

Communication

[Comunicação]

Prevalence of *Protostrongylus* sp. in ruminants in a semi-arid region of Northeastern Brazil

[Prevalência de *Protostrongylus* sp. em ruminantes na região semiárida do nordeste do Brasil]

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Lungworms are an important group of nematodes that infect domestic and wild animals throughout the world (Panuska, 2006; Bezerra-Santos *et al.*, 2020). In general, these parasites colonize the lower respiratory tract, resulting in a wide plethora of injuries and, occasionally, causing fatal pneumonia (Panayotova-Pencheva and Alexandrov, 2010). In livestock animals, aside from their pathogenic implications, infections by lungworms also cause significant economic losses, due to the reduction of milk production, as well as mortality of animals. For example, the estimated costs may achieve up to 167 € per cow, as demonstrated during an outbreak in The Netherlands (Holzhauer *et al.*, 2011).

Cattle are more commonly affected by *Dictyocaulus viviparus*, the causative agent of parasitic bronchitis in calves at first grazing season (Schunn *et al.*, 2013). On the other hand, small ruminants can be infected by different species such as *Dictyocaulus filaria*, *Muellerius capillaris* and *Protostrongylus rufescens* (Panuska, 2006). Morphologically, the differentiation of larvae of lungworms is difficult, and it is based on the observation of the posterior extremity and data of measure (Kafle *et al.*, 2015). It is known that several epidemiological factors (e.g., climatic conditions, rainfall, humidity, farm management systems) may influence the occurrence of lungworms in ruminants (Samadi *et al.*, 2019). Additionally, the presence of intermediate hosts (snails and slugs) may play an important role in the epidemiology of these nematodes (Kuchboev *et al.*, 2017; Tolossa, 2019).

Despite the scant data on distribution of these parasites, they have been reported in different continents such as Americas (Wapenaar *et al.*, 2007; Henker *et al.*, 2017; Macedo *et al.*, 2020), Europe (Kowal *et al.*, 2016), Africa (Bekele and Shibiru, 2017; Asmare *et al.*, 2018) and Asia (Lat-Lat *et al.*, 2007). Recently, a very comprehensive review of the literature on the occurrence lungworms in domestic ruminants in Brazil (Macedo *et al.*, 2021) cited only two reports of *P. rufescens* infecting small ruminants. Firstly, in the state of Rio de Janeiro, and almost four decades forward in the state of Pernambuco (Duarte and Miranda, 1984; Macedo *et al.*, 2020). The recent retrieval of *Protostrongylus* in Northeastern Brazil has stimulated researchers to study several aspects such as the economic and sanitary impact on ruminant populations. Any information obtained is crucial to improve the quality of the creation of these animals in this region, where the rearing of ruminants represent an important economic activity for subsistence of many families. In fact, it is believed that dairy farming is the main economic activity in the Northeastern region (Oliveira *et al.*, 2007), with an average production of 35 million liters of milk / year (Municipalities..., 2016). Therefore, the aim of this study was to determine the prevalence of lungworms in ruminants reared in a semi-arid region of Northeastern Brazil.

A transversal study was performed in farms (n=30) of ruminant rearing, located in the Garanhuns microregion (8°53'25" South and 36°29'34" West), which is comprised by 21 municipalities, located in state of Pernambuco,

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Northeastern Brazil. There is a predominance of a semi-arid climate with an annual average of temperature of 22°C (from 17°C to de 30°C), rainfall mean of 147 mm (from 25mm to 295 mm), and air relative humidity of 90%.

The Ethics Committee for Animal Experimentation (ECAE) of *Universidade Federal Rural de Pernambuco* approved all procedures herein performed (approval number: 21/2019).

The minimum sample size (n=384) was estimated based on the cattle (n=307,347), goat (n=35,770), and sheep population (n=99,606) of the study area (IBGE, 2016). In addition, an estimated prevalence of 50%, confidence level of 95% and statistical error of 5% were considered (Thrusfield, 2004). The farms were randomly selected by convenience (Reis, 2003).

Animals from one month to 5 years old were included in this study, and all individuals of each farm were sampled. The historical of deworming was absent in these farms.

Animals enrolled in this study were from semi-intensive (n=343) and intensive (n=90) rearing systems. They were fed with *Cynodon dactyl* (Tifton grass) and *Brachiaria* spp. and supplemented with concentrated and mineral salt.

From March 2019 to December 2020, fresh fecal samples (n=429) were collected from the rectum of cattle (n=219), goats (n=122) and sheep (n=88) using plastic gloves. The material was stored in isothermal boxes at 4°C until laboratory processing.

Each sample was individually processed by the Baermann technique (Forrester and Lankester, 1997). Larvae found were morphologically analyzed and features of anterior and posterior ends were recorded (Boev, 1975). Measurements were obtained using the software TCCapture 4.3.

Descriptive statistical analysis was performed to obtain relative and absolute frequencies. In addition, the Lilliefors test was used to verify the normality of the data. The relationship between the number of young (≤ 12 months old) and adult animals (> 12 months old) parasitized, as well as the occurrence of parasites in male and female, were evaluated through the Fisher's Exact test,

with 5% significance level. The BioEstat software, version 5.3 was used to perform all the statistical analyses.

Lungworm larvae were detected in 8.19% (10/122) of goat samples, whereas all cattle and sheep scored negative. Larvae (n=25) of *Protostrongylus* sp. with an average length of 351 μm ($\pm 29.06 \mu\text{m}$) and width mean of 19 μm ($\pm 1.46 \mu\text{m}$) were detected. Morphologically, they presented the tip of the tail typical of subfamily Protostrongylinae, consisting of an elongated, thin, pointed, and slightly undulating process; the dorsal spine at the insertion of the tip of the tail (typical of the protostrongylid subfamilies Muelleriinae, Varestrostrongylinae, and Elaphostrongylinae) was absent. In addition, the presence of small granules was observed in the intestine.

Young goats (30.0%; 6/20) were statistically more parasitized than adults (3.9%; 4/102) ($p=0.0013$). Conversely, no significant difference was observed in the parasitism of females (7.3%; 8/110) and males (16.7%; 2/12) ($p=0.2854$). All positive animals were concentrated in three different farms in the municipality of Paranatama (Microrregion of Garanhuns). They were reared in a semi-intensive production system and did not present any clinical signs suggestive of the infection by lungworm nematodes. The overall positivity according to the age, sex and rearing system of animals is reported on Table 1.

Table 1. Prevalence of *Protostrongylus* sp. according to age, sex, and rearing system in goats from the Microregion Garanhuns, Northeastern Brazil

	Goats % (n/N)
Age	
Young	30.0% (6/20)
Adult	3.9% (4/102)
Sex	
Female	7.3% (8/110)
Males	16.7% (2/12)
Rearing system	
Intensive	0.0% (0/58)
Semi-intensive	15.6% (10/64)

This study provides laboratorial evidence of the parasitism by *Protostrongylus* sp. in goats in Northeastern Brazil, as well as the absence of other lungworms parasites in cattle and sheep raised in the same region. In Brazil, data about this

parasite are limited only to two reports (Duarte and Miranda, 1984; Macedo *et al.*, 2020). The overall positivity (8.19%; 10/122) observed in this study is lower than that detected in previous research conducted in same area (i.e., 18.9%; 41/217) (Macedo *et al.*, 2020).

The dynamics of lungworm infection in ruminants is highly influenced by environmental conditions, which directly impact the development of larval stages and transmission opportunities (Habte and Simeneh, 2019). Therefore, the difference of parasitism between cattle/sheep and goats may be related to the period of the year, as well as the feeding behavior presented by these hosts. Cattle and sheep are known as grazers and are constantly challenged by parasites, developing a more effective immunity (Hoste *et al.*, 2010; Underwood *et al.*, 2015). On the other hand, goats are classified as intermediate selectors, and during the meal are less challenged by parasites, which difficult the development of resistance against nematodes (Hoste *et al.*, 2008).

Although, in grazing conditions both young and adult goats may be infected (Hoste *et al.*, 2008), in this study young animals were predominantly affected ($p=0.0013$). Immunological mechanisms against nematodes are differently expressed between young and adult animals (Hoste *et al.*, 2010). For this reason, clinical disease is more common at their first grazing season in young animals, whereas older goats developed a strong immunity over the time (Tolossa, 2019). Females and males were equally affected ($p=0.2854$). However, the association of positivity and sex requires additional analysis because in this study the number of males ($n=12$) evaluated was lower than females ($n=110$).

All positive animals were concentrated in three farms in a single municipality (i.e., Paranatama). This area is featured by a semi-arid climate with mean annual temperature of 22°C, mean rainfall of 147 mm and relative air humidity of 90%. It is known that the prevalence of lungworms is strongly influenced by different environmental aspects and climatic conditions (e.g., precipitation, humidity, and temperature) (Adem, 2016). From a climatic point of view, the region provides all suitable conditions for survival and development of *Protostrongylus* larvae (Fentahun

et al., 2016). Although, in this area the mollusk fauna has never been studied, it is known that the genus *Protostrongylus* uses different species of gastropods (e.g., *Vallonia* and *Helix*) as intermediate hosts (Kuchboev *et al.*, 2017; Tolossa, 2019). In Northeastern Brazil, the *Achatina fulica* may participate as an intermediate host for other nematodes (e.g., *Angiostrongylus* spp.) (Thiengo *et al.*, 2010); but the participation of this mollusk species in the development of lungworms affecting ruminants has never been evaluated.

All infected goats were raised in a semi-intensive production system and did not exhibit any clinical signs suggestive of the infection by lungworms. A common practice adopted in these farms is the confinement of animals at night, and the free grazing during the day. This kind of system favors the contact with contaminated pastures and putative intermediate hosts (mollusks) (Tolossa, 2019). The management of each farm associated with the nutritional status, level of immunity acquired by animals and the period of infection, may explain the absence of clinical signs, the reduced number of infected animals, as well as the low larvae excretion (Habte and Simeneh, 2019). Additionally, it is important to highlight that the use of anthelmintic compounds may contribute to the absence of clinical signs. However, the historical of deworming was absent in the farms herein sampled, which difficult this kind of analysis.

This study provides important data on lungworm infection in goats in Northeastern Brazil. Although, no clinical signs had been observed in infected animals, these data sound as warning to veterinary practitioners, which may include these parasites in the list of putative causes of respiratory diseases. Finally, it is imperative the adoption of appropriate sanitary measures, as well as a good nutritional management of animals reared in this area to prevent infection by these nematodes and to reduce the economic impact they may cause. Further studies focusing especially on the determination of intermediate hosts involved in the life cycle of *Protostrongylus* will be useful to fill in an important gap on the natural history of this nematode.

Keywords: goats, lungworms, epidemiology

RESUMO

Objetivou-se neste estudo determinar a prevalência de vermes pulmonares em ruminantes do semiárido, nordeste do Brasil. Amostras fecais (n=429), de bovinos (n=219), caprinos (n=122) e ovinos (n=88) foram coletadas e laboratorialmente analisadas pela técnica de Baermann. Larvas de *Protostrongylus* sp. foram detectadas em 8,19% (10/122) dos caprinos. Elas apresentaram o comprimento médio de 351µm ($\pm 29,06\mu\text{m}$) e largura média de 19µm ($\pm 1,46\mu\text{m}$). Todos os animais positivos eram mantidos em sistema de criação semi-intensivo e não apresentavam sinais clínicos sugestivos da infecção por nematódeos pulmonares. Bovinos e ovinos foram negativos. Este estudo fornece dados sobre a infecção por parasitos pulmonares em caprinos de uma importante área de criação de pequenos ruminantes no Nordeste do Brasil, onde informações sobre esses parasitos são quase inexistentes. Apesar da ausência de sinais clínicos nos animais deste estudo, medidas sanitárias são preconizadas para prevenir a infecção por esses nematódeos e reduzir o impacto econômico que eles podem causar na produção pecuária.

Palavras-chave: caprinos, vermes pulmonares, epidemiologia

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