, which may imply less production of active substances with toxic or repellent potential for parasite larvae.

The higher concentration of L3 in the lower strata of plants, found in this work, is in accordance with the findings of Troncha et al. (2019), who carried out an experiment with palisade grass in the same region, from August to November 2014. The results are also similar to those obtained by Santos et al. (2012) for *B. decumbens*, during July and August 2009, in the region of Botucatu, São Paulo. Gazda et al. (2009) also found a higher concentration of L3 in the lower portion of forage *Paspalum notatum* cv. Saurae and *Panicum maximum* (Syn. *Megathyrsus maximus*) cv. Aruana, in a study carried out in Paraná, from January to March 2007. This higher concentration of L3 in the lower stratum can be related to the most favorable microclimate existing at the base of forages, especially in the dry season, where air humidity is low, the presence of soil and feces concentrate the dew and provide milder temperatures for the larvae (Santos et al. 2012).

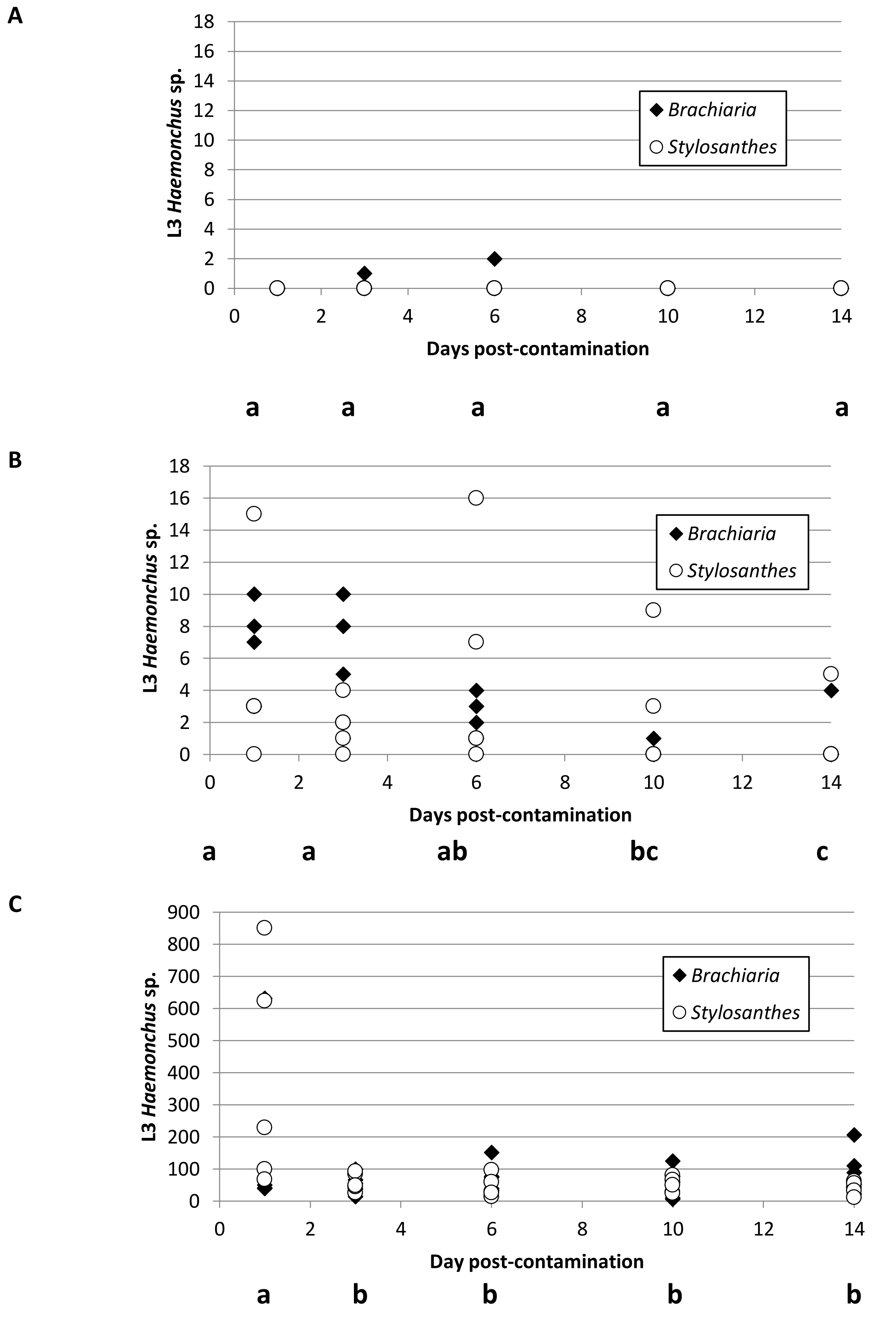

Figure 1. Number of *Haemonchus* sp. third stage larvae (L3) obtained from the superior strata (A), inferior strata (B) and feces (C) in *Brachiaria brizantha* cv. Marandu and *Stylosanthes* Campo Grande forages from 1 to 14 days after experimental contamination. Different letters represent different L3 means found over the days by MSD test ($p<0.05$).

Since the upper portion of the forage is preferred for grazing by small ruminants, these results show that the correct management of the pasture height may have an influence on the parasitic load of the animals.

A trend towards a gradual reduction in the number of larvae in relation to days observed in the present study may occur due to the dispersion of L3 in the environment or even due to death. Paniago et al. (2014) collect biweekly samples of Marandu palisade grass contaminated with sheep feces for up to 65 days and demonstrated a significant reduction in the number of larvae recovered over time, which can be represented by the equation L3 = 44,104 - 0.698 days. Several other authors observed a reduction in the number of infectious larvae after artificial contamination of the pasture (Gazda et al. 2009; Oliveira et al. 2009; Santos et al. 2012; Troncha et al. 2019)

Although the present study did not find any significant difference between the L3 mean found in different forage species, further studies are still required, seeking to identify factors favorable to the reduction of parasitic load in forages plants. This will imply a reduction in the use of chemical methods to control worms, reducing production costs and pressure on the selection of resistant parasites.

5. Conclusions

Under the conditions in which the present study was developed, there was no difference on the number of infectant larvae obtained from Estilosantes Campo Grande and Marandu palisade grass (*Brachiaria brizantha* Syn. *Urochloa brizantha* cv. Marandu). In both forage species studied, the upper stratum of the pasture remained with less contamination of infectious larvae and the contamination tended to decrease over the days.

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Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: Not applicable.

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