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Frequency of and risk factors associated to *Leptospira* spp. seropositivity in goats in the state of Sergipe, Northeastern Brazil

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ABSTRACT: The objective of this study was to determine the frequency and factors associated with the occurrence of seropositivity for *Leptospira* spp. in goat herds in the state of Sergipe, Brazil, on the basis of the analysis of 675 samples collected from 41 properties in 2013-2014. Analysis of anti-*Leptospira* spp. antibodies revealed that 25.74% goats (194) were seropositive and that 90.24% (37) of the properties had at least one infected goat. The most prevalent serogroup was *Icterohaemorrhagiae* (85.57%); although, the study animals were reactive to other serogroups: *Australis* (5.15%), *Pomona* (3.61%), *Sejroe* (3.09%), and *Pyrogenes* (2.58%) had titers ≤ 400 in 96.91% of cases. The origin of the water source [still water source (OR=3.86)] was associated with seropositivity. Results reflected the importance of adopting appropriate management practices for herds in Sergipe.

Key words: water source, leptospirosis, serogroup, *Icterohaemorrhagiae*, test microscopic agglutination test.

Frequência e fatores de riscos associados a caprinos soropositivos a *Leptospiras* spp. no Estado de Sergipe, Nordeste do Brasil

RESUMO: O objetivo deste estudo foi determinar a frequência e os fatores associados à soropositividade para *Leptospira* spp. em caprinos do Estado de Sergipe, Nordeste do Brasil, utilizando 675 animais de 41 propriedades no período de 2013 e 2014. A frequência de positividade entre os animais foi de 25,74% (194), e em 90,24% (37) das propriedades houve pelo menos um animal soropositivo. O sorogrupo reagente predominante foi o *Icterohaemorrhagiae* (85,57%), com presença também de animais reagentes para *Australis* (5,15%), *Pomona* (3,61%), *Sejroe* (3,09%) e *Pyrogenes* (2,58%) com títulos ≤ 400 em 96,91% dos casos. A origem da água fornecida (água parada) (OR=3,86) foi o fator associado à presença de caprinos positivos. Ressalta-se a importância da adoção de práticas de manejo adequadas nos rebanhos caprinos sergipanos.

Palavras-chave: fonte de água, leptospirose, sorogrupo, *Icterohaemorrhagiae*, teste de soroaglutinação microscópica.

INTRODUCTION

Leptospirosis is a major public health problem in Brazil with an annual average rate of 3,780 human cases between 2000 and 2015, 9.6% (364) of which were lethal. In the country's Northeastern region and in the state of Sergipe, lethality is as high as 12.8% (87/681) and 24.1% (10.6/44), respectively (SINAN/SVS/MS, 2017a, b). Leptospirosis is caused by spirochetes of the genus *Leptospira*. Twenty-five serogroups and approximately 300 serovars belonging to 25 serogroups can infect domestic animals, wildlife, and humans. On rural properties, transmission occurs primarily via the urine of infected animals (GOMES,

2013; HIGINO & AZEVEDO, 2014). Accordingly, rural workers in Jamaica who have contact with goats have a 3.52 times higher chance of presenting symptoms of the disease (KEENAN et al., 2010).

Goats usually present chronic infection, with a predominance of embryonic absorption, miscarriage, neonatal mortality, and hemorrhagic jaundice. In the acute form of the disease, a sudden onset of fever, anemia, jaundice, and hemoglobinuria occur, leading to death (MARTINS et al., 2012; HIGINO & AZEVEDO, 2014).

In the Northeastern region of Brazil, surveys were performed in the states of Rio Grande do Norte, Ceará, Paraíba, and Pernambuco. The rate of seropositive goats based on the microscopic

agglutination test (MAT) ranged from 1.5% to 33.3%. The most common serogroups in the Brazilian states were Icterohaemorrhagiae, which were positive in a sample from Ceará (FAVERO et al., 2002); Australis, Djasiman, Celledoni, Semarang, and Sejroe, which were reported in the state of Paraíba (FAVERO et al., 2002; HIGINO et al., 2013; COSTA et al., 2016); Autumnalis, which were detected in Rio Grande do Norte; (ARAÚJO NETO et al., 2010); and Canicola and Autumnalis, which were reported in Pernambuco (CUNHA et al., 1999). Surveys were conducted in other Brazilian states, including Rio Grande do Sul, Santa Catarina, Rio de Janeiro, Espírito Santo, and Minas Gerais, and the infection rates were 2.4%-35.47% (FAVERO et al., 2002; SCHMIDT et al., 2002; LILENBAUM et al., 2007b; LILENBAUM et al., 2008; DOS SANTOS, et al., 2012; CORTIZO et al., 2015; TOPAZIO et al., 2015).

The only serogroup isolated from goats in Brazil is Grippotyphosa, which has been reported in cultivated urine samples (LILENBAUM et al., 2007a). Studies involving goat herds from Brazilian states reported the following risk factors associated with the occurrence of *Leptospira* spp.: rodent infestations and a history of infertility in goats in the state of Paraíba (HIGINO et al., 2013); contact with sheep and the presence of concentrate in animal feed in the state of Santa Catarina (TOPAZIO et al., 2015); an intensive breeding system, presence of purebreds, animals aged >12 months, breeding systems involving other animal species, and farms that employed outside manual labor in the state of Minas Gerais (SANTOS et al., 2012); a semi-intensive farming system, a still water source (a well), and cases of miscarriage in the state of Espírito Santo (CORTIZO et al., 2015); and the tropical climate, absence and/or low frequency of veterinary care, and access to pastures in the state of Rio de Janeiro (LILENBAUM et al., 2008).

Knowledge regarding leptospirosis in goats is still scarce, and monitoring measures are still empirical because of the lack of information on infection, particularly in tropical conditions (MARTINS & LILENBAUM, 2014). Therefore, the aim of this study was to determine the frequency of *Leptospira* spp. seropositivity in goat herds in the state of Sergipe, Brazil, and to identify risk factors.

MATERIALS AND METHODS

The study was conducted in the Brazilian state of Sergipe (9°30'49" and 11°34'05" latitude and 36°23'40" and 38°15'00" longitude), which has a land area of 21,918,454km² and is divided into

three semiarbitrary mesoregions (the East region, the *Agreste* agricultural region, and the semiarid *Sertão* outback). The Northeastern region of Brazil has 8,109,672 goat heads, and Sergipe is the state with the smallest goat population in this region, with 23,647 animals. Of these, 8,265 are located in the *Sertão* mesoregion, 11,840 are located in the *Agreste* region, and 3,542 are located in the East region (IBGE, 2014). Sample size was calculated using the method described by Thrusfield (2007) according to the following parameters: a population of 23,647,000 goats (IBGE, 2014), a confidence interval of 95%, an error of 5%, and a prevalence of 11.83%, which represents the mean of serological surveys in goats in the Northeastern region of Brazil (CUNHA et al., 1999; FAVERO et al., 2002; ARAÚJO NETO et al., 2010; HIGINO et al., 2013; COSTA et al., 2016). This process resulted in a minimum sample of 369 goats (123 goats per mesoregion). Samples were collected in 2013 and 2014, and 675 blood samples were obtained from goats from 41 properties. Seven to 20 animals, of different breeds which were, apparently healthy and which were older than 6 months of age, were selected randomly from each herd. Properties were located in 15 municipalities of the three mesoregions: East, *Agreste* or *Sertão*. A total of 178, 233, and 264 samples were collected from each of these mesoregions, respectively. The choice of cities and properties was based on state divisions, convenience, ease of access, and the availability of goat ranches.

A structured questionnaire including closed-ended questions was designed to obtain information on the handling characteristics and the herd facilities to assess the factors associated with *Leptospira* spp. infection. Blood samples were collected from the animals' jugular veins using sterile vacuum tubes, centrifuged at 1,600g for 10min, transferred to 2-mL polypropylene tubes, and frozen at -20°C until processing. Microscopic agglutination tests were conducted in the Laboratory for Reproductive Bacterial Diseases in the Biological Institute of São Paulo to detect antibodies against *Leptospira* spp. using a collection of 22 serovars belonging to 18 serogroups (*Leptospira interrogans* serovars Australis, Bataviae, Bratislava, Butembo, Canicola, Celledoni, Copenhageni, Djasiman, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Pomona, Pyrogenes, Wolffi; *Leptospira borgpeterseni* serovars Autumnalis, Castellonis, Hardjibovis Javanica, and Tarassovi; *Leptospira santarosai* serovars Shermani; *Leptospira kirschneri* serovars Cynopteri; and *Leptospira noguchii* serovars

Panama) and leptospire were grown in a modified EMJH medium (ALVES et al., 1996).

Samples with visible agglutination at a dilution of 1:100 were considered positive (FAINE et al., 1999). Absolute and relative frequencies were determined using descriptive statistical analysis. The risk factors were evaluated by analyzing the univariate variables of interest using Pearson chi-squared test or Fisher exact test when necessary. Logistical regression analysis was performed, in which the dependent variable for *Leptospira* spp. infection was the MAT results (positive or negative). The independent or explanatory variables considered in the model were those that exhibited a statistical significance <0.20 . This probability was established so that possible risk factors for infection were not excluded from the analysis (HOSMER & LEMESHOW, 1989). The logistic regression model was considered as risk factor variables with $P<0.05$. The Epi Info software version 3.5.1 was used to make the statistical calculations.

RESULTS

Of the 675 goat sera assayed with the MAT, 28.74% (194) were positive, and 90.24% (37/41) of the properties had at least one positive animal (Table 1). Moreover, 194 serum agglutination reactions were positive, with titers of 100 in 60.31% (117/194), 200 in 29.38% (57/194), 400 in 7.22% (14/194), 800 in 2.06% (4/194), and 1,600 in 0.82% (2/194) cases.

Reactions against eight serogroups were observed, the most common of which was Icterohaemorrhagiae, in 85.57% (166) cases, followed by Australis (5.15%, $n=10$), Pomona (3.61%, $n=7$), Sejroe (3.09%, $n=6$), and Pyrogenes (2.58%, $n=5$). The only significant variables in the univariate analysis (Table 2) and that were confirmed in the multivariate logistic regression ($P<0.001$) were the animals' water source. The presence of goats seropositive for *Leptospira* spp. was associated with the ingestion of still water, including supplies from buckets, wells, dams, ponds ($P<0.001$, $OR=3.86$ and 95% $CI=2.11-7.05$).

DISCUSSION

Our results indicated a high frequency of goats exposed to *Leptospira* in the state of Sergipe, and this rate was one of the largest ever reported in a Brazilian study. These high values corroborated those of prior studies conducted in Pernambuco

(CUNHA et al., 1999), in the municipality of Uberlândia, Minas Gerais (SANTOS et al., 2012), and in the western and mountainous mesoregions of Santa Catarina (TOPAZIO et al., 2015), with rates between 31.3% and 35.47%. However, none of these studies collected samples from all the mesoregions present in their respective states, and in three cases, the number of samples assayed with MAT was much lower than that in the current study. Other surveys in the Northeastern region of Brazil reported lower rates of infection, ranging from 1.5% to 15.67% in the states of Rio Grande do Norte (ARAÚJO NETO et al., 2010), Paraíba, and Ceará (FAVERO et al., 2002; HIGINO et al., 2013; COSTA et al., 2016.).

The titers obtained were low, and this result agrees with those of other Brazilian studies (SCHMIDT et al., 2002; LILENBAUM et al., 2007b; ARAÚJO NETO et al., 2010; HIGINO & AZEVEDO, 2014; COSTA et al., 2016). This result also indicated chronic infection, which may go unnoticed, as well as low exposure to the agent, suggesting that serological studies are an essential part of control measures in goat herds. The chronic nature of infection indicated that goats eliminate the bacteria into the environment, which increases the risk of transmission of this agent to other animals.

MARTINS et al. (2012) reported high antibody titers (≥ 800) in a goat herd in the state of Rio de Janeiro, and the disease involved cases of miscarriage, embryo absorption, and neonatal death; although, the goats seemed healthy and only 3.28% (8/244) of the reactions had titers ≥ 800 .

Among the serogroups, Icterohaemorrhagiae was the most frequent (85.57%) in Rio Grande do Sul (SCHMIDT et al., 2002) and Santa Catarina (TOPAZIO et al., 2015), where farmers reported rodent infestations. The same situation may be occurring among herds in Sergipe because the positivity to this serogroup is associated with the presence of *Rattus norvegicus* and other rodent species from which this serogroup was isolated, including the black rat (*Rattus ratus*) and the Brazilian guinea pig (*Cavia aperea azarae*) (VIRGINIE et al., 2002; OLIVEIRA et al., 2013), indicating that the high transmission of *Leptospira* spp. in herds in Sergipe may be associated with the presence of rodents.

The presence of rodent infestations because of failures in health management, as proven by the substantial presence of serogroups associated with these reservoirs and with the consequent contamination of water sources, may explain the high rate of seropositive goat herds in Sergipe

Table 1 - Percentage of goats that tested seropositive to *Leptospira* spp. using a microscopic agglutination test (MAT), organized by municipality and mesoregion in the state of Sergipe, Brazil, 2013-2014.

Municipality	% (Number of properties with positive goats/total number of properties)	% (Number of seropositive goats/total number of goats)
Aracaju	100 (3/3)	21.53 (11/52)
Estância	100 (1/1)	55 (11/20)
Itaporanga D'ajuda	100 (1/1)	20 (4/20)
Nossa Senhora do Socorro	100 (1/1)	44.44 (8/18)
Salgado	100 (2/2)	32.14 (9/28)
São Cristóvão	100 (1/1)	45 (9/20)
Neópolis	100 (1/1)	5 (1/20)
Total East mesoregion	100 (10/10)	29.76 (53/178)
Itabaiana	100 (1/1)	60 (12/20)
Lagarto	100 (1/1)	35 (7/20)
Macambira	66.67 (2/3)	14.29 (6/42)
Poço Verde	81.82 (9/11)	24.5 (37/151)
Total Agreste mesoregion	87.5 (14/16)	26.61 (62/233)
Canindé de São Francisco	100 (6/6)	38.33 (46/120)
Nossa Senhora da Glória	83.33 (5/6)	22.83 (21/92)
Pedra Mole	100 (1/1)	7.69 (1/13)
Pinhão	100 (2/2)	28.21 (11/39)
Total Sertão mesoregion	93.33 (14/15)	29.92 (79/264)
Total	90.24 (37/41)	28.74 (194/675)

(90.24%), as also observed in Rio de Janeiro, Santa Catarina and municipality of Uberlândia, with rates of 100% (48/48), 90% (108/120) and 100% (11/11), respectively (LILENBAUM et al., 2007b; SANTOS et al., 2012; CORTIZO et al., 2015).

In Paraíba, HIGINO et al. (2013) reported that the presence of rodents in rural properties was a risk factor for infection of goats with *Leptospira* spp. (OR=2.78, 95% CI=1.22-6.31, and P=0.015). However, no positive reaction was observed for the Icterohaemorrhagiae serogroup. The most frequent serogroup was Australis, as reported by ARAÚJO NETO et al. (2010) in Rio Grande do Norte. This serogroup was the second most frequent in goats from Sergipe, and this finding confirmed the importance of rodents in the spread of the disease because the hosts include not only pigs and dogs but also the South American water rat (*Nectomys squamipes*) (GOMES, 2013; OLIVEIRA et al., 2013), a semiaquatic rat that typically inhabits the banks of streams, rivers, and flooded areas in the Northeastern region of Brazil, particularly on the basin of the São Francisco River from Bahia to Pernambuco (GENTILE, 2010).

The presence of these reservoirs, which were reported by 63.41% (26/41) of the goat owners in Sergipe, is usually associated with poor

sanitation practices such as the accumulation of waste, lack of hygiene during food and water handling, poor food storage practices, poor water quality, and presence of wild and synanthropic animals around the properties, favoring the increased contact of these animals with livestock and humans (SILVA et al., 2014). As a result, the facilities are constantly exposed to the etiological agent via the urine of infected animals, particularly in still water because it favors the survival of bacteria in the environment (GOMES, 2013).

These infection sources and the availability of still water (wells, dams, buckets) were associated (OR=3.86) with the presence of animals reactive to *Leptospira* spp. in the study herds. These findings are consistent with those of CORTIZO et al. (2015) in Espírito Santo (OR=1.8), who also reported high positive rates (97%) for the Icterohaemorrhagiae serogroup. Reproductive problems presented by goats infected with this serogroup on a property in the state of Rio de Janeiro led to the adoption of corrective measures for rodent control, including trapping, use of rodenticides, decreased access to stored feed, and restriction of goats access to flooded areas. After 1 year, there was a significant reduction in the rate of reproductive failure (MARTINS et al., 2012).

Table 2 - Percentage of *Leptospira* spp. seropositive goats from rural properties in the Brazilian state of Sergipe in 2013-2014 organized by variable and number of goats.

Variable	N	SAM (%)	Value p
-----Mesoregion-----			
East	178	53 (29.77)	0.673
Agreste	233	62 (26.61)	
Sertão	264	79 (29.92)	
-----Sex-----			
Female	566	176 (31.01)	< 0.001
Male	109	18 (16.51)	
-----Breed-----			
Anglo Nubiana	60	10 (16.67)	0.017
Boer	55	17 (30.91)	
Parla Alpina	16	3 (18.74)	
Saanen	215	65 (30.23)	
Undefined	321	94 (29.28)	
Toggenburg	8	6 (75)	
-----Objective of breeding-----			
Meat	298	86 (28.86)	0.509
Milk	377	108 (28.65)	
-----Production systems-----			
Extensive	114	41 (35.96)	0.016
Intensive	108	39 (36.11)	
Semi-extensive	453	115 (25.39)	
-----Installations-----			
Brick floor pen	162	41 (25.31)	0.509
Dirt floor pen	106	32 (30.19)	
Slatted floor pen	225	63 (28)	
None	182	59 (32.42)	
-----Availability of feed-----			
Covered trough	370	96 (25.95)	0.123
Uncovered trough	188	56 (29.79)	
Not available	97	34 (35.05)	
On the ground	20	9 (45)	
-----Water source-----			
Flowing streams	117	13 (11.11)	< 0.001
Still water	559	377 (67.44)	
-----Food Storage-----			
Open place	138	55 (39.85)	0.007
Place closed	517	135 (26.11)	
Place open and closed	20	5 (25)	
-----Rat access to the food place storage-----			
Yes	421	128 (30.4)	0.155
No	254	67 (26.38)	
-----Circulation of wild animals-----			
Yes	440	136 (30.91)	0.062
No	235	59 (25.11)	
-----Contact of healthy and sick animals-----			
Yes	413	122 (29.53)	0.359
No	262	73 (27.86)	
-----Disinfect installations-----			
Yes	515	155 (30.09)	0.128
No	160	40 (25)	

CONCLUSION

The occurrence of seropositivity for *Leptospira* spp. in goat herds in the Brazilian state of Sergipe demonstrates the wide distribution of this etiological agent in the state. Our results indicated that the primary method of maintenance and dissemination of spirochetes were sources of still water contaminated with rodent urine. Therefore, sanitary control measures should be adopted, particularly methods that include pest management practices, considering the predominance of the *Icterohaemorrhagiae* serogroup.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This study was approved by the Bioethics Committee of Universidade Pio Décimo under Protocol number 06/2011 from April 16, 2011.

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