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Serological study of the *Leptospira* spp. infection in sheep and goats slaughtered in the State of Paraíba, semiarid of Northeastern Brazil

Estudo sorológico da infecção por *Leptospira* spp. em caprinos e ovinos abatidos no Estado da Paraíba, semiárido do Nordeste, Brasil

Diego Figueiredo da Costa[1]; Aline Ferreira da Silva[2]; Areano Ethério Moreira de Farias[1]; Arthur Willian de Lima Brasil[1]; Fabrine Alexandre dos Santos[1]; Ricardo de Figueiredo Guilherme[1]; Sergio Santos de Azevedo[3]; Clebert José Alves[3*]

Abstract

The aim of this survey was to determine the frequency of anti-*Leptospira* spp. antibodies in goats and sheep slaughtered in different slaughterhouses in the State of Paraiba, in the semi-arid region of Northeastern Brazil. Blood samples were collected from 500 goats and 500 sheep randomly selected. To verify the presence of anti-*Leptospira* spp. antibodies the microscopic agglutination test (MAT), using 24 serovars as antigens, was used. From the 1,000 animals analyzed, 82 (8.2%; 95% CI = 7.0%-10.5%) were sero-reactive being 26/500 (5.2%; 95% CI = 3.5%-7.5%) in goats and 56/500 (11.2%; 95% CI = 8.7%-14.2%) in sheep. The most frequent serovars were Hardjobovis (14.6%) and Autumnalis (13.4%). In goats, the most frequent serovar was the Hardjobovis, and in sheep the Ballum, with frequencies of 19.2% and 17.9%, respectively. There was a significant difference in the frequency of positive animals among slaughterhouses, both for goats ($p = 0.035$) and for sheep ($p = 0.004$), with the county of Alhandra presenting the highest frequency of seropositive animals for both species. It is concluded that sheep and goats from the semiarid region of the Northeastern Brazil may have become adapted to the serovars Hardjobovis e Autumnalis, as well as wild rodents may be involved in the transmission of the disease. It is possible that the climatic conditions influenced in the transmissibility of the leptospirosis, especially in the Mata Paraibana mesoregion, however it was not considered as being sufficient to justify the low frequency of seropositive animals. In this way, it is possible to suggest the hypothesis that the rusticity of the small ruminants in the studied region contributed to the low sero-reactivity verified.
Key words: Adaptability. Goats. Leptospirosis. Sheep. Rusticity.

Resumo

O objetivo deste estudo foi determinar a frequência de anticorpos anti-*Leptospira* spp. em caprinos e ovinos abatidos em diferentes matadouros no Estado da Paraíba, região semiárida do Nordeste brasileiro. Foi coletado sangue de 500 caprinos e 500 ovinos aleatoriamente selecionados. Para verificar a presença de anticorpos anti-*Leptospira* spp. empregou-se o teste soroaglutinação microscópica (SAM), utilizando-se 24 sorovares como antígenos. Dos 1.000 animais analisados 82 (8,2%; IC 95%= 7,0%-10,5%) foram sororreagentes, sendo 26/500 (5,2%; IC 95% =3,5%-7,5%) em caprinos e 56/500 (11,2%; IC 95% 8,7%-14,2%) em ovinos. Os sorovares mais frequentes foram Hardjobovis (14,6%)

[1] Discentes do Curso de Doutorado do Programa de Pós-Graduação em Medicina Veterinária, Universidade Federal de Campina Grande, UFCG, Patos, PB, Brasil. E-mail: diegoveter@hotmail.com; areanomv@yahoo.com.br; arthurwillian7@yahoo.com.br; fabrinevet@yahoo.com.br; ricardo.figueiredo@hotmail.com

[2] Discente do Curso de Graduação em Medicina Veterinária, UFCG, Patos, PB, Brasil. E-mail: aline_ferreirabsf@hotmail.com

[3] Profs., Programa de Pós-Graduação em Medicina Veterinária, UFCG, Patos, PB, Brasil. E-mail: sergio.azevedo@pq.cnpq.br; clebertja@uol.com.br

[*] Author for correspondence

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e Autumnalis (13,4%). Na espécie caprina, o sorovar mais frequente foi o Hardjobovis, e na espécie ovina o Ballum, com frequências de 19,2% e 17,9%, respectivamente. Houve diferença significativa na frequência de positivos entre os matadouros, tanto para caprinos (p = 0,035) quanto para ovinos (p = 0,004), com o município de Alhandra apresentando a maior frequência de soropositivos para ambas as espécies. Concluiu-se que ovinos e caprinos da região semiárida do Nordeste podem estar adaptados aos sorovares Hardjobovis e Autumnalis, bem como roedores silvestres estarem envolvidos na transmissão do agente. Possivelmente as condições climáticas influenciaram a transmissibilidade da leptospirose, especialmente na mesorregião da Mata Paraibana, entretanto isso não foi considerado suficiente para justificar a baixa frequência de animais soropositivos. Deste modo, é possível sugerir a hipótese de que a rusticidade dos pequenos ruminantes na região estudada contribui para a baixa sororreatividade verificada.
Palavras-chave: Adaptabilidade. Caprinos. Leptospirose. Ovinos. Rusticidade.

Introduction

The economic exploitation of goat and sheep breeding is regarded as an economically viable alternative to farming in several regions of Brazil, particularly in the Northeast (HORA et al., 2013). At present, Brazil is home to approximately 9,384,894 goats and 17,662,201 sheep, of which approximately 90% (8,538,255) and 57% (10,110,352), respectively, are located in the Northeast region (IBGE, 2011). However, inadequate sanitization often results in reproductive loss, creating the need to replenish the animal stock (ESCÓCIO, 2009). In this context, leptospirosis is a cause for concern as it is widely disseminated and can lead to abortion, birth of weak and premature offspring, and decreased milk production (ELLIS, 2015).

Leptospirosis is a cosmopolitan zoonotic infection caused by pathogenic spirochetes of the genus *Leptospira* spp. (ADLER; DE LA PEÑA MOCTEZUMA, 2010). Its presence is associated with environmental factors and its incidence is strongly linked to periods of heavy rainfall (ALVES et al., 1996; LILENBAUM et al., 2008). Under favorable conditions and in the presence of suitable hosts, spirochetes can persist in the environment from weeks to months (FAINE et al., 1999). Leptospirosis is transmitted primarily via exposure to contaminated soil or water and contact with urine of infected animals (ADLER; DE LA PEÑA MOCTEZUMA, 2010).

Infection by *Leptospira* spp. is caused by serovars that have either adapted to the host or have not adapted (accidental infection). When a serovar is adapted to a particular species, the chronic form of the disease predominates and, in these cases, miscarriages, stillbirths, birth of weak offspring, and high mortality rates within the first few days of life are common (GERRITSEN et al., 1994). During accidental infection, the acute form of the disease predominates; in these cases, fever, anorexia, depression, jaundice, anemia, and hemorrhagic syndromes are the most perceived signs in small ruminants (MARTINS et al., 2012).

The identification of spirochetes can be performed by various clinical and laboratory methods (FAINE et al., 1999). In small ruminants, since the symptoms are less evident or even absent, the diagnosis is based primarily on laboratory tests (FAINE et al., 1999; MARTINS; LILENBAUM, 2014). The microscopic agglutination test (MAT) is the serological test recommended by the World Organization for Animal Health (OIE, 2012). The main advantage is its high specificity; however, it requires specific laboratory equipment and highly trained staff and, for this reason, can only be conducted in a few reference laboratories (BOURHY et al., 2013).

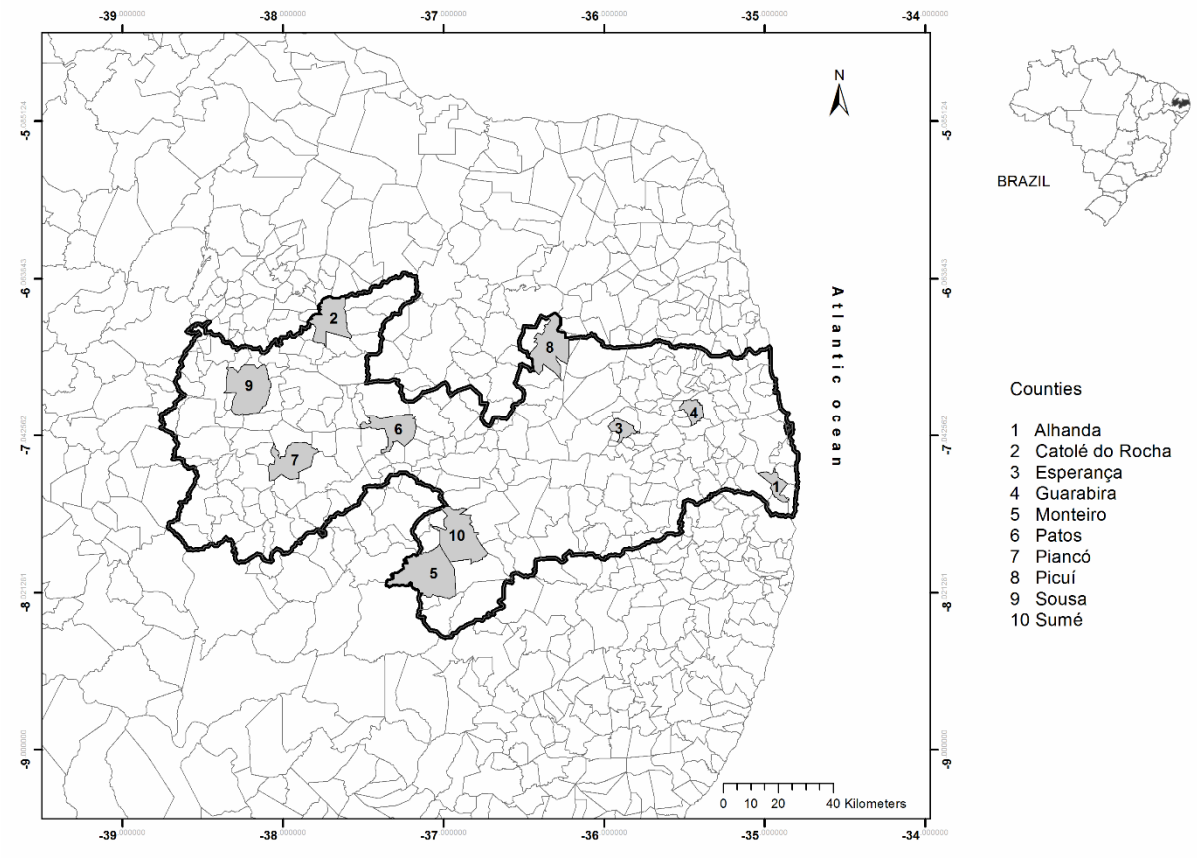
Despite their distinct variations and specific reservoirs, serovars that cause leptospirosis generally occur in regions and species to which they are most adapted; consequently, knowledge on these serovars and their reservoirs is necessary for

a better understanding of the disease epidemiology (ADLER; DE LA PEÑA MOCTEZUMA, 2010; GIANGASPERO et al., 2013). Slaughterhouses play an important role in the study of infectious diseases, particularly leptospirosis, as that is where the largest numbers of viable spirochetes are recovered from animals. For this reason, slaughterhouses are strategic environments for active surveillance in the identification of the origin of potential sources of outbreaks. Therefore, the aim of this study was to detect antibodies against *Leptospira* spp. in sheep and goats slaughtered within the state of Paraíba, a semi-arid region in Northeast Brazil.

Materials and Methods

Study design: The study was conducted in slaughterhouses from 10 municipalities located in the state of Paraíba, Northeast Brazil (Figure 1). The study population consisted of sheared sheep and adult goats of both sexes, which were intended for slaughter. To calculate the number of animals to be sampled, the following parameters were considered: (a) expected prevalence of 50% (used to maximize sampling), (b) absolute error of 5%, and (c) 95% confidence interval, according to the formula used for simple random samples (THRUSFIELD, 1995). According to these parameters, the minimum sample size would be of 384 goats and 384 sheep; however, 500 goats and 500 sheep were used.

Figure 1. Map of the Paraíba State showing the studied counties.



Sampling: Between April and December 2012, blood samples were collected from 1,000 animals on the slaughter line, including 500 samples from each animal species: 315 male and 185 female goats and 359 male and 141 female sheep. Blood samples were collected shortly before animal bleeding using accurately labeled, sterile, 8-mL vacuum blood collection tubes. Subsequently, the samples were transferred to the laboratory and centrifuged, and the serum was stored at –20°C until the serological tests were performed.

Serological test: Anti-*Leptospira* spp. antibodies were detected by the microscopic agglutination technique (MAT) (OIE, 2012) using the following antigens: *L. biflexa* serovars Andamana and Patoc; *L. interrogans* serovars Australis, Copenhageni, Bataviae, Bratislava, Canicola, Grippotyphosa, Hardjoprajitno, Pomona, Pyrogenes, Icterohaemorrhagiae, Hebdomadis, Wolffi, and Butembo; *L. borgpetersenii* serovars Autumnalis, Castellonis, Hardjobovis, Javanica, and Tarassovi; *L. santarosai* serovars Guaricura, and Shermani; *L. kirschnerii* serovar Cynopteri; and *L. noguchii* serovar Panama, provided by the Laboratory of Veterinary Bacteriology of the Fluminense Federal University (Universidade Federal Fluminense-UFF) and originated from the Pasteur Institute, France. All samples with agglutinating activity at a dilution of 1:100 were considered positive. The positive samples were titrated serially using a ratio of two. The cutoff point was the sample with the highest dilution that produced 50% agglutination compared with the control. The highest titer obtained was used to identify the causative serovar.

Statistical analysis: The association between seropositivity and the municipality of origin of the animals was determined using the chi-square test or G test at a significance level of 5% (ZAR, 1999) using BioEstat software version 5.03.

Results and Discussion

Of the 1,000 animals evaluated, 82 (8.2%; 95% CI = 7.0%-10.5%) were seropositive, and the prevalence rate was 5.2% in goats (26/500; 95% CI = 3.5%-7.5%) and 11.2% in sheep (56/500; 95% CI = 8.7%-14.2%) (Table 1). The most common serovars in the species studied were Hardjobovis (14.6%) and Autumnalis (13.4%). The most common serovars in goats were Hardjobovis (19.2%), Autumnalis and Patoc (15.4%), Bratislava, Castelonis, Pomona, and Icterohaemorrhagiae (11.6%), and Canicola (3.8%) (Table 2). The most common serovars in sheep were Ballum (17.9%), Autumnalis and Hardjobovis (12.5%), Castelonis (10.7%), Patoc and Pomona (8.9%), Icterohaemorrhagiae and Javanica (7.1%), Bratislava and Shermani (5.4%), and Canicola (3.6%) (Table 3). A significant difference was observed in the frequency of seropositives between the slaughterhouses in both, goats (p = 0.035) and sheep (p = 0.004), with the highest frequency of seropositives found in the municipality of Alhandra for both species.

The frequency of seropositive sheep in this study was lower than that previously reported for most studies conducted with this species in other Brazilian states: 34.3% in Rio Grande do Sul (HERRMANN et al., 2004), 33.3% in Rondônia (AGUIAR et al., 2010), 22.0% in Minas Gerais (SALABERRY et al., 2011), 47.4% in Rio de Janeiro (MARTINS et al., 2012), and 22.8% in São Paulo (RIZZO et al., 2014). However, these frequencies were higher than those found in surveys conducted in the Northeast region: 3.5% in Rio Grande do Norte (AZEVEDO et al., 2004) and 7.5% in Paraíba (HIGINO et al., 2010). In goats, these frequencies were lower than those reported in previous studies: 11.1% in Rio de Janeiro (LILENBAUM et al., 2007), 31.3% in Minas Gerais (SANTOS et al., 2012), 25.9% in Rio de Janeiro (MARTINS et al., 2012.), and 14.5% in Rio Grande do Norte (ARAÚJO NETO et al., 2010). A similar frequency (8.7%) was found in Paraíba (HIGINO et al., 2012).

Table 1. Frequency of seropositive animals to *Leptospira* spp., according to county of origin and annual rainfall, in sheep and goats slaughtered in the State of Paraíba, Northeastern Brazil, from April to December 2012.

County	Goats		Sheep		Annual rainfall (mm)*
	Nº of animals	Nº of positive (%)	Nº of animals	Nº of positive (%)	
Alhandra	50	7 (14)	50	14 (28)	1,511.1
Catolé do Rocha	50	4 (8)	50	6 (12)	410.0
Esperança	50	3 (6)	50	4 (8)	518.7
Guarabira	50	3 (6)	50	8 (16)	828.4
Monteiro	50	2 (4)	50	3 (6)	194.1
Patos	50	1 (2)	50	3 (6)	199.1
Piancó	50	4 (8)	50	4 (8)	232.6
Picuí	50	-	50	1 (2)	45.7
Sousa	50	-	50	9 (18)	406.5
Sumé	50	2 (4)	50	4 (8)	27.0
Total (%)	500 (100)	26 (5.2)	500 (100)	56 (11.2)	-

*Data from AESA (2014) (Agência Executiva de Gestão das Águas do Estado da Paraíba).

Table 2. Most prevalent *Leptospira* spp. serovars in goats from slaughterhouses of the Paraíba State, Northeastern Brazil, according to antibody titers, from April to December 2012.

Serovars	Titers				Total (%)
	100	200	400	800	
Hardjobovis	3	2	-	-	5 (19.2)
Autumnalis	2	2	-	-	4 (15.4)
Patoc	3	1	-	-	4(15.4)
Bratislava	2	1	-	-	3 (11.6)
Castelonis	3	-	-	-	3 (11.6)
Pomona	-	2	-	1	3 (11.6)
Icterohaemorrhagiae	2	1	-	-	3 (11.6)
Canicola	-	-	1	-	1 (3.6)
Total (%)	15 (57.7)	9 (34.7)	1 (3.8)	1 (3.8)	26 (100)

Table 3. Most prevalent *Leptospira* spp. serovars in sheep from slaughterhouses of the Paraíba State, Northeastern Brazil, according to antibody titers, from April to December 2012.

Serovars	Titers				Total (%)
	100	200	400	800	
Ballum	6	2	2	-	10 (17.9)
Autumnalis	4	3	-	-	7 (12.5)
Hardjobovis	6	1	-	-	7 (12.5)
Castelonis	5	1	-	-	6 (10.7)
Patoc	5	-	-	-	5 (8.9)
Pomona	1	2	-	2	5 (8.9)
Icterohaemorrhagiae	2	1	1	-	4 (7.1)
Javanica	1	2	-	1	4 (7.1)
Bratislava	2	1	-	-	3 (5.4)
Shermani	3	-	-	-	3 (5.4)
Canicola	1	-	1	-	2 (3.6)
Total (%)	36 (64.3)	13 (23.2)	4 (7.1)	3 (5.4)	56 (100)

The low frequency of seropositives found in this study, compared with studies in other Brazilian regions, may be associated with a possible difference in susceptibility between the breeds of the animal species evaluated. Some studies showed that race is a risk factor for leptospirosis in small ruminants, in which a greater susceptibility to infection was observed among purebred animals compared with crossbred animals (SILVA et al. 2012; SANTOS et al., 2012). In the semi-arid region of Northeast Brazil, most of the sheep comprise of crossbred races (CEZAR et al., 2004), while the goats belong to native and indeterminate breeds with remarkable robustness (SILVA; ARAÚJO, 2000). Therefore, we suggest the hypothesis that, in addition to environmental conditions, the robustness of the animal species evaluated contributed to the low seropositivity observed in this study. It should be noted that, although the relationship between rainfall and the frequency of seropositive animals is well established (ALVES et al., 1996; VANASCO et al., 2008), the same phenomenon was not observed in cattle in the same region, with 61.1% seropositivity (PIMENTA et al., 2014).

Hardjobovis and Autumnalis were the most frequent serovars of sheep and goats found in this study, a result that corroborates the findings of recent serological surveys conducted on small ruminants in Brazil (HERRMANN et al., 2004; LILENBAUM et al., 2007; ARAÚJO NETO et al., 2010; HIGINO et al., 2010; SALABERRY et al., 2011; CARVALHO et al., 2011; SANTOS et al., 2012; MARTINS et al., 2012; MORAES et al., 2012; ALVES et al., 2012; HIGINO et al., 2012; RIZZO et al., 2014). It was long believed that these animals acquired leptospirosis only by prior contact with other species (LEON-VIZCAINO et al., 1987), although the breeding of these species together with cattle breeding and the presence of rodents are important factors for the dissemination of the Hardjobovis and Autumnalis serovars, respectively (FAINE et al., 1999; SALABERRY et al., 2011; SEIXAS et al., 2011). However, reports of leptospirosis cases caused by the serovar Hardjo in sheep and goats without contact with cattle (COUSINS et al., 1989; GERRITSEN et al., 1994; DORJEE et al., 2008) and numerous reports of Autumnalis as the most frequent serovar among small ruminants raise the possibility that these animals may be reservoirs of these serovars. Considering that not even heavy rainfall in some regions was sufficient to boost the humoral immune response of these animals, with a predominant antibody titer of 1:100 (62.2%) in the species evaluated in this study, suggests their adaptability to these serovars and the plausibility of them being potential chronic carriers of the disease.

There are several risks associated with these serovars: Hardjo is considered the most frequent causative agent of reproductive disorders (abortions), subclinical infections, and death of young animals in small ruminants (FAINE et al., 1999; HERRMANN et al., 2004; LILENBAUM et al., 2009). Although Autumnalis has been isolated from sheep (SILVA et al., 2007), to date, commercial veterinary vaccines have not included this serovar in their composition and its virulence is not well established in sheep. Consequently, sheep, or in the general context the small ruminants, are important sources of infection of this serovar to other animal species and humans (ALVES et al., 2012), putting slaughterhouse workers at occupational risk. In New Zealand, infections in slaughterhouse workers accounts for approximately 50% of all cases of human leptospirosis and this rate is higher than that found in infected rural workers (DORJEE et al., 2011). Dorjee et al. (2008) reported that slaughterhouses pose a potential risk of transmission of leptospirosis because of the exposure of workers. Silva et al. (2012) reported that sheep can be reservoirs of *Leptospira* spp. that can transmit the infection to humans and other domestic animals and thereby spread the disease to slaughterhouse workers who handle blood and viscera without personal protective equipment.

The serovar Ballum, although rarely reported in prevalence studies conducted in Brazil, was the

most frequent serovar in sheep in this study, and is increasingly observed with other unusual serovars. Silva et al. (2010) reported that the appearance of new serovars and the decreased frequency of those previously considered common may be associated with the fact that the majority of the vaccines do not include these new serovars in their composition. As animals are not immunized, they act as sources of infection for other animals and humans when they are exposed to infection by these serovars. The Ballum serovar has gained more prominence in the number of human cases of leptospirosis, as the second-most frequent serovar in New Zealand (THORNLEY et al., 2002). This serovar was recently reported to be the leading cause of severe leptospirosis in humans in Cuba (SILVA et al., 2010). Small domestic mice or rats are the main reservoirs of this serovar in nature (BHARTI et al., 2003). Usually, these reservoirs do not exhibit clinical disease and can intermittently shed spirochetes through urine into the environment for long periods (SILVA et al., 2010). In Southern Brazil, the Ballum serogroup was isolated in four domestic rats (*Mus musculus*) captured in households and its strains exhibited distinct degrees of virulence (SILVA et al., 2010). It is possible that, owing to the limited food supply as a result of low rainfall in the state of Paraíba at the time of sample collection, the reservoirs of this serovar may have had access to sheep when seeking shelter and food near corrals and feed deposits, thereby possibly being in contact with contaminated urine.

The Ballum serogroup includes the Castellonis serovar, which was also identified in this study. This serovar is most common among wild animals (ALVES et al., 1996), whose presence has been recognized as a risk factor for bovine leptospirosis in the region (PIMENTA et al., 2014). The Brazilian guinea pig (*Cavia aperea*) is one of the most common wild rodents in Paraíba (ALVES et al., 1996) and is also an important reservoir of the Icterohaemorrhagiae serovar (CUBAS et al., 2007). Santos et al. (2000) isolated pathogenic spirochetes from the kidneys of these animals in Paraíba, demonstrating their importance as potential reservoirs of *Leptospira* spp. However, little can be done regarding wild species because many of them are protected by law and legal obstacles limit the access and capture of these animals for a more thorough investigation, favoring their involvement in the epidemiology of the disease and hindering their control.

The fact that the slaughterhouse in Alhandra, located in the mesoregion Mata Paraiba, showed the highest frequency of seropositivity in the species evaluated in this study, may be associated with the high precipitation index (1,511.1 mm) recorded in the municipality in 2012. This was above the minimum level established (500 to 550 mm) for the survival of spirochetes and for disease transmission in the region (ALVES et al., 1996). The fact that this mesoregion emerged as relevant, despite the fact that the state of Paraíba had lower than expected amount of rainfall (AESA, 2014), may be associated with its tropical Atlantic climate, unlike the rest of the state which is characterized by a semiarid climate (GIRARDI; ROSA, 2011). The occurrence of leptospirosis is already expected in herds located in tropical and subtropical regions with heavy rainfall (SALABERRY et al., 2011). According to Lilenbaum et al. (2007), the weather can be considered a risk factor for leptospirosis seropositivity, with animals in tropical climates showing a 2.63-higher likelihood of becoming seropositive.

Conclusion

It was concluded that sheep and goats in the semi-arid region of Northeast Brazil may be adapted to the *L. borgpetersenii* serovars Hardjobovis and Autumnalis, and that wild rodents are involved in the transmission of leptospirosis. The climatic conditions possibly affect the transmissibility of leptospirosis, especially in the mesoregion of Mata Paraíba; however, this factor was not considered sufficient to justify the low occurrence

of seropositive animals in this study. Therefore, we hypothesize that the robustness of the small ruminants in the study area contributed to the low seropositivity observed.

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