

Seroprevalence and predominant serogroups of *Leptospira* sp. in serological tests of ruminants in northeastern Brazil

Soroprevalência e sorogrupos de *Leptospira* sp. predominantes em exames sorológicos de ruminantes do Nordeste do Brasil

Carla LauiSe Rodrigues Menezes Pimenta¹; Camila de Sousa Bezerra¹; Davidianne de Andrade Morais¹; Maria Luana Cristiny Rodrigues Silva²; Denise Batista Nogueira¹; Diego Figueiredo da Costa¹; Carolina de Sousa Américo Batista Santos³; Severino Silvano dos Santos Higino³; Clebert José Alves³; Sérgio Santos de Azevedo^{3*}

Abstract

The objective of this study was to determine the seroprevalence of leptospirosis and the predominant serogroups in serological tests of ruminants performed at the Transmissible Diseases Laboratory of the Federal University of Campina Grande, Patos, Paraíba, northeastern Brazil, between 2010 and 2017. This research included the records of serological tests for leptospirosis from 5594 animals, including 1527 cattle, 1761 goats, 2170 sheep, and 136 buffaloes from four Brazilian states (Paraíba, Pernambuco, Maranhão, and Rio Grande do Norte). Of the 5594 serum samples, 662 were serologically positive, corresponding to a frequency of 11.8%. Serjoe (30.6%), Autumnalis (13.6%), and Icterohaemorrhagiae (11.3%) were the most frequent subgroups among all species. The individual frequencies in cattle, goats, sheep, and buffaloes were 20% (306/1527), 8.3% (147/1761), 7.9% (171/2170), and 27.9% (38/136), respectively, with titers ranging from 1:100 to 1:3200. The most frequent serogroups according to animal species were as follows: Serjoe predominated in cattle (62%), followed by Icterohaemorrhagiae (12.5%) and Tarassovi (6.6%); Autumnalis was the most frequent in goats and sheep (29.4% and 26.9%, respectively), followed by Seramanga (12.5%) in goats and Icterohaemorrhagiae (13.5%) in sheep; and Australis predominated in buffaloes (39.5%), followed by Pomona (31.6%) and Canicola (21.1%). *Leptospira* sp. infection, determined by serology, is widespread in ruminants (cattle, goats, sheep, and buffaloes) in northeastern Brazil, suggesting the existence of alternative transmission routes that are less dependent on environmental factors. Identification of the most frequent serogroups highlights the need to improve sanitary conditions, and implement efficient and targeted control measures directed at the primary infection sources.

Key words: Leptospirosis. Serology. Ruminants. Serogroups. Control.

¹ Discentes, Curso de Doutorado, Programa de Pós-Graduação em Medicina Veterinária, Universidade Federal de Campina Grande, UFCG, Patos, PB, Brasil. E-mail: carlalauiSe@hotmail.com; camilacstr.mv@hotmail.com; davidianneandrademorais@gmail.com; denise.bn.medvet@gmail.com; diegoveter@hotmail.com

² Pós-Doutoranda, Programa de Pós-Graduação em Medicina Veterinária, UFCG, Patos, PB, Brasil. E-mail: luacristiny@yahoo.com.br

³ Profs., Programa de Pós-Graduação em Medicina Veterinária, UFCG, Patos, PB, Brasil. E-mail: carolamerico@yahoo.com.br; higinoss@veterinario.med.br; clebertja@uol.com.br; sergio@vps.fmvz.usp.br

* Author for correspondence

Resumo

O objetivo deste estudo foi determinar a soropositividade para leptospirose e os sorogrupos predominantes nos testes sorológicos realizados no Laboratório de Doenças Transmissíveis (LDT) da Universidade Federal de Campina Grande (UFCG), Patos, Paraíba, Nordeste do Brasil, em bovinos, caprinos, ovinos e bubalinos no período de 2010 a 2017. Foram computados os registros dos exames sorológicos para leptospirose de 5.594 animais, que incluíram 1.527 bovinos, 1.761 caprinos, 2.170 ovinos e 136 bubalinos, provenientes de quatro estados brasileiros (Paraíba, Pernambuco, Maranhão e Rio Grande do Norte). Das 5.594 amostras de soro de bovinos, caprinos, ovinos e bubalinos, 662 amostras foram positivas no teste sorológico, resultando em uma frequência de 11,8%. Serjoe (30,6%), Autumnalis (13,6%) e Icterohaemorrhagiae (11,3%) foram os sorogrupos mais frequentes para todas as espécies. As frequências individuais de bovinos, caprinos, ovinos e bubalinos foram de 20% (306/1.527), 8,3% (147/1.761), 7,9% (171/2.170), e 27,9% (38/136), respectivamente, com títulos variando de 1:100 a 1:3200. Com relação aos sorogrupos mais frequentes por espécie animal, o Serjoe predominou em bovinos (62%), seguido de Icterohaemorrhagiae (12,5%) e Tarassovi (6,6%); Autumnalis foi o mais frequente em caprinos e ovinos (29,4% e 26,9%, respectivamente), seguido de Seramanga (12,5%) em caprinos e Icterohaemorrhagiae (13,5%) em ovinos; Australis predominou nos bubalinos (39,5%), seguido de Pomona (31,6%) e Canicola (21,1%). Conclui-se que a infecção por *Leptospira* sp., determinada por sorologia, encontra-se difundida em ruminantes (bovinos, caprinos, ovinos e bubalinos) do Nordeste do Brasil, o que sugere a existência de vias de transmissão alternativas menos dependentes de fatores ambientais, bem como a identificação dos sorogrupos mais frequentes sugere a necessidade de melhoria das condições sanitárias e implementação de medidas de controle eficientes e direcionadas para as principais fontes de infecção.

Palavras-chave: Leptospirose. Sorologia. Ruminantes. Sorogrupos. Controle.

Introduction

Leptospirosis is a zoonosis caused by spirochetes of the genus *Leptospira*, and affects many mammal species including humans. It occurs all over the world, and is particularly prevalent in tropical and subtropical countries, especially in periods of high rainfall rates (AGUIAR et al., 2010; VIEIRA et al., 2018).

In livestock farming, the disease is related to decreases in reproductive performance of the affected herds, resulting to economic losses (ELLIS, 2015). Leptospirosis is transmitted through indirect contact with contaminated water or soil, or through direct contact with the urine of carrier animals (PICARDEAU, 2013). The bacteria enter the host through skin lesions and mucous membranes, invade the circulation, spread throughout the body, and lodge in the renal tubules, being eliminated through urine, thus contaminating the environment and (ADLER, 2014; ELLIS, 2015).

Leptospira infection may be incidental or adapted. The incidental infection, which is highly dependent on environmental factors, is caused by unadapted serogroups transmitted by other domestic or wild animal species. In the latter case, the infection is determined by the adapted serogroups, and is less dependent on environmental conditions, with the maintenance host acting as a natural source of infection for its own species. This infection is associated with specific *Leptospira* serogroups (FAINE et al., 1999; LEVETT, 2001; SUEPAUL et al., 2011).

The World Organization for Animal Health (OIE) recommends serological diagnosis using the microscopic agglutination test (MAT), particularly for herd diagnosis of production animals (SUEPAUL et al., 2011; PINTO et al., 2015). Thus, seroepidemiological surveys are necessary to monitor and control leptospirosis in a given region. These surveys collect epidemiological indicators that are based on the elaboration of prevention

strategies and direct new public policies, with the aim of improving general public health.

Several serological studies investigating leptospirosis have been conducted using diagnostic laboratory data. Using data collected in Brazil between 1984 and 1997, a retrospective study by Favero et al. (2002) investigated the prevalent serological variants of leptospirosis in serological tests of sheep, goats, buffaloes, pigs, dogs, and horses from various Brazilian states. Martins and Lilenbaum (2013) evaluated several studies conducted in Rio de Janeiro over a period of 20 years to diagnose leptospirosis in dogs, rats, cattle, horses, goats, sheep, pigs, and wild mammals. Campos et al. (2017) analyzed serum samples from sheep, goats, and cattle from the state of Piauí between 2013 and 2015. In Italy, Tagliabue et al. (2016) epidemiologically evaluated leptospirosis based on serological data from cattle, buffaloes, horse, sheep, goats, pigs, dogs and wild animals from 10 laboratories between 2010 and 2011.

The objective of this study was to determine the frequency of leptospirosis and the prevalent serogroups in serological tests of cattle, goats, sheep, and buffaloes from 2010 to 2017 at the Laboratory of Transmissible Diseases of the Federal University of Campina Grande (UFCG), Patos, Paraíba, Northeast Brazil.

Material and Methods

Serological diagnosis of Leptospira sp.

The serological diagnosis of leptospirosis was performed using the MAT assay, according to Galton et al. (1965) and Cole et al. (1973). The tests used a collection of live antigens represented by the serogroups: Icterohaemorrhagiae (serovars Icterohaemorrhagiae and Copenhageni), Canicola, Pomona, Grippotyphosa, Serjoe (Wolffi, Hardjoprajitno, Hardjobovis and Guaricura), Australis (Australis and Bratislava), Andamana, Autumnalis (Autumnalis and Butembo), Bataviae,

Balum (Castellonis), Cynopteri, Djasiman (Sentot), Hebdomadis, Panama, Tarassovi, Javanica, Celledoni (Whitcombi), Pyrogenes, Shermani, and Seramanga (Patoc).

The sera were screened at 1:100 dilution, and those exhibiting agglutination $\geq 50\%$ were titrated by examining a series of two-fold geometric dilutions. Serum titer was the reciprocal of the highest positive dilution. The antigens were examined under a dark-field microscope before being tested for mobility and the presence of self-agglutination or contaminants. To identify the most frequent serogroups, the sera that presented ≥ 2 serological variants with the highest identical titer were discarded from the serogroup analysis but considered seropositive for *Leptospira* species (sp.).

Records

A survey of MAT results to diagnose leptospirosis was used in this study, and the information analyzed was compiled from the database of the Transmissible Disease Laboratory (TDL) of the Federal University of Campina Grande (UFCG), Patos, Paraíba, northeastern Brazil. The records of serological tests for leptospirosis of 5594 animals, including 2170 sheep, 1761 goats, 1527 cattle, and 136 buffaloes from four Brazilian states (Paraíba, Pernambuco, Maranhão, and Rio Grande do Norte) were recorded from 2010 to 2017, and distributed as follows: sheep - PB (94.5%); PE (5.5%); goats - PB (86.8%), PE (13.2%); cattle - PB (53.9%), MA (20.4%), RN (15.9%), PE (9.8%); and buffaloes - PB (100%).

Results and Discussion

Of 5594 serum samples from cattle, goats, sheep and buffaloes, 662 were serologically positive, corresponding to a frequency of 11.8%. The most frequent serogroups were identified considering 601 samples. Serjoe (30.6%), Autumnalis (13.6%), and

Icterohaemorrhagiae (11.3%) were the most frequent for all species (Table 1). The frequency of positivity (11.8%) can be considered high, which highlights that the infection is broadly spread among the studied species because immunization is not common in most rural properties in northeastern Brazil. Despite the unfavorable environmental conditions for the survival of leptospire in the Brazilian semi-arid region, this high percentage suggests the existence of alternative transmission routes that are dependent on environmental factors. In fact, studies conducted by our research group revealed the possible importance of female-male venereal transmission of leptospirosis in ruminants. Pimenta et al. (2018) investigated a leptospirosis outbreak

in cattle in the state of Maranhão and reported that, of 24 cows with reproductive problems (abortion, repeated heat cycles, and stillbirths), eight (33.3%) tested positive in polymerase chain reaction (PCR) testing using vaginal fluid, with positive genetic sequencing for *Leptospira borgpetersenii*. Costa et al. (2018) conducted an experimental infection in crossbred Santa Inês sheep, and reported that there was no statistical difference in the proportion of positive urine and vaginal fluid samples using PCR. In another study (SILVA et al., 2018), the DNA of pathogenic leptospire was detected in 54.9% (61/111) of genital tract samples (uterus, vaginal fluid, and ovary) of sheep slaughtered in Paraíba.

Table 1. Most frequent *Leptospira* species serogroups from 601 serum samples from cattle, goats, sheep, and buffaloes in northeastern Brazil between 2010 and 2017.

Serogroup	Number of positive animals	Frequency (%)
Serjoe	184	30.6
Autumnalis	82	13.6
Icterohaemorrhagiae	68	11.3
Andamana	43	7.2
Australis	31	5.2
Seramanga	30	5.0
Pomona	27	4.5
Djasiman	25	4.2
Tarassovi	24	4.0
Balum	22	3.7
Celledoni	17	2.8
Canicola	16	2.7
Grippotyphosa	12	2.0
Shermani	8	1.3
Javanica	7	1.2
Hebdomadis	2	0.3
Pyrogenes	1	0.2
Cynopeteri	1	0.2
Shermani	1	0.2

The present study revealed the Serjoe serogroup to be the most prevalent in ruminants. This result was expected because this serogroup is most

commonly observed in studies involving small ruminants and cattle (MARTINS; LILENBAUM, 2013; DIRECTOR et al., 2014). On the other hand,

reactions to the Australis and Icterohaemorrhagiae serogroups were related to incidental infections caused by strains found in other domestic and wild animals, suggesting the need to improve sanitary practices, including immunization, antibiotic therapy, environmental management, rodent control and increased environmental hygiene, with the objective of decreasing the spread of leptospirosis (FAINE et al., 1999; ZAKERI et al., 2010).

The positive cattle serotypes in the states of Paraíba, Pernambuco, Maranhão, and Rio Grande do Norte were 6.4%, 32.6%, 50.9%, and 18.5%, respectively. The positive buffalo serotypes were 27.9% in the state of Paraíba, while in sheep and

goats the frequencies were 7.5% and 8.5% for the samples from Paraíba, and 13.4% and 6.8% for the samples from Pernambuco, respectively (Table 2). These results reveal a lower number of seropositive small ruminants compared with cattle and buffaloes. This discrepancy can be explained by the rusticity and natural infection resistance attributed to small ruminants (COSTA et al., 2016). On the other hand, Costa et al. (2018) reported greater susceptibility in purebred sheep compared with crossbred animals, emphasizing the importance of the genital tract as a site of extra-urinary infection and the possibility of venereal transmission in sheep.

Table 2. Frequency of positive cattle, goats, sheep and buffaloes in the microscopic agglutination test to diagnose leptospirosis in northeastern Brazil according to state of origin between 2010 and 2017.

State (Brazil)	Species	Number of tested sera	Seropositive samples	Frequency (%)
PB	Sheep	2051	155	7.5
	Goats	1528	131	8.5
	Cattle	822	53	6.4
	Buffaloes	136	38	27.9
PE	Sheep	119	16	13.4
	Goats	233	16	6.8
	Cattle	150	49	32.6
MA	Cattle	312	159	50.9
RN	Cattle	243	45	18.5

The individual frequencies in cattle, goats, sheep, and buffaloes were 20% (306/1527), 8.3% (147/1761), 7.9% (171/2170), and 27.9% (38/136), respectively, with titers ranging from 1:100 to 1:3200. Among the most frequent serogroups according to animal species (Table 3), Serjoe was predominant in cattle (62%), followed by Icterohaemorrhagiae (12.5%) and Tarassovi (6.6%); Autumnalis was the most frequent in goats and sheep (29.4% and 26.9%, respectively), followed by Seramanga (12.5%) in goats and Icterohaemorrhagiae (13.5%) in sheep; and Australis predominated in buffaloes (39.5%), followed by Pomona (31.6%) and Canicola (21.1%).

Seropositivity was revealed to be high in cattle, similar to the results reported by Martins and Lilenbaum (2013) in Rio de Janeiro, with a frequency of 23%; Campos et al. (2017) in Piauí, with 50%; Pinto et al. (2016) in a systematic review in Latin America, with 44.2%; Pimenta et al. (2014) in Paraíba, with 61.1%; and Silva et al. (2012) in Maranhão, with 35.9%. These results are similar to those of the present study, and indicate that the disease, in fact, circulates in the studied cattle herds, emphasizing the impact of this agent on the health of these animals and, consequently, on public health.

Serjoe was the predominant serogroup in cattle, being frequently mentioned in several epidemiological surveys in Brazil and in other parts of the world (MARTINS; LILENBAUM, 2013; MENEGAS et al., 2013; PINTO et al., 2015, 2016; TAGLIABUE et al., 2016; CORREIA et al., 2017; CAMPOS et al., 2017). Bovine animals are recognized maintenance hosts of this serogroup, responsible for the development of chronic disease and subclinical persistent reproductive tract infection. Its presence in herds is related to direct

transmission among animals through urine and vaginal secretions (MARTINS; LILENBAUM, 2013, 2014). There were also reactions to the Icterohaemorrhagiae and Tarassovi serogroups, which have synanthropic rodents and pigs as maintenance hosts, respectively, suggesting that the presence of rodents plays a fundamental role in environmental contamination (CAMPOS et al., 2017), as well as the contact between cattle and pigs (STRUTZBERG-MINDER; KREIENBROCK, 2011).

Table 3. Prevalence of *Leptospira* sp. serogroups in cattle, goats, sheep, and buffaloes in northeastern Brazil according to animal species between 2010 and 2017.

Serogroup	Cattle	Goats	Sheep	Buffaloes
	Number of positive animals (%)	Number of positive animals (%)	Number of positive animals (%)	Number of positive animals (%)
Andamana	13 (4.8)	11 (8.1)	19 (12.2)	0 (0)
Australis	6 (2.2)	4 (2.9)	6 (3.8)	15 (39.5)
Autumnalis	0 (0)	40 (29.4)	42 (26.9)	0 (0)
Balum	1(0.4)	3 (2.2)	18 (11.5)	0 (0)
Canicola	1 (0.4)	3 (2.2)	4 (2.6)	8 (21.1)
Cynopeteri	0 (0)	0 (0)	0 (0)	1 (2.6)
Grippotyphosa	8 (3.0)	3 (2.2)	1 (0.6)	0 (0)
Hebdomadis	2 (0.7)	0 (0,0)	0 (0)	0 (0)
Icterohaemorrhagiae	34 (12.5)	13 (9.6)	21 (13.5)	0 (0)
Javanica	0 (0)	0 (0)	7 (4.5)	0 (0)
Seramanga	7 (2.6)	17 (12.5)	5 (3.2)	1 (2.6)
Pomona	7 (2.6)	3 (2.2)	5 (3.2)	12 (31.6)
Pyrogenes	0 (0)	1 (0.7)	0 (0)	0 (0)
Djasiman	2 (0.7)	11 (8.1)	12 (7.7)	0 (0)
Serjoe	168 (62)	5 (3.7)	9 (5.8)	1 (2.6)
Shermani	3 (1.1)	3 (2.2)	3 (1.9)	0 (0)
Tarassovi	18 (6.6)	5 (3.7)	1 (0.6)	0 (0)
Celledoni	0 (0)	14 (10.3)	3 (1.9)	0 (0)
Total	270 (100)	136 (100)	156 (100)	38 (100)

The frequencies for goats and sheep were 8.3% and 7.9%, respectively. Studies conducted by Tagliabue et al. (2016) in Italy, and Suwancharoen et al. (2013) in Thailand, reported frequencies similar to the present study (4.7% for sheep and 7.9% for goats), while other authors reported

higher results in the same species, such as Aguiar et al. (2010), who reported 33.3% positivity in sheep in Rondônia; Salaberry et al. (2011), who reported 22.2% positivity in sheep in Minas Gerais; Martins and Lilenbaum (2013), who reported 47.4% positivity in sheep and 14.95% in goats in

Rio de Janeiro; Cortizo et al. (2015), who reported 50% positivity in both species in Espírito Santo; and Machado et al. (2016), who reported 19.5% positivity in sheep in Pernambuco. The discrepancy among results is probably due to the characteristic environmental conditions of each region studied, and the management and control measures adopted for the herds (COSTA et al., 2016; MACHADO et al., 2016).

The Autumnalis serogroup was the most frequent in goats and sheep, a result that corroborates the findings of serological surveys conducted in Paraíba, Minas Gerais and Pernambuco in previous years (HIGINO et al., 2010; ALVES et al., 2012; SALABERRY et al., 2011; COSTA et al., 2016; MACHADO et al., 2016), suggesting that this serogroup may be adapted to small ruminants. The serogroup Seramanga was the second most frequent in goats. It is considered non-pathogenic and associated with the presence of rodents and free-living animals in properties, such as white-eared opossums (*Didelphis albiventris*), acting as sources of infection (SILVA et al., 2013; PAIXÃO et al., 2016). The Icterohaemorrhagiae is considered the second most frequent serogroup in sheep and is generally related to the presence of rodents, responsible for incidental infections in other hosts (ESCÓCIO et al., 2010; GENOVEZ et al., 2011).

In buffaloes, the seropositivity was 27.9%, a result lower than reported by Viana et al. (2009) and Favero et al. (2002), who evaluated buffaloes in Amazonas and São Paulo and reported frequencies of 80% and 43.7%, respectively. Although these frequencies are considered to be high, they were lower than in other studies, probably because the state of Paraíba is located in a semi-arid region of Brazil and, consequently, presents adverse climatic conditions for leptospirosis, which results in low seropositivity.

The Australis serogroup was the most frequent in buffaloes, as observed by Viana et al. (2009), who evaluated buffaloes from the state of

Amazonas, although most serological surveys indicate the Serjoe serogroup as the most prevalent (FAVERO et al., 2002; SUWANCHAROEN et al., 2013; TAGLIABUE et al., 2016). The serogroups Pomona and Canicola were the second and third most frequent, respectively. The high pathogenicity attributed to the Pomona serogroup suggests that buffalo herds have an active immune response against leptospirosis (PAIXÃO et al., 2016), and pigs may be acting as reservoirs for both the Australis and Pomona serogroups because these animals are recognized hosts of these serogroups. Their presence in properties is strongly associated with the occurrence of incidental leptospirosis (LILENBAUM; SOUZA, 2003). As for the Canicola serogroup, the close contact between buffaloes and dogs may justify the identification of this serogroup as one of the most frequent in buffaloes, given that dogs are considered adapted and serve as leptospirosis reservoirs (MARTINS; LILENBAUM, 2013).

Conclusion

Serologically diagnosed *Leptospira* sp. infection is widespread in ruminants (i.e., cattle, goats, sheep, and buffaloes) in northeastern Brazil, suggesting the existence of alternative transmission routes that are less dependent on environmental factors. The identification of the most frequent serogroups enables us to infer that these bacteria are maintained in cattle herds through direct contact among animals, while in herds of other species, the bacteria are maintained through contact with other domestic and synanthropic animals, which highlights the need to improve sanitary conditions, and implement efficient and targeted control measures directed at the primary infection sources.

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