

# Spatial distribution of equine seroreagent to *Leptospira* spp. in Northeastern Brazil

## *Distribuição espacial de equinos sororreagentes à Leptospira spp. no Nordeste do Brasil*

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### ABSTRACT

Considering the importance of leptospirosis in both equine husbandry and public health, as well as the relevance of knowledge of current serogroup and the small number of studies addressing this disease in equines in Northeastern Brazil, the present study performed a serological survey of *Leptospira* spp. in a serum bank of 1,267 equines originating from 177 municipalities, located in four states in Northeastern Brazil: Ceará, Rio Grande do Norte, Paraíba and Pernambuco. The microscopic agglutination test (MAT) was used for diagnosis of leptospirosis using 24 serovars as antigens. The frequency of reagent equines was 29.7% (376/1,267), and 68.9% (122/177) of municipalities had at least one positive reaction. The serogroups reagent were Australis (37.2%), Icterohaemorrhagiae (29.3%), Tarassovi (9.6%), Sejroe (5.8%), Pomona (5.3%), Grippotyphosa (4.5%), Pyrogenes (2.4%), Bataviae (1.9%), Ballum and Hebdomadis (1.3%), Mini (0.5%), Celledoni, Shermani, and Javanica (0.3% each). There were significant statistical differences regarding sex and age, with a higher frequency in females ( $P=0.014$ ), and in animals  $\geq 6$  years ( $P=0.001$ ). We concluded that seropositivity to *Leptospira* spp. is high in equines in Northeastern Brazil, with a predominance of serologic reactions to the Australis serogroup in the border areas between the states, and the Icterohaemorrhagiae serogroup in coastal areas or their vicinity. The high degree of seropositivity found points to the need to implement prophylactic strategies, both intraspecies infection prophylaxis and rodent control. We also recommended avoiding animal crowding and separating animals according to sex and age group during handling.

**Keywords:** Epidemiology. Zoonosis. Bacterium. Spatialization. Animal health.

### RESUMO

Considerando a importância da leptospirose na equinocultura e na saúde pública, bem como a relevância do conhecimento dos sorogrupos circulantes e do pequeno número de estudos realizados sobre esta doença em equinos no Nordeste do Brasil, o presente trabalho foi delineado para realizar um inquérito sorológico de *Leptospira* spp. em um banco de soros de 1.267 equinos provenientes de 177 municípios distribuídos em quatro estados: Piauí, Ceará, Paraíba e Pernambuco, localizados na região Nordeste do Brasil. Os soros foram examinados com o teste de Soroaglutinação Microscópica (SAM), utilizando uma coleção de 24 sorovares como antígenos. A frequência de equinos reagentes foi de 29,7% (376 / 1.267) e 68,9% (122/177) dos municípios que tiveram pelo menos uma reação positiva. Os sorogrupos reagentes foram Australis (37,2%), Icterohaemorrhagiae (29,3%), Tarassovi (9,6%), Sejroe (5,8%), Pomona (5,3%), Grippotyphosa (4,5%), Pyrogenes (2,4%), Bataviae (1,9%), Ballum e Hebdomadis (1,3%), Mini (0,5%), Celledoni, Shermani e Javanica (0,3%). Houve diferenças estatisticamente significantes quanto ao sexo e idade, com maior frequência no sexo feminino ( $P = 0,014$ ) e no animal com idade  $\geq 6$  anos ( $P = 0,001$ ). Conclui-se que a infecção por *Leptospira* spp. tem ocorrência significativa em equídeos do Nordeste do Brasil, com predominância de reações para o sorogrupo Australis nas áreas de fronteira entre os estados e para sorogrupo Icterohaemorrhagiae nas áreas litorâneas ou em suas proximidades. O alto grau de soropositividade encontrado aponta para a necessidade da implementação de estratégias profiláticas tanto intraespécies quanto relacionadas ao controle de roedores. Também recomenda-se evitar superlotações de animais em piquetes e manejá-los em grupos de acordo com o sexo e a faixa etária.

**Palavras-chave:** Epidemiologia. Zoonose. Bactéria. Espacialização. Saúde animal.

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Received: October 01, 2019

Approved: January 23, 2020

**How to cite:** Morais DA, Bezerra CS, Anjos DM, Nunes BC, Nogueira DB, Pequeno NF, Costa DF, Higino SSS, Azevedo SS, Alves CJ. Spatial distribution of equine seroreagent to *Leptospira* spp. in Northeastern Brazil. *Braz J Vet Res Anim Sci.* 2019;56(4): e162784. <https://doi.org/10.11606/issn.1678-4456.bjvras.2019.162784>

## Introduction

Equine breeding is an economic sector that accounts for about 4.18 billion dollars annually (Lima & Cintra, 2016), producing animals of high breeding value (Coelho & Oliveira, 2008). However, despite its economic importance, the main use of equids remains agricultural work, mainly cattle handling (Caselani et al., 2012). Highly neglected diseases such as leptospirosis cause losses in horse breeding businesses and endanger public health (Pinna et al., 2008).

According to data from Notification Diseases Information System (SINAN) (Sistema de Informação de Agravos de Notificação, 2018), 66,569 cases of leptospirosis in humans were confirmed in Brazil from 2000 to 2017, of which 18% (11,702) were in the Northeast region. During this period, 6,365 people died of leptospirosis in Brazil, 24% (1,515) of them in the Northeastern area, the region with the second highest number of deaths due to leptospirosis, surpassed only by the Southeast region. Equidae are considered important sources of infection for leptospirosis due to their proximity to humans, especially when both share inadequate environmental, infrastructural, and sanitary conditions (Hamond et al., 2012a), in which the agent can spread (Hamond et al., 2012b).

In equines, seroprevalence depends on the geographical location and the existing serogroups of *Leptospira* spp. (Sellon & Long, 2007). In this species, the disease is most often asymptomatic and when clinical signs are present, they may be confused with other diseases (Hashimoto et al., 2007; Braga et al., 2011). Leptospirosis can cause reproductive disorders and physical weakness, as well as recurrent uveitis, also called periodic ophthalmia (Braga et al., 2011), and

is considered the greatest worldwide cause of blindness in equines (Artiushin et al., 2012).

Serogroups identification in the region helps raise awareness to improve control of the infection. However, there is a lack of studies addressing the situation of leptospirosis in horses in northeastern Brazil. Characterized as a crucial region, this area has specific characteristics to hinder the survival of leptospirosis agent. Specifically, these characteristics include a significant part of its territory in the drought polygon, with a predominance of semiarid climate, caatinga vegetation, shallow and stony soils, variable relief and seasonal rainfall (Araújo, 2011).

The objective of this study was to perform a serological survey of *Leptospira* spp. in equines in four states of Northeastern Brazil by serology, searching for the predominant serogroups and their spatial distribution.

## Material and Methods

The research was performed using serum bank and a diagnosis database of horses, provided from the Veterinary Laboratory Diagnostics, Ltd., located in the city of Catolé do Rocha, Paraíba state, Northeastern Brazil. The serum bank contained 1,267 samples collected from March to April 2015 in 177 cities from four states in Northeastern Brazil: Ceará (459 samples), Paraíba (478 samples), Pernambuco (77 samples), and Rio Grande do Norte (253 samples) (Figure 1). There were samples of equines from all mesoregions of the states, except for one in Rio Grande do Norte and three in Pernambuco, thus totaling 16 mesoregions distributed among the four states (Table 1).

Sera were obtained from clinically healthy equines, unvaccinated against leptospirosis, aged from six months (to avoid the interference of colostral antibodies) up to 26 years, and included 635 females and 632 males, all destined to be equestrian sport horses.

The presence of anti-*Leptospira* spp. antibodies was determined by the microscopic agglutination test (MAT) (Organização Mundial de Saúde Animal, 2014), using a collection of 24 antigens: *Leptospira biflexa* serovars Andamana and Patoc; *Leptospira interrogans* serovars Australis, Copenhageni, Bataviae, Bratislava, Canicola, Grippotyphosa, Hardjoprajitno, Pomona, Pyrogenes, Icterohaemorrhagiae, Hebdomadis, Wolffi and Butembo; *Leptospira borgpeterseni* serovars Autumnalis, Castellonis, Hardjobovis Javanica and Tarassovi; *Leptospira santarosai* serovars Guaricura and Shermani; *Leptospira kirschneri* serovar Cynopteri; and *Leptospira noguchii* serovar Panama. The antigens were kindly provided by the Veterinary Bacteriology Laboratory

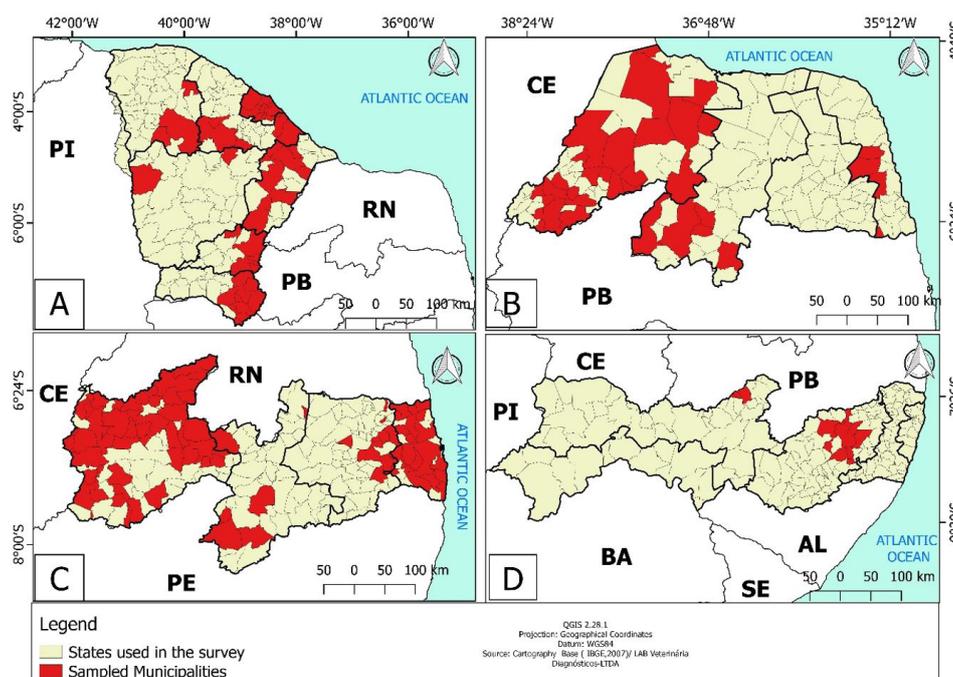


Figure 1 – Spatial distribution of the municipalities participating in the study in the year 2015 in the states of Ceará (A), Rio Grande do Norte (B), Paraíba (C), and Pernambuco (D).

Table 1 – Distribution and frequency of municipalities and equines from the Brazilian Northeast sampled and results of MAT applied to *Leptospira* sp., grouped by mesoregion, in March and April 2015 – Patos, 2019

STATE	MESOREGION	MUNICIPALITIES SAMPLED	MUNICIPALITIES WITH $\geq 1$ POSITIVE EQUINE (%)	EQUINES TESTED	POSITIVE EQUINES (%)	PREDOMINANT SEROGROUP	N (%)
CE	CENTRAL-SOUTH	6	5 (83)	58	13 (22)	Australis	6 (46)
CE	JAGUARIBE	7	5 (71)	69	21 (30)	Australis	11 (52)
CE	FORTALEZA M.	10	10 (100)	181	46 (25)	Icterohaemorrhagiae	17 (37)
CE	NORTHWEST	3	2 (67)	8	5 (62)	Australis	4 (80)
CE	NORTH	6	4 (67)	28	6 (21)	Icterohaemorrhagiae	3 (50)
CE	BACKLANDS	2	1 (50)	4	1 (25)	Icterohaemorrhagiae	1 (100)
CE	SOUTH	11	9 (82)	111	32 (29)	Australis	10 (31)
RN	CENTRAL	6	1 (17)	24	3 (12)	Icterohaemorrhagiae	2 (67)
RN	EAST	4	4 (100)	23	13 (56)	Australis	6 (46)
RN	WEST	27	18 (67)	206	53 (26)	Australis	22 (41)
PB	COUNTRYSIDE	11	4 (36)	25	9 (36)	Icterohaemorrhagiae	3 (33)
PB	BORBOREMA	5	3 (60)	9	4 (44)	Australis	2 (50)
PB	FOREST AREA	18	14 (78)	169	59 (35)	Icterohaemorrhagiae	25 (42)
PB	BACKLANDS	49	35 (71)	275	89 (32)	Australis	34 (38)
PE	BACKLANDS	2	1 (50)	11	4 (36)	Australis	2 (50)
	TOTAL	177	122	1267	376		

M = metropolitan;  $\geq 1$  = At least 1 positive; % = frequency by mesoregion. MAT = microscopic agglutination test to leptospirosis; CE = Ceará; RN = Rio Grande do Norte, PB = Paraíba; PE = Pernambuco.

of the Federal Fluminense University (UFF) and from the Pasteur Institute, France.

Sera were screened at a dilution of 1:100 and those having 50% or more agglutination were titrated by the examination of a series of geometric two-fold dilutions. The antibody titer was the reciprocal of the highest dilution that presented a positive result. Antigens were examined under a darkfield microscope prior to testing to verify mobility and check for the presence of autoagglutination or contaminants. For each sample, the most frequent

serovar was the one with the highest titer, and results were expressed by serogroups.

The mapping of the municipalities involved in the study and the spatialization of the seropositive equines for leptospirosis were carried out by digital processing of the territories from the municipalities using digital shapes provided by the Brazilian Institute of Geography and Statistics and processed by the QGIS 2.28.1 software.

The comparisons of seropositivity according to age and sex of each animal were performed by association analysis using the

chi-square test ( $\chi^2$ ), set at a significance level of 5% ( $P < 0.05$ ), using the BioEstat software version 5.3 (Ayres et al., 2007).

## Results

Of the 1,267 equine blood serum samples analyzed, 376 (29.7%) reacted positively to MAT and, of the 177 participating cities, 122 (68.9%) (Table 1) had at least one positive equine (Figure 2) with titers  $\geq 100$ .

The titers ranged from 100 to 800 and the serogroups found were Australis (37.2%), Icterohaemorrhagiae (29.3%),

Tarassovi (9.6%), Sejroe (5.8%), Pomona (5.3%), Grippotyphosa (4.5%), Pyrogenes (2.4%), Bataviae (1.9%), Ballum and Hebdomadis (1.3%), Mini (0.5%), Celledoni, Shermani, and Javanica (each 0.3%) (Table 2). Of the 376 positive reactions, 321 (85.4%) were among the 100 and 200 titer group. Among the serogroups tested, there were positive reactions in 14 (74%) (Table 2).

When grouping seropositive equines by state, we observed 124 (27%) in Ceará; 69 (27.3%) in Rio Grande do Norte; 161 (33.7%) in Paraíba, and 22 (28.6%) in

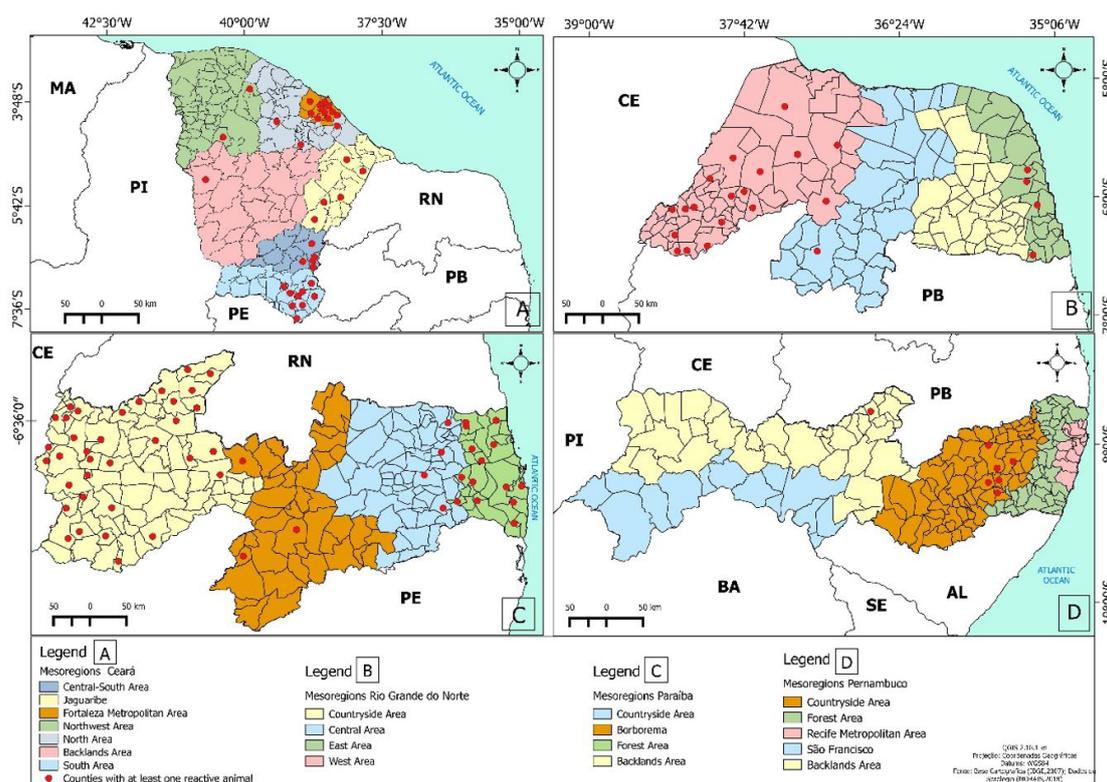


Figure 2 – Spatialization of the municipalities with at least one equine reagent to leptospirosis MAT in the states of Ceará (A), Rio Grande do Norte (B), Paraíba (C), and Pernambuco (D).

Table 2 – Distribution of MAT\* titers to *Leptospira* antibodies in equines from municipalities of Northeast Brazil in March and April 2015 – Patos, 2019

Serogroups	Titers					TOTAL (%)
	100	200	400	800		
Australis	77	51	10	2		140 (37.2)
Icterohaemorrhagiae	30	65	13	2		110 (29.3)
Tarassovi	16	17	3	0		36 (9.6)
Sejroe	5	8	8	1		22 (5.8)
Pomona	6	6	8	0		20 (5.3)
Grippotyphosa	4	9	4	0		17 (4.5)
Pyrogenes	5	4	0	0		9 (2.4)
Bataviae	4	2	0	1		7 (1.9)
Ballum	1	3	1	0		5 (1.3)
Hebdomadis	2	1	2	0		5 (1.3)
Mini	0	2	0	0		2 (0.5)
Celledoni	1	0	0	0		1 (0.3)
Shermani	1	0	0	0		1 (0.3)
Javanica	0	1	0	0		1 (0.3)
TOTAL (%)	152 (40.4)	169 (45)	49 (13)	6 (1.6)		376 (100)

\*MAT = Microscopic Agglutination Test applied to leptospirosis diagnosis.

Table 3 – Results of the univariate analysis with the most associated variables ( $P \leq 0.20$ ) with seropositivity for *Leptospira* spp. of equine blood samples, from March to April 2015, in municipalities of Northeast Brazil, Brazil – Patos, 2019

Variable	Category	Total of animals	Positive Animals (%)	P*
Sex	Male	635	168 (26.5)	0.014
	Female	632	208 (32.9)	
Age	1 to 5 years old	516	96 (18.6)	0.001
	6 to 10 years old	607	236 (38.9)	
	11 to 15 years old	122	38 (31.1)	
	16 to 26 years old	22	6 (27.3)	

\*Probability of Occurrence at Chance.

Pernambuco, respectively. There were significant statistical differences regarding sex ( $P = 0.014$ ) and age ( $P = 0.001$ ). In total, females presented with higher seroreactivity (32.9%) when compared to males (26.5%), and younger animals (1 to 5 years old) had lower seroreactivity (18.6%) than older animals (38.9%: 6 to 10 years old; 31.1%: 11 to 15 years old; 27.3%: 16 to 26 years old (Table 3).

## Discussion

The frequency (29.7%) of equines seropositive to *Leptospira* spp. in the present study was considered high, since they were animals destined for equestrian competition, theoretically being well cared for during rearing, although vaccination against leptospirosis was not a commonly adopted practice in the region. In addition, environmental factors that are adverse to the survival of the agent in the studied area, located in the Brazilian semiarid region, should be taken into account, as they may have negatively influenced seropositive frequency of exposed animals.

It was possible to verify a significant variation in the agglutinin titers, starting from the minimum 100 to 800. Usually antibody titers in equines remain in the range of up to 200 (Pilgrim & Threlfall, 1999), which also occurred in our study; 85.4% of the positive samples had titers  $\leq 200$ . Low titers, especially those  $\leq 200$ , may indicate either an adaptability to reactive serogroups or that these animals are in the chronic phase of the disease (Ellis, 2015).

The predominant serogroups in this study were Australis and Icterohaemorrhagiae, accounting for 66.5% of the positive reactions. We also observed that when the seropositive reactions were grouped among the 16 mesoregions studied, the serogroup Australis predominated in 10 of them and Icterohaemorrhagiae in six (Table 1). Martins & Lilenbaum (2013), analyzing studies performed in the last 20 years in Rio de Janeiro, found that the frequency of seropositivity varied between 27 and 42.9%, and that the main serogroups involved in the infection were also Australis and Icterohaemorrhagiae.

The variation in the occurrence of these serogroups is determined by the presence of reservoirs as well as by abiotic factors that directly influence the occurrence of the disease (Hamond et al., 2014). However, the serogroup Australis is the most common in equidae and is seen to be well adapted to this species (Ellis, 2015). According to Pinto et al. (2017), infections caused by incidental serogroups such as Icterohaemorrhagiae usually result in acute systemic disease, but when caused by the Australis serogroup, to which equines are adapted, the infections could be subclinical.

Reactions to the Australis serogroup (37.2%) predominated in the bordering mesoregions among the analyzed states, which are in a drought period most of the year. An exception is the Eastern Potiguar Region, where reactions were concentrated on the coast. Nevertheless, the frequency calculation in this region may have been influenced by the reduced number of cities and animals sampled (Table 1). It is worth mentioning that, although possibly adapted to equines, the serogroup Australis may still cause clinical disease in this species, and be a source of infection for other animal species (Pinna et al., 2008).

The Icterohaemorrhagiae serogroup, the second most frequent in this study (29.3%), has been widely reported in horses in tropical areas (Lasta et al., 2013; Teixeira et al., 2014; Dias et al., 2015) and is one of the main groups responsible for infections in humans (Oliveira et al., 2017; Polo et al., 2019). The serogroup stands out because of its high prevalence in equines in Brazil, especially in studies conducted in high density demographic regions and/or coastal areas (Teixeira et al., 2014; Dias et al., 2015). In this study, the occurrence of serological reactions to Icterohaemorrhagiae serogroup was observed in larger urban centers and coastal areas, such as the metropolitan area in Fortaleza and Mata Paraibana. This can be explained by inadequate sanitary conditions, as the main reservoirs of this serogroup are rodents (Alves et al., 2018), as well as the high humidity and rainfall, which favors contact between horses and urine (Hamond et al., 2012a).

There was higher seropositivity among females (33%) when compared to males (26.5%). These results reinforce the information reported by Langoni et al. (2004), Dashliboron et al. (2013), and Maleki et al. (2015), who verified that 61.4%, 58.3%, and 87.5%, respectively, of positive reactions in their studies were females ( $P < 0.05$ ). The greater frequency in mares can be attributed to the different management of females and males (Langoni et al., 2004). A possible hypothesis is that most male equines, especially those destined for sport, are castrated to make them more docile (Finger et al., 2011), are excluded from reproductive management, and when whole, most of the time are trained intensively, which reduces their contact with other groups and the possibility for contamination (Ellis, 1994). In turn, females are usually managed in large groups and actively participate in the reproductive sector of the estates.

We observed a higher frequency in animals older than six years when compared to younger ones (1 to 5 years old), a finding that coincides with Rocha et al. (2004), who attributed this higher occurrence to the longer exposure time of older equines to *Leptospira* spp. in the environment. The highest frequency of seropositive animals (38.9%) occurred in equines that were in the second age category (6-10), and in the older age group, the frequency tended to decrease to 27.3%, which can be attributed to a possible adaptation of the species to the serogroups to which they had been exposed throughout their lives. Dashliboron et al. (2013) found a significant relationship between aging and the prevalence of leptospiral infection ( $P < 0.05$ ); 4.87% for equines aged 1 to 3 years old, 20% for the 3-6 years old and 6-9 years old, and 0% in the group with animals older than 9.

Taking into account the significant level of occurrence of the Icterohaemorrhagiae incidental serogroup, it is important to analyze how the animals come into contact with rodents and how to proceed with the implementation of rodent control (Verma et al., 2013), adequate sealing of waste and waste depot sites, as well as periodic cleaning of feeders and drinkers used by the animals (Alves et al., 2016). However, for infections of the Australis serogroup, control

becomes more complex, since the main form of transmission is intraspecies infection (Ellis, 2015). General measures are indicated, such as avoiding conglomerations of animals and managing them in groups according to age and sex, allowing only the import of animals proven to be seronegative or who had been quarantined, monitoring of seropositive animals, correct disposal of sewage, placental, and abortion remains, and hygienic use of zootechnical equipment.

## Conclusion

The results of this study allowed visualization of the extent and distribution of leptospirosis infection in equines in the Brazilian Northeast region, as well as the most reagent serogroups. Intraspecies transmission seems to be the main route of dissemination of the disease in equines, although rodents are still important sources of infection. The introduction of hygienic-sanitary measures is suggested, as well as management of the animals in groups according to sex and age.

## Conflict of Interest

The authors state that they have no conflicts of interest to declare.

## Ethics Statement

The article was approved by the Research Ethics Committee (CEP) of the Center for Health and Rural Technology from the Federal University of Campina Grande (CSTR/UFCG) under No. 085/2017, and it was carried out in accordance with the technical standards for biosafety and ethics.

## Acknowledgements

National Council for Scientific and Technological Development (CNPQ) for the research grant. Veterinary Laboratory Diagnostics, Ltd. for providing equine sera, and Communicable Disease Laboratory (CFU) and its members for all assistance and support in the diagnostic analyses and in the preparation of the article.

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**Financial Support:** The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for the financial support (number 302222/2016-2).

**Authors Contributions:** Davidianne de Andrade Moraes participated in the planning, serology, analysis and spatialization of the results, and elaboration of the scientific text. Camila de Sousa Bezerra participated in the planning and serology. Denize Monteiro dos Anjos participated in the analysis and spatialization of the results. Bruno Cesar Nunes participated in the serology and revision of the manuscript. Denise Batista Nogueira participated in the serology. Nebson Fernandes Pequeno provided the serological samples. Diego Figueiredo da Costa participated in the serology and revision of the manuscript. Severino Silvano dos Santos Higino participated in the review of the manuscript. Sérgio Santos Azevedo participated in the planning, analysis of the results and in the review of the manuscript. Clebert José Alves participated in the planning, analysis of the results, elaboration and review of the scientific text.