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.
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 endangers public health (Pinna et al., 2008).

According to data from Notificação 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 (Artiu shin 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 semi-arid 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, Pyrogens, 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.

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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 ≥ 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    | BORBOREA | 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                  |                     | 177    |

M = metropolitan; ≥ 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.

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

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

| Serogroups          | 100  | 200  | 400  | 800  | TOTAL (%) |
|---------------------|------|------|------|------|-----------|
| 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.
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).

**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)           |        |
|          | Female            | 632              | 208 (32.9)           |        |
| Age      | 1 to 5 years old  | 516              | 96 (18.6)            | 0.014  |
|          | 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)             | 0.001  |

$^*$Probability of Occurrence at Chance.

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.

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