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Pasture contamination levels by infective gastrointestinal nematode larvae in small ruminants

[Níveis de contaminação em pastagens por larvas infectantes de nematoides gastrintestinais de pequenos ruminantes]

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Abstract: In this study, we aimed to quantify the levels of pasture contamination by third-stage larvae (L_3) of gastrointestinal nematodes in small ruminants. Pasture samples comprising *Panicum maximum* grasses (cultivars Massai, Aruana, BRS Zuri, and Tamani) were collected over a 12-month period at the Sheep and Goat Production Unit of the Maracanã Campus of the Federal Institute of Maranhão in São Luís, Maranhão. Samples were collected at ground level, individually labeled, and placed in plastic bags. To recover the L_3 larvae, the samples were initially immersed in a solution of water and neutral detergent in plastic containers for four hours and then transferred to a fresh solution for three additional hours. The grass was then removed, packed, and dried in an oven to determine dry matter content, and the remaining solution was allowed to settle for 24 h; the supernatant was then siphoned off, and the sediment was transferred to a conical sedimentation glass. Parasite densities ranged from 42 to 1,130 per collection, with *Haemonchus* sp. and *Trichostrongylus* sp being the most prevalent, accounting for 50.31 % and 43.67 % of the total larvae recovered across all collection months, respectively. The highest larval counts coincided with the months of peak rainfall. Larval counts ranged from 74 to 2,749 L_3/kg of dry matter. These findings are valuable for guiding management strategies aimed at controlling gastrointestinal nematodes.

Keywords: forage; helminths; goats; sheep; rainfall.

Resumo: Este trabalho teve como objetivo quantificar os níveis de contaminação da pastagem com L_3 de nematoides gastrintestinais de pequenos ruminantes. Amostras das pastagens, constituídas pelas gramíneas *Panicum maximum* cv. *Massai* e *Aruana* e *Panicum maximum* cv. *BRS Zuri e Tamani*, foram coletadas no Instituto Federal do Maranhão, Campus São Luís (MA)-Maracanã, no setor de Ovinocaprinocultura, no período de 12 meses. As amostras foram coletadas próximo ao solo, identificadas individualmente e acondicionadas em sacos plásticos. Para a obtenção das L_3, foram, inicialmente, imersas por quatro horas em solução de água e detergente neutro em recipiente plástico, onde, posteriormente foram novamente submergidas durante três horas. Logo, foram retiradas dos recipientes, embaladas e secas em estufa, para determinação de matéria seca. A solução dos recipientes decantou por 24 horas e então, o sobrenadante foi sifonado e o sedimento

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transferido para o cálice de sedimentação. Após a identificação e quantificação das larvas, foram observadas densidades variáveis de 42 a 1130 parasitos por coleta, sendo os gêneros com maior prevalência *Haemonchus* sp. (50,31 %) e *Trichostrongylus* sp. (43,67 %) do total de larvas encontradas nos diferentes meses de coletados. Os meses onde foram encontrados o maior número de larvas coincidiram com um maior índice pluviométrico na região. A quantidade de L_3 encontrada foi convertida por quilograma de matéria seca (L_3 kg MS), o que resultou em um número variável de 74 a 2749 L_3 kg de MS. O conhecimento desses dados é importante pois auxilia na escolha de estratégias de manejo visando o controle dos NGI.

Palavras-chave: forragem; helmintos; caprinos; ovinos; pluviosidade.

1. Introduction

The population of small ruminants has increased [1], with goat and sheep flocks growing by 3.9 % and 4.7 %, respectively, totaling 12.4 and 21.5 million head, respectively. Northeastern Brazil continues to be the national leader in small ruminant production. Most flocks in Brazil are pasture-raised, primarily due to the low cost and practicality of producing and supplying forage, which helps reduce overall production costs [2].

Despite the steady growth in animal populations, significant losses persist due to gastrointestinal nematode (GIN) infections. Although tropical and subtropical climates favor agricultural activities, such as pasture cultivation and ruminant production, they also create optimal conditions for the development and survival of GINs, which adversely affect ruminant performance [3,4].

Several GIN species parasitize goats and sheep, with *Haemonchus contortus* being particularly notable for its high infective potential, intensity of infection, and pathogenicity. This species has a direct life cycle consisting of a single host-dependent developmental phase (the parasitic phase) and an environmental phase (the free-living phase). The free-living phase begins when infected animals shed eggs onto the pasture through their feces. Under ideal conditions—temperatures of 18–26°C and a relative humidity of 80–100%—the eggs hatch, and the larvae develop into the L_3 (infective) stage [5-7].

Environmental factors, such as high temperatures, significantly influence population dynamics, reducing L_3 contamination on pastures during the hottest parts of the day, regardless of the season. In rainy weather, larvae disperse across the pasture, becoming available to infect hosts. They migrate to the rumen, shed their protective sheath, and move to their target sites, either the abomasum or the small intestine, where they mature into adults after molting from L_4 to L_5 [5].

Most small ruminants are infected by two or more GIN species, a condition known as mixed infection [8]. In tropical and subtropical regions, these nematodes are more prevalent during warm, rainy periods, with rainfall being the primary factor regulating their life cycle. Conversely, extreme temperatures and low humidity impede the development of the free-living stages, leading to lower survival rates during droughts in northeastern Brazil and harsh winters in southern Brazil [9]. An estimated 90 % of GINs are found on pastures, with only 10 % residing within the host [8]. Understanding the life cycle of GINs is crucial for implementing effective control programs [10].

Therefore, it is essential to study pasture contamination levels by assessing seasonal variations throughout the year and identifying critical periods for L_3 presence within pastures. This knowledge can inform better strategies for controlling GINs in small ruminants, thereby mitigating negative impacts on animal welfare and economic losses related to production and profitability in farming systems. Consequently, this study aimed to quantify GIN L_3 larvae and determine pasture contamination levels on a small ruminant production unit over a one-year period.

2. Material and methods

2.1 Study site

This study was conducted at the small ruminant production unit of the Federal Institute of Maranhão (IFMA), São Luís-Maracanã Campus, in São Luís, Maranhão (2°36'31.8" S, 44°16'04.8" W), from October 2021 to September 2022. The region has a hot and humid tropical climate, characterized by two distinct seasons: rainy season from December to July and a dry season from August to November. Minimum temperatures range from 22°C to 24.7°C, while maximum temperatures vary from 30°C to 34°C. The average annual precipitation is 2,200 mm, with the majority occurring between February and May [11].

2.2 Pasture description

The pasture used in this study covered approximately 5,000 m^2 and consisted of mixed grasses, including *Panicum maximum* cv. Massai (1,414.9 m²), *Panicum maximum* cv. BRS Zuri (1,588.6 m²), *Panicum maximum* cv. Aruana (1,468.3 m²), and *Panicum maximum* cv. BRS *Tamani* (537.25 m²). Forty adult animals (18 sheep and 22 goats) grazed the area for nine hours (8:00 a.m. to 5:00 p.m.) each day.

2.3 Quantification of infective GIN Larvae

The identification and quantification of L_3 larvae in the study area followed the methods described by Molento et al. [12] and Carneiro and Amarante [13], with modifications.

2.3.1 Field sample collection

A sampling schedule was established over a 12-month period. A trained technician walked a predetermined W-shaped path across the site, manually collecting approximately 100 g of grass every four paces (about 3.5 m). At each collection point, the grass species was identified and cut close to the ground (collecting the bottom 5 cm) using a blade [12,14]. The samples were labeled and stored in plastic containers until processed at the Federal Institute of Maranhão (IFMA) Animal Health Laboratory (LASA).

2.3.2 Recovery of infective larvae

During laboratory processing, the grass samples were submerged in 4 L of water mixed with approximately 0.5 mL of neutral detergent for four hours and then transferred to a second bucket under the same conditions for an additional three hours [15]. The detergent reduced the water's surface tension, facilitating the separation of L_3 larvae from the grass. The grass was subsequently removed, placed in paper bags, and dried in an oven at 60°C for 72 hours for dry matter (DM) analysis.

The liquid in the buckets was allowed to settle for an additional 24 hours. The supernatant was then removed, and the sediment was transferred to a conical sedimentation glass. The larvae were separated from the sediment, recovered, and quantified using the method described by Carneiro and Amarante [13].

2.3.3 Identification and quantification of infective larvae

After recovery, the L_3 larvae were inactivated and stained with Lugol's iodine for identification at LASA-IFMA using the keys proposed by Keith [16]. The larvae were quantified by genus, and the DM data for each grass species, along with the total L_3 count for each sample, were utilized to estimate the average L_3 concentration per kilogram of DM (L_3/kg DM).

2.4 Meteorological data measurement

Precipitation levels and environmental records were obtained from the IFMA and the Geoprocessing Center of the State University of Maranhão (UEMA), respectively.

2.5 Data analysis

Larval count data were recorded in preformatted Microsoft Excel© spreadsheets. Following normal distribution testing of the variables using the Shapiro–Wilk test, a correlation analysis between L_3 counts and rainfall levels was performed. Statistical analyses were conducted using R software [17], with additional packages utilized for figure generation [18].

3. Results and discussion

Figure 1 illustrates the quantification of GIN larvae on pastures grazed by goats and sheep over a one-year period, along with local meteorological data (NuGeo/UEMA) collected during the sampling period. The total larval counts recovered in each monthly collection from October 2021 to September 2022 in São Luís, Maranhão, were 243, 145, 365, 241, 879, 1,130, 424, 625, 170, 43, 69, and 42, respectively. Notably, the months with the heaviest rainfall corresponded with the peak larval counts recovered from the pasture, while the months with the lowest rainfall levels corresponded with the fewest larvae.

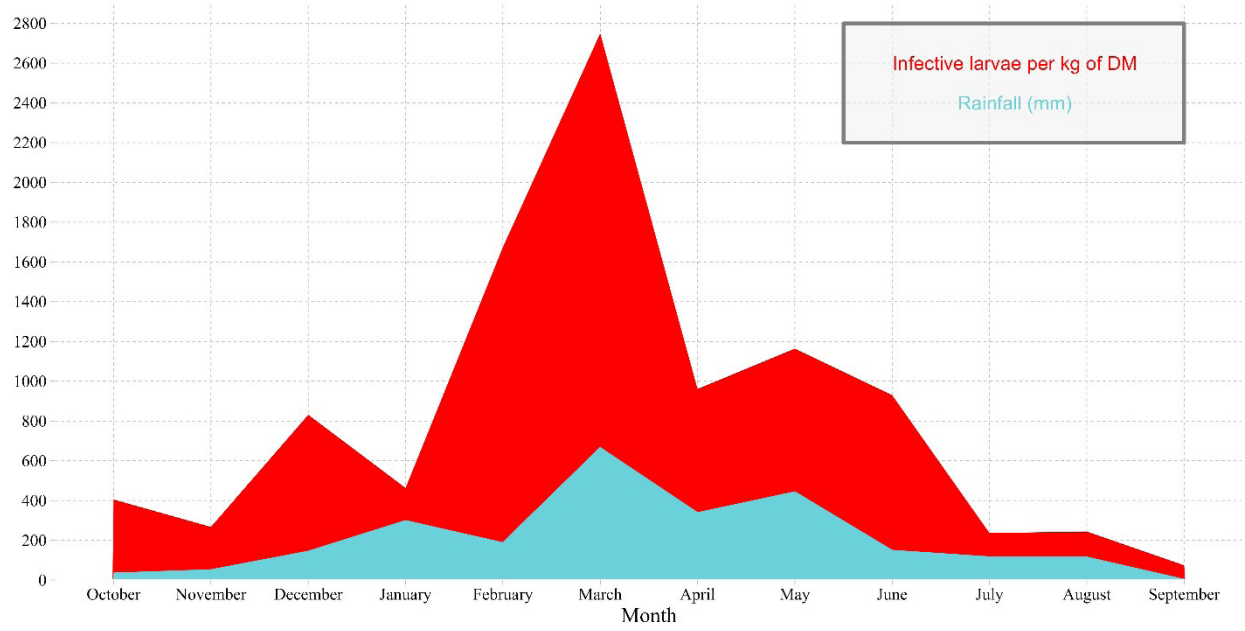

Figure 1. Quantification of recovered gastrointestinal nematode larvae (L_3) alongside rainfall levels during the collection months at the Federal Institute of Maranhão, São Luís-Maracanã Campus, from October 2021 to September 2022, on pastures grazed by goats and sheep.

Following larval identification, the genera of gastrointestinal nematodes affecting small ruminants were recorded, as depicted in Figure 2.

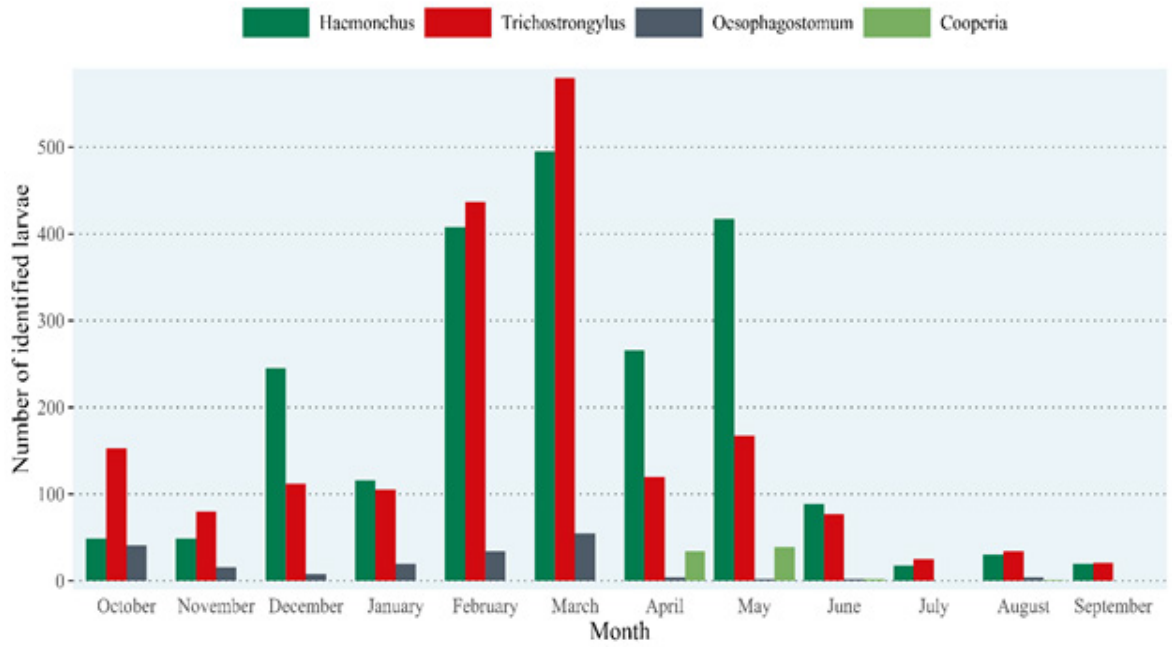

Figure 2. Genus identification and quantification of gastrointestinal nematode larvae from goats and sheep recovered from *Panicum maximum* grass pastures (cv. Massai, Aruana, BRS Zuri, and BRS Tamani) at the Federal Institute of Maranhão, São Luís-Maracanã Campus, from October 2021 to September 2022.

The initial quantification of L_3 larvae and the estimated L_3/kg DM throughout the study year are presented in Table 1. L_3 counts in the pastures varied from 42 (September 2021) to 1,130 (March 2022), with a mean ± standard error of 365 ± 100. When converted to L_3/kg DM, the values ranged from 74 to 2,749 L_3/kg DM over the same period, with a mean ± standard error of 833 ± 221 L_3/kg DM. Due to the non-normal distribution of one of the variables, Kendall's and Spearman's correlation tests were employed, revealing a positive correlation between precipitation levels and L_3/kg DM values (Kendall's correlation coefficient $\tau = 0.67$; P-value < 0.001; Spearman's correlation coefficient $\rho = 0.85$; P-value < 0.001). This correlation is depicted in Figure 1, which indicates higher L_3 counts during months with increased rainfall.

Figure 2 illustrates the GIN genera responsible for natural infections throughout the study year. *Haemonchus* sp. and *Trichostrongylus* sp. were recovered in all 12 months evaluated. In lower numbers, *Oesophagostomum* sp. was present in 10 months (84% of the study period), followed by *Cooperia* sp., which was identified in four months (33% of the study period).

Table 1. Quantification of infective gastrointestinal nematode larvae (L_3) recovered from grass pastures grazed by small ruminants over a one-year period in São Luís, Maranhão, Brazil.

Collection month	Grass species	Total L_3 count	L_3/kg DM
October	*Panicum maximum* cv. Massai	243	405
November	*Panicum maximum* cv. BRS Zuri	145	267
December	*Panicum maximum* cv. Aruana	365	830
January	*Panicum maximum* cv. Aruana	241	463
February	*Panicum maximum* cv. Massai	879	1,674
March	*Panicum maximum* cv. Tamani	1,130	2,749
April	*Panicum maximum* cv. BRS Zuri	424	960
May	*Panicum maximum* cv. BRS Zuri	625	1,164
June	*Panicum maximum* cv. BRS Zuri	170	930
July	*Panicum maximum* cv. BRS Zuri	43	235
August	*Panicum maximum* cv. BRS Zuri	69	243
September	*Panicum maximum* cv. Massai	42	74

L_3/kg DM: Infective gastrointestinal nematode larvae per kilogram of dry matter.

This study employed a pasture L_3 counting methodology, which serves as a qualitative and quantitative approach to inform parasitological and epidemiological management decisions on ruminant farms [12]. Although previous studies have identified the genera responsible for natural GIN infections in small ruminant production units in Maranhão, this study utilized a quantitative approach to assess pasture infectivity levels. This is highly relevant, as approximately 90% of GINs in a production unit reside in the pasture, external to the host [8].

The results indicated the presence of GIN larvae at the L_3 stage within the pastures, with densities fluctuating across the various collection months. The monthly larval counts from October 2021 to September 2022 were recorded as follows: *Haemonchus* sp. (49, 49, 245, 116, 408, 495, 266, 417, 89, 18, 30, 20); *Trichostrongylus* sp. (153, 80, 112, 105, 437, 580, 120, 167, 77, 25, 34, 21); *Oesophagostomum* sp. (41, 16, 8, 20, 34, 55, 4, 2, 2, 0, 4, 0); and *Cooperia* sp. (0, 0, 0, 0, 0, 0, 34, 39, 2, 0, 1, 1), respectively. The consistent presence of *Haemonchus* sp. and *Trichostrongylus* sp. across all cultivars aligns with the existing literature, particularly for northeastern Brazil, which is characterized by a predominantly hot and humid tropical climate [19-21]. According to Simões et al., *H. contortus* exhibits a preference for warmer temperatures between 25°C and 37°C, which may account for the elevated larval counts for this genus observed in the current study, as local temperatures fall within this range. Additionally, the high larval counts during the region's rainy season may be attributable to increased rainfall, which enhances pasture moisture and fosters the development and survival of infective L_3 GIN larvae [22].

The methodology of collecting samples close to the ground likely facilitated the recovery of the larvae from the grasses, enabling their subsequent identification in this study. Studies by Pegararo et al. [23] and Gazda et al. [24] demonstrated that higher larval counts were recovered from the lower 5 cm of the grass than from the upper portions of the plants. Furthermore, the impact of ultraviolet (UV) radiation on larval survival must be considered; a study by Van Dijk et al. [25] found that direct UV exposure increased L_3 mortality, with *Trichostrongylus* sp. larvae exhibiting higher mortality rates than those of *Haemonchus* sp.

As anticipated, environmental conditions contributed to the development, migration, and prevalence of L_3 larvae on the studied grasses. Their numbers rose in conjunction with rainfall, particularly between January and May. Correlation analyses yielded positive coefficients (Kendall's $\tau = 0.67$ and $\rho = 0.85$) and statistically significant values, confirming the relationship between rainfall levels and the larval counts retrieved from the pasture. This pattern has been documented in several Brazilian states, including Bahia [26], Rio Grande do Norte [3], and São Paulo [27, 28]. Initially, rainfall facilitates the formation of a water film on fecal pellets, which aids in the development of GIN eggs and larvae. Subsequently, moisture in the pastures promotes the migration and establishment of L_3 larvae, representing the final step in the ecology of the free-living stages of GINs [29].

Additional factors, such as the type of grass, might have compounded the effects of weather conditions to create a conducive environment for larval development within the pastures. Short, creeping, stoloniferous forage plants generate an ideal microclimate for the parasitic population and compel animals to graze close to the ground, facilitating L_3 migration [30].

DM analysis was conducted to determine the larval counts per kilogram of DM for each grass type. The L_3 larval counts in the grasses varied throughout the study year, ranging from a minimum of 74 L_3/kg DM in September to a maximum of 2,749 L_3/kg DM in March. Several factors, including rainfall, temperature, and humidity, can influence the free-living stages of GINs in small ruminant production systems. In Brazil, several studies have quantified L_3 larvae to evaluate

pasture infectivity levels. For instance, Silva-Roberto et al. [3] identified 8,181 larvae at various stages (L_1, L_2, L_3) per 100 grams of fresh *Brachiaria brizantha* grass in Rio Grande do Norte. Another study in Bahia [26] reported 22 L_3/kg DM on a small ruminant farm where animals grazed on *Panicum maximum* in the municipality of Barreiras. In São Paulo, Yamamoto et al. [31] documented considerably high counts during the summer (~14,000 L_3/kg DM) and winter (~18,000 L_3/kg DM) in the upper third of pastures consisting of *Cynodon dactylon*, *P. maximum*, and *Paspalum notatum*. In Rio Grande do Sul, L_3 counts in a *P. maximum* cv. IZ-5 pasture varied between 2,000 and 6,000 L_3/kg DM, and a more recent study using the same grass species recorded 532 L_3/kg DM [26].

Establishing criteria for assessing the L_3 contamination risk within pastures is crucial for both academic research and practical applications on farms. In this regard, the review by Molento et al. [12] provided guidelines based on pasture management, pasture rest periods, animal removal, and anthelmintic treatments to aid researchers and veterinarians in decision-making. Based on one year of L_3 recovery data from the pastures, we concluded that the GIN L_3 contamination level on a small ruminant production unit in São Luís, Maranhão, was low from October to January and again in April, June, July, August, and September; therefore, the pasture is considered “safe” during these periods. In contrast, L_3 contamination levels in the same production unit are considered “moderate” in February, March, and May.

4. Conclusion

The highest larval counts were recorded during the rainy season, coinciding with peak rainfall, particularly in March. The most frequently recovered genera throughout the study were *Haemonchus* sp. and *Trichostrongylus* sp. In summary, environmental conditions facilitated the development, migration, and presence of L_3 on the evaluated grasses, as their numbers increased alongside rainfall, particularly between January and May. This study enhances our understanding of the dynamics arising from the interactions between parasites, the environment, and hosts. These findings can inform the development of strategies to prevent L_3 ingestion by livestock, thereby reducing economic losses and aiding in the control of GINs in small ruminants.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Data availability statement

The complete dataset supporting the findings of this study is included within the article.

Author contributions

Conceptualization: Soares, S. C. P. Data curation, Investigation, Project administration, and Writing: Pinheiro, Y. S. A., Buna, N. H. S., Castro, R. L. P., and Bernardino, M. V. L. Investigation: Reis, A. C. and Silva, C. R. Investigation and Methodology: Fajardo, R. A. T. Formal analysis: Júnior, L. M. C. Conceptualization, Resources, Funding acquisition, Project administration, and Supervision: Brito, D. R. B.

Generative AI use statement

The authors did not use generative artificial intelligence tools or technologies in creating or editing any part of this manuscript.

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